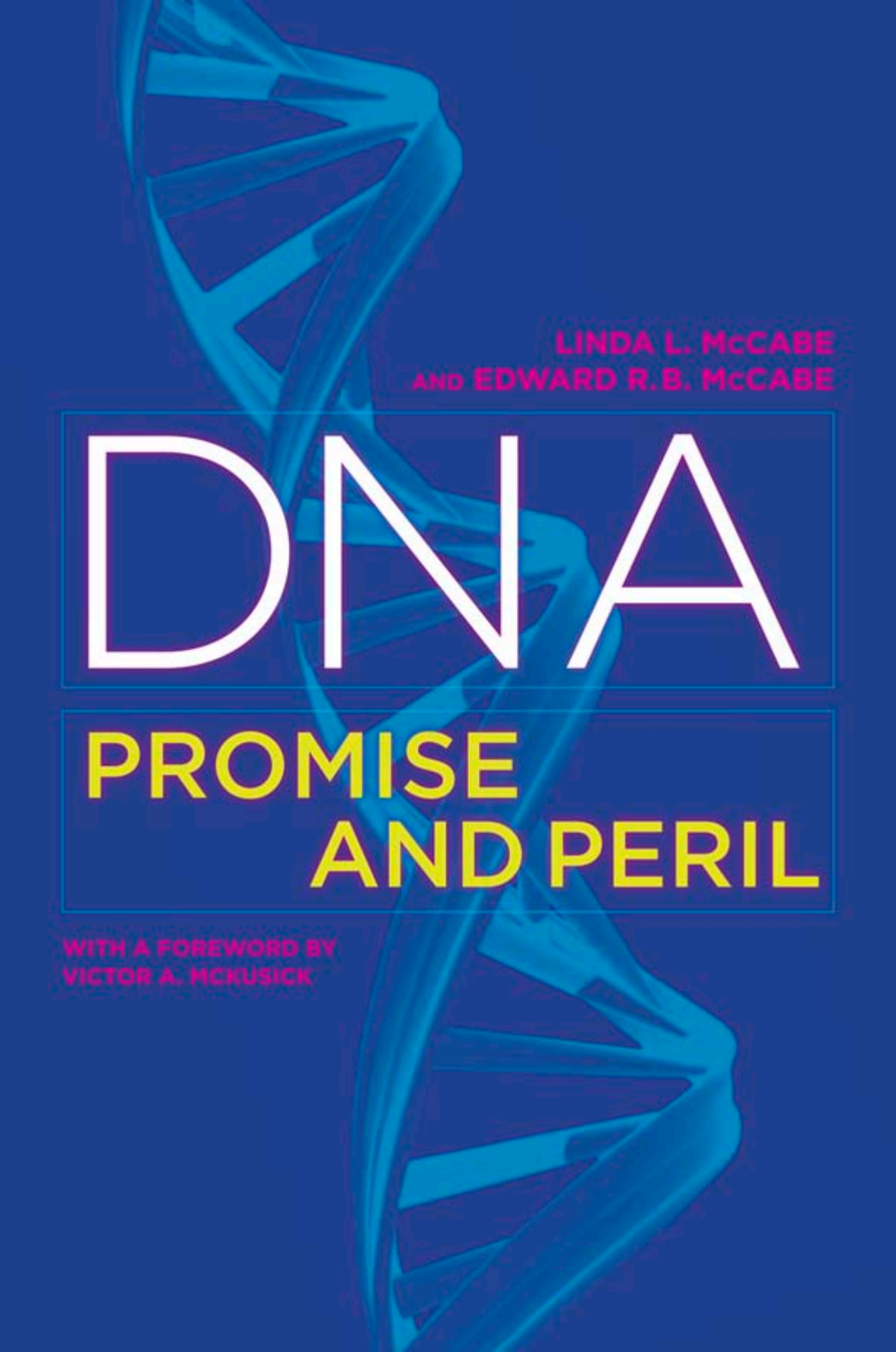

LINDA L. McCABE
AND EDWARD R.B. McCABE

DNA

PROMISE AND PERIL

WITH A FOREWORD BY
VICTOR A. MCKUSICK

DNA: Promise and Peril

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DNA: Promise and Peril

Linda L. McCabe
and Edward R. B. McCabe

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To Heather and Kevin

Contents

Foreword

Twenty years ago, when the proposed project to sequence the entire human genome was under consideration, both the promise and to some extent the peril were subjects of active discussion. The promise was seen to lie in the potential for deducing the way the human organism is formed and works. From that could come understanding of what goes wrong, leading to birth defects as well as ailments of later life. In turn, that understanding could lead to precise diagnoses, including prenatal diagnosis, prediction of late-onset disorders, and design of preventive measures, specific treatments, and even gene therapy. The promise captured the imagination of both the scientific and the lay community, leading to the public funding of the Human Genome Project here and abroad, with formal initiation of the National Institutes of Health (NIH) project on October 1, 1990.

The peril was discussed at greater length after the establishment of the Ethical, Legal and Social Implications (ELSI) Research Program as part of the NIH project. The Human Genome Project's first director, James Watson, determined that 3 percent of his budget (and more if necessary) should be devoted to the ELSI Research Program. Thus, beginning in 1990, research grant applications were submitted to the NIH not only by scientists for conduct of the technical aspects of the project but also by sociologists, legal scholars, science historians, philosophers, theologians, genetic counselors, and others for support of ELSI studies and conferences.

We are now in the postgenomic era. The Human Genome Project was

declared "completed" on April 25, 2003. The precise date was picked because it marked the fiftieth anniversary of the publication of the famous paper by James Watson and Francis Crick in the journal *Nature* in which they outlined the double helix structure of DNA. The McCabes give us a postgenomic evaluation of both the promise and the peril, particularly the latter. They examine how the information forthcoming from the Human Genome Project and related work in genetics might change one's concept of oneself. They also point out how the results of the project have changed the view of where humans fit into the entire biosphere. They call it the Copernican Revolution in biology, so called because it deconstructs the human-centered view of life, just as Copernicus deconstructed the earth-centered view of the universe. The genomes of many nonhuman "model" organisms that were sequenced in parallel with the human genome have demonstrated an evolutionary continuum in the DNA sequence over all forms of life, with the human exhibiting an "advanced" but not unique position.

Many of the perils reviewed by the McCabes were already recognized in the early days of ELSI. One was that, when the last nucleotide was determined, we would think we knew everything it means to be human. This followed from the twin fallacies of determinism (that the phenotype is hardwired to the genome) and reductionism (that resolution into all component parts is sufficient to understand the whole). We might come to think we know much more than we really know. The significance of weak associations between DNA changes and particular characteristics of single individuals or groups might be exaggerated, leading to discrimination and other problems.

The McCabes thoroughly trounce genetic determinism and at the same time reductionism. They make it clear that your genome, or any part of it, is not you. They point out, for example, that even identical (monozygotic) twins are not in fact identical, even though they share the same genome. Thus, human clones could not be expected to be identical. As an identical twin, I find the differences both interesting and informative. Some of these differences probably can be explained by chance (stochastic) variation in early development. This is illustrated by the differences between DNA "fingerprints" and dermatoglyphic fingerprints. DNA "fingerprints" are identical in monozygotic twins; dermatoglyphic fingerprints are, it seems, distinctive in all human beings, including identical twins. True, the dermatoglyphics of identical twins are more alike than those of ordinary siblings, but there are differences which are thought to be the result of stochastic events in the development of the

finger pads. Similarly, for example, in development of the brain, chance differences in cell migration may make differences in the eventual structure and function of the brain. At a "fork in the road," a particular neuron in twin A may go left, whereas in twin B it goes right.

The McCabes provide an insightful review of some of the leading societal and personal issues that have come to the fore with completion of the Human Genome Project and with advances in what in the past was called the New Genetics. The issues include gene patenting, discrimination in insurance and employment, use of assisted reproductive technology, pharmacogenomics for picking the right drug for the right patient in the right dosage, stem cells, therapeutic versus reproductive cloning, and so much more. Their presentations are interlaced with instructive vignettes. The whole makes a thought-provoking read.

Victor A. McKusick
Johns Hopkins University
April 2007

Acknowledgments

We wish to thank the many individuals who contributed to this work in ways that neither they nor we may ever remember. We know that we could never list all of you nor recognize your enormous input. For after all, this treatise represents very personal interpretations of the way in which the genomic initiatives of the late twentieth and early twenty-first centuries are influencing our consideration of who we are as individuals and population groups. Therefore, discussions with family, friends, colleagues, and students over the courses of our lives have undoubtedly influenced these interpretations.

While we are not able to thank everyone who has touched us, there are a few individuals and groups whom we wish to recognize. Sam and Alice Bessman, beginning early in our lives, forced us to think about not only the meaning of our scientific endeavors but also the relationship of our investigations to the scientific context and to society. In addition, they served as outstanding role models for the two-professional couple and the mutual respect essential to the successes of the individuals and the dyad.

We have known David Sadava, the quintessential professor, for almost thirty years, and our families have shared much over the years. David has influenced us through our many discussions, in which he always forced us to clarify our thinking and our arguments. On a visit to the Huntington Library, Art Collections, and Botanical Gardens, organized by David so that we could see the exhibition of illuminated manuscripts on the history of astronomy, he showed us Galileo's *Dialogue of the Two Chief World Systems* and commented on the amount of scientific and

philosophic history contained in the single illustration combining the images of Aristotle, Ptolemy, and Copernicus. During the afternoon's walk through the gardens and continuing discussions, it became evident that we are experiencing a similar revolution in our biological thinking (discussed in chapter 4). In addition, David has been generous with his time as a reviewer and with his support.

Tom Caskey and Art Beaudet welcomed us into their new Institute for Molecular Genetics at Baylor College of Medicine in 1986. Tom introduced us to the Human Genome Initiative, and Tom and Art led us through an exciting period in our professional and personal development at Baylor, which we still consider the "Camelot of Human Genetics."

Colleagues within the genetics professional societies, including the American Society of Human Genetics (ASHG), the American Board of Medical Genetics (ABMG), and the American College of Medical Genetics (ACMG), have discussed these issues with us formally and informally on many occasions. The influence of the members of the boards of directors and the officers of the ABMG and ACMG and their discussions, both formal and informal, cannot be underestimated. Similar input derives from the members of the Committee on Genetics and the board of directors and officers of the Section of Genetics and Birth Defects of the American Academy of Pediatrics. The considerable overlap of the individuals within these groups has led to a continuity of discussion spanning more than two decades.

The members and staff of the Secretary's Advisory Committee on Genetic Testing (SACGT) and Secretary's Advisory Committee on Genetics, Health, and Society (SACGHS), as well as those who made presentations to the SACGT and the SACGHS, also influenced the consideration of these topics. The intensity of the efforts, organized so capably by Sarah Carr, executive secretary of the SACGT and the SACGHS, have been tremendously influential. We must note, of course, that the opinions represented here are completely independent of the SACGT, SACGHS, and the federal government and are solely those of the authors.

Our colleagues at the University of California, Los Angeles (UCLA), including those at the Center for Society and Genetics (CSG), UCLA's chancellor emeritus Albert Carnesale, Norton Wise, Victoria Sork, Sally Gibbons, the planning group leading to CSG's formation, the CSG faculty, the CSG trainees, and our spectacular UCLA students have had a potency of effect that can never be fully appreciated. In discussions of the various themes we address in this book, everyone involved in CSG, and particularly our students, have broadened our perspectives and helped us

to more fully understand the issues that we consider here. Norton Wise identified the concept of coevolution of science and society, which is a central tenet of the CSG that we discuss in this book — the science does not drive changes in society or vice versa, but the two coevolve dynamically and inextricably. We thank Wayne Grody and Eric Vilain for their careful review of specific chapters. Special thanks are due to members of our spring 2006 and 2007 classes, who constructively edited earlier versions of the book. We also appreciate Don Ponturo's critical review and his help with the illustrations, and artists Lauren Forsythe and Genine Smith. We wish to acknowledge the support and hard work of Vince Romero and Lisa Calanni. Chuck Crumly, our editor at UC Press, has been a great support and advisor through this process. We wish to thank Victor McKusick for his thoughtful edits of the manuscript, and we are honored that he wrote the foreword.

Finally, to our family and friends who have asked us about genetics, the Human Genome Project, what it is that we do, and similar questions, we thank you for your candor as well as your inquisitiveness. Through your queries you have forced us to probe these issues more deeply and to present our discussions more accessibly.


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the print version of this title.]

CHAPTER 1

DNA Sequence Does Not Equal Destiny

Genetic determinism
The Dutch Hunger Winter
The Barker hypothesis

The concept that our DNA sequence — our genome — does not equal or predict our destiny has been extremely difficult for some geneticists to accept. We were attracted to this new field of molecular genetics in the 1980s because of the belief that disease-causing mutations would predict patients' futures. If geneticists could identify the genes responsible for their patients' diseases and the genetic changes or mutations in those genes, then, we believed, geneticists would be able to predict the clinical courses of their patients' diseases. Translating into individual terms, physicians would have the information from the laboratory analysis of a patient's specific mutation to inform the patient and his or her family whether they might expect, for example, a mild or a catastrophic clinical course. Many of us in the genetics community sincerely believed that DNA analysis would provide us with a molecular crystal ball that would allow us to know quite accurately the clinical futures of our individual patients.

We were committed to this belief in ways that would be life-changing. In 1986, we packed up our family, left the University of Colorado and our ranch outside Denver, and moved to Houston to be part of Tom Caskey's recently established Institute for Molecular Genetics at Baylor College of

Medicine. We made this move to learn how to identify the genes and mutations responsible for disease.

This was an exciting time in genetics, particularly at Baylor. Caskey's institute was growing rapidly from six to more than twenty investigators. Caskey was traveling the world, bringing back news about the new disease-causing genes that were being identified. We refer to this period at Baylor as the "Camelot of genetics," because if you had a reasonably good new idea, you could implement it. For example, we received funding from the National Institutes of Health (NIH) to create the Mental Retardation Research Center, which was developed around the idea of identifying the genes and mutations responsible for mental retardation and developmental delay. This center brought together faculty from many different departments at Baylor. They all joined enthusiastically because of the wonderful possibilities the research evoked. The faculty in this center would have the opportunity to be at the forefront of understanding diseases responsible for mental retardation at the most fundamental level — the DNA changes. Identification of these new genes and their mutations would give physicians novel insights into diseases previously understood only at a descriptive level. It was an exhilarating era in our professional careers and in those of colleagues at Baylor and around the world.

Caskey was involved in the Human Genome Initiative of the U.S. Department of Energy even before the NIH became the primary source of support and the effort was renamed the Human Genome Project. The discussions of the thrilling possibilities and phenomenal challenges in sequencing the human genome were incredible. The laboratory methods and informatics, or information-processing technologies, that were needed to complete the project did not yet exist. In addition, there were tensions between those who wanted to identify the entire genomic sequence and those who thought the early focus should be on genes associated with diseases.

Many geneticists believed that biomedical science would be revolutionized when the human genome sequence — the complete 3 billion base pairs of an individual's DNA — would become available. We will show you that there have been dramatic and truly revolutionary changes that have occurred as a result of the Human Genome Project. But we also intend to show that the rhetoric accompanying the sequencing of the human genome has been and continues to be excessive in its promises. Each of us has seen headlines and media reports attributing extremely ambitious results to this project — for example, "Human Genome: The Book of Life," and articles referring to the human genomic sequence as "the master blueprint of a human."

Such characterizations may have been intended as analogies to describe a complex subject in simpler terms. Too often, the result has been a perception that individual human futures are fully described in the sequences within each individual's genome, a concept referred to as genetic determinism. The Human Genome Project is a major triumph, but to suggest that investigators understand human life or have a blueprint for the construction of a human based on this sequence would be a wild overstatement. James Watson, the initial head of the NIH's Human Genome Project, stated, "The goal of the Human Genome Project is to understand the genetic instructions for human beings. . . . Getting the instructions is a big job; understanding those instructions can consume many hundreds of years."

It is essential to understand that the genetic instructions are not immutable, but quite plastic. An individual's unique experiences can permanently alter the expression of genes in that individual's genome. If this is the case, then it is easy to see how identical twins, with distinct experiences, may have very different patterns of gene expression and therefore different states of health and disease. Since identical twins are shaped by individual experiences, then the concept of a clone — be it human or another animal — as a "Xerox copy" of the original is also invalid.

Each individual is composed of complex and dynamic biological networks encoded by his or her genome. Environmental experience can influence those networks. If you think of those influences as pressures on the system, then you might anticipate that a push from the environment would be met with a push back from the genes that would be transient and would cease when the environmental pressure dissipated, or shortly thereafter. It is now recognized, however, that some environmental experiences may have specific and measurable effects that permanently and chemically modify specific genes within an individual's genomic DNA, resulting in lifelong alterations in the expression of these genes. These permanent alterations in gene expression are referred to as imprinting and can have serious health consequences that may not become obvious until some future time in one's life or the lives of one's offspring. This influence of the environment on expression of the genes in the genome is referred to as an epigenetic effect.

• • •

Observations of those who suffered through the Dutch "Hunger Winter" of 1944–45 demonstrated that, through imprinting, environmental

influences might program biological systems to behave in specific ways many decades into the future. Near the end of World War II, as Allied troops advanced across northern Europe in the summer and fall of 1944, quick liberation of the Netherlands was anticipated. However, when the Allied offensive stalled at the Rhine River, and the Dutch government in exile called for a strike of railway workers to support the Allies, a German reprisal banned all food transport to the Netherlands. This food embargo was in effect from October until early November 1944. By that time, however, the winter of 1944–45 had begun, the waterways were frozen, fuel was in short supply, and food could not be transported from the rural eastern to the urban western portions of the Netherlands.

From November 1944 until liberation from Nazi occupation by the Allies in May 1945, there was an incredible shortage of food in the western part of the Netherlands. Because of substantial rationing of food throughout the war, people did not have reserve stores of foodstuffs, and the famine was worse as a result. To survive, the Dutch even ate tulip bulbs, being careful to remove the bulbs' poisonous centers. Caloric restriction was extreme, compared with the typical adult requirements of 1,600 to 2,800 calories per day, depending on age, gender, and level of activity. Official daily rations for most adults fell from 1,800 calories in December 1943, to 1,400 calories in October 1944, to less than 1,000 calories per day by late November 1944. At the peak of the famine, the official daily rations for adults were 400 to 800 calories per day, well within starvation range. Although infants under one year of age were supposedly protected by an officially sanctioned ration that never decreased below 1,000 calories per day, there was an extremely high death rate from malnutrition and infection in the first year of life during the famine. From this tragedy, the Dutch Famine Birth Cohort was developed to study the effects of the famine on fetuses.

Individuals who had been exposed as fetuses to extreme undernutrition during the Dutch famine experienced increased risks to their health when they became adults. A glucose tolerance test measures the regulation of glucose uptake and metabolism, and adults who were still in the womb in the second or third trimester during the famine had abnormal glucose tolerance tests consistent with insulin resistance. Insulin resistance is seen in those with or at risk for adult-onset, or type 2, diabetes mellitus. The uterine environment had changed the expression of genes in their genomes, which led to the development of insulin resistance and a much higher incidence of type 2 diabetes in adulthood.

Additional adult health problems or predispositions have been noted in the Dutch Famine Birth Cohort. Fifty-year-old individuals who were exposed to the Hunger Winter when they were early in their gestation

had a blood lipid profile with increased LDL ("bad cholesterol") and decreased HDL ("good cholesterol"). This profile predisposed them to developing blockage in their arteries, including those of the heart (coronary arteries), referred to as coronary artery disease. Therefore, they were at increased risk for high blood pressure and heart attacks. Exposure to the famine in early gestation was also associated with an increased body mass index (BMI) indicative of obesity. The association of obesity, diabetes, abnormal blood lipid profile, and high blood pressure is referred to as the metabolic syndrome. It is intriguing that all of these features were observed in the Dutch Famine Birth Cohort.

The Dutch Hunger Winter came to a relatively abrupt halt when the Allied forces liberated the Netherlands. Some of the most striking findings were in those adults who had been small for gestational age at birth, consistent with starvation in the womb, and who then put on weight rapidly in early childhood following liberation. This phenomenon may have relevance for populations around the world who are emerging from disadvantaged economies and suddenly experience more abundant food resources. Individuals conceived in more calorie-restricted situations and then reared where food is more available may be at risk for a variety of common complex diseases in adulthood. They may develop individual disorders like obesity, diabetes, hyperlipidemia (high levels of fat in the blood), or cardiovascular disease; or they may develop all of them together, the metabolic syndrome.

Evidence from the Dutch Famine Birth Cohort also suggests that there may be intergenerational effects from the Hunger Winter. Investigators looked at a group of females whose mothers became pregnant with them during the famine and gave birth after the Allied liberation. When these individuals reached adulthood, they achieved normal height, but when they gave birth, their babies were small. This would suggest that the environment experienced by their grandmothers during the Hunger Winter could be influencing a second generation. Judith Hall, a prominent geneticist from the University of British Columbia, has pointed out that the egg that became you was developing in your mother when she was a fetus in your grandmother's womb. So there could be second-generation effects that are the direct result of influences on the developing egg, or ovum. Alternatively, there may be as yet unknown epigenetic influences that are maintained for many generations. Studying multigenerational effects will require accurate medical records, and it will require more than family lore. While we know some things about our mothers' environments during their pregnancies with us, we know almost nothing about the environmental experiences of our grandmothers during their pregnancies with our mothers.

The Dutch famine was a severe form of food restriction. While perhaps

not as striking, we would anticipate more subtle effects from less severe environmental influences. For how many generations the effects of such subtle influences on our genomes will persist has yet to be investigated.

• • •

These observations from the Dutch Hunger Winter are consistent with what has come to be known as the Barker hypothesis — that there are fetal origins of adult disease. David Barker, from the University of Southampton in England, hypothesized that many adult diseases arise from fetal environmental experiences. Barker was searching for archives that would provide detailed, accurate birth weights and living conditions from sixty years previously so that he could examine associations with cardiovascular health. With some luck and fortunate coincidence, he happened upon and gained access to records generated by an "army" of midwives organized and trained by Ethel Margaret Burnside in the county of Hertfordshire.

In his initial study in 1995, Barker was able to use the National Health Service Central Registry to find 15,000 men and women born in Hertfordshire prior to 1930. One-fifth had died. Nearly one-half of those died from coronary artery disease or related problems. Those with a low birth weight had a disproportionately large share of deaths.

The midwives returned when the babies were one year old to check on household conditions and weigh the infants again. Males who weighed 18 pounds or less at one year of age had a threefold increased risk of death from a heart attack compared with those who weighed 27 pounds or more. Those who were smaller at birth and had a slower weight gain in infancy might be at higher risk for cardiovascular disease.

Subsequent studies by Barker's team and others have confirmed the fetal origins of adult disease. The most concerning pattern of growth is low weight for gestational age at birth, slow weight gain in early childhood, and then a rapid increase in body weight. The increased risk for obesity, diabetes, hyperlipidemia, hypertension, and coronary artery disease has been confirmed in many different populations. In addition, other disorders have been proposed to have fetal origins, including kidney disease, lung disease, liver metabolic abnormalities, unipolar (depression or mania) and bipolar (depression and mania) major affective disorders, and schizophrenia. There is also growing evidence that experiences after birth may also have significant influences on the risks for adult disease.

The knowledge that adult diseases may have their origins in fetal exposures indicates that nurture can influence nature. These effects can be profound and long-lasting. It is important to recognize, however, that not all

individuals who were exposed to undernutrition in the womb developed adverse outcomes. These were epidemiologic investigations of groups of individuals and showed relative increases or decreases in risks associated with fetal environment.

The Barker hypothesis shows that two individuals conceived with identical genomes may have different fates after they are born and achieve adulthood. The concept of fetal origins of adult disease, or durably programming the reading of the genome in the womb, indicates that these eventual fates will be based on environmental exposures. Identical twins with identical genomes occupying the same womb could still experience different uterine environments, if, for example, the vascular delivery of nutrients differed between the twins. Clones developing in different wombs would be even more likely to have different environmental experiences and therefore would be less likely to have identical patterns of gene expression.

The plasticity of the genome would have been impossible to consider until very recently. However, evidence is accumulating that there are dynamic interrelationships between our genomes and our environments. The nature-nurture debates often seem to lose sight of the powerful and continuous interplay between these forces. Such plasticity and dynamism is exciting to watch unfold and argues ever more strongly against genetic determinism. Genetic imprinting provides scientific evidence for the biological uniqueness of individuals with identical genomic DNA, including identical twins and clones. It is not the sequence of the individual's genome that is important, but how it is read, and the reading of the genome is influenced by imprinting, or epigenetic environmental events.

• • •

In the chapters that follow we will explore the recent history of genetics and genomics and how the advances of the past and present may play out in the future. We will share with you examples of the thrilling and provocative insights that enable us to better understand population dynamics and the diseases of individuals and groups. Just as science and society are coevolving, the science is taking place in a social context and is influenced by the voices expressed in that context; we will introduce you to thought-leaders in these fields and examine their influence. We propose to be your guides through this journey and hope that you will find the trip as enjoyable, stimulating, and surprising as we have found the last thirty years in genetics and genomics.


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the print version of this title.]

CHAPTER 2

What Is Genomics?

In this chapter we will describe genomics and explore why it developed as a new discipline in science and medicine in the latter part of the twentieth century. Why did genomics capture the imagination of the population and the media so visibly in the early twenty-first century? Consideration of the scientific development and public visibility of genomics is essential to understanding how all of humankind may be influenced by genomics in what is being referred to as the postgenomic era—the period after the sequencing of the initial genomic DNA samples. In this exciting time, the ability to obtain an organism's DNA sequence is no longer speculative. Sequencing has become a powerful tool not only for biology and medicine, but also for the social sciences, such as psychology and anthropology.

• • •

Genomics is much more than the Human Genome Project; it includes the sequencing of genomes from many bacterial, plant, and animal

species. All of these data are essential to understanding the biological matrix in which humans are embedded and the roles humans play as a species and as individuals in this biology. We propose that the sequencing of the genomes of humans and other organisms is redefining perceptions of the relationship between any individual and other individuals or groups, as well as the concept of the position of humans in the biosphere.

So, then, what is genomics? The origin of the word *genome* is described by *Merriam-Webster's Collegiate Dictionary* as deriving from the German *genom,* which represents a compounding of *gen* (gene) and *-om* (chromosome). Its meaning has evolved from one complete set of chromosomes to all of the genes on those chromosomes, or one complete set of genes. More recently, with the Human Genome Project relying so heavily on the sequencing of the entire DNA complement of the genome, the term has come to include in its meaning the complete set of DNA in an organism, which in humans represents approximately 3 billion base pairs of DNA.

Nearly every cell in the body contains that individual's genome. Cells are quite complex and are not just a bag of liquid in which the chromosomal DNA and proteins float around. A eukaryote — the class of life to which humans belong — is defined as any organism in which the cells are partitioned into subcellular organelles, or compartments, including a membrane-bound nucleus. Two of these organelles contain DNA: the nucleus and the mitochondria.

For humans, as well as any eukaryote with a nucleus and mitochondria, the genome includes two types of genomic DNA, and it may be thought of as containing two separate and distinct genomes. The larger of these genomes, and the one usually thought of as *the* human genome, is the nuclear genome. Except for fully developed eggs or sperm, which are the reproductive, or germ, cells and have only one of each pair, twenty-three chromosomes (chapter 11), the typical human nucleus of a nongerm, or somatic, cell contains a pair of each chromosome, or a total of forty-six nuclear chromosomes.

The smaller of the two human genomes, or subgenomes — only about 16,600 base pairs (bp) in length — is the mitochondrial genome. Even though the smaller of these subgenomes is only approximately five-millionths of the size of the nuclear genome, this small circular DNA molecule is critical for the propagation and function of the mitochondria. Mitochondria are called the powerhouses of the cell and are essential for aerobic, or oxygen-based, energy metabolism. Those knowledgeable

about exercise are familiar with the concept of aerobic exercise, such as long-distance running, in which the muscles begin to use energy generated by the more efficient metabolic system in the mitochondria.

The mitochondrial genome is similar in structure to bacterial chromosomes. The mitochondria derived originally from bacterial endosymbionts. Bacteria were internalized in cells of an ancient, single-celled, evolutionary ancestor because of a symbiotic, or mutually beneficial, relationship. The internalization of these bacteria resulted in more efficient energy production than was available in the cytoplasm (the liquid gel surrounding the nuclei) and gave them a significant metabolic advantage. These ancestral cells containing the bacterial endosymbionts would develop into the eukaryotic cells of yeast, plants, and animals. Reflecting its origins, the genetic code of the mitochondrial genome shares some characteristics with bacteria and differs from the nuclear genome. Each mitochondrion contains many mitochondrial DNA molecules, and each human cell contains many mitochondria. Since each human cell has hundreds of mitochondria, it has thousands of copies of mitochondrial DNA.

With the human genomic sequence so large, extremely small error rates in sequencing would result in a surprising number of errors. For example, an accuracy of 99.9999 percent, which is incredibly accurate by any medical testing standard, would translate to an error rate of one in a million. That would be three thousand errors in a 3-billion-bp genome.

The ability to study the totality of DNA and all of the genes in an organism was recognized as a powerful tool in biology. Investigators proposed that the comprehensive investigation of other cellular components would yield similar power and should be undertaken. The interrogation of each of an organism's RNA transcripts, proteins, and metabolites has been given a separate name derived from the root word *genomics.* Since these areas are derivative, we will use the term *genomics* as an all-encompassing one.

A major difference exists between genomics and its derivative disciplines, dramatically increasing the complexity of the latter. The genome of an individual is essentially identical or nearly identical in all cells in that individual. Such is not the case for the RNA, proteins, and metabolites. The complement of molecules in each of these groups will differ not only among tissues, but even for individual cell types within tissues. There are differences among cells belonging to the same cell type but at different positions within the tissue. For example, being closer to or farther from a blood supply affects access to oxygen and other nutrients. In addition, these other components will differ for the same cell type at different stages

of development and under different external environmental influences. If the size of the human genome is impressive, then the amount of information that will be required for a full accounting of human RNAs, proteins, and metabolites will be truly enormous. The magnitude of the effort needed to apply these investigations to personalized health care (chapter 15) will be astounding.

The technical approaches to acquiring these data, and the information systems needed to accumulate and track all these data, are under development. As was true with the Human Genome Project, the informatics infrastructure, including computer data storage and analysis, will be as critical as the laboratory technologies to the interpretation, comparison, and understanding of the information. Raw data are not sufficient — it would be like trying to understand a text if we had only the computer code with which it was constructed.

• • •

Some have referred to the twenty-first century as the "century of biology." Humans are an egocentric and controlling species, and the nineteenth and much of the twentieth centuries saw people attempting to conquer the physical environment. Individuals sought to control waterways by dredging channels and building dams. They expanded farms by draining wetlands and felling forests on massive scales. They bridged chasms and built roads and railroads up the sides of mountains. The growing knowledge of physics fueled these engineering feats. People were able to split atoms and build lasers. They developed satellite communications and sent humans into space and to the moon. Humans sought to control nature for their own benefit by applying fundamental physical science to master the environment.

Everyone learned, or should have learned, that information and speculations were frequently incomplete. Unintended consequences were the penalties paid for technological blunders. For example, ecological systems could be drastically changed by deforestation and flood-control projects. Humans are now going back and undoing engineering efforts that may have taken decades to plan and execute. As one example, major efforts are under way in the area of wetland restoration. Anticipated benefits are not only ecological but also societal, because wetlands are natural buffers that protect areas from flooding, and their preservation and restoration save lives and property.

The twentieth century saw remarkable advances in biology and med-

icine. Researchers began to understand the workings of bacteria. They were able to identify and design antibiotics to target disease-causing organisms with little or no damage to the human host. Other investigators began to understand cancer, though much of cancer chemotherapy is still a brute-force attack. Cancer therapies are toxic to normal cells, but somewhat more toxic to the cancer cells. More recently, however, detailed analyses of these processes have facilitated the development of "smart drugs" that target the biology of the cancer cells with much less toxicity to the patient.

In the past, humankind sought to control its destiny by reordering the physical environment and energy options with engineering interventions. People now have the desire to be in command of their own biology using tools that undoubtedly will appear very rudimentary when those in the future look back at our early twenty-first-century attempts. The very assertion of a desire to control the biological destiny of humans denies the complexity of biology, indicates a naive assessment of the state of our biomedical understanding, and is a tacit assumption of genetic determinism. Some have attributed the collective wish of humans to play a greater role in their biomedical future to a basic human drive for autonomy. Extrapolation from experience with past mammoth engineering feats would suggest that this autonomy is being driven by fundamental human traits of egocentricity and control.

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It is always difficult to establish a beginning for an effort as large as the Human Genome Project. The formulation of fundamental principles governing the genetics of plants and animals were critical for the establishment of this discipline. In 1865, an Augustinian monk, Gregor Mendel, reported his experiments with peas and described what are now known as the "laws" of Mendelian inheritance. Mendel's meticulously reported data remain controversial, however, because his results were unaccountably so close to the theoretical calculations based on his laws. Mendel's work was not utilized during his lifetime and was rediscovered in 1900. While not all inheritance follows Mendelian patterns, these principles remain fundamental to our understanding of genetics across plant and animal species, and of inherited disorders, more than a century later. A Mendelian inherited disorder is still described as dominant — only one mutant gene from one parent required — or recessive — requiring a mutation in the gene from both parents.

Archibald Garrod, who was knighted for his work in 1918, described a rare familial disorder, alkaptonuria, as an "inborn error of metabolism" in 1902. The urine of individuals with this disease changes color from the normal yellow to brown and even black when exposed to air. Patients also develop arthritis due to an associated deposition of dark pigment in cartilage. Garrod observed that the inheritance of alkaptonuria followed the rules for a recessive disorder described by Mendel, the first such description of a human disease. Garrod also formulated the concept that each individual has unique and distinguishing genetic characteristics based on metabolic variation that he referred to as "chemical individuality."

Mendel laid down the principles of heredity, and Garrod showed that these principles applied to humans. The structural and chemical mediation of these principles needed to be established. Early in the twentieth century, chromosomes and genes were recognized as the units of heredity. In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty showed that DNA was the hereditary material by demonstrating that it mediated the transformation of cellular properties when it was introduced into cells.

In 1953, working in the Cavendish Laboratory at Cambridge University, James Watson and Francis Crick reported the double helical structure of DNA. Their description, though very brief, was a tour de force in structural biology that included X-ray diffraction of crystallized DNA. They recognized from the outset that the DNA structure reflected function, stating, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

In 1962, Crick, Watson, and Maurice Wilkins shared the Nobel Prize in Physiology or Medicine for their work to solve the structure of DNA. Some feel that Rosalind Franklin, who obtained the X-ray diffraction images that provided the initial experimental evidence for the helical structure of DNA, was never credited appropriately for her contributions. Wilkins and Franklin worked at King's College, London, but never collaborated effectively. In 2003, Watson wrote that Franklin focused on data collection and careful analysis, while Wilkins favored speculation and modeling. Wilkins showed Watson the X-ray photographs of the more heavily hydrated B form of DNA taken by Franklin's group. This gave Watson the critical information about DNA's crystal structure, since the cross pattern of the reflections immediately indicated to him that the structure of DNA was a helix.

Franklin's work, shown to Crick and Watson without her knowledge,

was critical to their formulation of their model. Franklin has been described as "the dark lady of DNA" and the "wronged heroine," and it is thought most likely she knew of the use of her photographs but did not complain. Ever the empiricist, when she saw the famous Watson and Crick model, Franklin is reported to have commented, "It's very pretty, but how are they going to prove it?"

In 2003, Watson wrote that Franklin and Crick had become close friends and colleagues before Franklin died from ovarian cancer in 1958 at the age of thirty-seven. The Nobel Prize is not given posthumously, and the award to Crick, Watson, and Wilkins in physiology or medicine was not made until 1962. Therefore, Franklin could not have shared in the prize even if her contribution had been recognized by the Nobel committee. The committee will not split a prize more than three ways, and Watson speculated that if Franklin had been alive in 1962, the Nobel committee would have needed to choose between Franklin and Wilkins. Alternatively, they may have awarded Franklin and Wilkins the 1962 Nobel Prize in chemistry, with Watson and Crick sharing the prize in physiology or medicine.

How did the double helix become such an iconic symbol for the field of genetics? As noted by Robert Olby in the journal *Nature* in 2003, the debut of DNA's structure was remarkably quiet. Many have argued that it was the personality of its protagonists, particularly Watson, that generated the visibility required of an icon. Soraya de Chadarevian noted in the journal *Science* in 2003 that the story, for which the famous iconic photographs were taken by Anthony Barrington Brown in May 1953, was not published in *Time* magazine and the negatives were returned to the photographer. Brown stated that he found the model to be neither impressive nor photogenic, and that is why he staged the shots with Watson and Crick. There was little interest in these photographs until the publication of Watson's book *The Double Helix* fifteen years later, with all of its insider intrigue to bring human interest to the research and the images. Therefore, the reason why we celebrate the double helix as an iconic symbol is in part science, but also in no small part personality.

Watson has been referred to as a "rock star of science." He even has his own bobble-head doll with the double helix in place of a rock star's guitar. On one of his visits to UCLA, Watson was mobbed by students seeking his autograph in books, on pictures, and even on their lab coats. Watson's persona made him a strong and effective advocate for the Human Genome Project.

Despite having ascertained the structure of DNA in 1953, it took until

1955 to determine the correct number of human chromosomes in which the DNA was packaged. Prior to that time everyone who had looked at human chromosomes had counted forty-eight. In 1955, Joe Hin Tjio finally counted the correct number: forty-six chromosomes in somatic cells. This history of the erroneous human chromosome number shows that too many of us see only what we are told we should see — we do not question prior authority. We have seen this more recently in the estimates of the number of genes in the human genome. A decade ago it was guessed at 50,000 to 100,000. This estimate supported our belief in our higher complexity compared with other organisms, to which we ascribed lower numbers of genes (chapter 4). The number of genes has fallen steadily with the completion of the Human Genome Project, and current estimates are 20,000 to 25,000 genes.

Creation of the tools for the implementation of the Human Genome Project began in the 1970s and 1980s. In the mid-1970s, Frederick Sanger, Allan Maxam, and Walter Gilbert developed methods for rapid DNA sequencing. In 1982, GenBank, the first genomics research database containing DNA libraries from individual human chromosomes, was established as a publicly accessible resource based at the U.S. Department of Energy's (DOE's) Los Alamos National Laboratory in New Mexico.

The polymerase chain reaction (PCR) was essential for amplifying, or copying, genomic DNA to facilitate sequencing, determining the order of the nucleic acids (A, C, G, T). PCR was invented in 1983 and reported in 1986, and its inventor, Kary Mullis, shared the 1993 Nobel Prize in Chemistry. PCR uses two primers that are complementary to and flank a target genomic DNA region, referred to as template DNA. It is a chain reaction because the product of the one cycle becomes the template for the next cycle, and the number of copies of the DNA in the region of interest increases exponentially:

Copies of Product = (Initial copies of double-stranded template) (2^N), where N is the number of PCR cycles.

If one started PCR with a single copy of double-stranded DNA, then after twenty cycles there would be more than one million copies of the target DNA. PCR, automated DNA sequencing, and yeast artificial chromosomes for large-insert genomic clones provided the technological tools for consideration of the sequencing of the human genome.

These methods alone would have been insufficient for the Human Genome Project. Automated technology was essential for individuals to begin to conceptualize science on the scale that would be required to

sequence a human genome. Leroy Hood had a major impact by developing the technology necessary for this project, including the automated DNA sequencer and synthesizer. Both of these replaced extremely labor-intensive and slower manual laboratory methods. The role of the sequencer for the Human Genome Project is clear, but perhaps less obvious is the role of the synthesizer. In order to achieve PCR amplification of DNA, the primers need to be specific for sequences flanking the region of interest. Therefore, there is a constant need for synthesis of new PCR primers. The development of automated robotic equipment to facilitate genomic sequencing was absolutely necessary for the stage to be set for the Human Genome Project.

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Early champions of sequencing the human genome were Robert L. Sinsheimer, Renato Dulbecco, and Charles DeLisi. Less well known is the role of Victor McKusick, from Johns Hopkins University, who first proposed the complete mapping of the human genome in 1969. McKusick had begun to catalog human genetic diseases in his book *Mendelian Inheritance in Man,* first published in 1966. He saw the immense benefits that would accrue to medical genetics if more complete knowledge of the human genomic sequence could become available.

Sinsheimer was chancellor at the University of California, Santa Cruz (UCSC), when in 1984 he proposed to the president of the University of California that UCSC develop an institute to sequence the human genome. He understood the powerful resource that this sequence would provide for biology and medicine. Sinsheimer's proposal was not funded but did result in a 1985 workshop to discuss his idea. The leaders in molecular genetics who attended the workshop became convinced that this project was feasible and important.

Independently, without knowledge of Sinsheimer's proposal or the UCSC workshop, Dulbecco, Nobel Prize–winning cancer investigator then at the Salk Institute in San Diego, saw the advantage that the human genome sequence would bring to cancer research. He began to discuss his vision in public lectures during the autumn of 1985. His commentary on this topic for *Science* in 1986 is considered the first real public presentation of the concept of a human genome sequencing project.

Because of its involvement with nuclear energy, the DOE was interested in the effects of radiation on DNA and protection against radiation-induced DNA damage. When DeLisi became director of the DOE's

Office of Health and Environmental Research in 1985, he began to propose the complete sequencing of the human genome, initially without knowledge of Sinsheimer's or Dulbecco's efforts. DeLisi was a computational biologist who was originally trained as an experimental physicist. He worked on the first mathematical modeling of RNA structure as a postdoctoral fellow and then joined a group at Los Alamos that used mathematical approaches to address biological problems, specifically in immunology. Subsequently, he moved to the National Cancer Institute, where he founded a section in theoretical immunology. Early in the 1980s, DeLisi began to recognize that the emerging problem in DNA sequencing was data analysis. He developed algorithms involving computationally intense approaches to detect regions of functional DNA. After ten years, DeLisi returned to the DOE, where he became a strong proponent of sequencing the human genome. He had the vision to understand the importance of mathematics to the success of the project. He also understood the culture of "big science," involving large teams working in an extremely goal-directed, product-oriented fashion. DeLisi was a successful advocate, and the DOE funded a nascent Human Genome Initiative in 1987 with involvement of the National Laboratories and academic institutions.

Congress authorized funding in 1988 for the DOE and the National Institutes of Health (NIH) to pursue the feasibility of this initiative. James Watson was hired by the NIH to direct the Office of Human Genome Research, which became the National Center for Human Genome Research (NCHGR). Watson's stature within the scientific community and with the public made him a persuasive spokesperson for the Human Genome Project, which was officially launched in 1990 with an anticipated duration of fifteen years. The very conceptualization of this project represented extreme confidence in innovation, because the tools to complete the project did not exist at the time.

This sequencing effort was not limited to the United States. In 1987, Italy initiated a human genome program that is reported to have had its origins in a lecture given by Dulbecco to the Italian Embassy in Washington, D.C., on Columbus Day in October 1985. The USSR began to discuss the possibility of a human genome sequencing project in 1987. Approved in 1988, the project was one of the few biology programs to survive the dissolution of the USSR. Additional efforts began in the United Kingdom, France, the European Community, Japan, and Canada. Coordination of efforts began with Latin American investigators collaborating with each other as well as scientists in North America and

Europe. The United Nations Educational, Scientific, and Cultural Organization (UNESCO) subsequently established a program for international genome coordination. In 1988, the International Human Genome Organization (HUGO), founded by Victor McKusick, had its inaugural meeting in Switzerland. The HUGO committees, organized by individual chromosome, had hearings in which investigators presented their new genes and the diseases they wanted to be assigned to that chromosome. If a researcher was successful, then there was discussion of the nomenclature, with HUGO being the final arbiter of the name. The hurly-burly of genomics at these early meetings was quite a commotion from which order seemed to emerge mysteriously.

Publicly funded biomedical research was typically hypothesis-driven and investigator-initiated and was usually carried out by relatively small laboratory groups. The Human Genome Project was goal-directed, centrally driven "big science." The Human Genome Centers in academic institutions began to look more like factories than laboratories. There were enormous rooms containing many replicates of the various pieces of equipment required for robotic sample handling and automated sequencing. The volumes of data generated in these sequencing centers required monumental advances in bioinformatics, including new software programs to analyze sequence data. In fact, many feel that the informatics contributions of the Human Genome Project may be more impressive and enduring than the laboratory bench sequencing technologies, which are already beginning to change to reduce sequencing costs.

The "big science," the goal-directed central coordination, and the finite fifteen-year lifespan were features of the Human Genome Project that led to considerable discussion and controversy among academics. Some faculty members expressed serious concerns about the coexistence of these sequencing centers in academic institutions with their traditional educational and hypothesis-driven research missions. Others stated that postdoctoral fellows trained in these sequencing centers would be mere "cogs in the wheels" of these mammoth efforts and would not have job security once the project was completed in 2005. The perceived siphoning of NIH money from investigator-initiated research grants to the Human Genome Project was also a source of criticism.

Some major academic institutions made conscious decisions to avoid any involvement with the Human Genome Project. Extremely bright and talented individuals who are innovative leaders in their own fields still asked at the end of the twentieth century why a research university would want to be involved in genomic research. We responded that an

institution should engage in genomic research only if it wished to be a part of the future — sequence-based medicine.

Whether or not the Human Genome Project would be successful was still unknown in the early 1990s. Sequencing remained extraordinarily slow and enormously expensive. The initial strategy involved extensive physical mapping to have the large-insert clones aligned for the time when high-throughput sequencing would be feasible.

When the Human Genome Project was proposed, the cost of DNA sequencing was approximately $10 per base pair. The goals established for the project were 500 million base pairs per year and a cost of less than $0.25 per base pair, and the goals for both sequencing volumes and costs were surpassed. By November 2002, the sequencing volume exceeded 1.4 billion base pairs per year, and the cost was less than $0.09 per base pair.

Despite the magnitude of the effort, individuals were ready to align their futures with this project. In 1992, two major figures in genome sequencing emerged. Francis Collins left the University of Michigan to direct the federal NCHGR, which would be elevated to the National Human Genome Research Institute (NHGRI) by the NIH in 1997. And in 1995, Craig Venter left the NIH to establish the Institute for Genomic Research (TIGR — pronounced *tiger*), which would eventually sequence a number of bacterial genomes beginning with *Haemophilus influenzae,* which causes pneumonia and meningitis.

In 1998, Venter formed a new company, eventually named Celera Genomics, with its business plan to complete the sequence of the entire human genome within three years using a strategy different from that of the NHGRI. The NHGRI approach was termed hierarchical shotgun sequencing. Hierarchical shotgun sequencing assembled contigs — contiguous DNA fragments — to cover an entire chromosome. Individual contigs were shredded randomly — hence the shotgun analogy — and individual shredded fragments were sequenced. Overlaps at the ends of these fragments allowed assembly of the sequences of the individual components of the contigs and the assembly of the sequences of the chromosomes from the contigs. With this approach the genome was deconstructed into a physical map made up of the contigs and reassembled through this hierarchical, organized structure.

Celera's new strategy, termed whole genome shotgun sequencing, had been used successfully by TIGR for microorganisms. With whole genome shotgun sequencing, the chromosomes are shredded and the fragments are sequenced. The sequences of the overlapping fragments are used to assemble the chromosome. Whole genome shotgun sequencing had

been rejected by the NIH as unfeasible for the size and complexity of the human genome. The NIH's concern about this nonhierarchical approach was that the similar repetitive sequences throughout the human genome would make assembly of the complete genome difficult and would lead to errors in the assembly. Celera's sequencing efforts would become known as the "private project," in contrast with the "public project" that represented an international consortium including, in the United States, the NHGRI and its funded sequencing centers.

The public project responded to the competition introduced by Venter and Celera with reorganization and refocusing of the large-scale sequencing activities within five centers: Baylor College of Medicine in Houston, Texas; the Joint Genome Institute of the DOE in Walnut Creek, California; the Sanger Centre in Cambridge, England; Washington University in St. Louis, Missouri; and the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts. However, while the large-scale sequencing efforts were focused in these institutions, the public consortium continued to include a total of twenty centers in six countries.

On June 26, 2000, the announcement of the draft sequence brought Collins and Venter together with President Clinton in the White House. This historic ceremony was conducted live and simultaneously with Prime Minister Tony Blair in Downing Street and other colleagues internationally. As those of us attending assembled outside the east entrance, the energy and anticipation were palpable; we knew that we were about to participate in an event that would have lasting importance as a milestone in genomics. Victor McKusick was there with his constant companion and wife of more than fifty years, rheumatologist Anne McKusick, about to see his idea from more than thirty years earlier celebrated at the highest levels. Hamilton Smith, a senior molecular geneticist who shared the Nobel Prize in 1978 for identifying bacterial restriction enzymes, had joined Venter, first at TIGR and then at Celera Genomics. He was surrounded by young scientists also from Celera. They were in an extremely festive mood with lots of laughter and photographs. The group that had been considered the mavericks of the Human Genome Project would see their leader, Venter, share the stage with the president of the United States and Collins, the U.S. director of the public project. In the East Room of the White House, President Clinton noted that nearly two hundred years earlier Thomas Jefferson and Meriwether Lewis "spread out a magnificent map" that was the product of the "courageous expedition across the American frontier" and "defined the contours and forever

expanded the frontiers of our continent and our imagination." We were being told that we were all present at an event that had the long-term value and impact of the Lewis and Clark expedition, in fact a "map of even greater significance."

The publication of the approximately 90 percent completed draft sequence in February 2001 by the public and private projects in *Nature* and *Science,* respectively, were received with additional fanfare and controversy. The release of the "complete" human genome sequence occurred in April 2003 to coincide with the fiftieth anniversary of the Watson and Crick publication of the double helix structure in 1953. Celebrations spanning several months were held in many countries and included schoolchildren, patient advocacy groups, and members of the research community. Consistent themes were how far science had moved from solving the structure of DNA to sequencing the entire 3-billion-bp human genome, the future promise of genomic medicine, and the ethical, legal, and social issues raised by the availability of this sequence.

We can give a very personal view of the acceleration of biomedical research brought about by the Human Genome Project, and the associated advances in technology and bioinformatics. As the sequence-coverage of the human genome became more complete, the amount of labor required to identify a new gene decreased dramatically. One area of our research focuses on genes in the midportion of the short arm of the X chromosome adjacent to the gene for muscular dystrophy. It took seven years (1986–93) and at least fifty person-years (a person-year is the amount of work done by one person during one year) for our laboratory to identify the gene for glycerol kinase. A mutation in this gene causes a disorder that can lead to coma and death if not diagnosed and treated. It took two years (1992–94) and five person-years for us to identify the gene that when mutated leads to adrenal hypoplasia congenita, or underdevelopment of the adrenal cortex, the tissue that makes stress hormones. Adrenal hypoplasia congenita can lead to death from the stress of a cold or flu if not diagnosed and treated. It took only one ten-week quarter in 1998 for a graduate student on a first-year lab rotation working with a postdoctoral fellow (0.2 years and less than 0.4 person-years) to find a gene that causes mental retardation when mutated. In fact, the graduate student identified the gene *in silico* — by computer, using the Human Genome Project's databases — during her first week on the rotation, and she spent the remaining nine weeks characterizing the gene.

Our anecdotal experience gives us a rough calculation of the acceleration of research productivity as a result of the remarkable increase in

sequence information from the Human Genome Project. Progress has been accelerated by orders of magnitude: roughly tenfold in the early 1990s and another tenfold by the latter 1990s, or by a factor of one hundred in the first eight years of the Human Genome Project before the really impressive acceleration of sequence acquisition had been achieved.

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The Human Genome Project is having an enormous impact by changing the nature of gene identification and disease diagnosis, and even the culture of biomedical research. The evolution of its influence will undoubtedly result in an even broader reach as we will explore in the subsequent chapters.

The Human Genome Project has been called the "moon-shot of biology." One analogy is the confidence from the outset that the requisite technologies would be developed in the course of the project. But the analogies run much deeper. The space program required processing and coordination of flight data in real time with previously unprecedented amounts of information. The computers had to be light enough to be carried into space. At least initially, payload requirements helped to drive the miniaturization of computers from room-sized operations to the present guidance systems for passenger airliners with the capability of hands-free takeoffs and landings. The space program impacted our lives with ever more powerful and lighter laptops and palmtops, and in ways we do not even realize, including computer-driven applications in our cars and homes.

The growth in computing has been exponential. Rapid improvements in DNA sequencing will continue to be seen, according to George Church of Harvard University and his colleagues. Moore's law, or the Kurzweil/Moore law, states that computer processor speed, measured as instructions per second per U.S. dollar, doubles approximately every eighteen months. Sequencing speed as a function of cost is also growing exponentially, measured as the number of base pairs of DNA accurately sequenced per U.S. dollar. The trajectory for sequencing somewhat parallels the curve for computer speed, at least in part because sequencing speed is heavily dependent on advances in computing and bioinformatics. Church and colleagues have argued that open source technologies can spread rapidly through an established infrastructure and therefore have the potential to accelerate innovation quite impressively, seen, for example, in the increase in Internet Web sites. The open source innovation in

sequencing that they cited was a novel technology using PCR amplification in a dense gel that gives a "colony" of DNA used for sequencing and is called polony (contraction of polymerase colony) sequencing. Measuring the advances in this sequencing technology in base pairs per minute, they observed an almost vertical rise in the sequencing rate with a doubling time of only one month. Using microbeads and the polony strategy, this group has reported sequencing the genome of the common bacteria *E. coli* with less than one error per million bases and a cost reduced to nearly one-tenth of that for conventional sequencing.

Genomics is already influencing the fundamental practice of medicine (chapter 15). Medicine is becoming sequence-based. For more than a century, microbiology laboratories relied on the culture of microorganisms. The rapid diagnosis of herpes encephalitis, a viral infection of the brain that is potentially devastating and for which early treatment is effective, no longer requires a brain biopsy and now relies on nucleic acid amplification by PCR from cerebrospinal fluid obtained by a spinal tap. Confirmatory follow-up for positive newborn screening tests for sickle cell disease and cystic fibrosis have been improved by DNA-based methodologies. Companies are developing sequence-based technologies for point-of-care diagnostics. They anticipate portable devices that will permit home genetic testing. The technology will be there, but the targets for marketing must be defined. Direct-to-consumer marketing has been initiated for breast cancer testing using the *BRCA1* and *BRCA2* genes. There are many sites on the Internet that claim to provide DNA-based personalized care ranging from nutrition to skin creams.

The Human Genome Project has changed the fundamental culture in biomedical research to make it more open. Early in the public genome project, the participants began to discuss the need to share reagents and information. The proponents of openness argued that the comparatively brief time frame for this massive endeavor demanded that there be no barriers to collaboration or data access. They recognized that their collective success would rely on interdependence, and they could not function as effectively or efficiently if they were competitive independent investigators.

A meeting of the international collaborators involved with the public project was held in February 1996 in Bermuda. Open access to the genomic sequence was formalized. The "Bermuda principles" included release of all data from the sequencing centers into publicly accessible databases within twenty-four hours of acquisition. These data would include errors and would not be considered "finished sequence," but it

was felt that the benefits of rapid accessibility outweighed any risks associated with unconfirmed data.

Celera, as a private company founded with a business plan that relied on marketability of proprietary sequence for its capital, did not agree to the Bermuda principles. This led to significant controversy around the publication of Celera's report on its draft sequence in February 2001. The journal *Science* had a policy that required data cited in any articles that it published to be freely available. Celera, however, refused to accept this requirement, and *Science* agreed to publish Celera's article anyway. The publication of the public and private projects' draft sequences were to have occurred simultaneously in *Science.* Lack of public release of the Celera data was at least in part the reason why the public project reported its results in *Nature.*

James Shreeve wrote a book titled *Genome War: How Craig Venter Tried to Capture the Code of Life and Save the World*. On May 10, 1998, Venter, then with TIGR, announced that he would found a company, Celera, that would use a different, much faster approach to sequencing the human genome than that used by the public project—and would complete the project in 2001, four years ahead of the proposed completion for the public project. This fight cast as protagonists the vociferous maverick Venter against the quieter academic Collins and included the controversy surrounding the publications in *Nature* and *Science* in February 2001.

In March 2003, despite the preparations made by both the public and the private genome groups to announce successful completion of the sequencing in time for the fiftieth anniversary of the Watson and Crick double helix, vigorous debate continued in the pages of the *Proceedings of the National Academy of Sciences.* There, in a point-counterpoint exchange, leaders in the public project continued to question the fundamental strategy that Celera had used for its sequencing, arguing that Celera could not have been successful without the data from the public project. Leaders from Celera asserted that their work was indeed independent of the public project. In our own research, we found identical errors in the public project's and Celera's databases, suggesting an unacknowledged interdependence.

This rivalry, perceived as epic by those close to the action, had all the elements of successful fiction. In fact, Paul Mullin wrote a play, *The Sequence,* based on the open and contentious rivalry of the protagonists as told by a fictional science reporter. Collins attended the premiere reading of the script and described the experience as "totally spooky." John

Russell, reviewing the play for *Bio-IT World,* noted: "Spookier still was the April 26 [2005] staging of the performance, the same day that Celera announced it was abandoning its dwindling business of selling access to its proprietary sequence information. Celera will now donate much of its remaining data — about 30 million base pairs — to the public domain."

Let's look briefly at some of the battles within the genome war. In 2002 Venter was replaced as Celera's CEO. It was a bold move to fire the founder of the company, who had invested so much of his personal energy as well as his own DNA in the private genome project and who is credited with accelerating the completion of human genome sequencing by at least three years. Venter has even questioned whether Celera was supposed to be successful. Celera maintains that its business plan was already eroding with progressive loss of value for its sequence database due to the increasing information available in the public databases. Celera's focus shifted to drug development as it moved from control of intellectual property in the form of sequencing information (chapter 9) to application of this information in the field of pharmacogenomics (chapter 15).

Another, less visible battle was being waged within the public project. Leslie Roberts, in the journal *Science,* reported on the outcome of a two-day meeting in mid-January of 2003 that brought an end to "the DNA Data War." As the sequencing of the human genome sped up, the sequencers could not write papers fast enough to keep up with the flood of information. Bioinformaticians mined the publicly available data and published manuscripts, sometimes not even acknowledging the sequencers. Members of the sequencing community became concerned that their ability to publish the fruits of their scientific labors would be seriously jeopardized. Accusations were made that the sequences were not being deposited in a timely manner as mandated by the Bermuda convention as a way to control access and publications. Francis Collins, ever the diplomat, orchestrated a reaffirmation of the Bermuda principles. However, the statement also argued that appropriate credit be given to the sequencers who had generated the data and that their labors be recognized by all concerned, including funding agencies.

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Why was the Human Genome Project undertaken at this time and in this manner? This is an extremely complex question that we are unlikely to answer completely or perhaps even adequately. But let's begin and attempt to dissect a bit of this complexity.

Why was this the right time? Embedded in our question is why this was the time for the Human Genome Project to capture the imagination of scientists and the public, including the government officials who provided the funding for it. Like all significant endeavors, it had to begin with individuals who saw its promise. They had the self-confidence to proceed, when others felt the task was too daunting. Humans are a controlling species and are also explorers. Genetics was an area in which people thought that with new information they could exert more control and improve health. The sequencing of the human genome was a new frontier.

Advances in public health and medicine over the course of the twentieth century had increased life expectancy and made health an expectation rather than a hope. Infectious diseases were brought under control in the developed world. Many of the residual chronic disorders affecting children and adults, and the more common complex diseases, including heart disease, high blood pressure, diabetes, and cancer, have significant genetic contributions.

Citizens throughout the developed world were ready for a new challenge. The expectations for the space program of the 1960s had not been achieved. Humans had neither returned to the moon nor made it to Mars. People felt that if they could not exert additional controls on their physical environment, including space, then perhaps they could have greater control on their collective genetic future. Watson described the secrets that the human genomic sequence would reveal in his testimony to Congress to obtain funding for the Human Genome Project. In addition, he recognized the need to attempt to anticipate the consequences of the Human Genome Project by investing a portion of the costs of the project to investigate its ethical, legal, and social implications. The recognition of the need for and dedication of resources to the Ethical, Legal and Social Implications (ELSI) Research Program helped to increase political support for the Human Genome Project.

Why did the Human Genome Project capture the public's interest? In addition to being a controlling species, humans are an introspective species. Each individual wonders at some time why he or she feels or acts in certain ways. To many, the genomic sequence represents the height of introspection. They feel that the genomic sequence of humankind contains the residuum of human evolution and possibly a look into the future of humans as a species. People were tantalized by comments from scientific leaders that this sequence might tell them about their individual futures — their predispositions to disease.

As we follow the public press, we have seen an increasing coverage of

genetics and genomics. Clearly, members of the public have a strong interest in the latest genetic and genomic advances, and they embrace this new frontier with enthusiasm.

We recognize the appeal of genomic medicine to the public and professional communities, for it promises to transform the paradigm of medical care. Western medicine has focused on acute interventions, and we have become very effective in supporting failing and even completely failed organ systems, for example, with kidney dialysis. Various scientific leaders have argued that when we are able to determine an individual's DNA sequence, we will be able to know that individual's genetic future. The genetic patterns a person carries will predispose him or her to future catastrophic illnesses before those illnesses become evident (chapter 15). With this information such individuals — really all of us — would be able to prevent the development of these disorders with medications and lifestyle changes. At least this is what some geneticists have proposed.

But is this proposal too ambitious to be true? It is difficult to change human behavior. Examples are seen in the challenges faced in preventing and managing obesity and smoking. In addition, some individuals may not want to know their genetic futures. Others may use the information in destructive ways, arguing, for example, "If my genes say I am going to die before my time, then why take responsibility for my actions." Such an approach endorses genetic determinism by saying, "I am my genome." A change in the paradigm of medicine will require major changes in the thinking of the public and physicians.

It is also possible to misuse sequence information. Every study of public opinion about genetics indicates significant fear of genetic discrimination by use of genetic information to limit employment or insurance (chapter 10). President Clinton signed Executive Order 13145 on February 8, 2000, reducing the risk of genetic discrimination in the federal workforce. States have laws with varying effectiveness to address this issue. However, instances of genetic discrimination continue to raise concerns. The public will require strong reassurance to embrace genetic testing without fear.

Why did we as a population stick with the Human Genome Project and not abandon it for some other opportunity? The 1990s were years of economic prosperity in the United States, and the resources initially identified for this project remained dedicated to it. The early results of human genomic sequencing resonated with the politics and philosophies of key leaders. For example, the observation that the sequence variation within ethnocultural groups exceeded the variation between groups, and

the conclusion that there was no biological basis for race or racism (chapter 5), was very appealing to President Clinton. This resulted in the leaders in the Human Genome Project being invited to the White House for a Millennium Evening at the end of 1999. Participants had the opportunity to discuss and to question the policies of the U.S. Patent and Trademark Office, eventually leading to significant changes in the requirements for gene patents (chapter 9).

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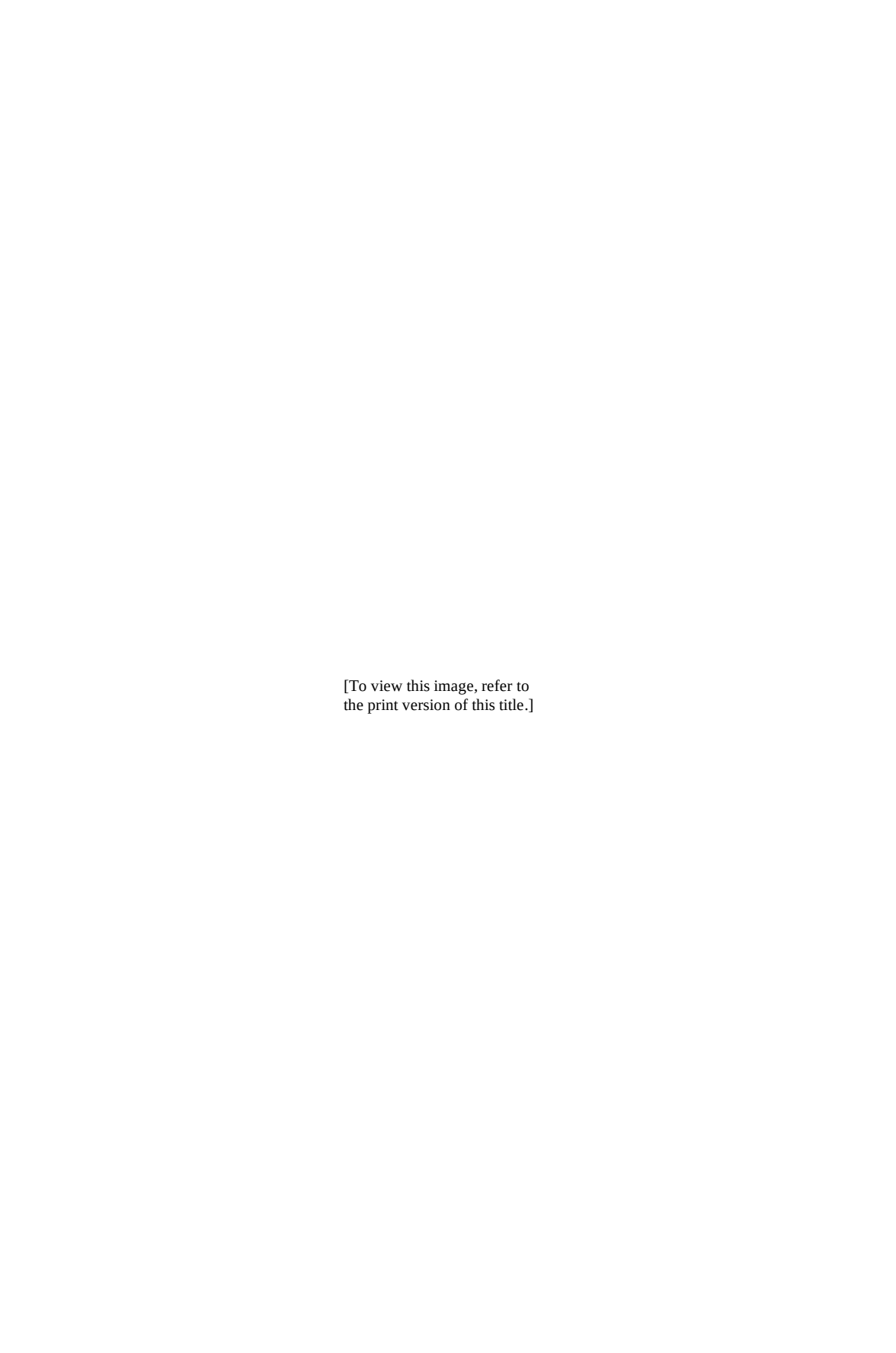
But why did genomics develop and not some other area of big science biology? The answer to this question will probably never be known, since it is difficult to know what other areas were ignored and did not come to fruition. As a population we were fortunate to have had visionary individuals like McKusick, Sinsheimer, Dulbecco and DeLisi, who were credible advocates for the Human Genome Project. Watson was able to pitch the idea to Congress successfully and obtain a long-term funding commitment. Aggressive leaders in the persons of Collins and Venter, for all of their battles, pressed the accomplishment of this venture two years ahead of schedule. And there were many others in the sequencing centers, as well as those more peripheral to the primary efforts, who were creative and effective and who got the job done.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CHAPTER 3

Genetic Determinism

Genetic determinism argues that you are your genome — your future is written in your genes. We introduced the topic of genetic determinism in chapter 1, and it will be a thread woven throughout this book, because this concept seems to be a by-product of the Human Genome Project or at least has been given greater visibility because of the project. Taken in its extreme, genetic determinism would deny the possibility of environmental influence on an individual's development and would suggest that each of us has a genetic blueprint that is set and cannot be modified by our experiences.

This chapter's exploration of genetic determinism and its roots will indicate how pervasive the concept is in modern thinking. The quotations assembled here, as well as many others readily available in print and on the Internet, show that genetic determinism is omnipresent and insidious.

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You might ask, does anyone really believe in genetic determinism? Some have asserted that although the concept of genetic determinism is often attributed to scientists, most in the sciences would consider this concept to be a straw man, that is, set up only to be struck down. Ample evidence suggests, however, that scientists and other thought-leaders do give credibility to genetic determinism.

If you explore the popular media surrounding genetics, biotechnology, and the Human Genome Project, you will find it rife with references, many involving quotations from leaders in genetics and genomics, that would appear to give credibility to the deterministic concept. An often-cited quotation from Nobelist James Watson in 1989, when he was advocating for the Human Genome Project, was published in *Time* magazine: "We used to think our fate was in the stars. Now we know in large measure, our fate is in our genes." Watson also made the following statement at a conference held at the University of California, Los Angeles (UCLA), "Engineering the Human Germline," which was quoted in a book edited by Greg Stock and John Campbell and published in 2000: "And the other thing, because no one has the guts to say it, if we could make better human beings by knowing how to add genes, why shouldn't we? What's wrong with it? . . . Evolution can be just damn cruel, and to say that we've got a perfect genome and there's some sanctity to it? I'd just like to know where that idea comes from. It's utter silliness." These quotations from Watson, set more than a decade apart, indicate a strong belief that genes describe our individual futures and that our fates could be improved by adding genes to our germline.

Others have expressed the opinion that through genetic manipulations, we will alter our futures individually and collectively. In 1998, Francis Fukuyama, who is currently at Johns Hopkins University, wrote: "Biotechnology will be able to accomplish what the radical ideologies of the past, with their unbelievably crude techniques, were unable to accomplish: to bring about a new type of human being. . . . Within the next couple of generations . . . we will have definitively finished human history because we will have abolished human beings as such. And a new post-human history will begin." Fukuyama's statement would suggest that the deterministic power of genetics is so potent that it would be able to change us irrevocably into a new kind of creature and in only "the next couple of generations" — perhaps as soon as fifty years from now!

A similar opinion was expressed by Greg Stock, an individual frequently involved in public debates with Fukuyama. In 1993, Stock wrote in his book *Metaman: The Merging of Humans and Machines into a*

Global Superorganism: "Once people begin to reshape themselves through biological manipulation, the definition of human begins to drift. . . . Altering even a small number of the key genes regulating human growth might change human beings into something quite different. . . . But asking whether such changes are 'wise' or 'desirable' misses the essential point that they are largely not a matter of choice; they are the unavoidable product of . . . technological advance." Stock indicates that manipulating even "a small number" of genes, if they are the critical ones, would change the meaning of what it is to be human. This statement defies the complexity of the biological networks that form the operating systems for our cells and tissues.

Arthur Caplan, who directs the University of Pennsylvania Center for Bioethics, was quoted in an interview for an *ABC News* item in 2000 entitled Babies of the Future: "Absolutely, somewhere in the next millennium, making babies sexually will be rare. . . . Many parents will leap at the chance to make their children smarter, fitter, and prettier. Ethical concerns will be overtaken by the realization that technology simply makes for better children. In a competitive market society, people are going to want to give their kids an edge. They'll slowly get used to the idea that a genetic edge is not greatly different from an environmental edge." The time frame of his speculation is much longer than Fukuyama's, and he accepts that there are environmental as well as genetic influences on outcomes. Caplan still asserts that genetic manipulation will provide a predictable "edge" that presumably will be sufficiently reproducible — meaning deterministic — to appeal to parents.

Superficially somewhat more frivolous, and phrased as questions rather than statements, but implicitly just as deterministic, is the following quotation from Gregory Pence, a philosopher at the University of Alabama at Birmingham: "Many people love their retrievers and their sunny dispositions around children and adults. Could people be chosen in the same way? Would it be so terrible to allow parents to at least aim for a certain type, in the same way that great breeders . . . try to match a breed of dog to the needs of a family?" We have heard variations on this comment numerous times, that since we observe consistent behaviors in certain breeds of animals, then could we not identify the genes for these behaviors? Once identified in these animals, then these genes could be studied in humans and the genetic variations could be correlated with human behaviors. The argument continues that it would be possible, using assisted reproductive technologies (chapter 11), to generate humans with predictable behavioral phenotypes.

This string of speculations, however, is based on faulty observation and logic. With apologies to the fanciers of various retriever breeds — and we are included among you — not all retrievers have perfectly "sunny dispositions"; there is variation of behaviors even for highly inbred domesticated animal breeds. The fault in observation and logic is the tendency to generalize about breed behaviors and to ignore exceptions to the inferred norms. We briefly had a Chesapeake Bay retriever that had a disposition dramatically different from the norm. We ended up returning him to the breeder because he was so dangerously aggressive.

How will parents respond if they do not get the behavior that they desired and for which they invested considerable time, money, and energy? A family who gave birth to a beautiful child with a treatable biochemical genetic disorder gave the child up for adoption because she was not the "perfect child" they had anticipated. (She was adopted by a truly wonderful and loving family.) What will be the response of parents who "order up" a child of perfect health and sunny disposition if the child does not completely match what they paid for?

Inherent in these questions is the concept that behaviors and other complex traits are genetically determined. We will consider the status of knowledge about behavioral genetics later in the chapter. But as we will discuss now, even the phenotypes (clinical features) of "simple," or "single-gene," Mendelian disorders are complex traits, meaning that the patients' phenotypes cannot be predicted with absolute certainty. Therefore, complex traits, including most certainly behavioral features that undoubtedly will have multigenic and environmental inputs, will not be specified by individual genes or even by whole genome genotypes. As was learned from the Dutch Hunger Winter and the work of Barker and others, the ways our genomes are expressed are influenced dramatically and permanently by environmental experiences (chapter 1). Such epigenetic influences have been demonstrated for behavioral traits as well.

Also implicit in these questions is the idea that with careful attention to genetics, people can improve the human race or even develop a "posthuman race." The analogy with domesticated animals and the corollary — that if people can improve animals scientifically, then they can apply the same principles to improve humans — were fundamental to the eugenics movement, as we will consider later in this chapter.

Some individuals have said to us that individuals' remarks that are quoted so as to indicate that they subscribe to genetic determinism have been misquoted, taken out of context, or represent an oversimplification of concepts. Even if these explanations have substance to them, scientists

should recognize these traps and beware so as not to mislead the public. The frequency of such comments suggests, however, that there are individuals who really do believe in genetic determinism.

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Uncertainty, unfortunately, is part of the postgenomic era. While he was a law student in 2001 at the University of California, Berkeley, Sherwin Chen wrote: "The conditional nature of information provided by genetic tests needs to be underscored, while the perceived infallibility and inevitability of genetic diagnoses needs to be de-emphasized and exposed as untrue. It is unlikely that an absolute diagnosis will ever be possible." This is an important insight into the true nature of genetics, genetic testing, and genetic information. For most of the common complex disorders like cancer, diabetes, and cardiovascular disease, a mutation present in a specific gene in an individual's genome indicates an increased risk, but not an absolute or 100 percent certainty that the individual will develop the disease. Therefore, individual patients who know their own genetic profile must live with the anxiety associated with this uncertainty. Many physicians and other health care professionals will also be uncomfortable with the probabilistic — as opposed to the absolute and unqualified — nature of the information provided by genetic and genomic testing.

We can show you the role of this uncertainty in an example from our own research. We introduced this area of investigation in chapter 2 when we described the dramatic impact the Human Genome Project had by accelerating gene identification. The patients we originally described in 1977 have a contiguous gene syndrome caused by a deletion or loss of DNA in the midportion of the short arm of the X chromosome. Genes that are lost or deleted are those associated with a muscle protein that when mutated causes Duchenne muscular dystrophy with deterioration of skeletal and heart muscle; the enzyme glycerol kinase; a transcription factor that regulates expression, or the reading, of genes involved in development of the adrenal cortex and the normal response to stress; and a protein involved in normal brain development that when mutated causes mental retardation.

Patients with the contiguous gene syndrome allowed us to map these diseases to this region of the X chromosome, and their DNA deletions were critical for our group and others to identify the genes encoding the glycerol kinase enzyme and adrenal development transcription factor. Some patients have large deletions and a combination of the clinical fea-

tures from each of the genes that are lost. Other individuals have mutations that involve only one of these genes. Having a single mutated gene is referred to as an isolated deficiency of that gene product or protein.

Our investigations of the protein glycerol kinase and isolated glycerol kinase deficiency led us to appreciate the complexity of biological systems and the difficulty in predicting the clinical features, or phenotype, from the DNA mutation, or genotype. Glycerol kinase is so named because it was first shown to function as an enzyme to catalyze or accelerate the chemical reaction that introduces a phosphate group from the high-energy compound ATP, produced by the mitochondria (chapter 2), onto the three-carbon sugar glycerol. This reaction is essential for the metabolic activation of glycerol eaten in our diet or generated in our cells, so that it can be used in carbohydrate or sugar metabolism, or in fat metabolism as the backbone of triglycerides.

Glycerol kinase deficiency is a particularly interesting disorder because individuals with the isolated enzyme deficiency will have one of two very different phenotypes: they may be completely asymptomatic, or they may have life-threatening symptoms. Those with the asymptomatic form are identified incidentally, often not until middle age or older, when their physician orders a blood lipid profile. Their high blood glycerol level is interpreted erroneously by the clinical laboratory to be high triglycerides, and subsequent workup reveals the elevated free glycerol concentrations. In contrast, those with the symptomatic form present clinically as young children with episodes consisting of severe central nervous deterioration that may progress to coma, abnormally high levels of lactic acid in their blood referred to as metabolic acidemia, and low blood sugar (hypoglycemia). We thought that although this enzyme deficiency is quite rare, it would be an instructive disorder in which to begin to understand the relationship between genotype and phenotype.

We were naive when we began this project, and we really thought that we would be able to sequence an infant boy's (as an X-linked disease it is seen nearly always in boys) glycerol kinase gene before he had time to develop clinical features and predict his phenotype. This information would be important because then we and the parents would know whether the boy would have the symptomatic form and need to be followed closely when he became ill with viral infections to keep his glucose and lactic acid levels in the normal ranges. If his mutation indicated the asymptomatic form, then this could be simply noted in his medical record to assist with interpretation of his lipid profile at some time in the future.

We cloned the glycerol kinase gene, and we took the standard reductionist scientific approach to understand the interrelationships between patients' mutations (genotypes) and their clinical features (phenotypes). Katrina Dipple, who was then a pediatric resident and medical genetics trainee in our laboratory and now is on the faculty in Human Genetics and Pediatrics at UCLA, determined the mutations in a series of patients who had symptomatic or asymptomatic glycerol kinase deficiency. Our original hypothesis was that those with the symptomatic form would have much more serious mutations, perhaps with complete absence of the glycerol kinase protein, whereas those with the asymptomatic form would have milder mutations that would be associated with significant residual enzyme activities.

Dipple found that patients with both forms had similar types of mutations that allowed for protein synthesis and changed a single amino acid. She next developed a model of the human glycerol kinase protein, mapped the mutations onto the protein model and found that there was no distinction between the positions of mutations in the two clinical groups. In fact, a mutation from an asymptomatic individual could be right next to the mutation from a symptomatic individual in the protein model. She measured glycerol kinase enzymatic activities and again could not distinguish the two groups. Whereas we had anticipated higher activities in those with asymptomatic clinical courses, some of the residual activities from symptomatic patients were in fact higher. From these data, we concluded that for at least this enzyme deficiency, genotype could not be used to predict phenotype.

We began to realize that the situation with glycerol kinase deficiency, where there was a lack of correlation between genotype and phenotype, was not unique among "simple" Mendelian "single-gene" disorders. In fact, we could find few if any examples where there was an absolutely robust prediction of phenotype from genotype. This led to our proposal that the phenotypes for simple Mendelian disorders are genetically complex traits, meaning that they rely on the broader genomic background and environmental experiences of the individual in whom the single gene mutation is expressed. We began speaking and writing about why such an absence of genotype-phenotype correlation might exist. The simplistic view with which we entered this project, that genotype would predict phenotype, as we noted previously was incredibly naive, and yet this view had been accepted without question by many human geneticists. We argued that genetic and environmental modifiers would influence the function of the protein within its biological network.

The consideration of these biological networks was also essential to understanding the nature of the modifiers. There are two alternatives in thinking about networks: they could be constructed in such a way that stability would be preserved by exquisitely accurate quantitative control of flow through each of the components; or, alternatively, their architecture could be such that they maintained stability over considerably larger ranges of variation in flow through the network components. If the second is achievable, then it would seem to be preferable, because it would tolerate variability that would be imposed internally, by changes within the organism, or externally, by influences from the environment. Hub-and-spoke networks, also referred to as scale-free networks, provide robust stability in the face of considerable variability. Individual networks can be assembled into modular structures, and it is these modules that ultimately build toward a working cell, tissue, and organism. These modular networks form the complex systems that underlie our biology, and these systems can be modeled and quantified. The investigation of these biological networks represents a new area of science called systems biology.

It is helpful to recognize that these robust networks are found outside of nature. For example, the Internet has the architecture of a hub-and-spoke network. It has been estimated that at any time at least 5 percent of the nodes on the Internet are not functioning, and yet the system works well as long as the highly connected servers continue to operate. The Internet teaches us that these complex systems are changing from moment to moment, and this dynamic quality is also characteristic of biological networks.

If even the simplest single-gene Mendelian disorders are so complex that we cannot predict phenotype from genotype, and if we are just beginning to develop the most rudimentary understanding of the dynamic coupling of networks in systems biology and the effects of genetic and environmental modifiers, then how can anyone simplify genetics to be a deterministic discipline? Humans are simply too complex biologically and too exposed to environmental influences to be reduced to a single gene (e.g., a disease gene), a group of genes (a primary disease gene and its genetic modifiers), or even our entire genome, in isolation from all of our external environmental experiences.

Biology is even more complicated because one gene may produce multiple proteins by using different coding blocks, or exons, and a protein may have more than one function in the organism. Proteins with multiple functions are referred to as "moonlighting" proteins, meaning that just like a worker who moonlights, one protein can have very different

jobs in the cell. Many proteins were named for the first activity that was identified for the protein, but as the protein was studied in different ways, additional unanticipated functional activities emerged. For example, glycerol kinase is now recognized to have additional moonlighting functions. A group of Japanese researchers from Osaka University — Ichiro Okamoto, Hiroshi Hirano, and Fumihide Isohashi — were investigating a protein they had named the ATP-stimulated glucocorticoid-receptor translocation promoter (ASTP). ASTP participates in moving the glucocorticoid receptor from the cytoplasm of the cell into the nucleus, where it interacts with specific DNA sequences to regulate the expression of these genes. When these investigators cloned the ASTP gene, they found that it was identical to the glycerol kinase gene. Therefore, this classic enzyme also functions in gene regulation and has other actions as well. It attaches to DNA-binding proteins in the nucleus called histones, and it is involved in programmed cell death, termed *apoptosis,* which is so important in modeling our tissues during development and in ensuring that older cells that accumulate mutations as we age will be programmed to die and not stay around to cause cancer. We and our collaborators at UCLA argue that the complexity of phenotypes may be due to mutations affecting the diverse moonlighting functions of proteins. Work of this type in systems biology represents the efforts of multiple individuals from very different but merging disciplines, like engineering, genetics, and mathematics, bringing their collective expertise to bear on biological questions.

If proteins have multiple functions involving very diverse cellular networks, then the complexity of the organism denies the possibility of genetic determinism. One might argue that science is too ignorant of systems biology at this time to understand how genes influence phenotypes. We respond that the dynamic operations of biological systems interacting with a dynamically changing environment will defy the possibility of genetic determinism even as we become more knowledgeable. Perhaps with more knowledge, we eventually will be able to specify the probability of a biological outcome based on an individual's whole genome sequence, and individual and familial experiences, but such predictions, if possible, will be inherently statistical chances and not absolute.

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Twins have always been intriguing to humans, particularly identical twins. As scientist, journalist, and author Matt Ridley points out in his book *Nature via Nurture,* twin studies have been central to the gene-

environment debate going back at least to Francis Galton in the nineteenth century. Let's look at a few twin studies to see how they help to separate genetic from environmental influences on diseases and other traits.

Identical twins are created by the spontaneous splitting of an embryo formed by a single sperm fertilizing a single egg. Once the egg is fertilized, it is referred to as a zygote. The scientific term for identical twins is monozygotic twins, meaning they originate from a single zygote. At the time the zygotic fission, or splitting event, occurs, the cells of the embryo can develop into any tissue — the cells are said to be pluripotent or totipotent. Since identical twins start out as a single embryo, the DNA sequences of identical twins are identical, or essentially identical, with extremely rare somatic differences that occur after the separation of the two parts of the embryo to form the twins. Monozygotic twins share essentially 100 percent of their DNA sequence. They are as identical genetically as human clones would be.

Fraternal, or nonidentical, twins are dizygotic, meaning that they are the products of two independent fertilization events — two separate eggs are each fertilized by a separate sperm. Therefore, dizygotic twins share 50 percent of their DNA sequences. They have the same genetic relationship to each other as parents have with their children and as full siblings born at different times have between them. Since fraternal twins are carried in the same womb, the in utero environment is as similar as it possibly can be. We learned in chapter 1 how important the fetal environment can be for later development.

If we investigate identical twin and fraternal twin pairs who were reared together, compared with pairs reared apart, we can begin to get an idea of the influence of genetics and environment. For example, we can look at the relative risks of a specific disease for monozygotic twins reared together or apart. A significantly lower risk for twins raised apart compared with those raised in the same household indicates an effect of the environment, and the degree of difference would be a measure of the strength of this environmental effect. By similar logic, a higher risk of disease among monozygotic twins compared with dizygotic twins indicates a genetic influence on this disease.

Coronary artery disease (CAD) is caused by constrictions in the arteries that bring oxygen and other nutrients to the heart muscle. When the constriction becomes limiting, it may be indicated by severe chest pain, called angina pectoris, or simply angina. Death from CAD is due to a heart attack, also known as a myocardial infarction, caused by starvation of the heart muscle for oxygen.

Because a family history of CAD had been thought to increase the risk of this disorder, Marjorie Marenberg and an international group of colleagues investigated the risk of death from CAD in 21,004 Swedish monozygotic and dizygotic male and female twins. They controlled for additional risk factors for CAD, such as smoking, high blood pressure, obesity, diabetes, level of education, and marital status. They looked at the relative risk of dying if the other twin had died at a younger age compared with an older age, using fifty-five and sixty-five years as the dividing line ages for men and women, respectively. For men, if the twin died before age fifty-five, then there was an 8.1-fold increased risk of death from CAD among the monozygotic twins and a 3.8-fold increased risk among dizygotic twins. For women whose twin died before age sixty-five, the risks were a 15.0-fold and 2.6-fold increased risk of dying due to CAD for monozygotic and dizygotic twins, respectively. The researchers concluded that among these Swedish twins, both men and women, a positive history of early death from CAD in one's twin was a robust predictor of death from CAD for the surviving twin, with the risk greater in monozygotic than dizygotic twins. Although they did not control for all risk factors associated with CAD, such as diet and physical activity, they speculated that the differences between monozygotic and dizygotic twins suggested that at least a portion of the increased risk was genetic. They noted that a study of Swedish twins reared apart had shown that the blood total cholesterol value, which is associated with CAD risk, had significant genetic influences.

A subsequent study by Slobodan Zdravkovic and colleagues using 20,966 twins from the Swedish Twin Registry sought to evaluate the relative effects of genetic and environmental influences on death from CAD. They examined the genetic effect using a quantitative measure termed heritability, which varies between 0 and 1.0. A heritability of 0 would mean that there is no genetic contribution, and a heritability of 1.0 would mean that the trait is 100 percent genetic and there is no environmental contribution. They found the heritability for death from CAD to be 0.57, nearly 60 percent genetic, among the male twins and 0.38, nearly 40 percent genetic, among the female twins. They concluded that there is a moderate genetic influence on CAD-associated mortality.

There are several points made by these studies that generalize to many common diseases that have a genetic component, including cancer. Individuals with these disorders include those with familial and sporadic forms, and those with a familial form are more likely to develop the disease at a younger age. In addition, these studies are epidemiologic

investigations, and they show us associations but cannot tell us about causation. Perhaps most important for our discussion of genetic determinism, these studies tell us that certain factors may increase one's risk of the disease, but they do not indicate that the individual will or will not get the disease. Such investigations leave the individual with probabilities surrounding risk and uncertainty about their individual outcome. From such studies more becomes known about genetic and environmental disease associations, but the knowledge is probabilistic and not absolute.

Let's move now from heart to skeletal muscle and consider the heritability of skeletal muscle fiber types and fiber-type proportions. Skeletal muscle is made up of type 1, or slow-twitch, fibers, and type 2, or fast-twitch, fibers. The proportion of type 1 and type 2 fibers varies considerably among individuals, with 25 percent — the lower quartile — of North American white men and women having less than 35 percent type 1 fibers and an equal percentage — the upper quartile — having more than 65 percent type 1 fibers. There are reported associations between fiber-type proportions and insulin resistance, obesity, and high blood pressure. Heritability estimates for fiber-type proportion in the literature run as high as 0.97, which would indicate that this trait is nearly 100 percent genetically determined. Other reports suggest that this proportion can be altered by training. Juleen Zierath and John Hawley, from Sweden and Australia respectively, argue that there is considerable plasticity in fiber-type composition and cite earlier work on elite runners. Although there seemed to be some correlation between performance and fiber-type proportion, that was not the entire explanation. For two runners who had similar marathon personal best times, one had 50 percent type 1 fibers and the other 98 percent.

Jean-Aimé Simoneau and Claude Bouchard from Quebec undertook a study to determine the influence of genetics and environment on fiber-type proportion. They examined muscle biopsies from pairs of brothers, and male and female dizygotic and monozygotic twins. They looked at biopsies from the right and left thigh muscles to identify variability that was due to sampling or technical variance, and they referred to this variance within the same individual as the "error component." They concluded that approximately 45 percent of the proportion of fiber types in human muscle was attributable to genetic factors, 40 percent to environmental factors, and 15 percent to the error component. Interestingly, they found that correlations in type 1 fiber proportions were similar for monozygotic and dizygotic twins, and lower for brothers, and they

concluded that since the twins shared contemporaneous environments their experiences had greater similarity.

From these studies, it would appear that the proportions of type 1 and type 2 fibers do not fully determine the individual's performance and that both genetics and environment influence fiber-type proportions. The environmental similarity that explained differences between brothers and twins in the Quebec study did not specify effects of the fetal versus postnatal environment. However, with reported correlations of fiber type with diabetes, obesity, and high blood pressure, it is intriguing to consider the effects of the fetal environment on fiber-type composition and possible relationships with these subsequent features of the metabolic syndrome — the fetal origins of adult disease, or the Barker hypothesis (chapter 1).

Let's now look at obesity and its possible relationship to arthritis. A number of studies have suggested an association between obesity and osteoarthritis, particularly involving the knees. There were two competing theories to explain this association. One, called the biomechanical theory, argued that there was increased wear and tear on the knee joints of obese individuals, because of the increased weight their knees were bearing. However, there was no consistent increase in arthritis in other weight-bearing joints, like the hips, and there was an increase in arthritis of some non-weight-bearing joints, like the fingers, associated with obesity. The second school of thought, referred to as the metabolic theory, argued that metabolic factors associated with obesity, such as diabetes and high cholesterol, also led to alterations in the connective tissues of the knee and arthritic joint deterioration.

Nisha Manek and colleagues from the United Kingdom and Rochester, Minnesota, examined genetic and environmental influences on obesity and X-ray findings of osteoarthritis of the knees in adult twins. They used healthy volunteer twins who were enrolled in the United Kingdom Adult Twin Registry. They found a strong relationship between obesity and knee osteoarthritis. The highest quartile for body mass index (BMI — a measurement that looks at weight in relation to height), or the most obese 25 percent, had a 3.9-fold increased risk of osteoarthritis compared with the lowest quartile, or leanest 25 percent. However, when the investigators looked at relative risk for arthritis across BMI quartiles for monozygotic and dizygotic twins, they saw no difference. If there was a genetic association between obesity and osteoarthritis, they would have expected to have seen a higher risk in the monozygotic compared to the dizygotic twins. The authors concluded that heritability of knee

osteoarthritis was 0.50 and heritability of BMI was 0.56, but there was no association between the arthritis and BMI. They argued, therefore, that environmental factors influence the BMI, and environment and BMI influence the risk of developing knee osteoarthritis. They suggested that identifying the environmental contributors and modifying these environmental risk factors could reduce obesity and thereby decrease the risk for osteoarthritis of the knee.

This research shows that twin studies can refute genetic influences that have been inferred by less rigorous clinical observations and association studies. We will see the value of twin studies when we examine the even more complex topic of behavioral genetics.

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Behavioral genetics involves the identification and characterization of genes that are related to personality and intellect and genetic alterations that lead to disorders in these areas, including, at the extremes, severe psychiatric disorders and profound mental retardation. It has been interesting for us to watch the evolution of behavioral genetics, particularly as it relates to genetic studies of personality and psychiatric disorders. This segment of behavioral genetics was a small and nascent field at the beginning of our careers, and in the 1980s and early 1990s it became almost taboo in the more "mainstream" human genetics community — meaning those who studied single-gene disorders. Beginning in the mid-1990s, there appeared to be a surge of interest in research on psychiatric diseases as some of the stalwarts showed increasing successes. The broader genetics community began to recognize that complex mental characteristics and their disorders were not dissimilar from the common complex "physical" traits like cancer and diabetes that were getting so much attention.

Huib Looren de Jong, from Amsterdam, the Netherlands, is a philosopher of science and technology. In an article published in 2000, he argued that genetic determinism had resulted in the misinterpretation of behavioral genetics. He attributed this at least in part to the "Gene Myth: the view that our nature (or even our fate) is in the genes, that genes determine behavior like a puppeteer his puppets." He stated that development was a dynamic, interactive process involving genes and environment. He asserted that the concept of duality of genetics and environment denied the interdependence and constant influence of each on the other in the function of the integrated organism. Finding a single gene that would be related to personality or mental capacities would simply be finding one

component in a complex system. He argued that genetic, neurophysiological, and psychological interactions needed to be considered.

Steven Rose, a neurobiologist at the Open University in Milton Keynes, England, expressed concerns similar to those of Looren de Jong about the nature-nurture dichotomy and how the deterministic underpinnings of this dichotomy influence behavioral sciences. He argued that an individual's genetic makeup was but one component of the organism that was on a trajectory through time and space. He called these trajectories "lifelines" and maintained that the constant interaction of the individual's genome and environment—the coevolution of these influences—is what leads to uniqueness. The dynamic quality of this coevolution means that the lifelines of any two individuals, even identical twins, will diverge and result in their unique individuality.

Individuals like Looren de Jong and Rose would argue that there is much more to behavioral studies than investigating the isolated influence of the genome or environment. To study the nature-nurture issue, Thomas Bouchard, at the University of Minnesota, investigates twins who were separated early in life. Many of these individuals were older adults when Bouchard brought them together for the first time in many years. He noted many similarities within twin pairs, even though the twins were raised in very different environments. He and others have concluded that these investigations support the importance of genetic factors influencing behavior.

For example, divorce has a moderate heritability, and this appears to be mediated at least in part by personality traits that are inherited. Bouchard cautions, however, that behavioral genetic investigations are intended not only to estimate heritability, but also to estimate environmental influences on phenotypic variations. In addition, harkening back to previously noted taboos about behavioral genetics, Bouchard and his colleague Matthew McGue noted in 2003 that "the task of science is to work out the links [between genes, environment, and phenotype] at every level, not to declare domains 'off limits.'" We find it deeply concerning that fears, many of which are grounded in a belief in genetic determinism, could restrict meaningful research.

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Eugenics is a topic that makes many of us in genetics extremely anxious. Historically, eugenics dates to the very beginning of our discipline and has been used as an excuse for the atrocities of the Holocaust and the mis-

treatment of individuals determined to be "unfit" by their communities. After World War II, because of its association with the Holocaust, *eugenics* became a term that was to be avoided and an area with which almost no one wanted to be associated.

The origins of eugenics date to the late nineteenth century. The word *eugenics* was coined by a cousin of Charles Darwin, Sir Francis Galton, who is considered the founder of the eugenics movement. In his 1985 book, *In the Name of Eugenics: Genetics and the Uses of Human Heredity,* the historian Daniel Kevles, now at Yale University, explores the origin of Galton's ideas and terminology. Kevles notes that although Galton began to publish his concepts of the hereditary basis for talent and character and the advantages of selective human breeding in 1865, he did not coin the term *eugenics* until 1883. Galton derived the term from a Greek root that meant "good in birth" or "noble in heredity." From his writings it is clear that he was coming to his conclusions not only based on observations of humans and their family histories, but also due to agriculture and the practices farmers used to breed plants and animals. Born into wealth and educated in medicine and mathematics, Galton used statistical approaches to support his concepts about heredity and eugenics. Eugenics had as its inventor and strongest early proponent an individual who cloaked his arguments in mathematics and science. Galton and his work in eugenics were highly regarded: he was knighted in 1909 and received the highest honor of the Royal Society of London, the Copley Medal, in 1910.

Eugenics was embraced by progressives and became quite popular in the United States, with the American eugenics movement extremely active in the early decades of the twentieth century. Garland Allen, at Washington University in St. Louis, whose areas of interest are the history and philosophy of biology, uses this movement as a case study to examine how eugenics grew out of the economic and social conditions of that period, what its political consequences were, and how these lessons might apply in this era of genomics.

The leading advocate for eugenics in the United States was Charles Davenport, who convinced the heavily endowed Carnegie Institution of Washington to fund an experimental station to study evolution at Cold Spring Harbor on the north shore of New York's Long Island. It opened in 1904, with Davenport as director. In 1910, supported by the Harriman family railroad fortune, Davenport established the Eugenics Record Office (ERO), also in Cold Spring Harbor, and appointed Harry Hamilton Laughlin as superintendent of the ERO. The scientific board of the ERO consisted of prominent academicians from top universities

including Harvard, Johns Hopkins, and Yale, as well as the inventor Alexander Graham Bell.

Groups interested in eugenics were established around the United States, including the American Eugenics Society, founded in 1923, and its many local chapters. The society even had contests for the "fittest family," and the winners were awarded medals. One medal read "YEA, I HAVE A GOODLY HERITAGE" and showed parents passing on the torch of hereditary fitness to their son. Eugenics was popularized in magazines, books, and film. Exhibits were prepared for the American Museum of Natural History and the U.S. Capitol rotunda and traveled the circuit of state fairs. Thousands of individuals filled out family trait questionnaires and sent them to the ERO.

Eugenics became a part of national and state policy and law. Laughlin was appointed eugenics expert witness for the U.S. House of Representatives Committee on Immigration and Naturalization in 1921 and testified in support of the Immigration Act of 1924. He and other eugenicists argued that the immigrants coming into the United States after World War I were genetically inferior to the existing, more Anglo-Saxon stock and would degrade the U.S. gene pool. This testimony was consistent with eugenics in the United States, which has had a strong history of racism.

Laughlin penned "model sterilization" legislation for the states and worked with the American Eugenics Society's chapters for passage. These laws specified mandatory sterilization for "genetically inferior" individuals in institutions. By 1935, sterilization laws had been enacted in thirty states and more than twenty-one thousand sterilizations had been performed. A 1925 challenge of the Virginia law was eventually appealed to the U.S. Supreme Court, where the law was upheld, with Chief Justice Oliver Wendell Holmes reportedly saying, "Three generations of imbeciles are enough."

Eugenics had already become a public passion in Germany in 1936, when both Laughlin and Davenport became associated with German eugenics. Laughlin was given an honorary doctorate of medicine for his work to preserve "the purity of the germ plasm." Davenport arranged for an invitation for German eugenicists to attend the three-hundredth anniversary celebration for his alma mater, Harvard University.

Allen attributes the ascent of the American eugenics movement to the social and economic turbulence of the time and the rise of progressivism. The progressive philosophy supported scientific approaches to improve efficiency. Since behavioral traits such as criminality, poverty, prostitution,

and unruliness were being attributed to genetic defects, it was argued that the most efficient strategy was to prevent the reproductive passage of these genes to future generations. Allen is concerned that the current economic polarization; the emphases on cost-effectiveness, the "bottom line," and public services reductions; and the public perception of genetic determinism and the power of genomics could lead to a resurgent eugenics movement, though with a different name. He asserts that our best defenses against such a resurgence of eugenics are informed and assertive public and biomedical communities.

Lionel Penrose was a prominent British psychiatrist and geneticist who was installed as the head of the Galton Laboratory and as Galton Professor of Eugenics at the University College, a title that he disliked profoundly and considered embarrassing. In 1954, he changed the name of a journal published by the laboratory to *Annals of Human Genetics* from *Annals of Eugenics.* In 1961, he convinced the provost to change the name of his chair to the Galton Professorship of Human Genetics.

In 1966 Penrose stated: "The social and biological values of hereditary differences are continually altering as the environment changes. . . . Our knowledge of human genes and action is still so slight that it is presumptuous and foolish to lay down positive principles for human breeding. Rather each person can marvel at the prodigious diversity of the hereditary characters in man and respect those who differ from him genetically. We all take part in the same gigantic experiment in natural selection." Penrose's comments are remarkably similar to those of Looren de Jong and Rose, despite having been written thirty years before. He asserts that the understanding of biology is inadequate to support genetic determinism or eugenics. The individual's development, with the interdependency of heredity, environment, and biological and social contexts as they coevolve dynamically over time, is too complex to be understood and used to shape human breeding effectively.

Medical geneticists have been accused of practicing eugenics, the argument being that genetic testing leads to eugenics. This concerns many of us who entered this field to help individuals by informing them of the prognosis of genetic disease in themselves or family members, treating patients with rare genetic diseases that are amenable to intervention, and assisting individuals in their reproductive decision making. The latter two areas, population screening and prenatal genetic counseling, have both been labeled as eugenic practices. One of the fundamental principles of genetic counseling is that it is nondirective, meaning that medical geneticists provide information, including various alternative courses

of action, but do not indicate which option they would choose for themselves or the individuals consulting them. This is because, for example, with prenatal counseling, different individuals will perceive identical disease risk and burden quite differently. For example, a 25 percent risk of disease in one's offspring is considered acceptably low by some, and a 5 percent risk is thought to be unacceptably high by others. Two leaders in human genetics have directly addressed the issue of whether geneticists are the modern eugenicists.

In 1998, Neil A. Holtzman, at Johns Hopkins University, who has long been concerned about the social implications of genetic technologies, published an article he titled "Eugenics and Genetic Testing." He proposes that if personal autonomy is respected in genetic testing, then the individual using this technology is not at risk of interference with their "procreative choices in order to attain a societal goal." As described above, the eugenics movement was an attempt to impose society's will on individual choices. Holtzman maintains, however, that interference with autonomy would be considered eugenic, and he asserts that the pressures to reduce health care costs by insurers, including governments and private corporations, can result in eugenic practices. One such example reported in the United States involved a health insurer telling a mother that if she did not have prenatal diagnosis, or if she had the testing and refused to terminate the pregnancy and an affected baby was born, then the company would not provide coverage for the baby. Such efforts are clearly coercive and would be considered eugenic by Holtzman.

Holtzman argues that there is a "new" eugenics that makes use of modern technologies. For example, in an autosomal recessive disorder in which both parents have to be carriers for them to have an affected pregnancy, carrier testing would be possible and selective mating could avoid the risk of having an affected child. Tay-Sachs disease is a disorder in which the baby is normal at birth and in early infancy but has progressive mental deterioration and seizures and dies in the first decade of life (chapter 6). The Tay-Sachs gene mutation is higher in frequency in the Ashkenazi (Eastern European) Jewish population, and carrier screening programs are available so that a couple can determine whether they are at risk and then can have prenatal diagnosis and prevent a child from being born who will surely die. Certain groups of Orthodox Jews, however, such as the Hasidim, do not accept abortion as an alternative and have a tradition of using matchmakers, who they feel help to achieve optimal compatibility for a couple. A group based in Brooklyn, New York, Dor Yeshorim (in Hebrew, "generation — straight/reliable"), provides genetic

testing for Tay-Sachs and other diseases with an increased frequency among Ashkenazim and makes the results available to matchmakers to include in their decision making about the best match. In this way, two-carrier couples who would be at risk for affected offspring are avoided. The question would be whether this is coercion if it is a high-tech extension of a practice that is well established and part of the belief system in this culture.

Holtzman maintains that prenatal diagnosis followed by abortion of affected fetuses has replaced the sterilization of the eugenics movement. He suggests that the coercion of eugenics has been replaced by voluntary acceptance, but he questions how voluntary the process is, given the economic and social pressures. He contends that those who refuse prenatal testing or abortion of affected fetuses face ostracism. Holtzman cautions that these processes are even more questionable when the disorder being tested for is not a single-gene disorder but instead an adult-onset, common, complex disease. This is because it is more difficult to predict who will develop these disorders, and therefore there is the risk of genetic determinism influencing decisions.

Charles Epstein, a renowned geneticist at the University of California at San Francisco, in his presidential address to the American College of Medical Genetics in 2003, posed the following question: "Is modern genetics the new eugenics?" After reviewing accusations that the answer is the affirmative, as well as the history of the eugenics movement, Epstein focused on prenatal diagnosis. He argued that although the goal of prenatal diagnosis is a "well-born" child, the strategy is individual and is not aimed at populations or gene pools, as was the original eugenics. He then addressed considerations introduced by Holtzman regarding whether there is coercion and loss of reproductive freedom. Epstein felt that while the process is not intended to be coercive, there are social pressures external to the counseling and a momentum toward abortion that might have this effect.

Another issue has to do with the role of government in population screening, even if not by setting a blatantly eugenic policy, but perhaps by funding the screening. Epstein cited the example of the prenatal screening program in California to reduce the risk of specific birth defects and quoted a section of a report about this program: "It is useful to reflect on the missed opportunities for the avoidance of birth defects." The document makes it clear that the missed opportunities are the women who did not elect termination after diagnosis of an abnormal fetus, and it raised concerns about cost-benefit analyses of these genetic services. After con-

sideration of issues expressed by the disabilities community and antiabortion advocates, Epstein concluded that prenatal diagnosis does not have the characteristics that made eugenics fundamentally wrong. He noted, however, that the medical genetics community must be sensitive to its critics and not ignore discussions of eugenics.

Both Holtzman and Epstein conclude that the practice of medical genetics is not intrinsically eugenic. But they both identify potential risks and the "slippery slope" that could lead us toward a modern institutionalized eugenics. They agree that genetic determinism was a factor in the original eugenics thinking and would be a risk in a resurgence of eugenics.

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Another issue to consider is the role of the media in communications surrounding genetic determinism. We know that our ideas can be shaped by the media, and subtle differences in stories can have a major influence on how the subject is perceived. The "spin" on the piece can be influenced by how a story is crafted, the types of words used, information that is left out, and the biases of the storytellers. Information on genetics and genomics has been increasing in the media, and it is important for us to consider whether this information is influencing public perceptions of genetic determinism.

On June 26, 2000, world leaders, including President Bill Clinton from the East Room of the White House and Prime Minister Tony Blair from the State Dining Room at 10 Downing Street, in satellite-linked press conferences announced and celebrated a working draft sequence from the international Human Genome Project (chapter 2). A group of investigators led by Brigitte Nerlich at the University of Nottingham in England took the day's events as an opportunity to evaluate how this information was revealed to the public. They analyzed the texts of the speeches by Clinton and Blair, comments by U.S. and U.K. scientists reported in official press releases, and news coverage in the British press in the days that followed. They concluded that the "skillful stage management of the official announcement" and the rhetoric, which was clearly designed to anticipate and neutralize or deflect criticism, were extremely effective. They found the metaphors and images in the announcements were intended "to steer the discourse towards public euphoria" and were effective as determined by the manner in which the statements were picked up by the press. These researchers noted, however, that some of these metaphors, as they were taken out of context and cir-

culated within the public domain, took on lives of their own that were much more deterministic than originally intended. For example, the "book of life" metaphor was one of the most often cited in the press. Though it was not used by Clinton, the president did use words like "letters" and "code," and stated, "Today, we are learning the language in which God created life." The investigators felt that the juxtaposition of *book of life* and *language of God* could be interpreted as a grandiose and magical revelation and possibly as a challenge to individual autonomy. They also thought that the "rhetoric of health" was important for positive public acceptance of this announcement.

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When we teach undergraduates at UCLA about ethical and social issues surrounding genetics, inevitably the students bring up the movie *Gattaca*. This 1997 production takes its name from the four letters of the DNA code: G, A, T, and C. The story is set in a future society in which one's genomic DNA is sequenced at birth, disease risk and life expectancy are determined at that time, and there are constant DNA tests for individual identification — a sequence-based biometric security system. Technological enhancement of the DNA sequence of the fetus is encouraged before birth for those who have access to the technology, and there is strict stratification of society based on genetics and technology. The story involves intricate deception at all levels, perhaps most importantly in the area of DNA biometric testing, which allows the main character to elude a fate determined by the blatant policies of genetic discrimination and determinism.

David Kirby, a Mellon postdoctoral fellow at Duke University who is interested in how the entertainment media communicate science, examined this film from the perspective of its messages about genetic determinism and the new eugenics. He argued that the filmmakers assumed the role of bioethicists by portraying "a world dominated by genes" and through its extremes indicated the risks of genetic technologies. He maintained that we are approaching an era when technology will have the ability to fundamentally change our genetic sequences individually and collectively. He felt that this film is "a bioethical text" that shows the public the problems of the new eugenics that will be generated by this technology, including "genetic discrimination, genetic prophecy, and the homogenization of society." This social construct would be the consequence of a belief in genetic determinism — that parents can give their

babies the genetic edge referenced by some of the thought-leaders discussed earlier in this chapter. Evidence that the filmmakers intentionally cast themselves in the role of bioethicists is provided by the tagline for the movie: "There is no gene for the human spirit."

We assert that individuals are not simply the products of their genomes, and a technology-based society of the type portrayed in *Gattaca* would not be able to achieve what its leaders would promise. Individual genomes are more malleable than such deterministic views profess, and the expression of the genes in an individual's genome is influenced — permanently for some of those genes — by the dynamic coevolution of the genome and the environment.

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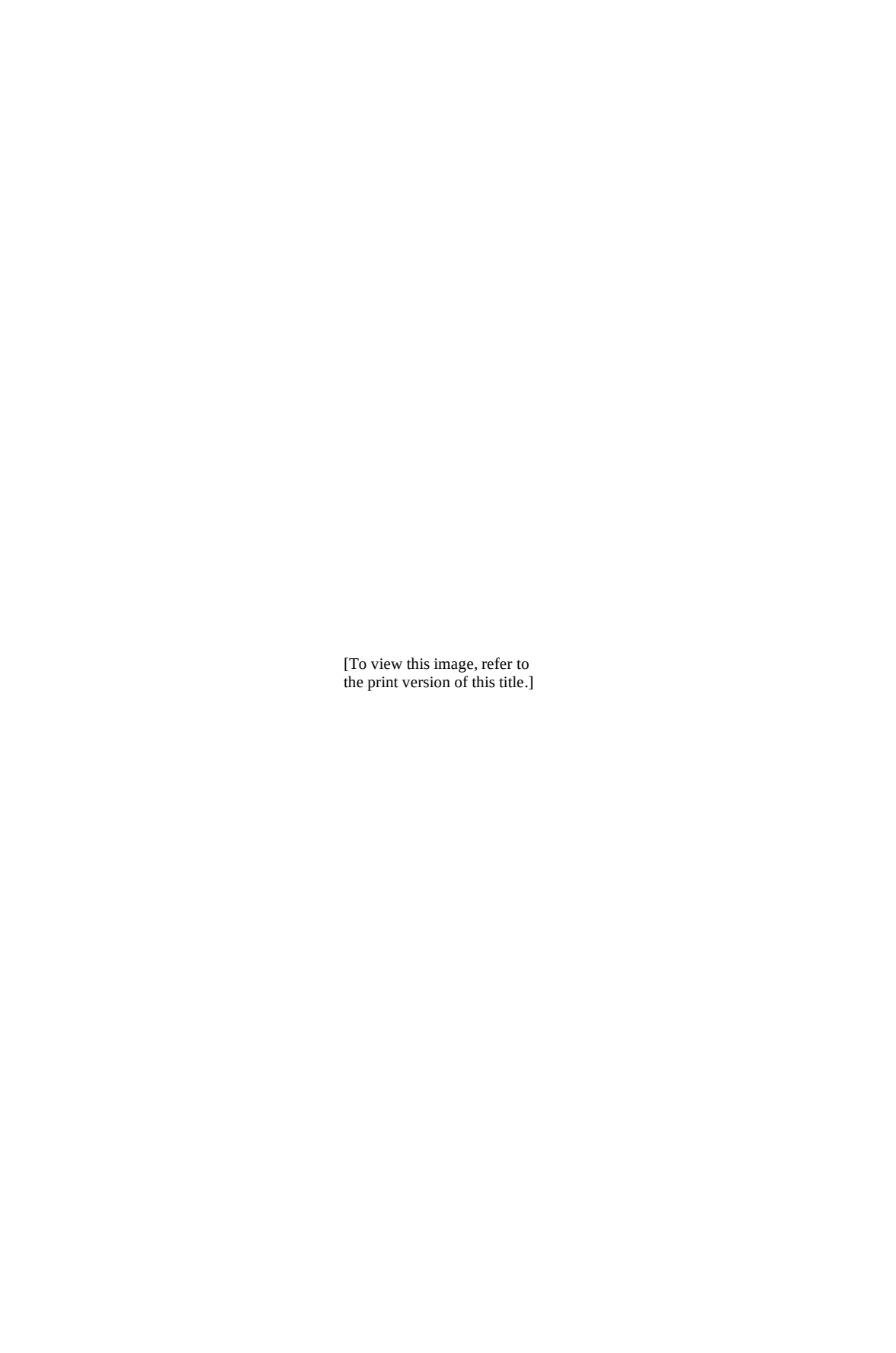
Ask yourself the question, Am I a different person today than I was yesterday? Ten years ago? At one year of age? At one hour of life? We expect that you answer in the affirmative. We are all the products of our genes, environment, and the many experiences that we have as we travel through our lives — we each have a unique lifeline. If you agree that your experiences have influenced who you are, then you have just refuted the fundamental premise of genetic determinism and of a society like that portrayed in *Gattaca*.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CHAPTER 4

The Evolution and Deconstruction of Human-Centered Biology

Perhaps one of the most lasting conceptual changes to be brought about by the discoveries of the Human Genome Project will be the deconstruction of a human-centered biology. The ability to sequence whole genomes from organisms across evolution has shown that genomes of humans are more similar to those of other organisms than many scientists had appreciated before these sequences became available. Rather than being at the central point in all of biology, humans are learning that biology is a matrix in which they themselves are embedded as integral, but not extra-special, parts. We refer to this as the Copernican Revolution in biology.

If humans have a special place in biology, it is only because they have the capability to impose their will on themselves and other organisms, for better or for ill. Humans also have the desire to control what is around them without recognizing that they cannot foresee all of the results —

there are almost always unintended consequences of actions intended to control nature.

In this chapter we will explore how the sequencing of genomes of humans and other organisms is influencing concepts in evolution.

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Before discussing the concept of a Copernican Revolution in biology, it is necessary to understand what is meant by this terminology in its original astronomical and cosmological contexts. A heliocentric, or sun-centered, model of the solar system is generally attributed to Copernicus but was originally proposed by Aristarchus, a Greek mathematician from the island of Samos who lived from 310 to 230 B.C. Aristarchus estimated the size of the sun using an ingenious geometric approach, determined that the sun was much larger than the earth, and concluded that it was unlikely that the larger object, the sun, would rotate around the smaller object, the earth. He explained that an observer on earth would see the apparent movement of the sun across the sky each day if the earth were spinning on its own axis. This rotation of the earth on its axis, Aristarchus said, also explained the movement of the stars in the night sky. Aristotle, though recognizing that the earth was round, rejected the concept of a rotating earth and consequently the heliocentric model. He argued that rotation would generate a strong wind and would cause an object thrown skyward to fall in a different place. Aristotle's influence was powerful and was reinforced by Ptolemy. As a result of these authoritative rejections, it would be more than seventeen centuries before the heliocentric model would be reasserted.

In 1543, Nicolaus Copernicus published his *De Revolutionibus Orbium Coelestium,* or *The Revolution of the Celestial Orbs,* in which he laid out his heliocentric model. He knew that his model would be received unfavorably by the Catholic Church in Europe, which interpreted the Bible to indicate that the earth was at the center (a geocentric model), and so he waited until he was near death for formal publication of his work. However, he had cautiously circulated an outline of his thoughts in his *Commentariolus* as early as 1514.

Galileo was a less cautious proponent of the heliocentric model. In 1615, he traveled to Rome to advocate for the Copernican model, but in 1616 Copernicus's *Revolution of the Celestial Orbs* was banned by the church. In 1632, Galileo published *A Dialogue Concerning the Two Chief World Systems,* and though it passed the church censors, in 1633 he was put

on trial by the church and forced to disavow his support for the Copernican system.

The earth-centered model was really a human-centered, or egocentric, view of the universe. Humans have had a similarly egocentric view of the biosphere with themselves at the center. The Human Genome Project and other genome-sequencing projects have shown, however, that the human sequence is far more similar to the genomic sequences of other organisms than perhaps would have been anticipated.

An example from our own laboratory shows this sequence similarity. The first gene that our lab isolated from the human genome in the 1990s encoded the enzyme glycerol kinase (chapter 2). When we translated the DNA coding sequence to determine the amino acid sequence of the protein, it was 50 percent identical and 65 percent similar to the glycerol kinase protein sequence in the bacterium *E. coli,* a normal inhabitant of the large intestine. This means that for the human protein, the positions of the amino acid building blocks and their identities are exactly the same for half and chemically similar in nearly two-thirds of these amino acids in the protein of an organism from which humans diverged in evolution at least 550 million years ago. To us this was an amazing degree of evolutionary conservation.

If humans are so closely related to the biology that is all around — and even within their own intestines — then they cannot be the central organism within the biosphere. This recognition is what we have called the Copernican Revolution in biology. This revelation is as fundamental to the perception of the place of humans within nature as the original Copernican Revolution in cosmology.

While perfectly understandable and acceptable to the scientific community, the idea that humans are not the central species within all of biology is still perceived as a significant challenge to certain religious beliefs, for example, in strict interpretation of the biblical foundation for Judaism and Christianity. This concept is one facet of "human exceptionalism" — the idea that there is something extremely special about humans that sets us apart from the rest of biology. We will consider other aspects of human exceptionalism in this chapter.

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Comparative genomics is the field that looks at similarities and differences between the genomes of different species. It is a relatively young field that has grown out of the genome projects that have looked at increasing

numbers of organisms from a broad range of species, including bacterial prokaryotes, and eukaryotes, such as yeasts, plants, and animals. Comparative genomics seeks to understand the evolution of genomes and has moved beyond the earlier interest in finding genes in different organisms to more recent attempts to understand the regulatory features within genomes that govern the expression of genes.

One example of the conservation of genomes between species is a comparison of the chromosomes of human and mouse, which shared their last common ancestor 75 million years ago. While there has been a reshuffling and repackaging of the DNA into different chromosomes, the relatively large blocks of conserved sequence are remarkable given the evolutionary divergence between these species. Sequencing of genomes from different species provides insights into their evolutionary similarities and differences and the process of speciation.

Assembling the sequence data for the human genome into the final finished product was a huge undertaking (chapter 2). The challenges generated by this problem attracted a sizable number of highly talented individuals in bioinformatics into the genomics community. They have been able to apply their creativity and skills to the challenges that have arisen in the course of these projects, including sequence assembly and gene finding that was strengthened by comparisons across species. Their tools have also improved, so that they are able to look beyond the coding sequences of genes to attempt to understand why certain noncoding blocks of DNA tend to be highly conserved across species and what the functions are for these conserved blocks. They are learning that many of these conserved regions outside of the coding sequences are involved in the regulation of gene expression.

Let's look at one example of studies in yeast that involved developing the tools for a set of comparative genomics investigations led by Eric Lander, who was trained in mathematics and was on faculty at the Harvard Business School before moving to the Massachusetts Institute of Technology, where he is now director of the Broad Institute. Lander's team examined the genomes of a series of four *Saccharomyces* yeast species. They compared *S. cerevisiae,* or baker's yeast, with three others that are evolutionarily distant from *S. cerevisiae* by approximately 5 million to 20 million years. In addition, the investigators argued that *S. cerevisiae* was the most studied eukaryote and therefore an ideal model organism, and the tools that they developed would have additional applications in comparative genomics.

The predictions of genes from sequences alone are based on mathe-

matical algorithms, and Lander's group improved the algorithms by comparing genomes. They eliminated approximately five hundred sequences that had been previously called genes and would have encoded proteins equal to or greater than a hundred amino acids long. This led to a 9 percent reduction of the gene complement for proteins of this size in *S. cerevisiae*. They also identified 188 genes encoding proteins of 50 to 99 amino acids in length and included an additional 43 genes that had not been recognized previously. Therefore, the comparative genomics analyses among these yeast species increased the power of the computational biology approaches over previous strategies and demonstrated the importance of comparing species to improve identification of genes from genomic sequences.

This research also examined DNA sequences that did not code for proteins but instead were used to determine when and where the proteins would be made; these are called regulatory sequences, or regulatory motifs. These investigators examined intergenic regions, or the DNA stretches between known genes. They found seventy-two conserved motifs that were present in all the yeast species and were distributed throughout the genome, including forty-two previously unrecognized motifs. They wanted to identify motifs that occurred together more frequently than would be anticipated by chance to look at how they might be working together to regulate gene expression. This would not have been possible to do with a single species, because these motifs are relatively short and can occur by chance. However, by finding them in conserved positions across species, the possibility of random occurrences could be reduced or eliminated.

DNA incorporates errors when it duplicates, and external factors damage DNA. Therefore, the sequences of progeny cells begin to differ from the original yeast from which the progeny derived. Scientists can estimate how many changes of this type happen over time, and therefore these changes represent a "molecular clock." The use of a molecular clock facilitates the study of the evolution of genomes over time.

Comparative genomics permits the estimation of evolutionary distance based on sequence changes in the intergenic regions, since these are not as tightly constrained by evolution as are the regions that code for genes. The authors of this study pointed out that the evolutionary divergence between *S. cerevisiae* and *S. bayanus,* the most distant relatives among these species, with their last common relative 20 million years ago, was 62 percent nucleotide identity after alignment. This is similar to the molecular distance between the human and mouse sequences, with 66 percent

identity after alignment, despite the fact that these two species shared their last common ancestor 75 million years ago.

The investigators noted that one of the reasons for initiating these analyses in yeast was the relative simplicity of the yeast genome compared, for example, with the human. They concluded that "comparative analysis offers a powerful and precise tool for interpreting genomes." Clearly bioinformatics approaches will not replace laboratory experiments, but with so much information coming forth from the genome projects, there is need for initial evaluation using bioinformatics to help direct the laboratory experimentalists.

If the DNA sequence of a protein-coding gene changes enough, the protein will be nonfunctional. The organism may die as a result, and this would be an evolutionary dead end. One way to avoid this terminal process and still permit evolution to proceed is to have multiple copies of a gene in an organism's genome. That way changes in one copy will not be lethal. Since the other copies are still intact, this possibility of evolutionary "experimentation" by gene duplication is accepted by many biologists.

Lander and his group tested the long-standing hypothesis that genomic duplication followed by massive loss and gene specialization in the residua would be a powerful evolutionary mechanism. Two competing processes had been proposed: whole genome duplication and independent local duplication events. Before the genomic era, it was impossible to look at entire sequences to differentiate between these proposals. They compared the sequence of *S. cerevisiae* with a yeast that diverged from their common ancestor before the genomic duplication event occurred. The investigators showed that *S. cerevisiae* derived from whole genome duplication of the ancestral yeast chromosomes followed by the loss of almost 90 percent of the duplicated genes through small and localized deletional events. The research team then looked at the evolutionary rate of the 457 duplicated genes that remained in the *S. cerevisiae* genome and found that 76, or 17 percent, evidenced rates of amino acid substitutions more than 50 percent higher than those observed for the related gene in the yeast with the unduplicated genome. In 95 percent of these, the accelerated rate of evolution was observed in only one of the two duplicated genes. The authors interpreted these results to support the hypothesis that evolution, at least in yeast, occurred by whole genome duplication, which was followed by massive loss and specialization of remaining genes.

Lander's group collaborated with a much larger group of colleagues at

the Massachusetts Institute of Technology and combined informatics with laboratory methods to evaluate the regulation of gene expression in *Saccharomyces*. They had identified regulatory motifs in their comparative genomics work described above. The group used high-throughput (large-capacity) binding assays that looked at where regulatory molecules bound to the yeast genome, a method termed "genomewide location analysis." They determined the specific DNA sequence motifs that bound the regulators as a test of their computational conclusions from prior work. They looked at different conditions, for example, the availability of nutrients, to begin to ascertain the more specific roles of these regulators in adjusting to environmental conditions. The investigators concluded that just as there is a three-letter code that permits transcription from DNA to mRNA and translation into protein, there is a "transcriptional regulatory code" that they were able to identify by the combination of bioinformatics and laboratory assays.

These investigations in yeast are one set of examples of the type of work that is being carried out in comparative genomics using computational tools. While these methods can be extremely powerful, they do not provide their optimal information in isolation — they must be validated in laboratory experimentation. But the laboratory analyses also must change to accommodate experiments that are based on studies of entire genomes. These investigations require high-throughput methodologies of the type used in genomewide location analysis described by Lander and his colleagues.

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Just as the studies in yeast have informed the understanding of evolution in these organisms, so would additional studies in animals improve evolutionary knowledge even more broadly.

In December 2002, the Mouse Genome Sequencing Consortium published its initial sequence and comparative analysis. This group found the size of the mouse genome to be approximately 14 percent smaller than the human genome at 2.5 compared to 2.9 billion base pairs, respectively. The group attributed the difference not so much to expansion of the human genome as to an increased rate of deletion in the mouse. The investigators could identify conserved blocks of identically ordered genes — referred to as syntenic regions — in more than 90 percent of these two genomes and indicated the order of genes in the last ancestor common to mouse and human. Similarly, they could identify the genomic

sequence of this last common ancestor. They found a rate of sequence conservation for short segments (50 to 100 base pairs) across the genome to be about 5 percent, which is a larger value than could be explained by the protein-coding sequences. This indicates that there are substantial regions outside of these coding regions that are being protected from sequence changes, presumably because these sequences have essential biological functions. The consortium found that approximately 80 percent of the genes in the mouse genome coded for a protein of identical function in the human, and less than 1 percent of genes in the mouse had no identifiable related coding sequences in the human genome.

Let's look at another model organism, the laboratory rat. *Rattus norvegicus* was domesticated for biomedical research prior to 1828, and a rat breeding colony was established in 1856, so this is a well-established animal model. Although the mouse became the dominant rodent model used in genetic research, the rat continues to dominate other areas of investigation such as physiology, nutrition, and psychology. To improve their research capabilities, investigators desired to have the genomic sequence for their model organism, which became available from the Rat Genome Sequencing Project Consortium in 2004 and provided additional insight into mammalian evolution.

The rat genome at 2.75 billion base pairs was intermediate in size between mouse and human and had roughly the same number of genes as mouse and human. Genes were identified in the rat that did not appear to be present in the mouse, and these were thought to have arisen due to expansion of families of genes that had similarities in their structures, such as those for immunity. There was an alignment of approximately 30 percent of the rat with the mouse genome, much of which was in repeat sequences specific to rodents, and at least 50 percent of the portion that did not align with mouse consisted of rat-specific repeats.

The consortium described a "eutherian core" of the rat genome, meaning the part of the rat genome that was shared with other placental, or eutherian, mammals, and they used comparisons with mouse and human to define this shared core. They found that this eutherian core consisted of one billion base pairs and estimated this core to represent approximately 40 percent of the portion of the genome devoted to actively functioning genes. The core included nearly all of the known exons (the coding sequences that are strung or spliced together to form the messenger RNA that is translated into protein) and regulatory sequences that together make up 1 to 2 percent of the genome. A larger portion of this core, however, representing 5 to 6 percent of the

genome, appeared to be evolutionarily constrained, suggesting that these sequences have functions that are important but remain unknown at this time.

One of the values of the rat as a model organism is how well the more than 230 inbred strains of *R. norvegicus* have been characterized, or phenotyped. This phenotypic information is being annotated and cataloged along with genomic variations associated with the different strains in the Rat Genome Database. In this manner, investigators have compiled not only a genomic repository but also a phenome database.

Anton Nekrutenko at Pennsylvania State University was interested in identifying rodent-specific exons. Genes are made up of exons that code for amino acid sequences and introns, or intervening sequences, that separate the individual exons in a gene. A gene may have many exons, and it may not use all of the exons in the gene at any one time. This is a process called alternative splicing. In a hypothetical example of a three-exon gene (1, 2A, and 2B) that uses exon 1 and either 2A or 2B as the second exon, proteins encoded by 1–2A and 1–2B have different amino acid sequences. The different proteins produced by alternative splicing are referred to as isoforms, and the different isoforms of a gene may have distinct functions, and the relative proportions of the isoforms coded by the same gene may vary, for example, in different tissues or at different stages of development.

Comparing the genomes of rat and mouse, Nekrutenko found 2,302 putative exons that appeared to be rodent-specific and were not present in the human genome. He found that 1,116 of these exons, or approximately 50 percent, mapped to genes that were present in humans, though these exons were not present in the human sequence. A similar number, 1,186, mapped to genes that did not have a human counterpart. He speculated that these rodent-specific exons were "the few successful lineage-specific survivors of the constant process of gene evolution by birth and death of individual exons." Evolution is a dynamic process and gene structures are not "frozen in time." The dynamics of evolution include "acquisition of novel exons" that can provide new functions to the proteins modified in this fashion.

Chris Lee is a professor at the University of California at Los Angeles, whose interests include alternative splicing. He and Barmak Modrek noted in 2003 that their group and others had shown that approximately 40 to 60 percent of identified human genes were alternatively spliced. They examined nearly 9,500 genes encoding the same proteins in human and mouse. Modrek and Lee divided the products of alternatively spliced

forms into the major, or higher-proportion, transcript form and the minor, or lesser, transcript form. They found that although the vast majority of exons were conserved between mouse and human, the exons included in only the minor forms were generally not conserved. They confirmed this with comparisons between the human and rat genomes. They speculated that the lack of conservation of these minor-form exons suggested that these lower-expression exons resulted from "recent exon creation or loss events."

These investigators suggested a process by which the acquisition of new exons could influence evolution. The exons are brought together in the mature messenger RNA by the process of splicing, and this involves splice signal sequences at the intron-exon boundaries. A new exon would probably have less robust splice signals than those in the preexisting exons. Therefore, with weaker splice signals, the proportion of the transcripts with the new exon would be less, and the original function of the gene would be maintained by the preexisting, predominant transcript. This would leave the new exon "free to evolve (accumulate mutations), with essentially no loss of the original activity. . . . If the rapidly evolving [new] exon acquires a useful function, positive selection can strengthen its splice sites (perhaps becoming the major form), or it can render them tissue-specific, if the new function has only local benefits." Thus, we see that comparative genomics is not simply a descriptive discipline that annotates the molecular changes that occur during evolution. This field can illuminate the mechanisms that result in evolutionary changes.

With the increasing numbers of organisms that have had their genomes sequenced, and with improved computational approaches to comparisons, articles on comparative genomics frequently report results from many different species. Some of these studies might almost appear to have a "Noah's Ark approach."

One group, led by Peter Stadler from the University of Leipzig, compared sequences from a series of vertebrates: human, mouse, rat, fugu (the poisonous fish that is a Japanese delicacy), and the zebra fish (a teleost with transparent, externally developing embryos that facilitate developmental studies in this model vertebrate). These researchers used the Comparative Regulatory Genomics database that cataloged conserved noncoding sequences upstream of identical genes in the species listed above. The investigators were interested in finding noncoding RNAs that are involved in functions other than transcription and translation, such as regulation of expression and X-chromosome inactivation

(chapter 6). They combined sequence comparisons with computationally based measurements of RNA structure and the stability of that structure. Their method was rapid and identified many more noncoding RNAs than had been appreciated previously. They described their approach as "computational RNomics" and proposed to use their approach "to draw genomewide maps of significant RNA structures." This work blends sequence comparisons with tools of the structural biologist to add new dimensions to comparative genomics.

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Comparative genomics has also been used to investigate evolutionary relationships among primates. An important decision in this research was to pick the primate genomes to sequence for comparison with the human.

As the race for the human genome between the public and private efforts heated up, resources were focused on the efforts in human sequencing and the end was in sight. It remained to be determined, however, which additional organisms would be selected to have their genomes sequenced. The cost of sequencing was still such that choices would have to be made. Some argued that one primate's genome — ours — was enough, and investigators needed to look more broadly at the diversity of biology, since there would be additional power from comparing genomes that were separated by longer evolutionary intervals. Advocates began to take strong positions in this debate.

Edwin McConkey of the University of Colorado, Boulder, and Ajit Varki from the University of California, San Diego, argued in the journal *Science* in 2000 that there were several reasons to complete at least one nonhuman primate genome. They felt that comparisons between the human genome and the mouse genome were helpful but could never explain why we humans differ from other primates. A comparison of the human genome with a nonhuman primate genome would aid in identifying uniquely human characteristics. They argued that a comparison of the human genome with that of the chimpanzee was important due to differences in the frequency and severity of disorders such as AIDS, Alzheimer disease, cancer, and malaria between these species. Sequencing of one or more nonhuman primate genomes might increase world awareness of the need for protection of other primates from extinction.

Maynard Olson, from the University of Washington in Seattle, and Varki, in articles in 2003 and 2004, discussed the lessons that the chim-

panzee-human genomic comparison would teach and already were teaching scientists about the types of genetic changes that are involved in developing a new species. These primates shared their last common ancestor only about 5 million years ago, and therefore these investigations would provide information on rapid evolutionary change of novel phenotypic differences. In addition to the disorders cited earlier, they noted other conditions and diseases to which the other great apes were less susceptible than humans, including menopause, influenza A, and myocardial infarction. Olson and Varki also cited disorders that were rare in humans and common in the other great apes, such as simian foamy virus infection and *Escherichia coli* K99 gastroenteritis. When comparisons of proteins were made, the differences in amino acid sequences between human and chimpanzee were less than 1 percent. Of the chimpanzee genome, 95 percent could be aligned directly with the human genome, and in these regions of direct alignment, the sequence differences averaged 1.2 percent. The lack of complete alignment was due to a number of differences, including the fact that the chimpanzee has one more chromosome than the human. In addition, genes on human chromosome 2 were split between chimpanzee chromosome 12 and 13. There were also large inversions on human chromosomes 1 and 18 relative to the chimpanzee.

Olson and Varki discussed the more rapid evolution of genes involved in metabolizing nutrients and in host defense in humans and the importance of nutrition and pathogens in driving evolutionary selection. They were interested in speech acquisition by humans compared with other primates, and they described the work on the transcription factor, or regulator of gene expression, FOXP2. When this gene is mutated in humans, it causes an autosomal dominant disorder of speech articulation. There is evidence that the FOXP2 gene has been the subject of positive evolutionary selection in humans, a story with intriguing implications for speech. However, Olson and Varki stated that understanding human uniqueness simply by comparing genomes would be quite difficult.

How similar is the human genomic sequence to that of our closest primate relatives? This is a question that is intriguing to many and key to understanding evolution among the great apes, a group that includes humans. Morris Goodman and his colleagues at Wayne State University in Detroit published a paper in 2005 entitled "Moving primate genomics beyond the chimpanzee genome." In summarizing the literature on genomic similarity based on comparisons of noncoding nuclear sequences

that could be readily aligned between species of the great apes, they provided the following average approximations of sequence identities: human with chimpanzee, 98.9 percent; human with gorilla, 98.5 percent; and human with orangutan, 97.0 percent. The evolutionary distances between human and gorilla, and chimpanzee and gorilla were identical, and similarly the distances between each of these three primates and orangutan were identical. These estimates are based on incomplete sequence information that will be remedied when the chimp genome sequence is completed.

These authors noted, however, that many of the abilities of humans, like making and using tools, extend deeper evolutionarily than the great apes into our primate ancestors. They argued, therefore, that while whole genome comparisons between human and chimpanzee will be informative, these would not be sufficient. Goodman and colleagues maintained that if these analyses are extended to additional primates, then humans are more likely to understand how they evolved and differ from the other animal species — what it means to be human.

In 2003, Evan Eichler and colleagues compared the genomic sequences of lemur, baboon, chimpanzee, and human. They showed that although rates of single-nucleotide substitutions were similar across these primates, the rates of retrotransposition, or new insertions of mobile genetic elements —"jumping genes" — were very different. Mobile genetic elements, or retrotransposons, have certain features in common with retroviruses of the kind used in gene therapy (chapter 14) and are embedded in our genomes. New retrotransposon insertions accounted for expansion of the human genome by 15 to 20 percent during the recent 50 million years of evolution. The role of mobile genetic elements in evolutionary change remains an area of active investigation.

Comparative genomic analyses are enhancing the understanding of the evolutionary relationships between humans and other primates. People are a long way, however, from identifying the complex genetic differences that distinguish humans from the other great apes and more distantly related primates.

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Let's now move from comparing genomes to comparing genes. This may appear to be counter to the current dogma that asserts that we are moving from genes to genomes. There has been so much excitement about comparing entire genomic landscapes that a focus on individual genes and

gene families may be considered old-fashioned by some. Let's look at two examples, among many, to see how instructive comparisons of genes can still be. The first will be the conservation of the HOX gene family and the execution of the "body plan," and the second will describe the persistent expression of the human lactase gene and it's coevolution with the "dairy culture."

David Angelini and Thomas Kaufman, from the University of Indiana, investigate the genetic basis of developmental programming using the fruit fly, *Drosophila,* as a model organism. They reviewed the role of HOX genes in arthropod body plans in 2005. Arthropods are a remarkably diverse group of organisms that include insects, crustaceans, spiders, scorpions, and centipedes and have a broad range of body plans. They pointed out that the terminology *body plan* was originally described as a structural plan or building plan that represented "the collection of homologous anatomical features seen across the natural history of a group" and was intended to indicate "an evolutionary context" and not "an anatomical grade of organization." In other words, it is the *plan* in *body plan* that should be emphasized, and this plan includes consideration of evolution across different groups.

HOX stands for homeotic complex, or group of homeotic genes, where *homeotic* is a gene product that causes a significant shift in the organism's structural development. For example, the HOX *Drosophila* mutant known as antenapedia has legs growing out of its head, where the antennae should be. Another mutant, bithorax, has a duplicated thorax, or body with two pairs of wings, instead of the usual single pair, and looks somewhat similar to a dragonfly. It is thought that these genes suppress the additional legs, like the centipede, and wings, like the dragonfly, of the protoarthropod ancestor.

The HOX genes code for proteins that are transcription factors that regulate the expression of other genes during development. These HOX genes show a very interesting feature of colinearity, meaning that within a HOX gene cluster, the series of HOX genes are arrayed in linear complex along the DNA. In addition, these genes are expressed sequentially during development in the order in which they are arrayed. This expression begins in the anterior portion and progresses toward the posterior portion of the organism. While there have been duplications of the clusters (*Drosophila* has one cluster and vertebrates have four), and within individual clusters (ancestral cluster, six genes; *Drosophila,* eight genes; mouse and human, range of nine to eleven genes in their four clusters),

the general organization of the HOX gene clusters and their colinearity are conserved from arthropods to humans.

HOX mutations are found in association with human disease. Mutations in patients with developmental abnormalities indicate the continuing importance of the products of these genes to the body plan. Jeff Innis, at the University of Michigan, studies these genes and has shown the particular roles of HOX genes in appendicular development, or development of specific bodily structures, the extremities. Mutations had been reported in three of these genes when Innis reviewed this topic, and all were autosomal dominant. Two of the genes were in the HOXA group, HOXA11 and HOXA13, and one was in the HOXD group, HOXD13. Mutations in HOXA11 are associated with a unique syndrome characterized by a low platelet count and bleeding disorder, together with fusion of the two bones in the forearm, called radioulnar synostosis with amegakaryotic thromocytopenia. HOXA13 mutations cause hand-foot-genital syndrome with small feet having very short great toes, abnormal thumbs, duplication of the genital tract including a bifid or double vagina in females and abnormal development of the penis in males. HOXD13 mutations are associated with a syndrome called synpolydactyly, meaning webbing between and duplications of fingers and toes. While researchers are still trying to understand the developmental mechanisms causing these syndromes, the phenotypes show the critical roles that the HOX genes play in development through a very long period of evolution from arthropods to humans.

Regulation of the HOX gene clusters is incredibly complex and has been difficult to dissect fully using more traditional molecular genetic approaches. Investigators are using comparative genomic approaches to identify regions that are conserved and therefore are potential regulatory regions. This powerful approach is already yielding some results for the HOX genes. Comparisons are not only between organisms but also between the various clusters among vertebrates. Combinations of computational approaches with investigations in mice are proving to be particularly fruitful in defining the importance of genomic context in expression of these HOX genes.

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The second example of evolutionary gene comparisons will be confined to studies of lactase gene variations in humans. Lactase is an enzyme that

is expressed in the intestine and breaks down the sugar, lactose, that is present in the milk of humans and cows. All mammals, except for a select group of humans, have a decreased expression of intestinal lactase after weaning. If individuals without lactase are fed a diet that contains lactose, like milk and related dairy products, lactose cannot be metabolized by the individual but can be metabolized by the bacteria in the intestine, and the result is gas, significant discomfort, and diarrhea. The evolutionary "normal" condition in humans, therefore, is the loss of lactase activity during childhood and adolescence, whereas the acquired condition is lactase persistence associated with a continuing expression of this enzyme. Joel Hirschhorn is a physician-scientist at the Children' Hospital and Harvard Medical School. Hirschhorn's group is contributing to our understanding of how lactase persistence coevolved with increased dairy farming and consumption of dairy products.

The frequency of lactase persistence differs dramatically in different populations. Not all populations rely on dairy products for nutrition into adulthood, but northern Europe is an area where the dairy culture has flourished over the last ten thousand years. The frequency of the high-expression form of the lactase gene — the high-expression allele — varies between approximately 50 percent and 85 percent among northern Europeans and U.S. Caucasians. This high-expression allele has much lower frequencies among Africans and Asians, ranging between approximately 5 percent and 20 percent.

When lactase persistence and dairy farming are mapped geographically, the two distributions correlate directly. Data in Europe produced by Albano Beja-Pereira and colleagues suggested a "gene-culture coevolution" between the dairy cattle, which showed "high allelic richness and genetic distinctiveness," and the human dairy farmers, who evidenced increased rates of the lactase-persistence allele. Therefore, it has been proposed that lactase persistence in European groups is a consequence of a positive selective advantage associated with the growth of the dairy culture. These data are epidemiologic, however, and show association but not necessarily causation.

Because the frequencies of lactase persistence vary so dramatically, even in populations other than northern Europeans, geneticists have questioned whether a single lactase-persistence allele arose once and spread worldwide or whether selective pressures caused different persistence alleles to arise in more than one place and time. Hirschhorn and his team used a group of more than one hundred single-nucleotide polymor-

phisms, or SNPs (pronounced "snips"), including 3.2 million base pairs around the lactase gene and two SNPs that had been shown previously to be associated with lactase persistence. SNPs can be used to define blocks of DNA referred to as haplotypes, which can be applied as tools in population genetics to examine associations with traits and the rate of evolution. The investigators found that there was a specific haplotype block, or DNA "signature," that was associated with lactase persistence, and it differed in frequency in the appropriate manner between populations, for example, in Europeans from the north with higher or the south with lower rates of persistence of the lactase gene.

The lactase-persistence haplotype is quite long, extending for more than one million base pairs. DNA is exchanged in blocks during the development of eggs and sperm by crossing over between the parental chromosomes. If a block of DNA remains large across many individuals, as is seen for the lactase-persistence allele, this suggests that it arose relatively recently, and if it spreads rapidly, this indicates a selective advantage. By looking at "decay," or loss of integrity of the normal lactase-nonpersistence haplotype, compared with the evolutionarily acquired persistence haplotype in European-derived U.S. pedigrees as the block is eroded over time, Hirschhorn's team was able to date the rapid rise in frequency of the persistence haplotype to between 2,000 and 21,000 years ago. This time span fits well with estimated 9,000 years ago when dairy farming is thought to have begun in northern Europe. When they analyzed small Scandinavian pedigrees, they found an even more recent estimate of 1,600 to 3,200 years ago. They took these estimates together to conclude that the lactase-persistence haplotype arose in the last 5,000 to 10,000 years.

Hirschhorn and his colleagues were able to calculate the magnitude of the selective advantage of the persistence haplotype. They found that this measure was relatively high and similar in magnitude to the well-known selective advantages of specific malaria-resistance alleles in regions where malaria is endemic. These malaria resistance alleles include glucose 6-phosphate dehydrogenase (G6PD) deficiency, which can cause anemia with jaundice but protects against malaria and sickle-cell trait, or the carrier state, which is asymptomatic and yet protects against malaria with one copy of this mutant allele, but causes life-threatening anemia with two copies (chapter 8). These investigators concluded, "Thus the added nutrition from dairy appears to have provided a selective advantage in northern Europe comparable to that provided by resistance to malaria in malaria-endemic regions."

Hirschhorn and his team were unable to determine whether the lactase-persistence haplotype is the only persistence allele worldwide. However, they discussed evidence from others, for example, in African populations, that would suggest that there are one or more additional persistence alleles. For example, a specific SNP that Hirschhorn's group and others have found to be associated with the persistence haplotype is not one that was observed in African groups with high rates of lactase persistence.

The story of the evolution of lactose tolerance in Europe is a lesson in coevolution. Evolution is a dynamic process that is a consequence of the constant and vigorous interactions between genes and environment. Behavior and culture are important ingredients in this coevolution. The dairy culture that improved nutrition through the ready availability of cow's milk and its products appears to have been associated with a change or changes in a specific region in the genomes of certain humans, in or around the lactase gene, to allow them to tolerate dairy products. These genetic changes provided certain humans with lactase persistence, distinguishing them from many people and all other mammals. This change has occurred extremely rapidly in human history, which suggests the magnitude of the selective advantage of improved nutrition from dairy products.

The rapid penetration of populations by the sickle-cell gene is understandable, because this was counteracting a severe disease, malaria. What was the selective advantage of lactose tolerance? The answer is that the ability to utilize the products of the dairy culture addressed a significant disease, malnutrition. Those of us in the developed world may not see this as the enormous problem it has been for humans over time and continues to be for many people. The speed with which the persistence allele spread through European populations with close contact to dairy farming is a measure of the magnitude of the severity of malnutrition as a disease and the dramatic health benefit of a convenient source of nutrition.

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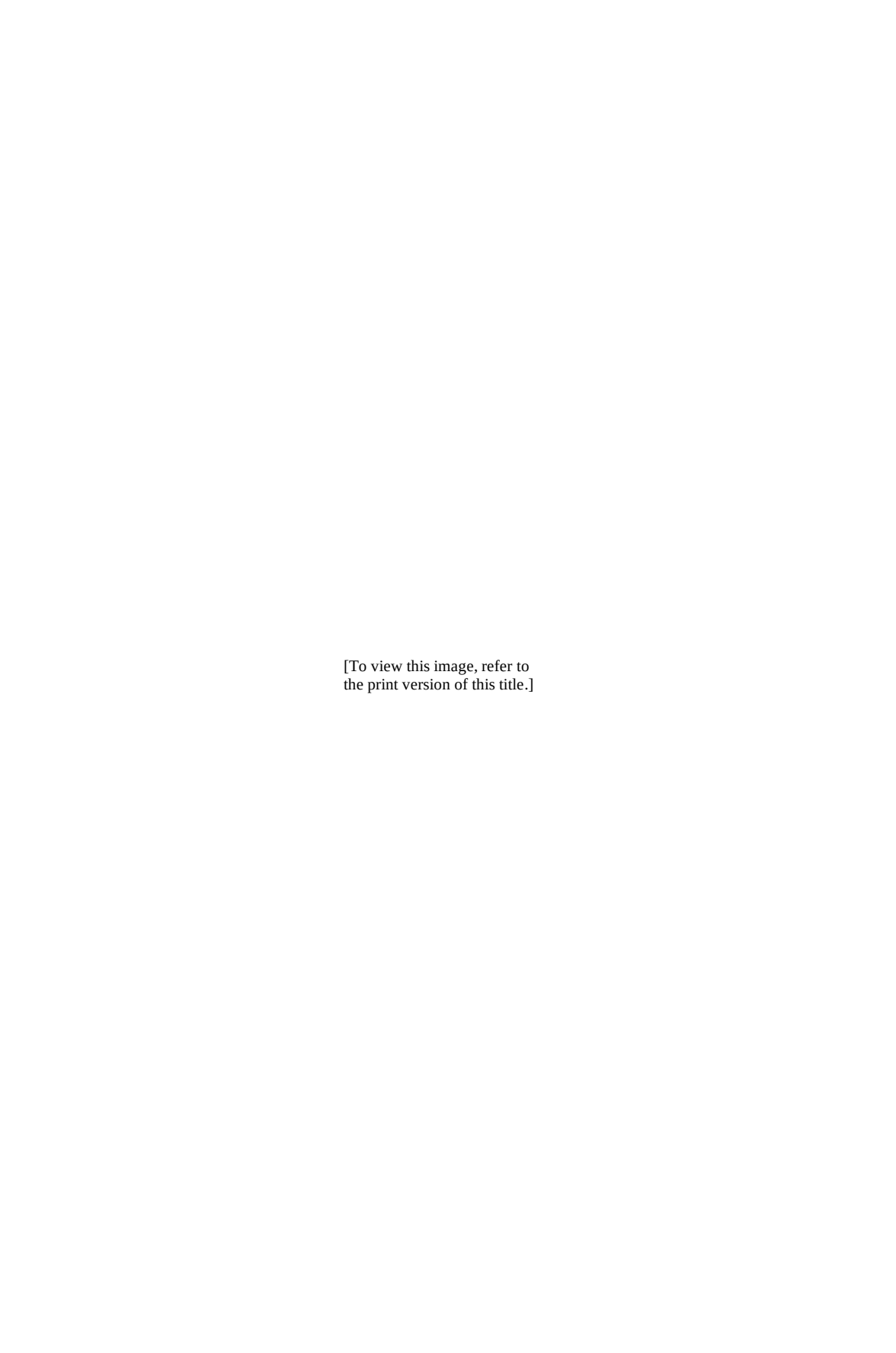
In this overview of comparative genomics, we have attempted to summarize some of the larger issues in the field and how this field is changing and acquiring new tools and strategies to decipher evolution as it is written in the DNA of humans and the organisms that surround us. Humans are incredibly similar to other species separated for hundreds of

millions of years of evolution. Given that similarity, it is difficult to understand the basis for human exceptionalism — that humans are the center of the biosphere. But this is part of our culture. We find individuals arguing for human exceptionalism all of the time, and we even found it creeping into our thinking as we wrote this chapter. So, we all must be on alert constantly to avoid this trap.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CHAPTER 5

Race and Ethnicity

Your History Is Written in Your Genes

Is the term race *valid for humans?*
Using "recreational genetics" to search for our roots
Ethnocultural group as proxy for individualized medicine
The Melungeon DNA study
Maternal lineages and mitochondrial DNA
Patrilineal relationships and Y chromosome DNA
Combining mitochondrial and Y chromosomal DNA analyses

For many individuals, their identity is dependent on their ancestry and the ethnocultural group to which they belong. But the knowledge within the family may be incomplete or influenced by inaccurate family lore. Clinical geneticists ask their patients for a three-generation pedigree in their initial evaluation. Can you do this for your own family?

Some individuals assume that racial or ethnic group membership of another person allow certain assumptions about that individual, perhaps even their religion or other shared values. As a couple who share an Irish surname, we are well aware of this. Many have erroneously assumed that we have a specific religious affiliation. Therefore, race and ethnicity are poor proxies from which to generalize social and behavioral characteristics.

In this chapter, we will examine the concepts of race and ethnicity and the extent to which genetics and genomics can inform us about these concepts. We will also consider whether these issues have any relevance to health care.

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Does the term *race* have any validity for humans? Biologically, *race* relates to a group that has diverged sufficiently, often by geographic isolation, so that it can be distinguished by appearance and genotype from another group within the same species. Since the two groups are part of the same species, they can continue to interbreed. Biological examples of races might be two subspecies of squirrels or oak trees that have adapted to different climates. They have become genetically isolated and may have taken on different characteristics of coat color or foliage, respectively. If given the opportunity, however, they could still breed successfully, so they are part of the same species. Therefore, if race has validity in humans as a biological construct, then a key issue will be whether the differences between human groups meet the standard in biology.

Genetics researchers say that the differences within ethnocultural groups are greater than the differences between these groups. The original statement of this principle is attributed to a 1972 chapter by Richard C. Lewontin of Harvard University. He maintains that there is tremendous genetic variation among individuals in the human species. On average, approximately 85 percent of this human variation occurs among individuals within local populations of the same national or linguistic group. The remaining 15 percent of human genetic variation is divided among different local populations within what have been classically considered to be the same racial groups (e.g., two different local groups of those of European descent), and between groups classically defined as races by visual appearance. Among that 15 percent of variation, the latter between-group variation represents only 6 to 10 percent of human genetic variation. Therefore, at least 90 percent of genetic variation is within the traditional racial groups. This is the original basis for the assertion that larger genetic differences exist within than between groups.

A. W. F. Edwards, from Cambridge University, refers to this assertion as "Lewontin's fallacy." Edwards argues that Lewontin's statistical approach is not flawed but is simply too superficial. Edwards states that analyzing data on a locus-by-locus basis ignores possible relationships between loci that are important in understanding structure within the data. Edwards notes that the classical work of Luigi Luca Cavalli-Sforza and colleagues uses more sophisticated statistical approaches, like cluster analysis, to provide a statistical definition of group membership that considers the importance of internal correlations and hidden structural features within the data. Edwards also asserts that Lewontin was using his

analyses to support a social position and undermine race as a scientifically valid construct.

Cavalli-Sforza, from Stanford University, defined the field of "genetic geography." He felt that genetics would offer new tools to explore human migration. He assembled an internationally diverse group of colleagues that included anthropologists, mathematicians, historians, linguists, and archaeologists. They adopted new genetic tools as they became available, and their results up to that time are summarized in *The History and Geography of Human Genes,* published in 1994. Their studies of gene frequencies across populations accounted for 65 percent of the variation between four ethnic groups—Africans, Australians, Caucasoids, and Mongoloids.

Cavalli-Sforza's group's investigations have been used by advocates for a biological basis of race to support their position. But the authors do not discuss four races but "four major ethnic groups." In addition, this analysis shows extensive gradients of admixture between these ethnic groups, indicating that they are not distinct populations.

The debate rages about whether race is any more than a social or political construct. This debate is evident not only in the formal scientific literature but also in the less formal venue of the Internet with its personal essays and blogs. Clearly this discussion will continue. The genetic evidence does not support the concept of distinct races within *Homo sapiens.* The differences between populations are not distinct and show extensive gradients of intermixture. The variation is significant within groups traditionally considered to be races. Our conclusion is that the data do not support more than one biological race, and we reject the biological concept of different races within the human species.

If we do not accept that there is a biological basis for race but do accept that all humans belong to a single human race, then we will not use the sociopolitical construct of race in our discussions here. We will use terminology such as *ethnocultural group* or *ethnicity* to reflect a more porous population structure than is usually reflected in concepts of distinct racial groups. While there is cohesion within these groups, we argue that this derives more from shared culture than from unique genetic features.

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Genetic tools may provide some insight into one's ethnocultural group identity. Web sites appealing directly to consumers assert that the secrets

of our ancestry are present in our DNA. Geneticists have referred to this as "recreational genetics," since it represents the use of genetic technology to satisfy an individual's curiosity.

As we begin to discuss ethnicity, it is important to recognize that these groups are not static entities. Individuals often look at ethnocultural groups as they exist in the present — frozen in the moment. But, in fact, these groups are changing constantly over time. This realization has critical implications for individuals searching for their genetic roots.

Human DNA has not been shuffled at the level of the individual nucleotide base pair, but identical nucleotides remain grouped together in what are referred to as haplotype blocks (chapter 4). It is not known whether the existence of these haplotype blocks represents the relatively young age of our species or is evidence of the biological importance of local DNA context within these blocks. Some haplotype blocks have remained intact from the mouse to the human genome, suggesting that there may be some functional or contextual relevance for these blocks.

Single-nucleotide polymorphisms (SNPs) within these blocks of DNA serve as "signatures" identifying specific haplotype blocks. Therefore, these SNPs might enable us to identify lineages among humans. There is a fundamental problem with this approach, however. If, for example, you wanted to trace your heritage back twelve generations, most likely you would not have representative DNA from three hundred years ago (twelve generations of twenty-five years per generation) representing the ethnocultural groups to which you might be related. You could obtain contemporary samples from the groups' representatives, but the DNA would have changed by in-migrations, out-migrations, armed conflicts, genetic bottlenecks — when small populations expand rapidly — and many other factors over the intervening three centuries.

Since ethnocultural groups are not stable entities and historically representative samples are rarely available, then your DNA results may be misleading. You might find that you are related to a contemporary group, but if you had historically continuous samples, then you might find that "your" group was very different. Perhaps "your" ethnocultural group from A.D. 1700 no longer even exists. Since history is recorded by the conquerors, if "your" group had been conquered and absorbed by the conquerors, then there may be no historical record of its existence.

Despite genetic similarities between different ethnocultural groups, differences can be identified. When the results of genetic studies are com-

pared with self-reports of ethnic group membership, the latter prove to be highly accurate.

An international group of population geneticists led by Marcus Feldman of Stanford University reported in 2002 what was then the largest study of human population genetic structure. They used 377 different short repeat sequences called microsatellites, each with at least four and up to thirty-two distinct patterns to generate genotypes for 1,056 individuals representing fifty-two populations. Their results were amazingly similar to Lewontin's: 93 to 95 percent of genetic variation was within populations, and only 3 to 5 percent of the variation was between major groups. Feldman and his colleagues identified six primary genetic clusters. Among these six clusters, five represented major geographically defined regions. These five regions included Africa, America, East Asia, Eurasia (encompassing Europe, the Middle East, and Central/South Asia), and Oceana.

The sixth group indicated consistency with its oral history. This group was largely composed of the isolated, relatively small, Indo-European-speaking, Kalash people of mountainous northwest Pakistan. Other groups from Pakistan were associated with the East Asia population and were distinct from the Kalash, and the Kalash had no affiliation with others within this geographic region. The investigators concluded that the Kalash results were consistent with previous genetic evidence of similarities that they shared with certain individuals from Europe and the Middle East. The oral history of the Kalash indicated origins from the armies of Alexander the Great, perhaps providing the source of the shared markers with the Europeans and Middle Easterners.

Small isolated groups like the Kalash experience genetic drift, or random genomic changes, over time that may change frequencies of genetic markers rapidly. Larger dynamic populations like the Europeans have broader input that may change their genomic sequences and in which small contributions could be lost. Therefore, even if two populations, for example, Europeans and the Kalash, were historically related, their genomic sequences would have changed substantially since the time of Alexander the Great in the fourth century B.C.

Historical complexity may result in population complexity. The Eurasian population was the group in which Feldman and his colleagues had most difficulty in assigning ancestry. Feldman attributed this difficulty to migration, conquest, and trade. Two groups within the Eurasian population were easier to separate, the Basques and the Sardinians, and both

were culturally and geographically isolated from the greater bulk of Eurasians. But what does this mean for other members of the Eurasian group? Two populations, for example, Swedes and Turks, each of which would probably consider its group to be quite different in appearance and culture from the other, were difficult to distinguish genetically.

In this large population study involving more than a thousand individuals, the population clusters were determined mathematically, and the analysis of molecular variance was carried out without knowledge of the origins of the individuals providing the specimens. When the mathematical analyses were complete, the investigators looked at correspondence between the mathematically defined clusters and the participants' self-reports of their ancestry and found the correspondence to be quite high. The authors concluded: "Thus, for many applications in epidemiology, as well as for assessing individual disease risks, self-reported population ancestry likely provides a suitable proxy for genetic ancestry."

In 2005, Neil Risch, then at Stanford, and his colleagues analyzed 326 genetic markers to assess ethnocultural groups among 3,636 unrelated individuals recruited from fifteen different geographic sites in the United States and Taiwan. The participants had self-identified their ancestry as white non-Hispanic, black non-Hispanic, Hispanic, Chinese, or Japanese. Cluster analysis using these markers assigned individuals to four groups. Chinese and Japanese were placed into a single East Asian group, since the analysis could not separate them. Only 5 of the 3,636 participants, or 0.14 percent, were assigned to a category different from the one they had identified themselves as belonging to. These investigators cautioned others about the interpretation of results of genetic marker analyses between different populations. They urged that not all differences between genetic clusters be assigned a genetic basis, because of the confounding influences of highly correlated nongenetic factors, such as social, cultural, economic, and behavioral influences. In other words, groups that separate genetically may have ethnocultural differences that influence their rates of diseases independently of their genetic differences.

A key question for anyone planning to participate in recreational genetics is, how accurate are the results? The answer is that laboratory technologies coupled with mathematical analyses to assess genetic and genomic differences are improving the ability to identify ethnocultural group membership. However, in general, these assignments are probabilistic and not absolute in nature. Accuracy will be influenced by the specific group or admixture of groups represented by that individual's

ancestry. The database of ethnocultural group DNA samples available to the laboratory will also determine the level of accuracy.

These laboratory analyses are expensive. Studies indicate that an individual's knowledge of his or her own ancestry is relatively accurate. In medical genetics, we know that one of the least expensive and most powerful genetic tools available to us is a good family history. The decision to carry out DNA testing for ancestry will be up to the individual. A far less expensive and excellent alternative available to many of us is to learn about our ancestry from the elders in our families. In addition, relatives' stories will have far more cultural meaning than biological measures of ancestry.

Although the tools are available to begin to dissect an individual's ancestry from his or her DNA, the differences that permit this dissection represent an extremely small part of the genome. The vastly larger part of genomic variation is within rather than between ethnocultural groups. In addition, the idea that analysis of a person's DNA will provide that individual with an understanding of her or his identity — that the secrets of ancestry are written in DNA — is another example of genetic determinism (chapter 3). For those individuals who move to a new home and a new culture, for example, by immigration or adoption, we would argue that the new culture will be at least as much a part of their identity as genes acquired twenty generations, or five hundred years, ago. Who among us is in the same place socially or culturally as one of our ancestors from five centuries ago?

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We have been examining the ability of genetic and genomic analyses to solve puzzles about our origins as individuals and groups. The next issue we address is whether ethnocultural group membership is a valid proxy for individualized medicine. A legitimate goal of genomic medicine is to determine predictive, preventive, and personalized approaches, but that is a goal that is beyond the technological capacity of medicine at this time. So, is there value in knowing an individual's ethnocultural identity as an intermediate step that can be used to improve that individual's health care?

The drug BiDil was approved in 2005 by the U.S. Food and Drug Administration to treat African Americans with heart failure. However, both Troy Duster from New York University and Jonathan Kahn from the University of Minnesota have noted that the study providing the basis for

this recommendation was criticized for focusing on an age range (forty-five to sixty-four years of age) with only 6 percent of heart failure mortality and for only studying African Americans. A 2005 editorial in *Nature Biotechnology* concluded that BiDil was more about money and political correctness than about a drug that was more effective for this ethnocultural group. The editorial further asserted that NitroMed, the manufacturer of BiDil, without having to conduct an expensive multiracial clinical trial, had added thirteen more years of market control for this drug with the "racially" focused patent. The first BiDil patent did not specify race and expired in 2007. The anonymous author of this editorial stated: "Race is simply a poor proxy for the environmental and genetic causes of disease or drug response," and added, "scientifically, race is a meaningless marker of anything. Pooling people in race silos is akin to zoologists grouping raccoons, tigers and okapis on the basis that they are all stripey." Clearly, this editorial's author did not approve of ethnic group membership as a proxy for individualized medicine.

Differences in disease associations among ethnocultural groups may provide insights into gene-gene and gene-environment interactions. Some proponents of the use of ethnic group membership in medicine have used the example of Alzheimer's disease — a progressive loss of mental capacity with age — and the risk associated with the variant of apolipoprotein E — a protein that is a component of certain plasma lipoproteins like very low-density lipoprotein (VLDL), also known as "bad cholesterol." The variant associated with increased risk of Alzheimer's disease is known as APOE ε4. Alzheimer's disease is a common complex genetic disorder, and scientists are still learning about the genes involved in it. Any insights into gene-gene and gene-environment interactions would therefore be important. The risk of Alzheimer's disease varied by a factor of 5 for both APOE ε4 heterozygotes (one dose of APOE ε4) and homozygotes (two doses of APOE ε4) across these populations. African Americans have a 1.1-fold increased risk if they are heterozygous for APOE ε4 and a 6-fold increased risk if they are homozygous. Caucasian Americans show a 3-fold increased risk if they are heterozygous and a 5-fold increased risk if they are homozygous. Japanese have a 5.6-fold increased risk if they are heterozygous and a 33-fold risk if they are homozygous. These results indicate that gene-gene and gene-environment interactions with APOE ε4 clearly differ in those three ethnocultural groups.

The ideal of genomic medicine would be to have each person's

genomic sequence available to guide his or her preventive health care (chapter 15). However, the affordability of that sequencing and the evidence-base to utilize that information effectively will not exist until well into the future. In the absence of that individualized sequence information, ethnocultural group may represent a proxy for some diseases and some groups. With any approximation, absolute accuracy cannot be achieved, and so it will be with ethnocultural group and genomic sequence. In addition, the genetic variation within ethnocultural groups argues for caution. Recognizing that it is an approximation, we would argue, however, that ethnicity is information that should be used when there is the evidence-base to support its credibility for a specific disorder. We must be cautious of the social risks of population stratification by ethnocultural group. We would argue that avoiding the inaccurate terminology of human races would be one possible step to consider.

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Studies of autosomal markers, such as SNPs, examine inheritance across the genome. The genetic heritage is predictable by the rules of Mendelian inheritance (chapter 2): half of these markers will be inherited from the mother and half from the father. One-quarter will be inherited from each of the four grandparents, and so it goes. While autosomal contributions from an individual's progenitors are decreased in each generation, there are ways to trace maternal and paternal lineages directly without such dilution.

Each of us, male or female, received our mitochondria (chapter 2) from our mother. Our mother received her mitochondria from her mother; and so on. A male, who has one Y and one X chromosome (chapter 6) received his Y chromosome from his father. His mother does not have a Y chromosome. His father received his Y chromosome from his father; and so on. We will explore the power of these approaches later in this chapter. Let's first examine potential pitfalls in studies of mitochondrial and Y chromosomal DNA.

The Melungeon DNA study illustrates these potential pitfalls. The Melungeon people, who inhabit relatively isolated areas of southern Appalachia in the border regions of Virginia and Tennessee, have mysterious historical origins. They are thought to be of mixed Native American, African, and European descent, and origin stories include genetic contributions by Turks, Portuguese, and many others. They

have darker skin pigmentation than is considered typical for those of northern European descent. During the period of slavery their legal status was "free," though they were subjected to significant discrimination. Because of the confusion and curiosity regarding their ethnic roots, a group of Melungeons began to work with a private company to elucidate their heritage at the DNA level. This Melungeon DNA study uses mitochondrial DNA (mtDNA) and Y chromosomal DNA markers to trace inheritance.

Problems with maternal and paternal inheritance studies are the extremely small contributions provided by those from whom the mitochondrial and Y chromosome markers derived. Paul Brodwin from the University of Wisconsin, Milwaukee, addressed the Melungeons on this topic. He cautioned that if, for example, one traced a Y marker back ten generations to a Native American male ancestor, that genetic contribution would represent only $1/1{,}024$ (calculated as $1/2^{10}$) of one's inheritance, a relatively small contribution. Therefore, following the Y chromosome markers would not give insight into complex genetic admixture.

James Elder, a researcher who is married to a Melungeon genealogist and author, criticized the Melungeon DNA project because of the potential effects of "lineal interruptions." He pointed out that if a woman in the maternal lineage, or a man in the paternal lineage, was not a Melungeon, then the results would be misleading. For example, let's say that you consider yourself a Melungeon because your mother and father both consider themselves to be Melungeon. However, if your great grandmother on your mother's side — your mother's mother's mother — was a German immigrant who met and married your Melungeon great grandfather, then the mitochondria would represent her matrilineal inheritance, and not the "Melungeon DNA." Similarly, if you are male, and your paternal great grandfather — your father's father's father — was Polish, even though he married your Melungeon great grandmother, then your Y chromosome would reflect the Polish, not the Melungeon heritage.

The unknown and mysterious origins of the Melungeons have led many within this group to be quite curious about their ancestry. They have used the traditional written historical records to trace their genealogies, and more recently they have turned to DNA markers to supplement these genealogies. The genetic studies may be quite accurate, but studying mitochondrial and Y chromosomal DNA may provide an incomplete story, particularly when a group's genetic background may be so complex.

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The maternal lineage can be traced by mitochondrial DNA. As we discussed in chapter 2, the mitochondria have their own circular DNA copies that resemble bacterial DNA. An individual's mitochondria derive from the mother's egg. While mitochondria are critical to sperm motility, the father's sperm do not contribute mitochondria to the embryo. Diseases resulting from mutations in genes encoded on the mtDNA or markers in the mtDNA are said to exhibit maternal, or mitochondrial, inheritance — they are passed from the mother to all of her offspring, and her daughters pass them to their offspring, but males do not contribute mitochondria or mitochondrial DNA to their offspring.

It is possible to estimate the age of mtDNA, since changes, including insertions, deletions, and single base pair alterations, occur over time. The predictable rate of change in these mtDNA, referred to as a genetic clock, is more rapid than the rate for the majority of the nuclear genome. If mitochondrial DNA from two different individuals have the same set of haplotype markers, then they are related. If they have some similar markers, but other different markers, then they share a common matrilineal ancestor, but their lines diverged at some point. The DNA-based genetic clock permits estimation of the relatedness of two maternal lineages using mtDNA.

In 1987, Allan Wilson and his colleagues at the University of California, Berkeley, reported their analyses of mtDNA samples from around the world and came to a conclusion that caught international attention. They examined mtDNA from 147 individuals representing five groups they defined as sub-Saharan Africans, Asians, Caucasians, aboriginal Australians, and aboriginal New Guineans. When they constructed an evolutionary tree for human mtDNA, they found that all of the mtDNA samples traced back to a single female ancestor, and the data indicated an African origin for this shared matriarch. By analyzing the rate of change in the mtDNA samples — the genetic clock — they determined that the common ancestor of all surviving mtDNA types lived 140,000 to 290,000 years ago. They also estimated when the human migration out of Africa took place but could not narrow the estimate any more closely than 23,000 to 180,000 years ago.

Since a single female ancestor can account for all humans based on these mtDNA analyses, she quickly became known as the "African Eve," and then, more correctly, the "Mitochondrial Eve." These data indicate that this woman is the most recent common female ancestor whose offspring survived to the modern era. There may have been other women, perhaps even older, whose lines were wiped out by disease or trauma

before their offspring could have daughters who could reproduce, and who had human female ancestors who shared her mtDNA. But, Mitochondrial Eve is the youngest common female ancestor whose offspring gave rise to the humans who survived until today.

Vincent Macaulay from the University of Glasgow and his colleagues attempted to determine the route of early human dispersal out of sub-Saharan Africa. The traditional model, referred to as the "northern route," would have involved migration from northeastern Africa through the Mediterranean southwestern Asian region, the area known as the Levant, approximately 45,000 years ago. A more recent model, known as the "southern route," would have involved an earlier time frame (approximately 60,000 to 75,000 years ago) and exit from the Horn of Africa followed by migration along the tropical coast of the Indian Ocean. Macaulay and colleagues sequenced thirty-one mtDNA coding samples from 260 aboriginal inhabitants of the Malay Peninsula, a group known as the Orang Asli, as well as additional samples from eastern Eurasian and African individuals. Archaeological records support a continuous hunter-gatherer occupation of this region by the Orang Asli for at least 40,000 years and perhaps as long as 70,000 years. These investigators determined the time to the most recent common female human ancestor, Mitochondrial Eve, to be around 200,000 years, consistent with the estimates of Wilson and colleagues in 1987.

The results of Macaulay and his colleagues support a southern route for the single dispersal out of East Africa by a founding population of perhaps several hundred individuals sometime after the appearance of a new mtDNA marker approximately 85,000 years ago. This exodus was followed by a period of mtDNA changes in this group, a band which presumably stayed intact or in close contact with its subgroups. Subsequently, around 65,000 years ago, there was a rapid dispersal from this group around the tropical coast of the Indian Ocean that took only several thousand years. Arrival times in India and Australasia are estimated to be only 3,000 years apart.

Macaulay and his colleagues compared mtDNA results from Malaysia with those from western Eurasia. They provided an alternative to the usual explanation that the Levant and Europe were colonized by individuals leaving Africa using the northern route. Their data fit better with an origin for modern Europeans in India. Both have a common ancestor who lived approximately 66,000 years ago. The researchers suggested that an "early offshoot" of the group that took the southern route out of the Horn of Africa stopped in India, where the ancestors of western Eurasians

remained until the climate improved. Then the ancestors to the modern populations of the Levant and Europe migrated from India to their new homes.

The mtDNA analyses reported by these investigators could rewrite history. Their results not only support the southern route for the human migration out of Africa but also give new insights into the origins of populations from Australasia through Asia and to Europe. An origin for modern Europeans in India fits well with the nature of the Indo-European language family spoken today by populations from central Asia — East Turkistan — through India, Iran, and Anatolia to Europe. This work also suggests that the cultural and creative innovations that appeared in the Upper Paleolithic period, the most recent subdivision of the Stone Age, was not a "revolutionary" response but the consequence of population dispersal and emerging modern humans meeting the challenges of their environments. By meeting these challenges creatively, they also had the leisure time to explore aesthetic interests more deeply and to enrich their culture.

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While mtDNA can be used to track matrilineal relationships, Y chromosomal DNA can be used to track patrilineal relationships. The Y chromosome, or male sex chromosome, is unusual among our chromosomes. It does not have an identical chromosome with which to pair, like all of the nonsex, or autosomal, chromosomes, and even the two X chromosomes in females. The male nuclear complement of chromosomes, or karyotype, is 46,XY. These two quite different chromosomes, the X and Y, do pair over portions of their lengths. Since the part of the Y chromosome outside of the X-pairing region does not pair and recombine, its changes happen over time and presumably by chance. Changes in the nonpairing region of the Y chromosomal DNA can be used to look at degree of relatedness and the time to the nearest common male relative, just as mtDNA permits assessment of female relatedness.

Certain cultural traditions follow patrilineal or matrilineal patterns, and if one of these patterns is dominant in a culture, then the culture may be referred to as a patriarchy or matriarchy, respectively. For example, the dominant traditional culture in the United States follows a patrilineal passage of surnames from the father to his offspring. This means that if we do not consider misattribution of paternity (generally estimated at 10 percent and as high as 30 percent based on studies in the 1970s through the

1990s, but with recent estimates in Western countries lower, at 1 to 3 percent), then males with the same surname, who are related to a common male progenitor, should have the same Y chromosomal DNA. If, however, the male progenitor lived long ago, then there may have been random genetic changes in the Y DNA of male offspring along the line. The accuracy of this cultural tradition of patrilineal surname continuity could therefore be traced by analyzing the DNA of the Y chromosome.

Examining the origins of the Jewish priests, the Cohanim, provides an opportunity to investigate Jewish priestly tradition using Y chromosome markers. Jewish identity has been transferred by matrilineal descent or by religiously ordained conversion, since at least the Talmudic period in the first millennium B.C. Certain traditions and responsibilities, however, are passed along in a patrilineal manner in Jewish culture.

According to biblical accounts and Jewish tradition, after the Exodus of the Jews from Egypt, three male castes were recognized — Cohen (Hebrew for *priest*; plural is *Cohanim*), Levi, and Israelite — and were determined by patrilineal descent. The Cohanim descend from Aaron, the brother of Moses, and have special duties, rights, and restrictions. For example, they are responsible for certain sacrifices and offerings. They have restrictions on choosing their spouses and against entering cemeteries.

The Levites, or descendents of Levi, who included Aaron and Moses, had some rights similar to, but did not have the special restrictions of, the Cohanim. The Israelites were all Jewish males other than the Cohanim or Levites. It has been estimated that the Cohanim and the Levites each represents approximately 5 percent of the male Jewish population worldwide of about 7 million individuals.

There are two geographically separate Jewish communities that, because of the time they have been isolated from each other, have developed certain distinct practices. The Ashkenazi Jews are thought to have originated in the "Ashkenaz" region of northwestern Europe at least as early as the sixth century A.D. and by the sixteenth century occupied communities throughout Eastern Europe. The term *Sephardim* originally referred to Jews who lived in Spain prior to the Inquisition and expulsion in A.D. 1492. However, it has come to include all Jews who trace their ancestry to those communities of the Near East and North Africa that followed the Sephardic religious traditions. It is generally accepted that there was minimal gene flow between the Ashkenazim and Sephardim until the mid-twentieth century.

Karl Skorecki from Technion, the Israel Institute of Technology, and

his collaborators examined Y chromosomal markers from 188 unrelated Jewish males and identified six different haplotypes, or distinct marker signatures. They asked each subject if he was a member of the priesthood, and if he was unsure or answered Levite, then he was excluded from this study. The investigators were able to clearly separate the Cohanim from the Israelites. They were also able to distinguish these groups as effectively in the Ashkenazim and Sephardim. They concluded that these results were consistent with the Jewish priesthood, or Cohanim, antedating the division into Ashkenazic and Sephardic groups more than a thousand years ago.

In a subsequent report, Skorecki and his colleagues confirmed the homogeneity of Y chromosomes in Ashkenazi and Sephardic Cohanim. They estimated the number of generations since the Cohanim chromosome arose to be 106 generations, or, with an average generation length of twenty-five years, approximately 2,650 years ago, between the Exodus and the destruction of the first Temple in 586 B.C.

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Combining mtDNA and Y chromosomal DNA analyses in the same population can provide very interesting results about not only population origins but also the mating patterns within populations. Ken McElreavey from the Pasteur Institute, S. Qasim Mehdi from the Dr. A. Q. Khan Research Laboratories, and Chris Tyler-Smith from the University of Oxford, with large international teams, have carried out these studies on individuals from southwest and central Asia.

The Kalash, whom we introduced earlier in this chapter, had admixture of Y markers with the Greeks of 20 to 40 percent, but this was felt to be due to genetic drift among the small Kalash population and not evidence of a Greek origin of the Kalash Y chromosomes. The investigators concluded, however, that "this conclusion does require the assumption that modern Greeks are representative of Alexander's armies," which as we have noted previously may not be the case. The mtDNA appears to be representative of western Eurasians with no detectable contributions from east or south Asian lineages. They speculate that the matrilineal evidence is strongest for the western Eurasian origin of the Kalash, who ultimately trace their origin to the Middle East, all consistent with their origin in their oral histories from the armies of Alexander the Great.

Another group, the Hazaras, claim male-line origin from a different army, that of Genghis Khan. Evidence from mtDNA and Y chromosomal

analyses indicate patterns consistent with origins in eastern Asia, including Mongolia, and a virtual absence of markers from the surrounding Pakistani populations. The investigators interpret these results as consistent with the Hazara origin story and indicate that Genghis Khan's male soldiers brought females with them of eastern Asian ancestry and that this group has continued mating largely within their ethnocultural group. This is a mating pattern defined as symmetrical.

Other groups, however, evidence asymmetrical mating patterns. The Parsis of southeastern Pakistan are documented by historical record to have emigrated from Persia in the seventh century A.D. following their prophet Zoroaster, arriving in Gujarat, India, approximately A.D. 900, with a group eventually settling in Pakistan. The Y chromosomal markers are essentially 100 percent Persian, but the mtDNA data show an estimated 100 percent admixture from Gujarat. This indicates a Persian origin for Parsi males, who then mated with local females in India, resulting in loss of the Persian mtDNA.

Another example of an asymmetrical mating pattern is found among the Negroid Makrani from the coastal Makran region of Pakistan. These individuals have physical traits of Africans and trace their origins to the slave trade between the coastal regions of eastern Africa, including Mozambique, and Makran. The DNA data, however, indicate that the maternal contribution to the Makrani from sub-Saharan Africans is estimated at 40 percent and the paternal contribution at only 12 percent. These results are attributed to a female bias in the East African slave trade, in which the female slaves were primarily domestics or concubines, and offspring fathered by the male owner were freed. Male slaves faced strong cultural challenges to fathering offspring and many of the male slaves were eunuchs. Therefore, the mating pattern favored African females mating with western Eurasian males.

In these examples of mitochondrial and Y chromosomal DNA analyses, the interplay of history and culture is clearly evident in the origins and mating practices of these peoples.

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DNA analyses of the mtDNA and Y chromosome provide powerful tools to dissect the migrations of humans and the interactions between different groups. But this technology is not only available as an archeological dig to explore our biology. As shown by the investigations of the Cohanim, these analyses also permit examinations of cultural traditions,

their stringency over time, and the influence these traditions have on genetics. You have seen how genes coevolved with culture in the work on lactose tolerance (chapter 4). In the examples presented in this chapter, you can see yet again that your genes are intricately intertwined with your culture and the histories of your predecessors. While we would argue that your genes do not perfectly predict your future, they do contain your past. Your history is written in your genes!


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the print version of this title.]

CHAPTER 6

Gender as a Spectrum, Not a Dichotomy

Just as ethnocultural group membership is important to many individuals' concept of their identity (chapter 5), we propose that for many, though not all individuals, their gender is at least as important to that self-identity. Our culture generally assumes gender to be dichotomous — male or female. As we will see, however, a simple dichotomy does not adequately address the many possible categorizations of gender and sex, and in fact the same individual may be defined as male in one category and female in another.

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The following scenario — the question in the delivery room — is one that occurs more often than one might expect. The Intersex Society of North America (ISNA) estimates that ambiguous genitalia — external genitalia

that cannot be described clearly as male or female — of sufficient concern for a specialist to be consulted occurs approximately once in every 1,500 to 2,000 births. With 4 million U.S. births per year, these numbers mean that at least 2,000 infants with ambiguous genitalia are born annually, or around five infants with this condition each day.

A couple, both thirty years of age, are in the delivery room for the birth of their first child. They had not elected to have prenatal testing, such as chromosomal analysis. The couple had no interest in knowing the sex of the fetus prior to delivery. They really had no preference for a girl or a boy and cherished the mystery of not knowing.

The labor and delivery proceed normally, with the father holding the mother's hand and encouraging her. As the baby appears, crying vigorously, both parents ask simultaneously, "Is it a boy or a girl?"

The obstetrician and pediatrician, after briefly taking note of the baby's genitalia, glance knowingly at each other. The pediatrician responds, "We are not always able to answer your question immediately in the delivery room. We will bring together our team of experts to give you the best answer as soon as we can. You have a lovely baby. Let me examine your baby carefully, and I will talk to you more in a few minutes."

When physicians are faced with this situation, they may feel considerable pressure from the parents and other health care providers to answer with a best guess. As difficult as it is to answer as the pediatrician did in this scenario, it is far better than having to raise the question once you have told the parents a specific gender.

Let's discuss the terminology we will use in this chapter. The word *gender* traditionally has been used in reference to grammatical categorization, such as a noun in some languages being masculine, feminine, or neuter, or cultural classification, such as masculine or feminine behaviors. The traditional use of the word *sex* has been for biological categorization. We will discuss social constructs as well as biological concepts in this chapter. Because the distinctions between the terms *gender* and *sex* are not widely followed, and to avoid confusion by continuously changing terminology, we will use the term *gender* more generally throughout this chapter and will reserve the term *sex* for biological discussions.

Gender is typically considered dichotomous: one is either male or female. This concept is concretized, for example, in the biblical origin story: humans were created as the two distinct sexes in the forms of Adam and Eve. This dichotomy is so ingrained in our consciousness that most individuals would not question it.

These distinctions, however, are not absolute. Gender is a continuum

rather than a dichotomy. There are many ways to categorize sex and gender. An individual, however, may not be consonant in all categories. One may fall into different positions on the continuum for the various categories. In other words, not only is gender a continuum, but also one individual might be categorized as more than one gender!

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Let's consider anatomic sex. Anatomic differences may be what many individuals consider to be the basis for dichotomous genders: a female has labia and a vagina, and a male has a scrotum and penis. But as we saw in the delivery room scenario, these distinctions are not always absolute or obvious. The traditional medical terminology for such a phenotype is ambiguous genitalia, implying an abnormal or incorrect appearance. Members of the advocacy community led by ISNA prefer the term *intersex*. A recent meeting of medical specialists from a variety of fields settled on the term *disorders of sexual development,* which is even more value-neutral.

Genital sex is determined long before birth. Until the eighth week after conception, a human fetus has bipotential external genitalia, meaning that the genitalia look the same in both male and female. Soon, the genitalia start to look different, and the key is a signal present only in males. If the signal is present, the fetus develops male structures, but if not, then female structures develop.

Testosterone is a male hormone that is made in the developing testis — the male gonad. Testosterone is converted by the enzyme steroid 5α-reductase to dihydrotestosterone (DHT). DHT activates the androgen receptor, regulates expression of developmental genes, and promotes development of the penis and scrotum.

Gonadal sex is also bipotential early in development. The development of the male gonads, the testes, can first be observed during the fifth gestational week. Primordial germ cells migrate out of the yolk sac wall to the gonadal ridge of the embryo beginning by the sixth week of gestation. Differentiation of the bipotential gonad into testes begins after the sixth week of gestation and is obvious by the end of the seventh week, much earlier than differentiation of ovaries. The testes begin to produce testosterone at approximately the ninth gestational week. Fetal circulating testosterone peaks at approximately the sixteenth week of gestation and then falls to prepubertal levels. Mature sperm do not develop until puberty, and they continue to be produced throughout the man's life. A

typical ejaculate contains approximately 200 million sperm, and it is estimated that a trillion sperm are formed during a man's lifetime.

Differentiation of the indifferent gonad into an ovary occurs in the twelfth gestational week, much later than the testes, and requires the absence of testes-determining genes. All of the ova that a woman will produce during her lifetime are formed and in an early stage of oocyte development by birth. These ova will not complete development until ovulation, when the egg is released to be available for fertilization. These oocytes number 2.5 million at birth, but only about four hundred mature to ova.

Individuals with one disorder of sexual development have both ovarian and testicular tissue. They may have an undescended testis on one side and an ovary on the other side, or they may have a mixed tissue structure, with features of both an ovary and a testis — an ovotestis. These individuals are distinguished from those with another disorder of sexual development, who may have the gonadal tissue of one sex and either the genitalia of the other sex or ambiguous genital structures. These individuals show us how there may be a discrepancy between sex categorization depending on whether they are classified by their external genitalia or their gonads. They could, for example, be female based on their external genitalia and male based on their gonads.

One common disorder of sexual development is congenital adrenal hyperplasia. In this disease, the adrenal glands that make stress hormones are dramatically enlarged or hyperplastic. The most common cause of this genetic disorder is an enzyme deficiency with abnormal concentrations of certain hormone intermediates that stimulate the androgen or male hormone receptor, and masculinize, or virilize, the external genitalia, so that they are male or ambiguous in appearance. These individuals have normal ovarian tissue.

Another disorder of sexual development, androgen insensitivity syndrome, leads to the appearance of female or intersex external genitalia and testicular tissue. The androgen receptor is the receptor for testosterone and DHT. If mutations render it resistant to activation by testosterone or DHT, then the genitalia remain female in appearance. These individuals have normal female external genitalia, as well as normal female secondary sex characteristics, including development of breasts and the female pattern of fat distribution. They do not have menarche, or onset of menstrual periods, and this feature is frequently the only reason why they come to medical attention, because all of their features are so typically female. At that time, medical examinations show that the undescended

testes are found in the abdomen, and the unfused halves of the scrotum appear anatomically to be labia majora.

The individuals with the disorders we have described so far may have different genital and gonadal sex assignments, but these features remain constant during their lives. Some individuals with gene mutations, however, can actually appear to change genders during development. While the disorder we will discuss is rare in most populations, it provides important lessons in cultural acceptance of gender change in an individual. Steroid 5α-reductase type 2 (SRD5A2) is an enzyme that converts testosterone to DHT, and the genetic deficiency of SRD5A2 blocks this conversion.

SRD5A2 is expressed in the external genitalia primordium before differentiation into penis and scrotum. If this enzyme is mutated, then DHT production is deficient, and the external appearance at birth and during early childhood is female. The individual is in fact a genetic male, and the testes will begin to make testosterone when she reaches puberty. While DHT is critical for the development of fetal male external genitalia, testosterone is important in the development of male secondary sex characteristics during puberty, including changes in the voice and virilization of the external genitalia. These individuals will begin to have such changes at puberty, and their phenotype will become increasingly masculinized as they progress through puberty.

SRD5A2 deficiency is an autosomal recessive disorder. This enzyme deficiency disorder was studied originally in an isolated population in the Dominican Republic in which it occurs at a relatively high rate due to the interrelatedness of the members of this community. The purpose of the investigations was to determine the relative influences of sex of rearing and androgens on the development of male gender identity.

Many affected individuals had a female appearance at birth. Eighteen of the affected individuals were reared "unambiguously" as girls. During puberty, seventeen of the eighteen developed male identities and sixteen of the eighteen assumed male gender roles. The phallic and testicular growth, and the development of secondary sexual characteristics at puberty were well known in the community. Gender reversal was completely acceptable within the culture of this community. The authors concluded that the normal exposure of the central nervous system to testosterone during development would appear to make a substantial contribution to male gender identity. The experiences of these individuals demonstrated that the influence of testosterone could override the influence of the individuals' sex of rearing as girls. The absence of socio-

cultural barriers to gender identity change in this community clearly facilitated the shift in gender identity and role.

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Genotype is an individual's genetic constitution. The genotypic sex is the entire genetic makeup of that individual as it influences sex determination and sexual differentiation. Sex determination is the biological decision-making process that takes the bipotential or multipotential early embryo capable of forming male or female gonads or genitalia and commits it toward a male or female phenotype. Genotypic sex can be subdivided into chromosomal sex and genetic sex.

For most individuals, the chromosomal sex will reflect the genotypic sex. The normal chromosomal complement, known as the karyotype, usually is 46,XX for a female and 46,XY for a male. The karyotype may be abnormal, such as: 45,X; 47,XXX; 47,XXY; or 47,XYY. A general but not inviolate rule is that the gonadal phenotype will be female in the absence of a Y chromosome and male if one or more Y chromosomes are present. This observation led physicians and investigators to speculate that a gene termed the testis-determining factor (TDF) resided on the Y chromosome. This proposal argued that the testis and maleness were conferred by the presence of this Y chromosomal gene. The ovary and femaleness were described as the "default" condition that would occur in the absence of TDF.

Individuals with a 45,X karyotype have a collection of findings called Turner syndrome. Most fetuses with this disorder do not reach term and are resorbed or spontaneously aborted. The surviving girls with Turner syndrome have short stature, poor drainage of lymph fluid resulting in "webbing" of the neck and puffiness of the hands and feet, a female phenotype with absence of development of secondary sexual features, and primary amenorrhea, or a total absence of menstrual periods. Some individuals will be mosaic, or have a mixture of cells; for example, some cells will have the 45,X Turner syndrome karyotype, and others the 46,XY typical male or 46,XX typical female karyotype. If we take the first example of 45,X/46,XY mosaicism, it is assumed that at the time the egg was fertilized by the sperm, the resulting cell was 46,XY. Then early in development a multipotent cell lost the Y chromosome, resulting in this mosaicism. That individual's sex determination and sexual differentiation will be determined by the relative representations of the two karyotypes,

not only in their whole body, but also in the individual organs, such as the gonads and the brain.

Some individuals may have an appearance that is opposite of their karyotype. This is caused by genetic alterations that cannot be visualized at the level of the chromosomes, leading to the concept of genetic sex. While we cannot explain these discrepant observations for all such individuals, our knowledge is increasing in the area of the genetics of sex determination. We can identify the causes for many of these discrepancies.

The gene for the testis-determining factor was assumed to be on the Y chromosome. The gene termed *SRY,* for sex-determining region Y, was identified and shown to be TDF. Transgenic XX female mice that had a mouse *Sry* transgene inserted into their genomes, to make them XX,Sry^+, developed as males, showing that the *Sry* gene was sufficient to result in male sex determination. The majority of 46,XX males who do not have sexual ambiguity have an *SRY* gene that has been translocated onto the X chromosome, and therefore their genotype is 46,XX,SRY^+.

A common cause of 46,XY females is a genotype of 46,XY, SRY^-, meaning that the *SRY* gene has been deleted from the Y chromosome. There can be other mutations in the *SRY* gene that inactivate the protein and do not completely delete *SRY* that result in XY sex reversal. The change of only one base pair in the DNA of the *SRY* sequence is sufficient to cause a female phenotype with a 46,XY karyotype. Frequently XY females come to medical attention when they have delayed onset of puberty.

In both of these sex reversal situations — 46,XY females and 46,XX males — while the phenotypes may be unambiguously reversed, the individuals are unlikely to be able to generate functional eggs or sperm.

An even more complex situation is a 46,XY,SRY^+ female; this can be due to a variety of alterations involving the X chromosome or the autosomes. It was recognized that certain individuals with this genotype had a duplication of the middle portion of the short arm of the X chromosome. This region, which measured 160 kilobases in length, was called the dosage-sensitive sex reversal, or DSS, region. As we discussed in chapter 2, our laboratory cloned the *DAX1* gene, and mutations in this gene caused adrenal hypoplasia congenita. Duplications of *DAX1* are responsible for dosage-sensitive sex reversal, and a double dosage of the DAX1 protein appears to block the male-determination network that includes *SRY*. Females with this disorder of sexual development have the following genotype: 46,XY,SRY^+,DAX1dup.

The gene *WNT4* maps to a region on the short arm of chromosome 1 that when duplicated is associated with sex reversal. From the work of Eric Vilain at the University of California, Los Angeles, and his colleagues using cell culture and mouse models, it appears that *WNT4* is one of several genes on autosomes and not on a sex chromosome that is involved with sex determination. Females with this form of XY sex reversal would have the following genotype: 46,XY,SRY^{+},WNT4 dup.

In these examples, the genetic sex of these individuals is different from their chromosomal sex, so there can be discrepancies even between one's chromosomal and genotypic sexes.

Most of the work to date has been on *SRY* and its role in promoting male sex determination. Female sex determination had been considered the "default" pathway, that is, due to the absence of SRY. One hypothesis is that *SRY* is not a positive regulator or effector for maleness, but rather a negative effector of an inhibitor of male sex determination. It is becoming clear in humans, like many other organisms including the fruit fly, *Drosophila,* and the nematode worm, *Caenorhabditis. elegans,* that sex determination involves the dosage of a variety of gene products. We have seen this with *SRY, DAX1,* and *WNT4*. Therefore, a single gene like *SRY* cannot be considered the "master" sex-determining gene or the "maleness" gene. Sex determination is regulated by a complex physiologic network (chapter 3) involving a number of proteins of which *SRY* is only one.

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In 1967, for the first time, an athlete was disqualified for failing a "sex test." It was reported she had "an extra chromosome" and "internal male-like characteristics." She was accused of competing "unwittingly as a man," and therefore, laboratory testing changed the definition of womanhood. Women whose chromosomal testing was abnormal would either leave voluntarily or submit to a physical, including a gynecological examination that would determine if they would be eligible to compete. In every Olympics except one since 1968, one or two women athletes have been banned. Many others have withdrawn rather than face scrutiny.

Many individuals with sex reversal will not come to medical attention. They will not be diagnosed until they have delayed puberty, and for the girls, delayed menarche. There is a well-known relationship between body mass and menarche, so it is not unusual for competitive women athletes to have amenorrhea. At the Olympics, if testing shows that her karyotype

is 46,XY, she would be publicly identified as a male masquerading as a female, and as a cheater, who has only achieved her competitive edge over her XX colleagues by having the XY karyotype. She undoubtedly would have serious concerns about her own sexual identity after this very public humiliation without the support of appropriate counseling.

The story of María Patiño, an unsuspecting twenty-four-year-old Spanish national hurdle champion, illustrates how the misunderstanding of the biology underlying disorders of sexual development led to inappropriate use of technology at the Olympics and other sporting venues. Patiño was banned from competition when she "failed" the screen at the World University Games in 1985 at Kobe, Japan. She was diagnosed subsequently with androgen insensitivity.

The circumstances under which Patiño learned of the abnormal genetic screening test are quite dramatic:

> On the way to her first race, she was told that she should fake an injury and withdraw — and if she didn't, her story would be leaked to the press. She didn't back down and she won her race, collapsing with physical and mental exhaustion after the finish line. The next day, her story was front page news. She returned to Spain to be stripped of her titles and lose her university scholarship and her boyfriend. "I knew I was a woman in the eyes of medicine, God, and, most of all, in my own eyes," Patiño told a reporter. "If I hadn't been an athlete, my femininity would never have been questioned."

After three years of "public humiliation and shock," Patiño was reinstated. Her actions brought attention to the lack of clinical validity of the testing, which did not provide the diagnostic accuracy attributed to it by the International Olympic Committee. (IOC). In addition, there was a growing realization that there was no quality control in the testing, particularly at some of the smaller sports venues.

There are historical examples of men competing as females in the Olympics. At least one of these was a deliberate masquerade forced by the Nazi leadership. Others were individuals with ambiguous genitalia, some of whom later had sex-reassignment surgeries. The IOC and the International Amateur Athletics Federation (IAAF) took measures they felt appropriate to ensure that no athletes had a competitive advantage based on gender. This was called gender verification testing.

Beginning in 1960, in non-Olympic events, there were physical inspections of women athletes, and gynecologic examinations were used in at least one venue. Complaints led the IOC to introduce microscopic examination of cheek cells for Barr bodies. Since males have only one X chro-

mosome and females have two, one of the female X chromosomes is inactivated to maintain equal dosage between males and females. An inactive X chromosome will condense to form a Barr body. XX individuals have one Barr body in each somatic cell, and XY individuals do not have any Barr bodies.

In 1991, the IOC replaced Barr body testing. Medical genetic laboratories had abandoned this test long before, in the 1970s, due to its inaccuracy. The IOC switched to amplification of Y-specific sequences, including SRY. The IOC did not question the ethical, legal, and social implications, or the biologic validity of this newer test, until 1999. The concept of unfair advantage is inaccurate as exemplified by the androgen insensitivity syndrome. Since the tissues of women with this disorder are unable to respond to androgen, they have no competitive advantage over other female athletes.

In 1990, the IAAF recognized the problems with gender verification testing. By 1992, the organization adopted an approach to block only "imposters" — males masquerading as females — from their competitions. In 1996, the Norwegian Parliament outlawed genetic testing of women athletes in Norway. In 1997, the Women's Sports Foundation, based in the United States, endorsed the IAAF model. In 1999, the IOC dropped its on-site laboratory testing for the 2000 Olympics in Sydney, Australia.

In May 2004, the IOC executive board announced that transsexual athletes would be eligible to compete after their surgery. The requirements for these athletes were legal recognition of their new gender and hormonal therapy for at least two years. This made high-level sports competition more widely accessible, not only for the transgender community, but also for individuals with disorders of sexual development who might undergo surgery after beginning their athletic careers.

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Let's next examine whether there is a difference between the brains of males and females. In normal males, androgens are produced in largest proportion from the testes. Therefore, a consequence of the sex of the individual could be the relative concentrations of circulating androgens in males and females.

In rodents, several regions of the brain and spinal cord differ in size between males and females and therefore are called sexually dimorphic areas. One of the best-studied is a region involved in control of mating behavior, called the preoptic area. The preoptic areas are substantially

larger in males than in females. In rodents, it appears that testosterone increases the expression of androgen receptors in sexually dimorphic areas. Higher levels of the androgen receptor result in the increased expression of an enzyme, aromatase, which converts testosterone to estradiol, and estradiol is responsible for the growth in the preoptic areas in males.

It might seem surprising that testosterone, the male sex hormone, is the source of estradiol, the major estrogen, or "female," sex hormone present in males. But this shows the flaw in archaic concepts and vocabulary. The terminology of estradiol as a female sex hormone refers to a simpler time in the understanding of physiology. It now appears that this hormone is a mediator of growth in the dimorphic nuclei in the male.

Songbirds give additional insight into sexual dimorphisms. The male zebra finch sings and has a larger song center in the brain, whereas the female does not sing and has the smaller song center. In the past, the predominant concept was that the differences between the song centers in males and females were mediated by gonad-derived steroids. Now, however, hormones released by the testis are not thought to be responsible. Administration of androgens during development will masculinize females so that they have larger song centers and sing, and just as in mammals, estradiol administered to young females will also masculinize this dimorphic nucleus, and these masculinized females sing when they become adults. It appears, however, that the estradiol responsible for masculinization of the song centers is due to estradiol intrinsic to the brain. Recent results suggest that the expression of genes encoded by the sex chromosomes — the genetic sex of cells in the brains of zebra finches — influence the levels of estradiol in the brain and the responses to estradiol of cells in the brain. Thus, it appears that it is the genetic constitution of the brain, and in some experiments, the specific sex chromosome makeup of the neurons in the song center, that are critical for development of this sexually dimorphic structure.

Eric Vilain is investigating sex determination and sex differences in developing mammalian brains. His research group and others are beginning to look at differences in gene expression between male and female mice using microarray technology. This technology is one of the current approaches to studying the transcriptome, or the mRNA transcripts that are expressed in a specific tissue at a specific time in development.

Vilain and his colleagues, in a report in 2003, detected more than fifty genes that were differentially expressed in the brains of male and female mice. Thirty-six genes showed enhanced expression in females compared

with males. Eighteen had increased expression in males. These differences were observed in fetuses at 10.5 days after fertilization, before evidence of sex-specific differentiation of the genital ridges, and before the gonads began to synthesize and release hormones. Two of the female-enhanced genes were located on the X chromosome, and two of the male-enhanced genes mapped to the Y, but many of the differentially expressed genes were autosomal. The investigators assumed that the expression of these autosomal genes was influenced by genes on the sex chromosomes.

In a 2006 report, Vilain and his colleagues challenged the dogma that sexual differentiation of the brain is under the influence of gonadal steroids that organize specific neural circuits. They demonstrated in mice that the *Sry* gene was expressed in a specific region of the brain called the substantia nigra, which is involved in sensorimotor function and possibly in learning which behaviors, for example, related to food or sex, are rewarded. Within the substantia nigra, *Sry* expression was specifically localized to neurons that synthesize the neurotransmitters DOPA and dopamine, and dopamine is known to be a associated with neural circuits involved in reinforcement and pleasure. When *Sry* expression was downregulated in the substantia nigra of rats, the investigators observed a decrease in an enzyme, tyrosine hydroxylase, in the DOPA and dopamine synthetic pathway and altered motor behavior consistent with alterations in this pathway. These researchers concluded that there is a direct effect of *Sry* on male brain function, without the involvement of hormones synthesized in the testes.

What does it mean if differences in brain development and function are influenced by an individual's sex chromosome complement? We cannot answer this question, because it is difficult to extrapolate from songbirds, rodents, and other model organisms to humans. There is considerable plasticity in central nervous system development and therefore a substantial role for the environment. However, the environment may not be able to completely overcome the effects of the underlying biology.

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Gender of rearing is the path selected by the child's caretakers for that child's gender. It is part of gender socialization, or scripting, for the child by environmental prompts including play, clothes, and many other factors. Some have believed that gender socialization would override a child's genetics. Individuals with intersex conditions argue that they know — their brains know — what their gender is. They say this is inde-

pendent of any surgery that may have been performed on them or their gender socialization. One individual's case history is quite informative.

Bruce (later renamed David) Reimer was born an identical twin in the mid-1960s. For the first eight months of his life he was reared as a boy, but he had complications from a circumcision at eight months of age that left him without a penis. His parents were convinced by experts, most notably John Money from Johns Hopkins University, to have him undergo castration and other sex-changing surgeries. He was raised for the next twelve years as Brenda, with intensive social, mental, and hormonal interventions. David described this process as "brainwashing."

Reimer's case was reported by Money and colleagues as an unqualified success. The 1970s feminist movement considered Brenda to be evidence that gender was a cultural construct resulting from social and not biological conditioning. As a teenager, however, Brenda was very uncomfortable in the role of a girl.

When he was a teenager, Reimer demanded that his parents tell him the whole story about what had happened. Upon learning the truth, Reimer was immediately convinced of the need to revert to his biological sex. With his parents selecting his new name, he became David Reimer at the age of fifteen. He initially argued against the need for reconstructive surgery and attempted suicide three times. At twenty-two years of age he underwent surgery to reconstruct his penis that began a twelve-stage process to restore sensation and function. He subsequently married and had three stepchildren, and then he and his wife were separated.

In 2004, at the age of thirty-eight, Reimer committed suicide. Reports stated that he was saddened by his brother's death the year before, his separation from his wife, and his unemployment.

The ISNA is arguing for the need to develop evidence to assess whether current practices are appropriate in dealing with infants with disorders of sexual development. Until recently, the absolute dogma was that it was important to determine the best, often meaning the most expeditious, sex for a child with ambiguous genitalia and have the surgery as early as feasible. The child then could be raised as that gender before she or he started preschool or were in other environments where the condition might be recognized. There are increasing reports from individuals with disorders of sexual development that, like David Reimer, they know their gender and know whether it was determined incorrectly. There has been a movement to wait until the individual can make the decision that is right for him or her. There is even a challenge to the concept that a dis-

order of sexual development is abnormal, with individuals saying, "If that is the way I am, then that is part of me and I do not want to change."

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There are several other categories of sex and gender that we will briefly discuss. *Gender identity* is terminology developed in the 1960s to refer to the conceptual development by the child that he or she belongs to one gender. Much of the writing and research on this topic seems aimed at understanding how individuals sort themselves into male or female. But this dichotomous sorting is not shared by all cultures. Other cultures have additional recognized gender groups, and whether they are a mixed or a third gender remains a matter of anthropological debate. Many Native American cultures had individuals named by the Europeans the "berdache," or by native terms, like "two spirits." These were individuals with male genitalia who took on characteristics of males and females. They were often not discouraged from these behaviors by their parents. They had special duties and ritual functions, and they are reported to have served the group by offering sexual pleasure to married men without any threat to the men's marriages.

Gender role represents the composite of attitudes, behaviors, and personality traits that a specific culture or community determines as masculine or feminine within its social context. Examples would include selected roles in play and toy interactions. "Tomboys" in our society are girls whose behavior is more like that typically ascribed to boys. The Tomboy Project is seeking to understand the behaviors of individuals labeled in this manner. These researchers find that in activities, playmate preferences, and gender identity, the tomboys rate higher on scales of masculinity than their sisters do, but lower than their brothers.

The legal gender is that which is assigned on legal documents, the most important usually being the birth certificate. Some states in the United States allow the birth certificate to be amended if there is surgery for a child with a disorder of sexual development, but many states do not. There is no category for those with a disorder of sexual development and no clear assignment as male or female, and this makes the legal gender decisions for these individuals very difficult.

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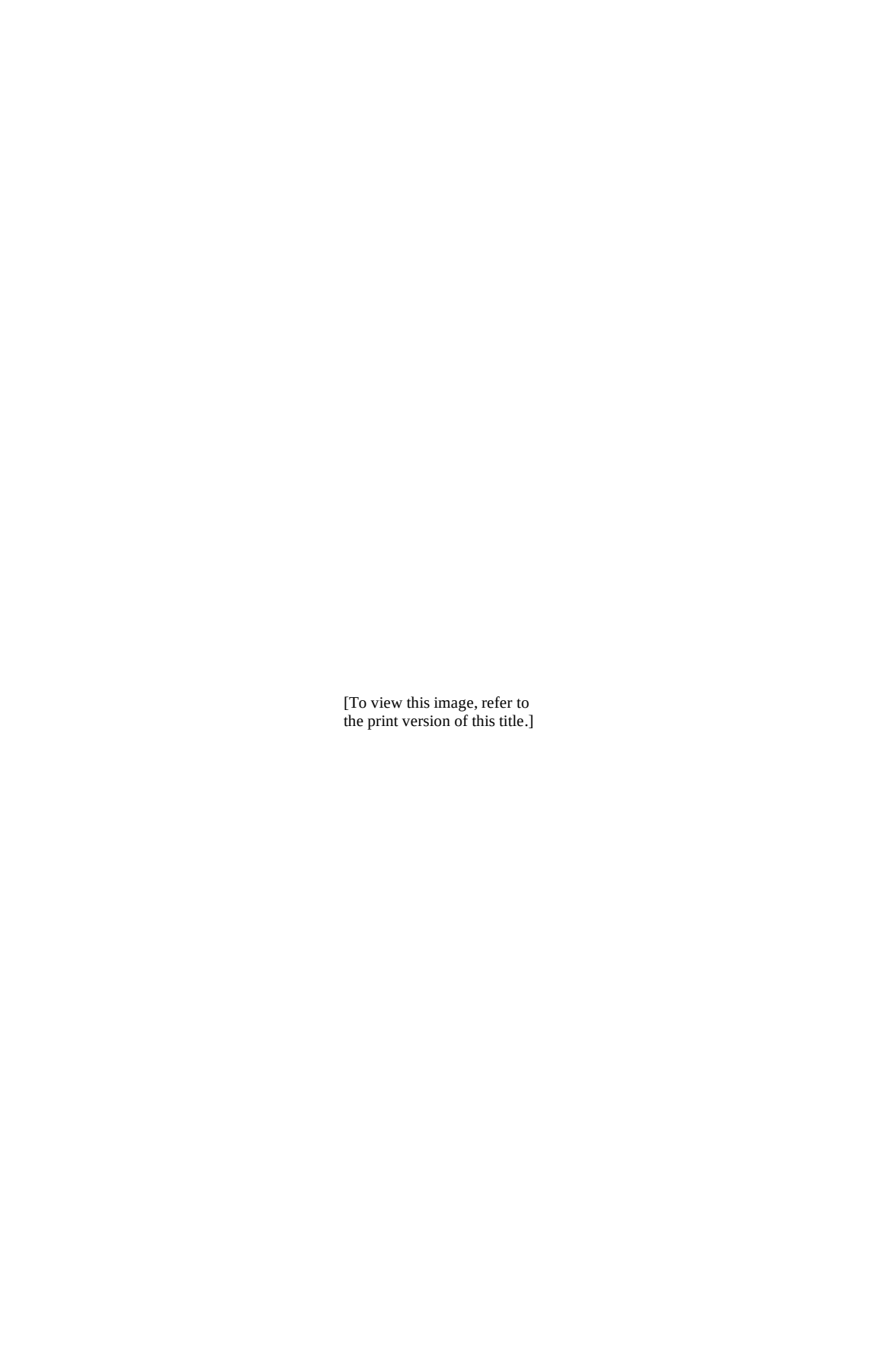
In the United States, there has recently been considerable interest in passage of a constitutional amendment requiring that marriage occur only between a man and a woman. The intention of this amendment would be to block states from recognizing gay marriages. As Vilain has pointed out, however, given the many different definitions of sex and gender, it may be difficult to judge who is a man and who is a woman. In fact, a couple that would seem to fit the traditional definition of a man and a woman might not fit so well by other categorizations. Would this mean that women who have androgen insensitivity would not be able to marry because they have a 46,XY karyotype? We hope that we will have convinced you that any attempt to define gender as simple and dichotomous is fundamentally flawed.

Similarly, although the technology exists for selecting the sex of offspring prenatally, the complex biology of sex determination indicates that errors will occur (chapter 11). From our discussion here it should be obvious that this practice is rooted in a belief in genetic determinism. The biologically fallacious argument would be that if the karyotype of the fetus after prenatal testing is 46,XX or 46,XY, the fetus must be a girl or a boy, respectively. Sex reversal and other disorders of sexual development would be ignored with this approach and could lead to surprises in the delivery room. Geneticists counsel families that the sex is highly likely to match the chromosomal sex, but there will be exceptions.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CHAPTER 7

Genome-Based Forensics

Forensic versus medical testing
Advantages and disadvantages of DNA analysis
Genetics of forensic analysis
Early forensic cases
DNA databases
"All felons" legislation
Indicting DNA
Reuniting families

We have been discussing topics related to an individual's identity, including ethnocultural group and gender. In this chapter we will discuss the testing for identity using DNA-based forensic analyses.

The courts have long recognized identification of suspects using phenotypic traits including eyewitness testimony of a suspect's appearance, matching of fingerprints to a database, and laboratory testing of blood type. DNA analysis provides an additional tool and may be used to identify the victim and the suspect and to determine family relationships.

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An essential distinction between genetic forensic testing and genetic medical testing is the very different use of the results. Forensic analyses are carried out with the purpose of providing evidence suitable for use in a court of law; medical analyses are intended for use in clinical decision

making. The same technologies may be used in both of these settings, and the same sequences — for example, single-nucleotide polymorphisms (SNPs) — may be targeted for analyses, but the differences in use have caused many geneticists to argue for firewalls around samples containing DNA. If a sample is drawn for a forensic purpose, it should not be used to determine health information, and likewise if the sample is obtained for medical reasons, it should not be used for a forensic purpose.

The American Medical Association's (AMA's) Council on Ethical and Judicial Affairs considered this issue regarding differences between forensic and medical genetic information in a report published in 2002. The report discussed the primacy of physicians' obligations to their patients and the importance of patient privacy in that relationship. It also noted the responsibilities physicians have to their communities as embodied in the AMA's code of ethics: "As a citizen and as a professional with special training and experience, the physician has an ethical obligation to assist in the administration of justice." The report points out that physicians have custody of genetic information, including patients' samples, which could be used for DNA analyses. This information could be useful to the authorities, for example, if there is crime scene evidence and the suspect has not yet been detained. The report concluded: "Confidentiality is a cornerstone of the patient-physician relationship. While release of genetic information constitutes a breach of confidentiality, adherence to the law and a societal interest in convicting criminals permit physicians to release a patient's genetic information with the consent of the patient or when requested by an order of the court through a warrant. When releasing genetic information, physicians should provide the minimum amount of information required, and should not permit general access to clinical or research sources of genetic information or other unauthorized access to confidential health information." The need for physicians to respect patient confidentiality while also honoring the orders of the court is clear.

The terminology *DNA fingerprinting* suggests that an individual's genotype is unique. What this methodology provides, however, is a statistical analysis yielding a probability that the suspect's DNA is a match with the evidence, or more correctly, that a match could have occurred by chance. In other words, like much of genetics, the result is not absolute but probabilistic.

If a higher number of loci are tested, then the likelihood is lower that a match could have been obtained by chance. For example, the results might indicate that there is only a one in a million probability that this suspect's DNA would match the DNA associated with the evidence by

chance, but with a higher number of loci the probability of a chance match might decrease to one in 100 million. Without having the entire genomic sequence from the suspect and the evidence, there is always the possibility, no matter how small, that there is a difference in a part of the genome that is not being interrogated by the forensic DNA analysis.

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What are some of the advantages and disadvantages of DNA analysis? One advantage of DNA for forensic analysis is that an individual's DNA is unique, unless she or he happens to be an identical twin (chapter 3) or a clone (chapter 12). In addition, with the rare exception of mosaicism (e.g., the 45,X/46,XY individual we discussed in chapter 6) or therapeutic chimerism (e.g., after a hematopoietic stem cell transplant, chapter 14), an individual's DNA is identical in all of the body's tissues, so any specimen from the individual can be used to identify that person.

DNA analysis is extremely sensitive, requiring less than a drop of blood, only the cells in saliva on the back of a licked stamp, or even the cells found in a traditional fingerprint. DNA is also very stable, lasting years, particularly when it is in a dried state, for example, in dried blood spatter on a surface, like a wall, or in fabric, like clothing or sheets. We know of a dried blood specimen on blotter paper from a seventeen-year-old residual newborn screening sample (chapter 15) from a deceased patient with cystic fibrosis that yielded DNA for carrier-testing the patient's brother. DNA has been retrieved from the fragment of a femur, or upper leg bone, dated by carbon-14 decay analysis to be more than 40,000 years old. Mitochondrial DNA (mtDNA) analysis (chapter 5) revealed that the DNA from this ancient femur matched other Neanderthal mtDNA specimens and was distinct from early modern humans. These results confirm that DNA is stable over extremely long periods of time.

Disadvantages of the forensic use of DNA analysis are technical. Laboratory issues include the significant expense of the reagents and equipment needed. DNA analysis is also labor-intensive, though the Human Genome Project has brought increasing automation to DNA analytical technologies. As with any forensic evidence, DNA must be collected in accord with chain-of-evidence procedures. These include labeling of each piece of evidence at the crime scene and documenting acceptance and transfer of the evidence by each person who handles it subsequently. Care in collection of samples is required to prevent contamination by those handling the evidence because the analytical methods are so sensitive. Those

collecting or handling the specimens must wear gloves to prevent their own DNA from being transferred to and incorporated into the sample. The samples must be stored in a cool place or in a freezer. DNA integrity is improved if specimens are maintained in as dry a condition as possible, so they are frequently stored with desiccant—a drying agent like silica gel—that will absorb residual water from the specimen and the humidity in the atmosphere of a closed storage container.

DNA evidence may place a person at a crime scene, but it does not determine that person's guilt. In June 2003, Bruce Budowle, senior scientist at the U.S. Federal Bureau of Investigation (FBI), in testimony before the U.S. Department of Health and Human Services Secretary's Advisory Committee on Genetics, Health, and Society, noted these limitations of DNA forensic evidence. He pointed out that this evidence is for identification only—it may indicate that the individual was at the scene where the DNA was deposited if there is a high-probability match. The evidence, however, could have been left at the scene by some mechanism other than the commitment of the crime for which they may be accused. It does not mean that a crime was committed by the person identified by the evidence. The decision regarding whether the identified individual committed the crime is not the responsibility of the forensic laboratory. The judicial process will consider all of the evidence, including the DNA forensic results, before deciding guilt or innocence.

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Now let's examine the genetics of forensic DNA analysis. The fundamental "rules" of genetics govern DNA forensic analyses. These are relatively simple, and we have considered them in the previous chapters. For any pair of nuclear genetic markers, referred to as alleles, one allele is inherited from each parent. Mitochondrial DNA and its markers come from an individual's mother. We can trace our matrilineal genetic pathway, eventually merging with all of humanity, to our common matrilineal ancestor from sub-Saharan Africa, the Mitochondrial Eve (chapter 5). Each male inherits his Y chromosome and its genetic markers from his father, and his patrilineal inheritance can be traced back in time by means of these markers (chapter 5). The typical female will be 46,XX, and the typical male will be 46,XY (chapter 6), so the genetic sex of a victim or an assailant can be determined by the presence of X and Y chromosomal markers. There may be exceptions or qualifications to each of these

"rules"; they are not absolute. These exceptions can lead to very interesting scenarios, because of unexpected misdirections.

Let's look now at how these markers are used in DNA forensic testing. First we will consider parentage analysis. Since all of our autosomal nuclear genes are paired, allelic markers within and around genes, like SNPs, will also be paired. Typically one allele in each pair is inherited from the mother and the other from the father. Therefore, these allelic markers can be used to determine or authenticate biological parental relationships. Examples of forensic parentage testing include determination of paternity in court cases regarding child support and ascertainment of parentage if there is concern that two newborns were switched in the nursery or if a child may have been kidnapped years before. If DNA alleles for a child and one parent are established, then the child's alleles that are not accounted for in the first parent should be observed in the second parent. The absence of a child's paired alleles in the parents' DNA samples for a series of markers would raise serious doubts about whether these are the biological parents of the child. The technical jargon for a "failed" paternity test is "nonpaternity," but since that is a biological impossibility, we prefer the terminology "misattribution of paternity." Similarly, "misattribution of parentage" is when neither the woman nor the man appear to be the biological parents.

A paternity test originally was a series of "autoradiographs," or Southern blots — named after British molecular biologist Edward Southern, who originated this method. These were typically prepared by using a gel in an electrical field to separate the DNA fragments according to their sizes. The DNA was transferred, or "blotted," from the gel to a thin membrane filter by capillary action, and the filter was "hybridized" with radioactively labeled "probes" — sequences complementary to or the opposite strand of the markers of interest. After washing the filter to remove the unbound radioactive probe, it was exposed to X-ray film. Where the radioactive probe hybridizes to DNA fragments on the filter, the energy from radioactive decay of the probe was transferred to the X-ray film, and when the film was developed, these spots would be dark. Other markers, including tandem repeats and SNPs, are now used.

To interpret the paternity test result, it is best to begin by looking at the child's markers. The parents give only one of each pair of their alleles to the child, so there will be alleles in the parental DNA samples that are not in the child's DNA. But if the parentage is correct, then all of the child's alleles should be present in one parent or the other. Any allele present in the child's DNA but not represented in the mother's DNA will have been inherited from the biological father. If the alleged father in this case

is the biological father, then the results from his DNA should complete the complement of alleles in the child. In an actual case, an adequate number of alleles would be tested to meet the requirement of most courts — that is, a probability greater than 99.5 percent (a one-in-two-hundred probability) — as evidence of paternity.

Alleles in the child's DNA that were not inherited from the mother and that don't match those of the alleged father unequivocally exclude him as the biological father. In forensic testing, it is generally easier to exclude an individual, whereas inclusion will always be a probability. With today's technology, the probability of inclusion can be quite high, but this probability can never be 100 percent.

What are some of the issues in paternity testing? Samples for parentage testing are easily obtained by swabbing the inside of the cheek, which contains mucosal cells. Collection sets can be mailed directly to customers, who can collect the specimens in their own home. We were told several years ago that the most common source for samples going to a commercial paternity-testing laboratory was fathers, who would send samples from themselves and their alleged offspring. Presumably the situation involved a father who had a custody relationship with the child. He would collect the samples and send them to the commercial laboratory as an "investment" to try to eliminate payment of child support by exclusion of biological paternity.

Presumably these results would not be acceptable in the courts because there was no chain of custody, and the samples could be from another male and not the individual whose name was on the specimen. But the commercial results could lead to "reasonable doubt" about the biological relationship and therefore to court-ordered and supervised collection and testing.

Another issue is lack of consent for this testing by the mother. Genetics professional organizations and federal advisory committees have recommended that all medical genetic tests should have informed consent. The mother's genetic information would have been accessed through the child's forensic analysis. But we have said that there are differences between forensic and genetic testing, so what are the mother's rights and protections in this area? This is clearly an issue for the courts.

In April 2004, in the United Kingdom, a criminal conviction was brought for the first time after a relative's DNA results were used to track the suspect. Craig Harman of Surrey received a sentence of six years in prison for manslaughter. Harman was convicted of throwing a brick from a bridge that went through a truck's windshield and struck the driver in the chest. The driver died of a heart attack in the truck after steering it to

safety. Blood from the presumed assailant was present on the brick, and a DNA profile from it was compared with the national DNA database for the United Kingdom. In April 2004, that database contained approximately 2.5 million profiles of individuals charged with a crime and as of that month would also begin to include those arrested for, but not charged with, a crime.

Harman did not have a criminal record, and therefore his DNA profile was not in the database. However, a close relative of Harman's, who had a criminal conviction, did have a profile in the database, and the relative's profile matched the blood on the brick with sixteen points out of twenty, where twenty points would be a perfect match for ten markers with two alleles for each. The system of "familial DNA searching" began in September 2003 and considered more than twelve markers in common to be evidence of a familial relationship.

This familial searching system in the United Kingdom is approved only for serious crimes, like sexual assault or murder, and then only with authorization from high levels within the police. However, concerns have been raised, arguing that the use of a relative's forensic DNA information would be a violation of the civil liberties of individuals who had no criminal history and had no cause for their DNA profile to be in the database.

The power of mtDNA testing is in determining matrilineal relationships, and we will see how that property can be used for forensic purposes later in this chapter in an example from Argentina. Mitochondrial DNA is used quite commonly in forensic cases because of its higher copy number per cell compared with nuclear DNA (chapter 2). Therefore, in samples such as skeletal remains in which DNA has degraded, it is possible to obtain results with mtDNA extracted from bones, teeth, and hair shafts even after extreme conditions of incineration by fire or decomposition in water. In 1991, the use of matrilineal mitochondrial DNA markers permitted identification of a child who had disappeared four years earlier based on DNA analysis from a skull found in a California desert.

In sexual assault crimes, the evidence is often a mixture of male semen from the assailant and female vaginal and blood cells from the victim. There are standard methods for separating sperm from female somatic cells, but the separation is often incomplete, leading to problems with amplification of the mixed sample, since one DNA component, for example, the woman's, might amplify more efficiently and overwhelm the analysis. In addition, interpretation of the mixed results may be complicated. Analysis of Y chromosomal markers would be more effective in such situations. In addition, this approach is useful in identifying men

who do not produce sperm, either because of a medical condition called azoospermia (absence of sperm) or after surgical vasectomy but who will still have somatic cells in their ejaculate. The use of Y markers is valuable when there are semen contributions from more than one male. Y sequences also can be useful in other situations where the forensic evidence is a mixture of specimens from individuals of different sexes, for example, skin of a male assailant under the fingernails of a female victim.

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Let's look at several of the early forensic cases that involved direct matches. The first involves disputed parentage in an immigration case. Alec Jeffreys, who was subsequently knighted for his contributions to basic research and DNA forensics, was on the faculty of the University of Leicester, where he and his colleagues were studying variable regions within the genome. They identified "core" sequences within these variable regions and developed probes that would hybridize with these core sequences for Southern blots. They recognized that these sequence variations would be extremely useful in assessing kinship relationships and identifying individuals. In one of the very early applications, in 1985, Jeffreys and his colleagues reported a case involving a boy who was born in the United Kingdom but traveled to visit relatives in Ghana. When he attempted to return to the United Kingdom, the authorities questioned the legitimacy of his residency, accused him of illegal immigration, and suggested that his aunt or an unrelated woman was his mother. Jeffreys showed that the boy and his mother shared all twenty-five maternal-specific DNA markers, and the boy was granted residence in the United Kingdom with his family.

The first use of DNA analysis in a murder investigation was described by Joseph Wambaugh in his book *The Blooding*. As a former police officer, Wambaugh recognized that DNA forensics would dramatically influence police work. Two teenage girls were raped and murdered in the mid-1980s near the village of Narborough in England. The University of Leicester and Jeffreys were near Narborough, and Jeffreys was asked to apply his new DNA forensic approaches to the investigation. His study of the evidence from the two crime scenes revealed that both murders were committed by the same person. A seventeen-year-old male had been charged with the murders, and he had even signed a confession. However, when Jeffreys compared the boy's DNA profile with the evidence, it showed that the seventeen-year-old was innocent. Wambaugh explained the next "revolutionary" step in January 1987: "All unalibied male resi-

dents in the villages between the ages of seventeen and thirty-four years would be asked to submit blood and saliva samples voluntarily in order to 'eliminate them' as suspects. . . . The cops quickly began to refer to their testing sessions as bloodings."

The police collected samples from more than 4,500 men, but there were no matches for any of these samples with the evidence. One man in a pub revealed to his friends that he had provided a sample twice. He had provided a blood specimen in his own name, and he had felt sorry for a baker, Colin Pitchfork, who claimed to be afraid of needles, and gave a second sample as Pitchfork. The friends of the sample donor notified the police, who arrested Pitchfork. He was not so much afraid of needles as he was of the results of the DNA testing, and quite rightly so, as his DNA matched the evidence. Pitchfork was convicted of both murders in 1988.

Three U.S. cases in 1987–88 brought additional visibility to DNA forensics. The first individual reported to have been convicted with the assistance of DNA evidence was Tommie Lee Andrews in Florida. In May 1986, a woman was attacked in her apartment by a man with a knife, who raped her and stole her purse. During a six-month period more than twenty-three rapes were attributed to this same individual, but he was able to hide his identity from the victims, and he always stole something that belonged to them. However, in one case he did leave two fingerprints at the scene. When another woman reported seeing a prowler, Andrews was apprehended and his fingerprints matched the evidence from the prior rape. The rape for which he was initially convicted occurred on February 21, 1987, when he entered a woman's home during the night, threatened her with a knife, raped her, and burglarized her home.

Tim Berry, who was an assistant state's attorney for Florida, contacted a company, Lifecodes, which had begun to develop DNA forensic testing commercially in the United States. Berry recognized that the authorities were going to need more evidence than they had to convict Andrews as a serial rapist. They had only a blood group match between Andrews and the semen, the one pair of fingerprints, and the one victim who was able to identify him. Andrews's blood, along with the semen evidence, was sent to Lifecodes, and the DNA was a match.

In Andrews's first trial, the judge called for a pretrial hearing to determine whether the science of DNA forensics met the rigorous criteria required for evidence and ruled that the DNA evidence could be admitted. The individual from Lifecodes who had analyzed Andrews's DNA said, "It's like leaving your name, address, and social security number at the scene of the crime. It's that precise." Unfortunately during the trial, the prosecutor

overstated the odds and could not substantiate them, and the result was a hung jury. In the second trial, for a different case, a guilty verdict was returned by the jury on November 6, 1987. Subsequently, the first rape case was retried and the DNA evidence was presented more clearly. Andrews was convicted, and his prison sentence was extended from the original 22 years for burglary, aggravated burglary, and rape to 115 years for serial rape.

The case *The People v. Wesley,* tried in Albany, New York, was one of the earliest U.S. court decisions on DNA testing and eventually, on appeal, would help to legitimize DNA forensics to full scientific validity. George Wesley had visited a seventeen-year-old girl on September 15, 1987, shortly before her body was found. She had been raped and murdered, and when police searched Wesley's apartment, they found bloodstains on some of his clothes. Forensic analysis showed this to be the victim's blood, and Wesley was convicted largely on the basis of the DNA evidence. The court's decision included the following: "DNA Fingerprinting, if accepted, will revolutionize the disposition of criminal cases. In short, if DNA Fingerprinting works and receives evidentiary acceptance, it can constitute the single greatest advance in the 'search for truth,' and the goal of convicting the guilty and acquitting the innocent, since the advent of cross examination." The New York State Court of Appeals ruled in *The People v. Wesley* in 1994 that DNA forensic data were admissible. In addition, this case resulted in acceptance of DNA testing by the courts as scientifically valid. No longer would New York require pretrial hearings with expert testimony to determine whether DNA forensic evidence was sufficiently reliable to be admitted as evidence to the court.

Another early use of DNA forensic data in a U.S. court was in Virginia for an individual who had been labeled the "South Side Strangler" by the press. In 1987, there were two rapes and murders in a relatively affluent area known as the South Side of Richmond, Virginia, and a third took place nearby. The first occurred in September, and the victim was an attorney and account executive with a private firm. The second occurred in October, and the victim was a resident physician-in-training at the Medical College of Virginia. In November, a high school freshman in Chesterfield County, Virginia, was the third victim. All three women had been bound and strangled in much the same manner, and DNA evidence indicated that they had all been raped by the same individual.

Joe Horgas, a police detective in Arlington, Virginia, realized that these murders were quite similar to two that had occurred very close to each other nearly four years before in Arlington, Virginia, and could be related to as many as nine unsolved rape cases between June 1983 and

January 1984 in Arlington County. One man, David Vasquez, had confessed and was convicted of one of the murders. Horgas remembered that the detectives had not been convinced of Vasquez's guilt at the time.

Another victim was murdered in Arlington in a similar fashion around Thanksgiving of 1987. A computer search of prison records revealed only one suspect who was free when all of these crimes occurred. The suspect, Timothy Spencer, had been imprisoned for a 1984 burglary and, upon release, lived in a halfway house for parolees near the South Side victims. Spencer had also visited his mother in Arlington over Thanksgiving. Spencer was very cautious, wearing gloves and wiping any surfaces he might have touched to remove fingerprints. But he had not recognized that his DNA would incriminate him.

Spencer's DNA matched the evidence from the murders; he was convicted of murder in Richmond, Chesterfield, and Arlington; and he was given multiple death sentences. Vasquez was released, and Spencer was executed in April 1994. This made Spencer the first person in the United States who was put to death primarily due to DNA forensic evidence. Largely as a consequence of the visibility of the Spencer cases, and the importance of DNA in solving them, Virginia was the first state to establish a DNA forensic laboratory and one of the first to establish a DNA database for felons.

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The cases we have discussed thus far used specimens that were obtained specifically to solve the case. Samples may have been collected in what was in essence a "DNA dragnet," as in the original "blooding," or they were obtained after a suspect was in custody. It was soon recognized, however, that development of DNA databases from convicted or accused or, in some jurisdictions, merely apprehended individuals would permit a more powerful implementation of this new forensic technology. If not only the DNA but also the profiles were on record, then the profiles could be compared against the profiles from evidence, much as traditional fingerprints are compared to the database collections of fingerprints. Thus, linking the evidence to the DNA profile database would permit a "cold hit," in which an individual would be identified solely by DNA and not through any other suspicion. Recidivism among violent criminals was one argument for developing these databases. Cases such as that of the South Side Strangler, whose career of increasing violence was interrupted by an incarceration for burglary, were used to support the inclusion of DNA from individuals convicted of nonviolent offenses.

To generate a database of DNA samples, it is necessary to collect and store a source of DNA, like blood or cheek swabs. Large collections of specimens in liquid form would require considerable space and special storage conditions, like freezing samples of liquid blood. In 1985, Jeffreys and his colleagues, in demonstrating the forensic application of their DNA "fingerprint" technology, showed that DNA of excellent quality could be extracted and processed for analysis from bloodstains, as well as semen stains, on cotton cloth that was four years old. Cotton swatches were a traditional vehicle for collection of liquid forensic specimens that were then air dried before storage. Evidence is still collected in this manner, and bloodstain standards are still available commercially on cotton swatches.

In 1987, our group reported that DNA could be extracted from dried blood spots on filter paper blotters of the type used in collection and transport of specimens for newborn screening (chapter 15). We recognized in that initial publication that there were forensic applications in the use of these dried blood specimens, though we focused on the utility of DNA profiles for correctly identifying newborn screening blotters. For example, we suggested that the original specimen could be "identified by comparing DNA 'fingerprints' between the original specimen and a freshly obtained specimen from the child."

We subsequently showed that the extracted DNA could be amplified by using a polymerase chain reaction and that pieces of the blotter paper containing dried blood could be directly amplified without extraction. Since the newborn screening specimen is being obtained for a medical test, this is a highly specialized, quality-controlled blotter collection paper that is approved by the FDA.

Around the time of the first Gulf War, Victor Weedn, a forensic pathologist and lawyer who was on active military duty in the Office of the Armed Forces DNA Identification Laboratory, envisioned the Department of Defense (DOD) DNA Registry, which became known as the "DNA Dog Tag," a label that Weedn always detested. His goal was that there should never again be an "unknown soldier." He was aware of our work with dried blood spots and contacted us to discuss the use of blood collection blotters for the registry. After the war, when the DOD implemented the DNA registry, Weedn used this paper blotter collection system. This actually caused a crisis in the newborn screening community because the DOD bought so much of the FDA blotter paper that a shortage developed.

Each member of the uniformed services provided a blood sample, spotted on two separate blotter papers, and a swab of the inside of the mouth, which was then placed in a tube of alcohol for preservation. The

samples were stored for DNA identification purposes in the event that the service person was killed and could not be identified in any other way.

Two members of the military sued to keep from providing the samples, because they did not want their samples used for medical or forensic purposes beyond identification of their remains. Two marines refused to provide samples, arguing that doing so would violate their Fourth Amend-ment rights against unlawful search and seizure, and they were court- martialed and separated from the Marine Corps while the case was on appeal.

As of December 2002, the repository held samples from approximately 3.2 million military service personnel. The use of high-quality blood collection paper facilitated the sampling and storage of DNA-containing specimens, particularly in the early days when collection was being done in the field. The system has been extremely useful in identifying the bodies of service personnel, not only those killed in action but also, for example, those dying in plane crashes in peacetime. In fact, in 1998, using DNA forensic testing, Weedn was successful in identifying the remains of a soldier killed in the Vietnam War who had been interred in the Tomb of the Unknown Soldier in Arlington, Virginia, in 1984.

From the beginning of the DOD DNA database, concerns had been expressed that these samples might be used in criminal prosecution, such as in cases of rape on or near a military installation. The armed forces originally agreed to use the DNA repository only for identification purposes in the event of death of their personnel. However, it appears that these protections began to erode with statements indicating that there is no blanket protection and that individual requests would be considered on their merits. In 2002, a bill sponsored by Representative John Culbertson (R-TX) passed without debate and permitted use of DNA in the repository for investigation or prosecution, if no other source of DNA was available. This law was passed in response to a rape of one soldier by another at Fort Hood, Texas, in which law enforcement was prohibited from having access to the DNA registry. Culbertson argued that there was need for uniformity of policy. This use of the DNA dog tag specimens would complement collection of samples from military personnel convicted of certain crimes, which are then sent to the FBI, where they become part of the Combined DNA Index System (CODIS).

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The use of DNA databases by civil authorities for apprehension of criminals has grown dramatically in the United States, since their beginnings

in the 1980s. Police authority in the United States is at the state level, and therefore implementation of these DNA databases requires legislation in every state where they are used.

Tim Schellberg is a lawyer and consultant who tracks the status of DNA forensic legislation at the state, national, and international levels and has documented the history of this legislation in the United States. In 1983, California became the first state to legislate collection of blood from criminals, though interestingly the terminology, DNA, was not included in that legislation. Five years later, in 1988, Colorado, presumably in response to the early cases in the United Kingdom and the United States, was the first state to enact legislation to obtain DNA from sex offenders. The Virginia legislature followed in 1990 but extended statutory reach to include what has become known as an "all-felons" law, meaning that DNA would be collected and stored from all individuals convicted of a felony.

In 1991, the FBI began to advocate for state laws for collection of DNA from sex offenders and developed the concept of a centralized resource that would include not only the repository but also expert consultation that they termed CODIS. In 1994, the U.S. Congress passed the DNA Identification Act, which formalized CODIS by statute.

By 2000, all fifty states included the DNA of sex offenders in their repositories, twenty-seven included DNA from those convicted of violent crimes, fourteen included DNA from those convicted of burglary, and seven were all-felons laws. A huge backlog of cases had resulted from the passage of these laws, and the U.S. Congress appropriated $140 million to assist the states in clearing the backlog.

The American Civil Liberties Union (ACLU) decided in 2001 that it would not fight all-felons legislation. Preliminary data from Virginia, which had this broad-reaching legislation, showed that only 16 percent of its "cold hits" came from those whose prior conviction was for a violent crime, including a sex crime, homicide, kidnapping, or wounding assault. These data meant that more than 80 percent of the cold hits would not have occurred with a more limited law. As a consequence, there was an increase in the passage of all-felons state legislation. Schellberg argued, however, that these were not truly all-felons laws but generally included only those felons who were in prison, resulting in the addition of approximately 5,600 DNA samples annually. Including felons in jails and community correction facilities, as well as juveniles, would add nearly 24,000 samples annually, and a one-time retroactive collection among those in prisons and jails and those on probation and parole would add more than 18,000 samples to the database.

In 2002, Schellberg discussed expansion of legislation for DNA databases to include DNA samples from those convicted of misdemeanors. Offenses that were being considered for addition were sex-related crimes, like solicitation of prostitution, and violence-related crimes, like fourth degree assault, stalking, harassment, and destruction of property. He estimated that 6,500 samples would be added annually if such samples were included. Since that time, some states have passed laws mandating collection of DNA from all individuals arrested for any crime, even if they are not convicted.

In a questionnaire to its 179 member states in 2002, the International Criminal Police Organization (Interpol) attempted to determine the status of DNA forensics among its members. It had responses from 127 member states (71 percent); 77 of the members (43 percent) performed DNA analyses, and 41 (23 percent) used DNA databanks. The number of member states with databanks had increased by 14 percent since 1999 and was anticipated to increase to 38 percent of members in the subsequent several years. Therefore, the use of DNA forensics and establishment of DNA databases represent international phenomena.

As discussed previously, DNA is a more powerful tool for excluding than including a suspect; exclusion is absolute, whereas inclusion is always a statistical probability. In a 2003 *Washington Post* opinion editorial, William Sessions, director of the FBI in 1987–93, discussed the early results from the FBI's DNA forensics laboratory:

> I was fully expecting the results to confirm the careful investigative and evaluative work that had gone into the decisions to prosecute these suspects. Instead, I was stunned by the results. In about 30 percent of the cases, the DNA gathered in the investigation did not match the DNA of the suspect. Fifteen years later, this rate remains virtually the same. Approximately 25 percent of DNA tests do not produce a match. . . . But with 137 post-conviction DNA exonerations on the books in the United States, I am increasingly concerned about recent news stories that suggest a growing resistance on the part of prosecutors across the country to allow post-conviction DNA testing, even in cases where there is strong evidence of innocence.

Prosecutors cite limited resources and concerns that exonerations, often many years later, can force victims to relive the crime. After convictions, police have been known to discard rape evidence kits, arguing a lack of space, but they are suspected of eliminating the possibility of future DNA challenges. A prosecutor in St. Louis wants inmates to pay the $2,500 cost of the testing, unless they are exonerated, in order to prevent "frivolous" retesting.

If DNA repositories are so powerful in solving crimes, the question has been asked, should they be expanded to include everyone? Two of the leaders in molecular genetics have advocated for developing databases of DNA profiles from the entire population. Alec Jeffreys first recommended this approach in a speech in 2002 and has continued to support it since then. In 2003, in a celebration of the fiftieth anniversary of the discovery of the DNA double helix, he said, "The real problem in a typical crime is that even if you get DNA from a crime scene, you can't pick up a suspect because they don't have a record, so one possibility is to extend the database to include the entire population." He felt, however, that this repository should not be controlled by the police, but rather "by a quite independent agency." It would be "not just a criminal investigation database but a personal security and assurance database as well."

Also in 2003, James Watson argued for an international DNA database for everyone in Europe and the United States. He felt that civil liberties objections were less concerning than risks from criminals and terrorists. He said, "The sacrifice of this particular form of anonymity does not seem an unreasonable price to pay, provided the laws see to a strict and judicious control over access to public data." He added that he was sure such an international program would be implemented within the next hundred years, largely driven by the desire to identify terrorists.

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Throughout this book we have been arguing against genetic determinism and for the concept that an individual is far more than her or his genome. But in the eyes of law, in a number of jurisdictions, you are your DNA. Beginning in 1999 in Milwaukee, Wisconsin, prosecutors filed indictments against "John Doe DNA." These indictments have been filed in New Mexico, California, Utah, and New York when assailants had not been identified, but there was DNA evidence left at the crime scene and the statute of limitations was about to run out.

A DNA warrant was issued for a serial rapist and burglar in Sacramento, California, on the day before the statute of limitations was to expire. Subsequently, Paul Eugene Robinson was identified by a "cold hit" in the California DNA database, and his DNA matched five cases named in the warrant. He was sentenced to sixty-five years in prison for these five counts of rape.

Michael Bloomberg, mayor of the City of New York, brought public attention to this strategy with his announcement in August 2003 that the

ten-year statute of limitations would soon expire for at least six hundred unsolved sex crime cases in which DNA evidence existed, beginning with those committed in 1994, and the authorities therefore intended to seek John Doe indictments in those cases. Part of the problem was the large backlog of DNA samples that had not been processed or analyzed, which was reported to number 12,000 in New York City at the time of Bloomberg's announcement.

In April 2004, the U.S. Congress passed a law authorizing federal prosecutors to use John Doe warrants in cases of sex crimes within their jurisdiction. Critics express concern that these indictments, by extending the statute of limitations, will compromise the case for the defense and violate the Sixth Amendment, which guarantees defendants the right to a speedy trial. So even though a person's biological fate is not determined solely by DNA, a person's legal fate could be determined by his or her indicted DNA.

Another approach to DNA identification when no suspect is available and there is no DNA for a cold hit in the database has been referred to as "ethnoforensics." This is based on claims that crime scene DNA will be able to give the physical characteristics of the suspect, including height, hair color, and ethnocultural group. From the discussion in chapter 5, it should be clear that although the analyses are getting more accurate, they are still probabilistic. It has also been pointed out that, for example, the prediction of red hair was only 70 percent accurate. Regarding height and other physical characteristics, our understanding of these complex traits is still in the future. We consider the idea extremely naive that DNA will be able to replace the police sketch any time soon.

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Forensic DNA analyses can also be used to reunite family members. Here we will describe two examples of this use of DNA testing, one from Argentina and the other from the United States.

Under the military dictatorship in Argentina in the 1970s and early 1980s, many families were removed from their homes and were never seen or heard from again. They were called *los desaparecidos* — the disappeared ones. The adults were killed, though frequently there was no evidence of their murders, and there were rumors that the children were adopted by the killers or their associates. Similarly, pregnant women were imprisoned and then killed after their babies were born, and it was thought that these infants were adopted by the perpetrators or their friends.

After a regime change, the *abuelas* — grandmothers — of these chil-

dren and infants began asking if their grandchildren were alive and how they could be identified. Mary-Claire King, a preeminent molecular geneticist at the University of Washington who was involved in the race to clone the breast cancer gene and many other projects of the highest profile, began working with the grandmothers to help reunite them with their grandchildren.

With the publicity the grandmothers were able to generate and because of their demonstrations in a Buenos Aires plaza, informants began to come forward. The informants suggested the location of many of the children, who had been adopted illegally by military families during the time of *los desaparecidos.* By testing the mtDNA markers of these children and comparing them with the markers from the grandmothers, many grandchildren were reunited with their grandmothers.

Linda Hammer of Sarasota, Florida, is a former private investigator who through her weekly radio show and newspaper column assists adoptees and birth families in finding each other. In August 2004, she proposed a DNA database that would contain samples from thousands of individuals who were trying to be reunited. The goal would be to increase the probability of reunions through "cold hits" of individuals who might not have been able to track down birth parents or offspring in any other way. Hammer hopes to raise the $1 million that it will cost to establish this project and permit the banking of the first five thousand specimens so that the service can be free to the users. She argues that there are medical reasons for learning about one's biological family. She cites an adopted woman whose child had an unusual disease, who spent $100,000 to try to find the cause of her child's disease. After her child died, the woman was able to find the child's birth mother and learned that the family had a rare disease that most likely caused her child's death. Thus, this registry would provide a technological approach to identifying family histories for illnesses when an adoption interrupts that history.

Trading on parental fears of kidnapping, several companies have offered to store DNA samples of their children, for substantial fees. What these companies do not point out is that DNA from the parents can be used to establish that they are the biological parents of the child. Mitochondrial DNA from the women in the mother's family can be used to establish maternal inheritance, and for a boy, Y chromosome markers can be used to establish patrilineal relationships. In addition, DNA is found in skin cells on clothing, in hair in hairbrushes and combs, and in fingernail and toenail clippings. An issue that has not been addressed is who owns the DNA if the company goes out of business or goes bank-

rupt, which did happen to one of the early aggressive companies that was recruiting newborns in nurseries and paying the physicians "finders fees."

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Is your DNA personal or community property? In chapter 8 we will consider this question from a cultural perspective, but here we address it from a legal perspective.

In the United States, it is assumed that individuals owns their own DNA. A recent case in the United Kingdom led to laws there and in Australia that prohibit the use of an individual's DNA without his or her permission. The case involved a man in London, who denied being the father of a child. A journalist removed the dental floss of the alleged father from his rubbish bin. The DNA on the dental floss was compared to the child's DNA, and the ensuing analysis indicated that this man was the child's father. The journalist had a front-page story with this finding.

There are no laws in the United States to prohibit DNA analysis without the individual's permission, but consider Governor Arnold Schwarzenegger's discarded cough drop that was offered for sale on eBay; it could be used to analyze his DNA. Such discarded DNA samples could be used to contaminate a crime scene or implicate the individual in criminal or civil proceedings. Your DNA is left behind whenever you touch an item, dandruff falls from your scalp, or you scratch an itch and dislodge some skin cells. We suggest that you picture Pigpen in the Peanuts comic strip and think of your DNA as the dust cloud that always surrounded him.

The discussion in this chapter about DNA databases is based predominantly on U.S. law and culture, which assumes the primacy of individuals and their rights. According to this concept, your DNA is your personal property, and unless you are in the military or you commit an offense against society, you can have complete control over how your DNA may be used in forensics.

Some cultures, including that of a European country to be discussed in the next chapter, have a very different conceptual framework. The blood and now the DNA of an individual in that country belong to the group, not the individual. Similarly, in some cultures DNA is considered a communal resource that cannot be sold to others for profit or used for research without the consent of the community. Perhaps we are seeing this value system in the comments of Watson and Jeffreys, who argue for universal DNA collections to protect the group as well as the individual.


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the print version of this title.]

CHAPTER 8

When Genes Belong to Groups and Not Individuals

DNA is incredibly personal. Unless you are an identical twin, your DNA is absolutely unique. In fact, even for identical twins there will be a number—a relatively small but very real number—of genetic changes that occur after the splitting of the newly fertilized embryo in the twinning process. So, even identical twins are slightly different at the level of the genomic sequence.

If your DNA is unique, and if you accept that it contributes to your uniqueness as an individual, then most people in the United States would argue that your DNA is yours. This cultural belief system would argue that you should have the right to make decisions about what is done with your DNA. But is this a universal concept? In this chapter, we will explore this question and consider cultures in which an individual's DNA is considered to belong to the community, at least to the extent that the group's leaders make decisions about the members' DNA. In addi-

tion, we will discuss the potential for stigmatization of individuals within the group or stigmatization of the entire group when certain genetic disorders are found to be concentrated within the group.

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Paul Rabinow, from the University of California, Berkeley, wrote *French DNA: Trouble in Purgatory,* in which he described the unraveling of a commercial deal that would have conveyed DNA collected from French families to a U.S. biotechnology company. The French government considered these DNA samples to be part of the collective patrimony and therefore not appropriate as items of commerce. Rabinow attributes this to the French concept of "gift" or "benevolence," meaning that useful bodily products, such as blood, belong to the community, cannot be bought and sold, and are provided as anonymous donations for the benefit of the group. This concept of an individual's body and body parts being part of the French patrimony is in contrast to the U.S. practice, in which blood donors, historically, were paid for the blood they provided.

Rabinow traced this French cultural bioethos to the French Resistance in World War II and the need for blood for transfusions. Members of the Resistance organized a "patchwork" of volunteers, or "*bénévoles.*" This system developed into a "powerful and enduring symbolism, a noble ethos — of the act of giving blood as the embodiment of resistance. After the war, for some this act symbolized resistance to capitalism; for others, resistance to evil; for others, to the materialism of the modern world."

Rabinow contrasted these ideas in France and the United States: "In the name of dignity of the person, French law basically refuses the individual the right to dispose of his or her body and its parts; American law has allowed greater latitude for proprietary and commercial relations concerning the body and person, privileging autonomy and value over inherent and inalienable dignity." According to this cultural construct, the genetic patrimony of the French population is a direct descendent from the Resistance and its concepts of dignity and benevolence. Rabinow argues, therefore, that there is a collective French DNA. An individual's DNA, which has the legal status of a body part, belongs to the community and not to that individual, and therefore, the French government could legitimately decide to block the sale of French DNA samples to a U.S. firm.

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One of the consequences of viewing a group in its entirety by any characteristic is generalization. A risk of generalization is stigmatization, particularly when there are perceptions of negative features associated with this characteristic, such as mutations or diseases.

Robin Gregory and Theresa Satterfield, from the University of British Columbia, investigate the social impact of technology. Their interests are in the stigmatization of products, such as genetically modified organisms; communities, such as those near technological hazards; and individuals, such as loggers. The principles they have identified also apply to "isolated" communities, with apparently increased concentrations of certain genetic disorders.

Gregory and Satterfield have suggested that there are social, psychological, and cultural impacts of stigmatization. They are concerned that stigmatization may result in community members being shunned and suffering social and economic hardships. They feel that public perceptions of stigma are impacted by lack of control, adverse intergenerational influences, and the scientific uncertainty regarding the risks. Stigmatized communities experience risks not based on their own experience of risk, but instead based on how they and the risks associated with their communities are viewed by others. Initiatives to deal with the source of the stigma may lead to social discord within the community because of disagreements about strategies. Worries about one's personal situation merge with concerns about the community and may be increased for certain individuals or segments of the community if there is stratification of risks in the community.

Traditional approaches to stigmatization focus on economic and social impact. Gregory and Satterfield cite the need to elicit opinions from members of the group about the fundamental issues of importance to them with regard to the stigmatizing issue. In addition to the routine tools of standardized questionnaires and workbooks to generate data for statistical analyses, they also suggest the addition of individual narratives to the assessment process. These personal narratives provide a depth of insight into the issues that might otherwise be missed. The narratives indicate how members of the community feel they are perceived by those outside of their community, including the media, which is often seen as manipulating and amplifying risk. These narratives can also capture the emotions of the community members, and their passions about the issues should not be dismissed, as this could further marginalize these individuals.

All of us need to think about these issues of stigmatization, and their

resolution, as we consider genomes being defined by ethnocultural group membership and sometimes even being held as the property of a particular community.

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Now let's consider one such group, the Ashkenazim, and, specifically, genetic testing and research focusing on them. Approximately 90 percent of the 6 million Jewish Americans are Ashkenazim, descended from central and eastern European ancestors (chapter 5). Until the middle of the twentieth century, the Ashkenazim remained largely isolated culturally and genetically. Therefore, they remain a relatively homogeneous group and have been amenable to participation in genetic research to detect disease-causing mutations. Some Ashkenazim were alarmed by research showing that 2.3 percent of Ashkenazi women carried mutations that predisposed them to breast and ovarian cancer. The rate of mutations in genes linked to colon cancer was 6.1 percent among the Ashkenazim. There was concern in the Jewish community that members would be subjected to stigmatization and discrimination due to these research results.

There are more than fifteen genetic diseases with typical Mendelian inheritance for which the frequencies appear to be increased among the Ashkenazim. These include progressive neurological disorders that are uniformly fatal in early childhood, familial deafness, and bleeding disorders, among others. Tay-Sachs disease is an autosomal recessive genetic mutation that requires both parents to be asymptomatic carriers. If they are both carriers, and therefore are considered "a couple at risk," they have a 25 percent chance with each pregnancy of having a child with Tay-Sachs disease. These children are completely normal at birth, but within the first year of life begin to show progressive neurological deterioration, with loss of developmental milestones, like head control and sitting. They develop seizures that are very difficult to control, lose fundamental abilities like swallowing, and die in the first decade of life. This progressive neurological disease is caused by the deficiency of an enzyme that is involved in the breakdown and recycling of cellular components in brain cells. The carrier frequency for Tay-Sachs disease in the U.S. Ashkenazi Jewish community is one in twenty-seven individuals, compared with one in two hundred in the general U.S. population.

Tay-Sachs disease was the first disorder with increased frequency among the Ashkenazim for which there was a carrier screening test that could be made widely available. In 1970, Michael Kaback, a physician

then at Johns Hopkins University, carried out the first public carrier screening in Baltimore, Maryland. These screening "clinics" frequently were housed in temples, Jewish community centers, and other sites central to the Ashkenazi community. As carriers were identified, and they in turn informed their family members, awareness of carrier status — who was and who was not a carrier — increased among the Ashkenazim. It is uncommon today, except among the ultraorthodox segments of the community, for a couple to become pregnant and not know whether they are at risk for giving birth to a child with Tay-Sachs disease. In fact, this is generally the case even for couples in which only one member is Ashkenazi Jewish. The acceptance of this population-based screening program by the Ashkenazim has shifted Tay-Sachs disease from being a disease of Jews to one primarily of non-Jewish couples. Among the ten babies with Tay-Sachs disease in North America with birthdates in 2003, none were born to Jewish parents. Preliminary data from Israel suggested that there were no babies born with Tay-Sachs disease in 2004, and only one in 2003.

Rabbi Josef Ekstein, founder of the organization Dor Yeshorim (chapter 3), is an Orthodox Jew living in Brooklyn, New York, and was the father of four children born with Tay-Sachs disease. In the Orthodox community, carrier screening was not performed, since prenatal diagnosis and preimplantation genetic diagnosis (PGD) (chapter 11) were forbidden. Ekstein knew the heartbreak of this disease and wanted to develop a way to prevent it in his community.

The system Ekstein developed, Dor Yeshorim, tests young men and women in the yeshivas and other high schools and colleges attended by young members of the Orthodox Jewish community. Some have done this through matchmakers, a part of the culture of this community, who include this information in their decisions about what constitutes a "good match." Other young people are tested and given a numerical code, and Dor Yeshorim's records include that number, the individual's birth date, and her or his telephone number. No names are kept and the individuals are never given their results. Before a relationship between two young people becomes serious, they contact Dor Yeshorim and are told whether or not they are "compatible." Those couples who are compatible include two noncarriers and those in which only one member of the couple is a carrier. "Incompatible couples" are provided counseling regarding their risks and the implications, and according to Ekstein, "except for some rare exceptions, most of them do not proceed with the relationship."

As of February 2004, Dor Yeshorim had tested nearly 170,000 individuals. As a result of Dor Yeshorim's activities in the United States and Israel, testing has now become part of the culture of the Orthodox Jewish community, and births of children with Tay-Sachs disease in that community have become extremely rare. Dor Yeshorim currently tests for ten disorders, including Tay-Sachs disease.

In 1998, Meredith Wadman, a reporter for the journal *Nature,* described a meeting of leaders from the Jewish community with Francis Collins, director of the National Human Genome Research Institute, and Richard Klausner, who was then the director of the National Cancer Institute. This meeting was sponsored by Hadassah, a Jewish women's organization, and the Jewish Council for Public Affairs and was intended to address growing fears of stigmatization within the Jewish community. There were forty Jewish leaders present representing Orthodox to ultra-liberal denominations.

Marlene Post, president of Hadassah and a nurse-educator, expressed the community's fear that concerns about stigmatization and discrimination could actually slow important genetic research. Concerns also arose that these fears could keep individuals from seeking information derived from genetic testing that could be extremely important in making decisions regarding health. Klausner stressed that ethnic groups do not equate with genetic groups. Collins emphasized that Jews appeared no more likely than other individuals to have mutations — they simply were the first to have been studied. Collins suggested that as a result of this early participation, they also would be the first to experience the benefits from drugs and other therapies that would follow from the research regarding their genetic disorders. Everyone present at the meeting expressed concern that federal legislation intended to protect citizens against genetic discrimination (chapter 10) in employment or health insurance continued to languish in the U.S. Congress.

Although Tay-Sachs disease is typically found among individuals of Ashkenazi Jewish descent, the carrier frequency is the same (one in twenty-seven) among French Canadians and the Cajuns of Louisiana, who derived from the French Canadian Acadian community. The late Emanuel Shapira, from Tulane University, identified a cluster of children with Tay-Sachs disease in rural Louisiana among the Cajuns, whose ancestors were exiled from the French Acadian area of Canada by the English and Scots between 1764 and 1788. This was a well-known disorder in the Cajun community, known as "lazy baby disease," and because of Shapira's clinical insight, these children now had a formal medical diag-

nosis. Shapira and his colleagues identified two mutations in six unrelated families in this community: a four base pair insertion, found in 70 percent of Ashkenazi Jews, was present in eleven out of twelve mutant alleles in the six Cajun families; and a second single base pair change found among those with origins in the British Isles, particularly the Irish, Scotch, and Welsh, was the remaining Cajun allele. Genealogical studies identified a couple of French descent common to pedigrees with the most frequent mutation and suggested the presence of that mutation in the Cajun community for two hundred years since it was founded in Louisiana. The less frequent mutation appeared to have been introduced into this population approximately one hundred years ago. Other accounts have suggested assimilation of Jews with the Cajun community, or a founder effect from a European Jew who immigrated to Louisiana, where he did not disclose his background, married a Cajun woman, and fathered numerous children and grandchildren.

The lesson from Tay-Sachs disease and many other genetic disorders is that diseases that are concentrated within one community may be present even at substantial frequencies in another. We must not consider these diseases to be unique to one ethnocultural group.

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The African American community has had serious concerns regarding sickle-cell disease and stigmatization. Sickle-cell disease is an autosomal recessive genetic disorder due to a mutation of the hemoglobin gene that causes the hemoglobin protein to form crystals in the red blood cells when the oxygen concentration is low. As these crystals enlarge, they deform the normally discoid red cell to form abnormal shapes that include what is described as a "sickle" form, like the hand-held sharpened metal sickles used to cut weeds. These deformed cells clog vessels and can cause strokes and other diseases and result in red cell breakdown and anemia. When testing for sickle-cell disease was introduced in the 1970s, individuals who were found to be unaffected carriers of this autosomal recessive disorder were fired from their jobs and charged higher rates for, or even denied, health and life insurance. Pilots for the U.S. Air Force and private airlines were restricted from flying if they were determined to be carriers by genetic testing, and carriers were denied entrance to the Air Force Academy. These flight restrictions apparently were based largely on a single report of an African American soldier who died while mountain climbing. Review of the scientific and medical data by the Carter admin-

istration in 1980 resulted in reversal of these discriminatory and stigmatizing practices.

It was not only the lay community that misunderstood the difference between heterozygous carriers and homozygous affected individuals with sickle-cell disease. A survey of 160 U.S. physicians in 1974 revealed that one in seven thought that the sickle-cell trait or the heterozygous carrier state was the same as the disease, and one in five had difficulty distinguishing the carrier from the disease clinically. There was considerable confusion among all involved.

A number of states enacted laws in the 1970s that mandated screening for sickle-cell carriers. There were many problems with these statutes, in addition to confusion between carrier status and disease. For example, some of these laws targeted the population to be screened as children entering public school, with the presumed argument that then at least one parent would be identified as a carrier and knowledge of their carrier status would help them with family planning. But this was an extremely inefficient approach to identifying carriers of child-bearing age. If one parent was a carrier, then only 50 percent of their children would be carriers and would be identified in school-based screening. In the interval between the child's birth and school entry, it was likely that siblings would have been born. In addition, many of these state laws did not include any provisions for confidentiality or counseling.

Twelve states passed laws between 1970 and 1972 mandating sickle-cell screening for African Americans. As a consequence of the serious problems with the screening laws and programs and concerns that this was a eugenic (chapter 3) or even genocidal strategy targeting African Americans, many leaders in the African American community recommended boycotts of these mandatory screening programs. The clamor created within the African American community resulted in the ninety-second U.S. Congress passing the National Sickle Cell Anemia Control Act of 1972. This legislation provided funding to the states for voluntary screening if these state programs included genetic counseling services. Grant support to states was designated for screening programs by this legislation "first to those persons who are entering their child-producing years, and secondly to children under the age of 7." This legislation opened the door to newborn screening for sickle-cell disease and other disorders involving hemoglobin, called hemoglobinopathies.

Newborn screening involves taking blood from neonates before discharge from the nursery to be tested for a variety of diseases (chapter 15). Phenylketonuria (PKU), a genetic disease for which early dietary treatment

can prevent severe mental retardation, was the initial disorder targeted for newborn screening in 1961. The first state to initiate a newborn screening program for sickle-cell disease was New York in 1975. These early newborn screening programs suffered from the previous bitter experiences of the African American community with mandatory carrier screening and had to overcome an emotional barrier to screening in general.

Colorado in the late 1970s had an advisory committee to consider expansion of the newborn screening program to add disorders beyond PKU. This advisory committee included representation from the African American community. The hemoglobinopathies were considered for addition to the screening battery and were added in 1979. The following issues were raised as potential barriers in the committee's discussions about initiating newborn screening for sickle-cell disease.

One argument was that there was no evidence of benefit that could justify newborn screening for sickle-cell disease. Data were beginning to come out of Graham Serjeant's experience in Jamaica, however, that indicated that sickle-cell newborn screening prevented death from early overwhelming infection. Admittedly this information was preliminary, but the benefits seemed to make sense from an understanding of the physiology and pathology of sickle-cell disease. In addition, there would be benefits in knowing which babies had sickle-cell disease so that their families could be educated about the disorder and therefore could be proactive to prevent or minimize early complications of sickle-cell disease. In addition, there was a need for additional states to mount field trials to assess hemoglobinopathy screening. In a review of their first ten years of experience published in 1990, the Colorado group reported no deaths among the forty-seven infants identified with sickle-cell disease by screening.

Another argument against screening was that sickle-cell disease was too rare in Colorado to justify screening. Census data provided the demographic structure of the population of Colorado, and the frequency of sickle-cell disease among African Americans was approximately one out of six hundred births. The calculations indicated that although the number of African Americans in Colorado was relatively small at that time, the frequency of the disease in that subpopulation was so high that the incidence of sickle-cell disease would be higher than the incidence of PKU. The Colorado experience after ten years showed the incidence of sickle-cell disease to be approximately one in 11,000 births, and if all babies with a hemoglobinopathy were included, then the incidence increased to one in 7,000 births, compared with the incidence of one in 20,000 live births for PKU.

Because of a misunderstanding of the incidence and population distribution of sickle-cell disease, some Coloradoans recommended targeted versus universal newborn screening. Targeted screening would involve identification of babies to be tested, usually by the nurses or physicians physically inspecting the baby and mother to determine if they were African American. Such targeting ignores the fact that this disorder has an increased incidence among those from the Mediterranean Basin, the Near East, and India, as well as Latinos from Central and South America and Puerto Rico. In addition, targeting denies the increased frequency of other hemoglobinopathies in some of these populations, as well as those from other areas of the world, such as Southeast Asia, including southern China. There was concern on the part of the advisory group that sorting the samples would lead to clerical errors, and subsequent analyses of newborn screening programs showed that 80 percent of errors were clerical in nature. The idea of segregating babies by physical features was abhorrent. Finally, the expense of sorting all of the samples to identify those for hemoglobinopathy screening, which at the time would have required human labor, was not that different in cost than testing all of the samples. Therefore, the recommendation of the advisory group was to proceed with newborn screening for sickle-cell disease and several other disorders in Colorado.

In June 1986, Marilyn Gaston, then at the National Institutes of Health (NIH), and her colleagues reported the very clear benefits of early identification of infants with sickle-cell disease in order to prescribe penicillin prophylaxis and to prevent death from overwhelming infections. This was a very carefully constructed multicenter, double-blinded, randomized, controlled clinical trial that compared penicillin to a placebo, or sugar pill. Deaths occurred in the placebo group, but none were observed in the penicillin group. The data safety monitoring committee had access to the unblinded data but were not otherwise involved, and they determined that there were adequate statistical differences between the two groups and terminated the trial early. Gaston and her colleagues concluded that children should be screened for sickle-cell disease so that those with this disorder could be started on penicillin before they were at risk of overwhelming infection.

The evidence was so compelling that the NIH held a Consensus Development Conference, chaired by Doris Wethers of Columbia University in April 1987, less than one year from publication of the paper by Gaston and her colleagues. Wethers and her panel stated their conclusions so clearly in the report that none should have been able to mis-

take the strength of their position: "In summary, the panel concludes that every child should be screened for the hemoglobinopathies to prevent the potentially fatal complications of sickle-cell disease during infancy." They did not recommend some screening or targeted screening, but screening "every child" — universal screening.

The recommendations of the Consensus Development Conference, together with funding from the federal government, accelerated the implementation of hemoglobinopathy newborn screening programs. However, there must also be the political will to implement policies, and it took more than fifteen years — until 2005 — for all fifty states and the District of Columbia to require universal neonatal hemoglobinopathy screening.

The story of screening for sickle-cell disease from the 1970s to the present is one rooted in discrimination and stigmatization. These issues arose from a poor understanding of the differences between carriers and affected individuals and from excluding the community from deliberations about the programs. There was also a fundamental distrust of programs thrust upon the community, based on hundreds of years of experience with discrimination. All of the identifying features of stigmatization noted by Gregory and Satterfield were present. This unfortunate experience with carrier screening created some initial barriers to newborn screening. However, the clear evidence of life-saving benefits and the involvement of African American community members in policy deliberations have overcome these initial concerns. The history of discrimination against African Americans led to unfortunate delays in implementation of universal newborn screening for sickle-cell disease and related hemoglobinopathies in all states.

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Collaborating with Paul Rabinow, Gísli Pálsson at the University of Iceland, has chronicled the progress of deCODE Genetics as an example of "a national human genome project," and they have developed a Web site that follows the project's developments. Iceland is a country with a population of 300,000 in the North Atlantic between Greenland and the British Isles. Iceland was settled beginning around A.D. 870 and has a relatively homogeneous population genetically. The settlers were primarily Norwegian chieftains and their families and Irish slaves to these chiefs. The population has remained fairly small, approximately 50,000 in the nineteenth century and 293,000 in 2004. The populace has a strong inter-

est in genealogy, with a desire to register as many living and deceased Icelanders as possible in a large computerized genealogical database that includes 80 percent of Icelanders who ever lived. In addition, dating back to 1915, Icelanders have medical records that document diagnoses of diseases. These records will enable researchers to ascertain the familial nature of phenotypes, such as common complex disorders, like cancer, diabetes, and cardiovascular disease. These features set the stage for the founding of deCODE Genetics.

Kári Stefánsson and Jeffrey Gulcher, both of whom had been on the faculty of Harvard Medical School, cofounded deCODE Genetics in August 1996. Stefánsson has described his vision for deCODE as improving the health of the Icelandic population, encouraging Icelanders trained in biomedical disciplines elsewhere to return to Iceland, and attracting the biotechnology industry to Iceland to improve the country's economic base. Though formally a U.S. company, deCODE has had majority ownership by and sole operation in Iceland since it was founded. A business association with prescription drug giant Hoffman La Roche helped to strengthen the financial status of deCODE in its first years.

In December 1998, following nine months of debate, the Icelandic parliament — the oldest parliament in the world, founded in A.D. 930 — passed legislation to establish a health sector database and an exclusive license with a private corporation. Health information is deposited without specific consent of the individuals: they can opt out, but in the absence of action, their information will be included by default. The database belongs to the Icelandic National Health Service, but the licensee has the exclusive right to commercialization of the database for twelve years. deCODE backed the legislation and, in a competitive process, obtained the contract for the database.

deCODE's lead projects include identification of genetic predispositions to heart attack, coronary artery disease, asthma, vascular disease and stroke, schizophrenia, noninsulin-dependent diabetes mellitus, and obesity. Their goal is not simply to identify the genes for these disorders but to use the gene products as targets for therapeutic interventions, and they already have drugs in trials. Thus, this is fundamentally a pharmacogenomic (chapter 15) business strategy and not simply a gene-finding exercise.

In a 2001 interview, Stefánsson discussed the speed with which a project can move forward because of the deCODE database, using its work on the genetics of obesity as an example. Once the deCODE researchers had received approval to launch their obesity project, they went into their database and found they already had genotypes on approximately nine

thousand Icelanders for whom they also had a body mass index (BMI). The BMI is calculated by dividing the weight in kilograms by the height in meters squared (BMI = W/H^2) and is considered an estimate of adiposity, with higher numbers indicating greater degree of obesity and an "ideal" BMI considered to be 20 to 25. With the genotypes and BMIs from these individuals, they were able to identify genes associated with a BMI greater than 30 in a matter of hours. They then queried the data in the opposite direction and found genes associated with a low BMI of less than 19. They then looked at diabetes, a condition typically thought to be associated with obesity, and found a gene associated with diabetes in thin individuals. Stefánsson concluded, "Normally, this would have taken you 10 years to work out, but we were able to do this in real time because we had the data and the data mining instruments."

One can see the obvious advantage of having health data linked to genotypes on a large population. This has appeal to pharmaceutical companies, and in 2002 deCODE and Merck developed a "major alliance" to identify targets for therapeutic intervention in obesity. In 2005, they had accumulated information on 17,000 participants for their obesity research program.

Iceland considers the DNA of its citizens to be a national resource. Melvin McInnis from Johns Hopkins University has looked particularly at the issue of consent versus assent in the construction of the database. The Icelandic Parliament argued that a requirement of informed consent for such a large segment of their community would be laborious and expensive and would compromise the collection and quality of the database. McInnis states: "With this law, the government [of Iceland] is proclaiming the assent of the nation." Estimates of the confidence of Icelanders in deCODE establishing the health database have been as high as 90 percent or more. However, the rules have changed for deCODE.

An eighteen-year-old student, Ragnhildur Guðmundsdóttir, sued to prevent transfer of her dead father's health information to Iceland's planned Health Sector Database. This case became known as *Ragnhildur Guðmundsdóttir vs. The State of Iceland,* since the Health Sector Database would be owned by the Icelandic government. The plaintiff argued that the inclusion of her father's information in the database would violate her privacy, since it would indicate that she was at risk for any genetic diseases identified in her father. The information in the database was described in the 1998 law and by deCODE as confidential and encrypted, although how this would work in practice if no one held the encryption code was unclear. If the encryption code was known, then the confidentiality of the information was not ensured.

Apparently, Iceland's Supreme Court agreed with this assessment and stated that there was a possibility of individuals being identified when information in the Health Sector Database was linked to other unencrypted data, such as that from other genealogical and genetic databases. In November 2003, the Supreme Court of Iceland issued a verdict in favor of Guðmundsdóttir based on Iceland's constitution (paragraph 1, article 71), which asserts that "everyone shall enjoy the privacy of his or her life, home, and family" and challenged the ability of Parliament's 1998 law to guarantee privacy. The verdict stated that "taking into account the principles of Icelandic legislation concerning protection of privacy, the Court recognises the right of the Appellant in this respect." Because of the fundamental guarantee of privacy in Iceland's constitution, this decision has been interpreted as a declaration that Parliament's 1998 law establishing the database is unconstitutional.

The construction of the database was already delayed because of a number of conflicts, including those of deCODE with the Icelandic National Bioethics Committee and Data Protection Authority about access to and use of the data. The University Hospital in Reykjavík, which cares for more than 50 percent of Iceland's population, also had concerns that its physicians would be doing much of the fieldwork for this research but would not be reimbursed for it either on the front end or through sharing in intellectual property from any discoveries on the back end. The Health Sector Database was already controversial in Iceland, and in May 2004, more than 20,000 individuals had exercised their right to opt out.

Meanwhile deCODE had moved ahead with DNA collection for fifty disease-specific projects without the national database and had collected samples from 110,000 Icelandic adults, or more than 50 percent of the adult population. Therefore, deCODE Genetics is going forward with large-population-sample collection projects that will be essential in genomic medicine (chapter 15). However, the company is doing this in a more traditional manner, using informed consent.

As we will see below, the Navajo approach to informed consent and to genetic testing involves Navajo leaders making decisions for tribal members about the use of their DNA.

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Steve Holve and Diana Hu are pediatricians who have spent their careers on the Navajo Reservation, most recently in Tuba City, Arizona. They observed five autosomal recessive genetic disorders that appeared to

occur at a higher frequency than they anticipated. Three of these disorders, metachromatic leukodystrophy (MLD), severe combined immunodeficiency (SCID), and microvillus inclusion disease had much higher frequencies among the Navajo than in the general population. One of these, SCID, seems to be a particular form, referred to as the Athabascan type, in reference to the language group to which the Navajo language belongs, and this form of SCID is also observed in the Athabascan-speaking Jicarilla Apaches in the southwestern United States and in Athabascan speakers in Alaska and northwestern Canada. One of these five disorders, Navajo neurohepatopathy, is unique to the Navajo, and another, Athabascan brainstem dysgenesis, is found in the Navajo and Apaches.

The Navajo tribe is the second-largest American Indian tribe in North America. Currently, 151,000 Navajo live on the 27,000-square-mile Navajo Nation Reservation in Arizona, New Mexico, and Utah. The Athabascan language group also includes the Chipewyan (Canada), Chasta-Costa (Oregon), Hupa (California), and Apache (southwestern United States), and at one time the Navajo were part of the Western Apache. The Athabascan tribes are thought to have originated in central Asia and arrived in Alaska across the Bering Strait around 10,000 years ago. The Apache, including the Navajo, migrated toward what is now the southwestern United States, and the first evidence of the Navajo in Arizona and New Mexico is seen in the fifteenth century. Holve and Hu began to examine Navajo history as a possible explanation for the increased frequencies of these usually rare autosomal recessive diseases among the Navajo, and particularly among those who live in the western part of the Navajo Nation.

Because of conflicts with European Americans over grazing lands in the mid-1800s, Colonel Christopher (Kit) Carson and his New Mexican volunteer infantry waged war against the Navajo beginning in 1863 by destroying their orchards, crops, and livestock. The Hopi, Pueblo, and Ute Indians, who were traditional enemies of the Navajo, having suffered raids by the Navajo for many years, took advantage of the Navajo's weakened state and joined Carson in his brutal economic war against them. Defeated, nearly eight thousand Navajo endured the Long Walk, in which they were forced to walk three hundred miles to Fort Sumner and the surrounding reservation, Bosque Redondo, on the Pecos River in eastern New Mexico. Approximately 40 percent died during their four years of forced exile.

An estimated one thousand Navajo avoided capture by Carson and his

troops, stayed behind, and hid out in the rugged territory of what is now the Western Reservation. The areas to which these Navajo fled were the most inaccessible: Navajo Mountain on the border between Arizona and Utah and the Little Colorado River in the southwestern portion of the Navajo Nation in Arizona. Holve and Hu examined the oral histories of local elders, which indicated extreme geographic and genetic isolation in these areas until the latter portion of the twentieth century. These regions did not begin to have paved roads until the early 1960s, and at the beginning of the twenty-first century, the only vehicular access to Navajo Mountain was a forty-mile-long dirt road.

During the four years at Bosque Redondo, the Navajo suffered disease from the brackish water of the Pecos River and experienced crop infestations and inadequate supplies. One Navajo wrote in 1865: "Cage the badger and he will try to break from his prison and regain his native hole. Chain the eagle to the ground — he will strive to gain his freedom, and though he fails, he will lift his head and look up at the sky which is home — and we want to return to our mountains and plains, where we used to plant corn, wheat and beans."

On June 1, 1868, the federal government signed a treaty allowing the Navajo to return to their Navajo Nation, and on June 18 a ten-mile-long column with U.S. cavalry escort began the Long Walk Home. Because of the distance back and the size of the Navajo Nation, those who returned tended to stay in the eastern part of the reservation, separating them from those who had stayed behind on the Western Reservation.

The Navajo population has grown considerably since the Long Walk. From the 6,000 members of the tribe at the time of the return from Bosque Redondo, the population has grown to 219,000 in the 1990 census and 253,000 in the 2000 census. The fertility rate has been twice the average U.S. rate over the last century. The consequence appears to have been a classical "genetic bottleneck" — a population shrinks because of external forces and then expands dramatically when those forces are removed. All of us are carriers for a number of rare autosomal recessive disorders, but since these mutations are rare, there is a low probability that we will mate with another carrier. However, in a genetic bottleneck the geographically and culturally isolated population will begin to intermarry, even when, as among the Navajo, there are clan barriers to attempt to prevent this. If one member of the group that survived the bottleneck is a carrier for a mutation, for example for MLD, then with the population explosion that mutant allele will increase in frequency. Since there is a relatively high degree of consanguinity, though unintentional, when a

population grows from 6,000 to over 250,000 in only five to seven generations of twenty-five years, these autosomal recessive disorders will increase in frequency. Those who survived the forces that caused the population to shrink will become the founders of the subsequently expanding population.

Working with Sean McCandless from the University of North Carolina, Chapel Hill, in 2001, Holve and Hu examined the mutation in the gene responsible for MLD in their Navajo patients, and it was identical in all, consistent with a founder effect. They then looked at where the patients were born and found that eight out of ten with MLD were born on the Western Reservation, but one was born in Salt Lake City and another in Phoenix. As they looked at the birthplaces of parents, grandparents, and great-grandparents, they saw a concentration in the Navajo Mountain and Little Colorado River areas. They concluded that the Navajo MLD mutation "was established at a new, higher frequency in the western Navajo people as a result of genetic drift and a significant population bottleneck in the mid-19th century."

Holve and Hu have had a number of geneticist-collaborators, including individuals who are interested in, and capable of carrying out, the molecular genetic studies to examine the relatedness of these families and the nature and age of the mutation. Let's consider why they have not pursued these investigations.

The Navajo Nation Council enacted a code to govern human research in 1998. The council had three goals: to protect the Navajo community, to protect the Navajo people, and to protect the Navajo Nation's heritage. This code was established in response to environmental researchers who wanted to investigate soil and water for contaminants left over from earlier mining operations. The code requires all data from any research in the Navajo Nation to be retained as the property of the Navajo people. Review of applications by the Navajo Nation Institutional Review Board, which has been named the Human Research Review Board (HRRB), is rigorous, with privacy highly valued, and is guided by the desire to ensure that the Navajo will not be exploited. The HRRB closely monitors all studies and requires that all manuscripts be submitted to the review board and all recommended changes made before the manuscript can be published.

In 2002, the Navajo Nation Council declared a moratorium on all genetic research that fell within its authority. Given that the Navajo Nation is not only a geographic designation but also the population of Navajo, one could argue that even those who have left the reservation are still within the jurisdiction of the Navajo Nation. The council indicated

that it would lift the ban at a time when its human research code had been amended to adequately address issues like genetic discrimination and gene therapy. So the additional studies could not be performed because of a fundamental distrust of genetics that was formalized in the 2002 moratorium.

The Navajo Nation Council code places as one of its three primary goals the protection of the Navajo culture. Genetics is a construct foreign to the culture and has a history burdened by discrimination and eugenics. In addition, the Navajo origin story involves emergence of the *Dine,* the Navajo people's name for themselves, from the underworld in the area of northwestern New Mexico bounded by sacred mountains. This is very different from a genetic description of migration from central Asia. Cultural values and social cohesion have maintained the Navajo for centuries in the face of considerable hardship, some very directly deriving from the dominant U.S. culture to which many geneticists belong.

Along with a colleague at the University of California, Los Angeles, Sean McGhee, we are developing a newborn screening test for SCID that would permit early identification and treatment with bone marrow transplantation that we feel would save Navajo children's lives. But we were told that at this time we would not be able to carry out any research using this test within the Navajo Nation because of the moratorium on genetic research. It is likely that we could do pilot testing of this SCID newborn screen in the Navajo community in Phoenix that numbered more than 15,000 according to the 2000 census. But what would that do to trust with the Navajo Nation Council when we reported our results? We feel that our goal should be to help the larger numbers of children with SCID who are on the reservation.

We have had to come to an understanding of the moratorium, which is so alien to our European American culture. The people of the Navajo Nation gave their leaders this authority and placed a high value on protection of their culture. We must be sensitive to these values and understand the cohesive nature of culture and the importance of that culture to survival of the group and individuals within the group.

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In this chapter we discussed some of the consequences of genes belonging to groups. We have reviewed several examples in which there are collective implications for the genetics of the group, including discrimination and stigmatization. We have also considered examples in which the

control of an individual's genome may not reside within that individual. All of these situations are in flux as some concerns are addressed and new challenges arise. Cultures, and their legal and regulatory structures, are coevolving with the science. The experience in Iceland will have broad-reaching implications for other populations that hope to use their genomes to develop intellectual property. In the next chapter, we will address the issues of that intellectual property and consider the commodification of genes and genomics.


[To view this image, refer to
the print version of this title.]

CHAPTER 9

Genes as Commodities

Ownership of Genes by Business Interests

Patenting oneself
Sample ownership
Informing patients and their families
Gene patents
Strategies of patient advocacy groups and governments

In chapter 8, we discussed groups, such as the Navajo, in which genes are not private entities but rather "belong to" or "are controlled by" the group and not individuals. For those who come from a different culture, for example, the dominant U.S. culture in which individuality is such a powerful value, it may be difficult to come to an understanding of group control of something that is so important to one's own biology. But as shown by the examples we selected, the group's leaders — be they French or Navajo — have the interests of the group, and therefore the individuals in the group, guiding their decisions.

In this chapter, we will explore what it means when there is true ownership of genes as commodities, and that ownership is not by cultural groups but by corporations that intend to profit as the owners. We will also see some strategies that group leaders, including governmental officials and advocacy groups, are developing to regain control of what they perceive as their genes and not corporate property.

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When we discuss gene patents in our classes, our students are incredulous that their genes can be patented and owned by a corporate entity. In 2000, Donna Mac Lean, a thirty-one-year-old poet and waitress from Bristol, England, attempted to do something about this situation — she paid £130 for her patent application that she titled, "Myself." The U.K. patent office gave her the application number GB0000180.0. Excerpts from her application follow:

Biotechnology companies have requested and received patents for different plant and tree species, and human genetic material has already been patented. The behavior of such biotech companies has led me to wise up to my own inalienable rights. Can I be described as the owner, the sole proprietor, of MYSELF, my being, both physically and metaphysically? Is it possible that a corporation might legally claim ownership of MYSELF, in whole or in part? Do my inalienable rights as an individual have any real meaning unless I can translate them into monetary terms, or determine and protect them in a hard-headed, businesslike manner? Very briefly, I came to the conclusion that no, they do not. I am therefore applying to patent MYSELF, Donna Rawlinson Mac Lean, in my entirety; that is, my physical reality including my genes, which are me, and all the other less tangible elements which constitute the wonder that is me. . . .

With reference to the guidelines, I can state: . . .

I am not obvious. . . .

I am not merely a discovery or an aesthetic creation. I am more than the sum of my abstract aspects. And quite clearly I have physical features.

I do not find MYSELF in your definitions of "excluded inventions." . . .

The accompanying illustrations show MYSELF to be an excellent example of a human being. The bones of MYSELF are all present and connected in the correct sequence. The limbs are supple and obedient to the will of MYSELF. The physical features on the face are my own. I am alive and my genes are MYSELF. Please do not hesitate to contact me if you need further diagrams or any additional proofs that I am that I am.

Mac Lean's patent application is a creative act of defiance, but it also demonstrates the passion and concern that people have about the patenting and ownership of genes. Her application was denied.

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First we will discuss the topic of biological sample ownership. Let's consider that you provide a blood sample to a molecular genetics researcher so that she can use it in her investigations. Who owns the sample? After all, it is your tissue. Perhaps it was a cheek swab and therefore giving it would have been painless and minimally invasive. But what if it was a blood sample, or even more painful, a sample requiring a surgical procedure? If you suffered to provide this sample, does that not give you reason to claim ownership? In fact, you do not own the sample. If this is a research specimen, then you most likely signed an informed-consent document. If this sample was provided in a University of California setting, because of the *Moore v. the Regents of the University of California* ruling, then the consent undoubtedly made clear in very specific language that you did not retain any rights of ownership.

The investigator leaves one institution to work at another institution. She thinks she owns your sample and can simply take it with her. That is actually not the case either. The university or research institute that employed this scientist when your sample was taken would assert that it owns the sample, though, as we shall see, not everyone would agree. In point of fact, a university or research institute will almost always release the samples for the scientist to take with her to the new institution, transferring ownership to the new institution. Occasionally, if these samples were used collectively by a group of investigators, then they may ask that a portion be left behind for use by the others. The legal precedents are still being determined in this area, however, particularly if there is commercial value to the samples.

Rina Hakimian and David Korn from the Association of American Medical Colleges reviewed the status of the ownership of tissue specimens used in research in 2005. They considered the regulatory and judicial framework in this area to be quite ambiguous. They noted, however, that "recent examination of these issues by a U.S. federal court resulted in a ruling that individuals do not retain rights of ownership or control of biological materials contributed for research, regardless of whether commercial benefit accrues." They concluded: "because the benefits of medical knowledge derived from tissue research potentially accrue to all individuals and future generations (rather than a single recipient), society may justify an expansive use of these valuable resources for future studies."

In 1987, the U.S. Congressional Office of Technology Assessment asserted that "there is great uncertainty about how the courts will resolve disputes between the human sources of specimens and the specimen

users." The case law remains very thin, though the cases with decisions have been consistent.

The U.S. Food and Drug Administration (FDA) is the agency within the U.S. Department of Health and Human Services that is responsible for the safety and efficacy of drugs and medical devices. Because of its role in reviewing clinical trials for approval of therapeutics and devices, the FDA has put out an information sheet to guide the institutional review boards that must approve all of the human research performed in their particular institution and the informed-consent forms that are read and signed by all research participants. Although the FDA allows the consent forms to acknowledge that samples will be used in research projects, it prohibits any use of the term "donation," because the FDA feels that if the subject is donating the sample, that would imply "abandonment of property rights."

The Office of Human Research Protections (OHRP) within the U.S. Department of Health and Human Services is responsible for enforcing the standards that govern human research. In 1996 and again in 2001, the OHRP took the position that language could not be used in a consent form that might make subjects think that they would be waiving any "property rights" associated with their tissue specimens. Following the 2003 settlement of *Greenberg et al. v. Miami Children's Hospital in Florida,* the OHRP was asked whether it would reconsider its position, and it refused. Thus, the consensus within the U.S. Department of Health and Human Services is that human research subjects may have ownership rights attached to their samples and should not be told that they must relinquish those rights.

Court opinions, though limited, do not agree with the Department of Health and Human Services. At this time three court decisions have addressed sample ownership, and all three determined that the research subjects did not have property rights to their samples. The first case, known as *Moore v. Regents of the University of California,* involves a cell line patent. John Moore was a patient at the University of California, Los Angeles (UCLA), with a form of leukemia called hairy cell leukemia. In the course of Moore's treatment, his physician recommended that his spleen be surgically removed, and Moore signed a consent form giving his approval for this operation. His physician had planned to use a portion of Moore's spleen for his research but did not inform Moore of his plans. Moore had the surgical procedure in October 1976, and he returned to UCLA from his home in Seattle for follow-up visits from 1976 to 1983. During these additional visits, other samples were taken, including blood and bone marrow. Sometime before August 1979, one

of his physicians established a cell line with unique properties from Moore's lymphocytes. In 1981, the physician, through the Regents, as the legal entity governing the University of California, applied to patent this cell line, and the patent was issued in 1984 with the Regents listed as the assignee on the patent. Two companies were interested in this patent, and they paid the physician and UCLA with stock options and at least $440,000 in cash.

Moore sued the University of California on a number of grounds, including "conversion," or legal protection, "against interference with possessory and ownership interests in personal property." Moore argued "that he continued to own his own cells following their removal from his body, at least for the purpose of directing their use, and that he never consented to their use in potentially lucrative research."

The California Supreme Court ruled that the sample was owned by the University of California. The majority opinion was that the patented cell product had been altered in the laboratory, and therefore, even if the original cells that had been removed were owned by Moore, the patented cell line would not belong to him—the cell line was "both factually and legally distinct from the cells taken from Moore's body." The justices used legal decisions supporting the patenting of living organisms, such as genetically altered mice, as precedents, since the position in those decisions was that the organism had been altered by human intervention and ingenuity. They also disagreed with Moore's argument of conversion, which is a principle of property law, and pointed out that in California statutes, tissues are not treated under property law but under the Health and Safety Code intended "to protect the public health and safety." They felt that extension of conversion into medical research "will hinder research by restricting access to the necessary raw materials."

One of the justices concurring with the majority wrote: "Plaintiff has asked us to recognize and enforce a right to sell one's own body tissue *for profit* [justice's emphasis]. He entreats us to regard the human vessel the single most venerated and protected subject in any civilized society as equal with the basest commercial commodity. He urges us to commingle the sacred with the profane. He asks much."

The Supreme Court justices did raise some concerns about the physician's behavior in this case, though that was not the issue before them. One even suggested that Moore "in this matter is not without remedy; he remains free to pursue defendants on a breach-of-fiduciary-duty theory" and other claims not before the Supreme Court. Apparently Moore did not pursue that course.

Since the decision in the Moore case in 1990, all informed-consent documents for research conducted within the University of California contain a paragraph that states that samples provided by subjects to research studies are owned by the University of California. This paragraph also states that the University of California can transfer ownership to individual investigators or to companies. These policies clearly contradict those of the U.S. Department of Health and Human Services described above.

The second case involves the Canavan disease gene patent, known as *Greenberg et al. v. Miami Children's Hospital*. Canavan disease is a progressive degenerative disease of the white matter of the brain that has its onset in early infancy, with death occurring usually by eighteen months of age. Affected children have poor growth, decreased muscle tone, severe mental retardation, blindness, and increased head size. Canavan disease is an autosomal recessive genetic disease that has an increased incidence in the Ashkenazi Jewish population.

Debbie and Dan Greenberg had a son in 1981 and a daughter in 1983, both of whom were diagnosed with Canavan disease. The Greenbergs founded the Chicago chapter of the National Tay-Sachs and Allied Diseases Association to assist other families with carrier screening for diseases for which testing was available. Testing was not available for Canavan disease at that time, and in fact the cause of the disease was unknown.

The Greenbergs met a biochemical geneticist, Reuben Matalon, and in 1987 they convinced him to refocus his career on Canavan disease. They provided him with samples from themselves and another couple and seed money from their Chicago chapter. Within a year, Matalon was successful in identifying the deficiency of a specific enzyme. In 1988, the Greenbergs began a pregnancy knowing that they would be the first couple to have prenatal testing (chapter 11) for Canavan disease. Debbie Greenberg gave birth to a normal, healthy child, and over the next two years at least nineteen families had similar prenatal diagnoses for this disorder. During this time, Matalon moved from Chicago to Miami Children's Hospital.

Unfortunately, there were difficulties with the enzymatic testing, and at least four pregnancies were misdiagnosed as normal, resulting in the births of children with Canavan disease and at least two lawsuits for misdiagnoses. Therefore, Matalon's group began work to identify the gene and mutations responsible for the disease as a more reliable testing strategy. The Greenbergs and more than one hundred families with a history of Canavan disease provided specimens, and Rabbi Josef Eckstein of Dor Yeshorim (chapters 3 and 8) provided approximately six

thousand samples. In 1993, the gene and mutations responsible for Canavan disease in the Ashkenazim were identified, and the Dor Yeshorim samples permitted estimations of the population frequency of the mutant alleles.

Unknown to any of those who supplied the samples, Miami Children's Hospital applied for a patent for the gene in 1994, and the patent was issued in 1997. The hospital took an aggressive position regarding licensing the patent. Licensing a patent for diagnosis means that the patent holder gives permission to one (an exclusive license) or more (a nonexclusive license) laboratories to use the patented gene for diagnostic testing, with the laboratory or laboratories providing fees to the patent holder. In the first stage of their marketing, Miami Children's Hospital proposed that a restricted number of laboratories at academic institutions be permitted to carry out a limited number of tests each year for a licensing charge of $12.50 per test, payable to Miami Children's Hospital. The limited number of annual tests made it very difficult to justify the cost of establishing this testing. In addition, these laboratories would go through the expensive phase of setting up this gene test only to have it shut down, since this period of nonexclusive licensing of academic laboratories would be followed by an exclusive license to a "market leader" that would be able to handle high-volume testing.

This marketing strategy ignored the experience of grassroots community-based organization and communication that had been so effective for Tay-Sachs (chapter 8) and early Canavan carrier screening. The result of Miami Children's Hospital's restrictive and expensive licensing approach was that testing became essentially unavailable to the families who provided the samples and funding for the research. The hospital and its representatives were extremely aggressive in their negotiations, in one case denying anyone at the University of Pennsylvania the ability to order the Canavan gene test, because the university refused to sign a licensing agreement that included among its clauses a "gag order" on one of its faculty members, who was president of a national professional organization that had been critical of gene patents. Miami Children's Hospital did not want this faculty member speaking out against gene patents in general, or the Canavan disease gene patent in particular.

There were failed attempts at dialogue between a group that included the Greenbergs and a consortium of community representatives, and Miami Children's Hospital. During this time, the hospital gave up its attempt at identifying an exclusive licensee. It offered the consortium

approximately $20,000 from annual estimated royalties of $375,000, with the offer including another "gag order" — this time on the members of the consortium. The offer was refused. On October 31, 2000, the Greenberg family along with several additional families, the National Tay-Sachs and Allied Disease Association, and Dor Yeshorim filed a suit in federal court against Miami Children's Hospital.

In 2003, a federal judge in the Southern District of Florida upheld the Canavan patent, denying the plaintiffs' suit. The judge found that the plaintiffs knew that they were volunteering for research and that the investigator did not have a responsibility to inform the participants of the possibility of future financial benefits, and therefore the investigator had not misused the samples or defrauded the participants. The judge used the same argument that appeared in *Moore v. UC Regents* and is accepted by the U.S. Patent and Trademark Organization to justify ownership by Miami Children's Hospital: the patented gene was altered from nature by the intervention of the investigators and therefore was "legally and factually" distinct from that in the original sample, justifying ownership by the investigators' institution. Florida state law establishes that exclusive ownership of genetic information is reserved for the individual. The judge argued that the statutory language in the state law that DNA test results are the "exclusive property of the person tested" were applicable only to these tests and not to research. He argued, therefore, that the Florida law did not apply in this case.

The judge also expressed concern that the involvement of individuals might have a chilling effect on medical research by allowing them to direct that research. Patient advocacy groups learned from this case and have taken a much more active role in ownership and direction, as we will see with the advocacy foundation, PXE International.

Before the federal court judge's decision was formalized, however, the plaintiffs and the defendants in this case reached a confidential settlement effective August 6, 2003, that they described briefly in a joint press release on September 29, 2003. Central to this settlement, apparently, was "continued royalty-based genetic testing for certain licensed laboratories and royalty-free research by institutions, doctors, and scientists searching for a cure." The plaintiffs agreed not to challenge ownership or licensure of the patent in the future.

The third case involves the question of whether samples are owned by an investigator or the university and is referred to as *Washington University v. Catalona*. The cases we have discussed so far have primarily centered on ownership of samples and the intellectual property derived

from them by patients or institutions. This case focuses on a faculty member, William Catalona, who was chief of Urological Surgery at Washington University in St. Louis and moved to Northwestern University in Chicago. He had a considerable collection of prostate samples and wished to move this repository with him to Chicago, but Washington University sued as the plaintiff to prevent this move. Catalona argued that the research subjects relinquished their ownership to their specimens through the language in the informed-consent document they signed. He also maintained that this document conveyed ownership to him, and not to the university, and therefore he should be allowed to take them with him to continue his research. Six thousand research participants signed a document to discontinue their participation in Washington University's research protocol.

On March 31, 2006, the U.S. District Court found in favor of the plaintiff, Washington University, determining that the defendants, Catalona and eight research participants named in the suit, had not shown that they had "any ownership or proprietary interest" in the specimens. The court used a now familiar argument: control of research samples by the participants would lead to a situation in which "highly-prized biological materials would become nothing more than chattel going to the highest bidder. It would no longer be a question of the importance of the research protocol to public health, but rather who can pay the most. . . . Accountability would no longer exist since institutions would merely be warehouses filling purchase orders." The decision continued: "More alarming is the great potential for prejudicial influences into medical research. . . . This kind of 'selectiveness' [in the storage and use of specimens being determined by the research participants] is repugnant to any ethical code which promotes medical research to help all of mankind."

The decision in this case again contradicts the position of the U.S. Department of Health and Human Services regarding patients' inability to abandon their samples and prohibiting language that would indicate such abandonment. This outcome, however, supports the position of most universities, that the institution, not the investigator, controls samples.

Another approach to sample management substitutes the concept of stewardship for ownership. A committee of the College of American Pathologists published a document in 1999 recommending policies for uses of human tissue samples in a variety of venues including research, education, and quality assurance. This committee argued that patholo-

gists, and any medical specialists who are the recipients of tissue and other medical specimens, should "regard themselves as stewards of the patient tissues and consider it their duty to protect the best interests of both the individual patient and the public." These interests would include the direct care for that patient from whom the sample was obtained, as well as that specified in the published court cases above, that is, improving knowledge in medicine more generally through research. This stewardship, or managerial responsibility for the samples, would replace the ownership relationship.

The concept that professionals should take responsibility for the samples in their care to ensure that they are used in the best interests of the individual and the group seems very straightforward. It might in fact seem that such a principle would be difficult to violate. But we will see that not all who have samples in their possession appear to understand their stewardship responsibilities.

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It would seem obvious that one way of avoiding conflict over the nature of investigators' and their institution's relationship to research samples would be to inform the research participant fully regarding the nature of the investigation and the management of the sample. Please notice that the terminology we have used here is "to inform," not "informed consent." The informed-consent document, as we noted previously, is the form developed by the lead investigator that a human research subject reads and signs in the presence of an individual knowledgeable about this project. This process is intended to ensure that the subject has had the opportunity to ask any questions of the investigator. These documents can stretch into tens of pages in length and can be very dense in content. We would argue, and many others would agree, that these informed-consent forms are no longer about informing, but rather their purpose is to protect the institution and the investigator from liability. The primary purpose of these documents is defensive and not educational.

Several professional organizations and federal advisory committees, including those on which we have served, have recommended that all genetic testing be carried out only after informed consent. These recommendations were made because it was considered important to educate individuals that information may be learned about other family members as well themselves and that genetic testing may have additional risks including genetic discrimination (chapter 10). These recommen-

dations have been described by critics as examples of genetic exceptionalism. These critics would argue that there is no difference between a genetic test and any other clinical test. We feel, however, that there is a very real difference between testing for a mutation responsible for an inherited disorder, such as breast cancer, and a routine clinical laboratory test, like a blood count. The blood count does not indicate that your siblings or children have a risk for a serious disease with potential consequences for their insurability. We feel that individuals have the right to be fully informed about what is being done to them and their tissue samples, and we are not convinced that informed-consent documents accomplish these purposes. We must learn how to put the "informed" back into informed consent!

We will now consider several examples in which individuals were not informed about the use and disposition of their samples. The first will describe the origin of a cell line in the mid-twentieth century at Johns Hopkins University. The cell line came from Henrietta Lacks. Born in 1920 in Roanoke, Virginia, she grew up on a tobacco farm worked by her family. At the age of twenty-three, she moved to Turner's Station, a community just outside of Baltimore, Maryland, to join her husband, who was working in the shipyards there. She gave birth to five children, and then on February 1, 1951, because of blood spots on her underwear, she went to Johns Hopkins where she was diagnosed with a malignant cervical tumor. She returned eight days later for radiation therapy. Another biopsy was excised for research by a resident physician-in-training before her cervix was covered with radium.

The tumor biopsy went to the laboratory of George Gey, who with his wife, Margaret, had been attempting to develop an "immortal" cell line that would grow indefinitely in the laboratory and facilitate their cancer investigations. For twenty years they had been unsuccessful, but with this cell line, which they called HeLa, they had cells that multiplied indefinitely and did not die after a few generations.

Despite surgery and radiation treatments, Lacks died on October 4, 1951. Her husband initially refused any postmortem studies, but with his cousins' urging, and thinking the information might help their children if they developed cancer, he agreed. He said that the doctors told him "it was the fastest growing cancer they'd ever known, and they were supposed to tell me about it, to let me know, but I never did hear." His wife's body was taken back to the family cemetery in Lacks Town, Virginia, where she was buried in an unmarked grave.

On the same day as Lacks's death, Gey went on national television to

show a vial of HeLa cells. He said, "It is possible that from a fundamental study such as this [using these cells], we will be able to learn a way by which cancer can be completely wiped out." These HeLa cells were so tough that he could send them through the mail, and he sent them to colleagues in the United States and around the world.

Twenty-four years later at a neighborhood dinner party attended by Barbara Lacks, a researcher from Washington, D.C., said that her name sounded familiar. He then added, "I think I know what it is . . . I've been working with some cells in my lab; they're from a woman called Henrietta Lacks. Are your related?" She whispered her reply, "That's my mother-in-law. She's been dead almost 25 years, what do you mean you're working with her cells?" The investigator explained that they were used extensively in medical research.

Barbara ran home to tell her family. They could not understand why they had never been contacted. Barbara said, "I wonder why they never mentioned anything to the family. They knew how to contact us." Deborah Lacks-Pullum puts it more succinctly: "I'm just tired of my family getting walked over. It hurts."

HeLa cells are a mainstay of laboratory research, growing rapidly (doubling every twenty-four hours) and robustly (taking over and replacing all other cells in culture dishes in a laboratory). We use them routinely in our laboratory for gene expression studies. HeLa cells have been used to study many areas of medicine, including radiation effects, cancer, and AIDS. These cells were critical for studying the polio virus, and Jonas Salk used them in developing the first polio vaccine.

One could argue that 1951 was a long time ago and ethical standards are very different now. But we will see that the right of individuals and families to know what is done with their specimens is still not recognized universally. It also might be argued that communal good—the public's health—is a transcendent value, as have the courts in the decisions discussed above. But does that justify not informing or misinforming individuals regarding the use of their samples?

Let's discuss two lawsuits, with damages totaling $60 million, that were filed in 2004 by Havasupai, a Native American tribe—one by Carletta Tilousi, who was elected to the tribal council in 2003, and the other by the tribal council. The defendants in these cases included Arizona State University (ASU), the University of Arizona, Stanford University, and investigators Therese Markow, John Martin, and others.

The Havasupai are a tribe that originally moved seasonally between the upper plateau above the Grand Canyon and the western canyon floor,

where they farmed in spring and summer. In the early twentieth century, their numbers had been reduced by diseases and natural disasters to approximately 165, and of these only 80 men and women were in reproductive age ranges. This was a significant genetic bottleneck, as we discussed in relation to the Navajo (chapter 8), since the current Havasupai tribal membership has expanded to 650, of which approximately 70 percent live in the tribal village of Supai at the base of the canyon. The group is culturally and geographically isolated, with access to Supai village still requiring a three- to four-hour drive from Flagstaff, Arizona, followed by an eight-mile "moderately difficult" hike, of which at least 1.5 miles are a series of switchbacks along the canyon wall. There are no paved roads or automobiles in Supai.

The rate of diabetes is quite high among the Havasupai, reaching levels of 55 percent among women and 38 percent among men. John Martin had worked with the tribe for decades, having spent more than a year with them as part of his doctoral research in anthropology in the 1960s. Because tribe members were concerned about their high rate of diabetes, and because of their trust in Martin, they requested his help to stem the "diabetes epidemic," and Martin enlisted the help of human geneticist Therese Markow. Martin invited Tilousi to a seminar by Daniel Garrigan, one of Markow's graduate students in 2003, where Tilousi learned of Garrigan's work, which included use of blood samples from approximately one hundred Havasupai. Tilousi said, "He spoke about how the DNA of this isolated, intermarried group of people — us — was unique, and how my people had migrated to Arizona from Asia."

Tilousi publicly interrogated the student about his right to have performed these investigations, which she felt went well beyond the diabetes studies for which consent had been given. She said, "I'm from the Havasupai tribe, and I want to know if you asked us permission to do this study." She remembered that Garrigan "was really nervous. He said no, not to his knowledge." The origin story of the Havasupai maintains that the Grand Canyon was created as waters receded from a global flood and became the birthplace for all humans. Tilousi felt that the blood of her people had been used to challenge their identity and to refute their religion, and all of this without their permission.

Other issues have arisen in the course of these cases. Documents in the suits argue that of the twenty-three scientific publications based primarily on the Havasupai blood specimens, fifteen focused "on schizophrenia, inbreeding and migration, not diabetes." Informed-consent

forms were described as misleading, with language that was difficult to understand by the tribal members, many of whom did not complete high school. Samples were to be stored without identifiers but were sent to other laboratories with names attached. At least one researcher who was in Supai obtaining specimens reported that he did not obtain informed consent. Another indicated that he would search the tribal members' confidential medical records at night looking for evidence of schizophrenia without permission of the individuals or Indian Health Service officials.

When asked what was desired beyond a cash settlement, tribal vice chair Dianna Uqualla answered tearfully, "First, I would like all of the blood returned to us. There are people, loved ones, who gave blood and who have passed away. But their blood is still out there somewhere, I think. Blood is very important to us. We need a ceremony with ASU officials present to bury that blood." In the meantime, the Havasupai have suspended all research among their people.

Let's now look at a state that sold specimens in its custody to a for-profit corporation. Newborn screening is a public health program designed to identify and treat disease in babies before they become symptomatic (chapter 15). When newborn screening tests are completed, some states throw the dried blood samples away, and others store them for varying periods of time, some indefinitely. These are valuable to genetics investigators, since they represent an unbiased sample of the genetic makeup of the community, at least for those being born into it. For example, we used newborn screening blotters that were to be discarded by the Texas program and were stripped of all identifiers except ethnicity; from those samples we learned that a mutation for a hemoglobin disorder was much more common than had previously been appreciated. This disorder is called β-thalassemia, and when the mutation is present along with one sickle-cell disease allele, it is much milder than sickle-cell disease (chapter 8). But it was masquerading as the full-blown disease in the newborn screening test. Babies were being misdiagnosed with sickle-cell disease when genetically their two hemoglobin alleles were sickle-cell and β-thalassemia. When Desiree Sylvester-Jackson was a postdoctoral fellow with us, she showed, using the discarded, anonymous specimens, that the β-thalassemia allele was at least twice as frequent in African American babies as anyone had previously appreciated. This work showed the public health community that β-thalassemia had to be considered in newborn screening programs.

The state of Utah decided to sell its residual newborn screening blood blotters to a commercial research and testing laboratory, Myriad Genetics, based in Salt Lake City. Myriad, the company that tests for the breast cancer gene mutations, wanted to study the frequency of specific breast cancer gene alleles in an unbiased sample. Those running the newborn screening program in Utah reasoned, presumably, that rather than simply throwing their specimens away, they could sell them and make money for the program. When the Salt Lake City press and the people of Utah learned of the sale of these neonatal samples, they were extremely concerned. Among the issues raised were the custodial responsibility of the state for these specimens and whether the state should profit from material given freely for a specific public health screening purpose.

It would appear that the concept of stewardship responsibility—doing the right thing for the individual and society—should be considered more often and more seriously by those who have been designated by society as the custodians of the samples, be they researchers, public health officials, or others. An essential part of this responsibility is open communication with the individuals involved about what is being done with their specimens or those of their loved ones.

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We will now consider the patenting of genes in the U.S. legal system. Article I of the U.S. Constitution lays out the powers of the legislative branch of the government, including the powers to collect taxes, to declare war, and to protect the rights of authors and inventors. The durability of our Constitution is in significant part due to its simplicity, and the language authorizing the Congress to establish the Patent and Trademark Office (PTO) is an example of that simplicity. In one clause of Section 8, Article I, without ever mentioning patents or trademarks, this document laid the foundation for the PTO: "To promote the progress of science and useful arts, by securing for limited times to authors and inventors the exclusive right to their respective writings and discoveries." The final clause of Section 8 provides the basis for making "all laws which shall be necessary and proper for carrying into execution the foregoing powers."

Congress passed the first U.S. patent statute in 1790, and the Patent Office became a separate and distinct bureau in the Department of State in 1802. It was transferred to the Department of the Interior in 1849 and

finally to the Department of Commerce in 1925, being renamed the Patent and Trademark Office in 1975. Responsibilities of the PTO with respect to patents include administering the patent laws, examining patent applications, publishing issued patents, and recording patent assignments. The jurisdiction of the PTO does not extend to questions of patent infringement and enforcement or to patent promotion or utilization.

Patents are legal documents issued by the U.S. government to the holders or assignees of the patents. A patent entitles the assignee the sole legal right to the invention described in the patent, meaning that the assignee can prevent others from manufacturing, using, or selling the invention for the duration of patent protection, which is generally a period of twenty years.

Patent rights are analogous to property rights and therefore can be licensed to someone else, giving this other party the right to make, use, and sell the invention, usually for a contractually specified period of time and amount of money, as we discussed with the Canavan disease gene.

When licenses are exclusive, competition is limited and monopolization is possible. The issue of monopolization is important in the tensions within patent law decisions. The judicial branch of the U.S. government is responsible for hearing cases related to patent law, and the U.S. Supreme Court, as the final arbiter of constitutional law, has the ultimate authority in weighing the issues related to congressional statutes and the Constitution. These decisions involve balancing what may appear to be competing tensions. On one hand, there are the interests of the public, including access to the newest technologies, while being protected from monopolies. On the other hand, there are the interests of the country as a whole, which include rewarding creative, inventive individuals and stimulating commerce.

The issue of monopolization has already come up in our discussion of Canavan disease and the early desire of Miami Children's Hospital to seek exclusive licensure. A very simplistic view of monopolization is that restriction of a commodity, like a genetic test, to a single vender tends to increase the value and, therefore, the profit margin for that commodity. As we saw in that case study, however, this view, which was fervently pursued by Miami Children's Hospital officials, may be too simplistic.

In 1999, Joan Merz at the University of Pennsylvania expressed concern that the rapidly growing number of disease gene patents, which claimed all methods for diagnosis of a particular genetic condition, would threaten physicians' abilities to provide medical care for their

patients. Previously, patented diagnostic tests were broadly available to members of the medical community as test kits or licenses to use the patented test. However, Merz argued that disease gene tests were being monopolized by a small number of providers. Her logic followed that this monopolization of medical test services would have the following consequences: it would restrict research, create conflicts of interest, reduce patients' access to testing, lead to extensions of patents on tests and related discoveries, and grant to patent holders the ability to interfere with the practice of medicine. Merz suggested that patent law should be amended to require compulsory licensure of the patents to permit physicians to provide medical services to their patients.

In August 1999, the American College of Medical Genetics presented a position statement on gene patents and accessibility of gene testing. The three points of the statement were as follows:

1. Since genes and mutations occur naturally, they "should not be patented."
2. Patents on genes with clinical import "must be very broadly licensed."
3. Licenses "should not limit access through excessive royalties and other unreasonable terms."

The college argued that without these protections, the monopolistic licensing practices on gene patents not only restricted patient access but also limited training for the next generation of clinical laboratory geneticists, who would not have experience with these tests until after the patent expired. In addition, if only one laboratory was carrying out this test, then controlling the quality of the test would be difficult.

As we discussed in chapter 2, the initial market strategy for Celera's private genome project was to patent sequences identified as genes using bioinformatics algorithms. Celera would then control the future uses of these genes and would profit from licensing the intellectual property from the genome. Celera submitted at least 6,500 patent applications specifying sequence with the function of the putative protein product of the gene based on sequence similarities with other proteins of known, demonstrated functions. Between Celera and other institutions, approximately 6,000 gene patents have been issued, and many of these were based on sequence information alone, with no demonstrated functional data.

A number of individuals in the genetics community, including ourselves, felt that this policy of patenting sequences without demonstrated function was a departure from the accepted practices of the PTO. Since

the Patent Act of 1793, the two principal requirements for an innovation to be patentable have been novelty and utility. Novelty means that the patent must describe something that is new, and therefore the PTO would not provide patent protection to the assignee for something that was previously known. Utility involves practical purpose and an industrial applicability. An additional requirement introduced by Congress in the Patent Act of 1952 was that the patent must be "nonobvious." Our concern was that a sequence was not a demonstration of utility, and we feared that even if the predictions differed from the function eventually demonstrated, the assignees would still own the additional uses. The company would own the sequence, since it was the sequence that had been patented. Subsequently defined uses would be dependent on the sequence, and therefore these uses would be owned by the original company.

Another concern has been that human genes are natural — products of nature — that cannot be patented. Such natural products are not new and therefore would not meet the requirement for novelty. The argument that human genes can be patented is based on patent precedents. A 1912 case, *Parke-Davis v. HK Mulford,* was brought by the company because it had patents on the isolation and purification of adrenaline and one of its salts from animal adrenal glands. Mulford sold adrenaline and argued that it was a natural product. The court decision maintained that this was a product modified from nature by the isolation and purification procedure, and it did not exist in nature in the exact form that was used in medical therapeutics. The PTO, by analogy, maintains that a gene in nature could not be patented, but once it has been isolated from the genome and purified and modified so that it can be sequenced, it is no longer in its natural state — it has been modified by human intervention.

The PTO has increased the requirement for utility in gene patents beyond simply having a sequence by demanding some demonstration of function. This increased requirement has reduced the rate of new gene patents, but with 6,000 human genes patented, approximately 25 percent of the 20,000 to 25,000 genes in the human genome have owners. This would mean that for whole genome sequencing approaches to genomic medicine (chapter 15), 25 percent of the coding sequence would be "out of bounds" and the sequences for these genes could not be reported without licenses negotiated with multiple organizations holding the patents. In addition, Genetic Technologies, an Australian company that holds global patents for noncoding DNA, has attempted to assert these patents by contacting academic research groups in Australia, New

Zealand, the United States, Japan, and Europe. Genetic Technologies has been asking for a license fee of one thousand dollars from basic researchers. This is a very aggressive policy, since license fees typically are charged to commercial laboratories for clinical tests. The company claims to have received fees from pharmaceutical companies for use of noncoding DNA.

Also, as we discussed in chapter 3, biology is not simple, with one gene causing a single disease; there are genetic and environmental modifiers. Does that mean that if there are five genes involved in a disease, one primary and four modifier genes for a Mendelian disorder, and patents on them are owned by or licensed to five different organizations, then to develop a predictive test for this "simple" Mendelian disorder you would have to negotiate with five entities? If there are twenty-five genes involved in a common complex disease, could that require you to negotiate with twenty-five entities? And if one of the negotiations failed, would that mean that you had to offer a compromised test? The economic and clinical implications are enormous!

There have been discussions about the PTO revisiting those patents and reconsidering their legitimacy under the newer guidelines of utility. There have also been legislative attempts to exempt health care providers, or researchers, from patent infringement suits.

Some individuals have gone even further in their recommendations. E. Richard Gold from McGill University in Montreal and Timothy Caulfield from the University of Alberta in Edmonton suggest that patents be subject to ethical review and be allowed only after such a review. Beth Arnold and Eva Ogielska-Zei with a law firm in Boston question whether current patent law adequately balances the beneficial role of patents in the development of new technology and products, as compared with the detrimental impacts on research and clinical testing.

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Patient advocacy groups and governments have developed strategies to regain control of gene patents. One early example represents a creative approach by the foundation PXE International. Pseudoxanthoma elasticum, or PXE, is an autosomal recessive genetic disease involving certain connective tissues throughout the body, with abnormal deposition of calcium in elastic tissue resulting in loss of resilience. The tissues and systems involved include skin, eyes, the gastrointestinal tract, and the cardiovascular system. For example, the skin of the neck and other parts of the

body becomes lax and relatively inelastic. There may be a skin rash and localized plaquelike thickened regions of the skin surface. Calcification of vessels in the retina of the eye may rarely lead to blindness. Bleeding from the gastrointestinal tract may occur. Cardiovascular abnormalities include abnormal vascular calcifications and blockage of arteries.

In 1994, Sharon and Patrick Terry's children, ages five and seven at that time, were diagnosed with PXE. Sharon, a former college chaplain, was incensed when different groups of investigators called her asking for repeated blood specimens to do DNA research, saying that they did not share samples with each other. The Terrys began a Web site and a tissue bank. In 1996, they formed PXE International and, anticipating a situation like the Canavan disease example, they worked with attorneys at Testa, Hurwitz & Thibeault, a Boston firm, working pro bono, to develop contracts with researchers to ensure that PXE International would be co-owners of any patents that would develop from the investigations. PXE International's goal was not the financial revenues from licensing royalties, but control of the intellectual property to ensure that it would benefit the patients and their families. They built their tissue bank to include samples from at least 1,500 individuals with PXE. Five research groups agreed to work with PXE International and accepted the terms established by the group.

Charles Boyd at the University of Hawaii, using samples from the PXE International tissue bank, called Sharon Terry in February 2000 to tell her that his group had identified the PXE gene. In addition, because of her critical involvement in obtaining the samples, and the genetic information associated with the tissue bank, Boyd was including her as a coauthor on the manuscript Boyd and his group were submitting to a medical journal. She was included as one of the five co-inventors on the patent application. PXE International agreed to pay the costs of the patent application process and to broker the licensing deals for this "orphan," or rare disease, so that these deals would benefit individuals affected with PXE. Sharon Terry says, "If you're a pharmaceutical person, we're an easier mark—we're not as hard-nosed [as universities] about profits. We're more interested in the search for treatments and patient support and research."

Sharon Terry subsequently became president and CEO of the Genetic Alliance, a group that describes itself "at the crossroads of the genetics community" as a consortium of hundreds of genetics advocacy organizations, health care professionals, hospitals, clinics, and corporations. One of her initiatives at the alliance is to assist other advocacy

groups in replicating the experience of PXE International by developing the Genetics Alliance Biobank, which the alliance describes as "a cooperative [collection of] biological samples and data repository that allows lay advocacy and community organizations to bank and manage samples and data, thereby accelerating research both within and across disease by providing access to fully protected, linked samples in a centralized collection."

PXE International found a way to maintain control of its samples and genetic information. This strategy might be called "Patent Thyself" — an approach in which Donna Mac Lean would surely take pride. Now other advocacy groups are learning from Terry and the Genetic Alliance how to become co-owners of genetic intellectual property that is important to them. This strategy is an extremely effective example of a group regaining autonomy over its own genetic information.

Governments are also regaining control over what they see as patent policies that negatively impact their citizens. The province of Ontario, Canada, challenged the Salt Lake City corporation Myriad Genetics. In the 1990s, scientists working at Myriad isolated a gene called *BRCA1* (for breast cancer, gene 1) that is mutated in some patients with breast cancer. In the overall population, genetic mutations in *BRCA1* account for less than 5 percent of all breast cancer and are found in about 10 percent of families with hereditary breast cancer. Myriad patented *BRCA1* as well as *BRCA2,* which is involved in breast and ovarian cancer.

In September 2001, the health minister of Ontario stated that the National Health System could not afford to pay Myriad's charges of $2,600 for *BRCA1* and *BRCA2* testing for breast and ovarian cancer. The nearly exclusive control that Myriad was exerting over the licensure of the patents for these genes made Myriad essentially the sole provider of this test. The minister felt that the charges were excessive and that laboratories within the National Health System were competent to perform the tests. Other provinces were also concerned that the cost of testing through Myriad was preventing many Canadian women from having this test performed.

In a 2005 report, the U.S. Institute of Medicine (IOM) noted that testing by the province of Ontario was being carried out for one-third of Myriad's price and with results available eight weeks sooner and concluded that Myriad's business strategy was limiting access to care and the company's testing strategy missed mutations in these genes. The IOM report noted that regional hospitals had disregarded the patent and continue to offer *BRCA*-gene-testing services. Ontario health minister Tony

Clement opposed Myriad's patent: "We do not accept their claim and we are disregarding that claim." In response to threats from Myriad Genetics to enforce its patent, Clement stated that he was willing to take the issue to court. Clearly the IOM was sympathetic to Ontario's claims and considered the monopoly created by Myriad in breast and ovarian gene testing to be detrimental to health care.

Myriad's claims were also disputed in Europe. In 2001 and 2002, the French Institut Curie challenged the European patents held by Myriad on the two breast cancer genes, *BRCA1* and *BRCA2*. It is not surprising that these challenges in Europe would be initiated by the French with their culture of a genetic patrimony (chapter 8). In their first attack on the *BRCA1* gene patent, the institute argued that the claims were not novel, since tests for breast cancer predisposition using other approaches, though less direct, were available before the gene test. The institute argued that the claims were lacking in inventiveness, since Myriad relied in part on public genome databases to identify the gene. It also maintained that the description in the application was incomplete because the original sequence in the patent application contained errors. In addition, the sequencing of the *BRCA1* gene, the approach that Myriad used in its testing, involving genomic amplification, missed large deletions of the gene on one of the two chromosomes, calling the sequence normal, when in fact the individual would be at significant risk for cancer. Other individuals and organizations joined the opposition to the patents, including representatives from eleven European countries.

In 2004, the European Patent Office revoked the first patent for methods to identify mutations in the *BRCA1* gene and granted a patent on *BRCA2* to Cancer Research UK. In 2005, the European Patent Office amended the original *BRCA1* patent, significantly limiting its scope.

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Human genes and the human genome have become marketable commodities and have been protected by patent authority. However, individuals and governments have developed strategies that appear to be increasingly effective in neutralizing companies' business strategies. As the science unfolds, it will be interesting to follow the progress of this issue and revisit whether commodification of the human genome will be successful from a business perspective. We anticipate that as the success of genomic med-

icine becomes more apparent and effective to individuals in their own health care, the public will rise up against what it perceives to be corporate impediments to that care. What began as an individual's creative act of defiance on the part of Donna Mac Lean in Great Britain is likely to become a broader call for freedom from genomic commodification.


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the print version of this title.]

CHAPTER 10

Protection against Genetic Discrimination

The New Civil Right

Genetic discrimination is the use or potential use of an individual's genetic information in decisions regarding employment and insurance, including life and health insurance. Discriminatory behavior includes actions against the individual's best interests based on this genetic information and without regard to the individual's merit. Discrimination occurs when information typically used to consider other individuals has been subordinated to, or supplemented by, an individual's genetic information. Decisions made by an employer or insurer involving the use of genetic information in a routine or blanket manner pertaining to all individuals concerned also represents a discriminatory practice.

Genetic information used in this discriminatory fashion could include a broad range of types of information, such as family history or phenotype, including a physical feature or clinical test result, or genotype, including a DNA sequence variation. In other words, this discriminatory practice could involve many different types of information, but the key

would be that it was being used specifically because it was perceived as giving insight into someone's genetic qualities and that these qualities permitted a specific categorization of that individual.

Genetic determinism is one of the fundamental drivers for genetic discrimination. Discriminatory behavior has often been based on a misperception that one characteristic, such as gender or ethnocultural group, has the power to subordinate all other characteristics of the individual. The misperception that genetic information is deterministic provides it with a power it does not deserve.

A feature of discrimination is that it is an arbitrary use of selected information in decisions about the individual. We contend that genetic discrimination is arbitrary because our understanding of genetics and genomics is so incomplete at this time. For example, the gene and the nature of the expansion responsible for Huntington disease — associated with neurological degenerative disease, with onset usually occurring after reproductive age and clinical findings in 100 percent of individuals with a trinucleotide repeat expansion — are known, so an employer or insurer would have the tools to discriminate against an individual with a Huntington disease gene expansion. Other individuals may have a DNA sequence variation, or group of sequence variants in different genes, that place them at very high risk for stroke. But at this time we don't understand, or perhaps even know, about that genetic association increasing the risk for stroke, so for those individuals, the employer and insurer would not have the tools for genetic discrimination. Therefore, in the face of incomplete information, the decisions that lead to genetic discrimination are completely arbitrary.

For behavior to qualify as discrimination in a legal sense, there must be a historical recognition of this discriminatory behavior over time. A panel at the California Institute of Technology that included three lawyers and two physicians discussed genetic discrimination. One of the lawyers very directly stated that he did not understand why anyone was concerned about genetic discrimination, because in fact there was no historical evidence of genetic discrimination. He said that the field of medical genetics was too young to meet the historical test required for a behavior to be called discrimination. We responded that a significant portion of individuals who have disabilities have a genetic basis for their disabilities. Since the U.S. Congress found that individuals in the disabilities community were being discriminated against systematically and enacted a statutory remedy for these individuals with the Americans with Disabilities Act (ADA), then our federal legislators recognized discrimination against individuals with genetic diseases. Therefore, we argued that there was a history of genetic discrimination that was recognized by Congress.

Efforts to eliminate genetic discrimination, including attempts to assist the passage of legislation against genetic discrimination, have been a major focus of our policy activities at the national level in the United States since 1998. This is a topic about which we are passionate, as will become evident in the course of this chapter.

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Let's first consider additional evidence of genetic discrimination and public concerns about and experiences with this form of discrimination. The U.S. Department of Health and Human Services Secretary's Advisory Committee on Genetic Testing (SACGT), which held meetings between June 1999 and July 2002, repeatedly heard comments from members of the public regarding their concerns about, and experiences with, genetic discrimination. At the January 27, 2000, public meeting in Baltimore, Maryland, the SACGT heard impressive reports of genetic discrimination against individuals. As part of the public outreach leading up to that meeting, the SACGT received insightful comments from many individuals. One college student wrote that if the problem of genetic discrimination could not be remedied effectively, then the Human Genome Project, including its products in the form of improvements in genetic testing, would have been wasted effort, since no one would be willing to use genetic testing. We appreciate this student's insight, and we agree completely.

Similar testimony was provided to the successor to the SACGT, the Secretary's Advisory Committee on Genetics, Health, and Society (SACGHS), in its meetings that began in June 2003. Having heard personal stories of genetic discrimination throughout the period of the SACGT and the SACGHS charters, and with federal legislation to ban this discrimination appearing to be at an impasse, the SACGHS requested oral and written comments from the public beginning in September 2004. At the SACGHS meeting on October 18, 2004, the committee heard comments from members of the general public, health care providers, institutions, and professional organizations. The presentations from the seven members of the general public were particularly powerful. As we very briefly review these comments, you will begin to recognize themes that we have discussed previously, such as confusion of unaffected heterozygous carriers of a disorder with affected homozygous individuals.

Heidi Williams is homozygous for mutations that cause the autosomal recessive disorder alpha-1 antitrypsin (AAT) deficiency. AAT deficiency leads to progressive lung disease with emphysema (massive ballooning and dysfunction of the normally microscopic air exchange sacks in the

lungs), liver disease, or both. Her husband is homozygous normal, and therefore their two children will be heterozygous carriers and phenotypically completely normal, as documented in the research literature.

In August 2003, Williams attempted to obtain health insurance from Humana for her two children. They were refused insurance, even though she explained that they would be unaffected. Working with the Genetic Alliance (chapter 9) and a prestigious legal firm in Washington, D.C., Williams appealed the August 2003 rejection from Humana and included documentation that her children would be normal. In February 2004, she received another letter from Humana again rejecting her children on her appeal. When a journalist from "a well-known and well-respected newspaper" approached Humana, the company finally reversed its decision.

Williams said that Humana's final decision "felt like a hollow victory." She added: "No one should have to force an insurance company to cover perfectly healthy children. . . . Humana, Inc. made me feel guilty and ashamed. . . . Furthermore, they made me feel guilty for needing a parent's peace of mind in regard to my children's future health, and for that I am angry."

Phaedra Malatek discussed a disorder, hemochromatosis (accumulation of iron in various organs in the body, including the heart and liver, leading to the potential for serious, life-threatening dysfunction), that was diagnosed in her father. She had been given information regarding the genetic status of her children and the health planning that could reduce their risks of severe disease and the concern that this could make them uninsurable.

Malatek stated the following: "It's remarkable to me to realize that the work my parents did for the Civil Rights Act in the sixties was not complete. Here I am forty years later, working on the same issue, equal rights and protection under the law, no matter the genetic makeup of the person. The fact that we can look inside of the DNA of a person to know more about them should not preclude them from the protection that was fought for so valiantly [in the civil rights movement]." It is important to note that Malatek is not alone in this assertion. A number of individuals have begun to refer to freedom from genetic discrimination as "the new civil right."

Rebecca Fisher has a family history of breast and ovarian cancer and is a breast cancer survivor who shares a *BRCA1* mutation with her daughter. She spoke about her experience and the emphases in the following quote are hers:

> The argument has been advanced that "seeking to ban DNA discrimination isn't really necessary," [Sharon Begley, *Wall Street Journal* (East Ed) Feb. 6, 2004; B10] because discrimination based on genetic information simply doesn't exist.

Actually, genetic discrimination *does* exist. But the fact that it exists only sporadically and anecdotally is a function of the newness of the technology . . . — it is *not* a function of insurance companies' and employers' decision to take the moral high road and . . . remain disinterested in this information in the same way that they are legally obliged to remain disinterested in information such as race, gender, creed, or sexual preference.

Fisher knew from personal experience that insurance companies did not operate in an altruistic manner. When she was ill, she worked as a medical librarian in a small hospital that like many small businesses was self-insured. She was told that her health care costs, including her bone marrow transplant, had exceeded the "calendar cap" in the previous year and was asked "if that's going to happen again this year." Fisher replied, " I *really* hope not!" She commented:

This experience taught me that there are people who are paid to look at me and see — not my ability to contribute to a community; not my honesty, integrity, or faith; not my education, hard work, or social conscience; not my family members and the ways in which I have helped each of *them* succeed — but dollar signs, costs, increased liability, and the odds of my dying an expensive death. . . .

I fear for my children — especially for my daughter — who must live not only with an exponentially higher risk of developing a terminal disease but also with the burden of never knowing whether or when she will *legally* be asked to take a genetic test as a condition of employment, be *lawfully* fired from a job because of her genetic condition, or be *legitimately* denied health or life insurance on the basis of her genetic predisposition to disease.

Fisher went on to discuss a friend's refusal to get genetic testing:

Last summer [2003], attorney Lawrence Lorber (representing the U.S. Chamber of Commerce, the loudest voice speaking against federal genetic information protections) told a House Education and Workforce Committee that "the threat of allegations of discrimination from both a liability and public relations perspective is enough to prevent . . . employers from ever contemplating acquiring genetic information." I would like Mr. Lorber to tell that to my friend Susan, whose 38-year-old sister is being treated for breast cancer, whose mother had premenopausal breast cancer, and whose aunt died of it. . . . I gently asked whether *she* had considered speaking with a genetic counselor. "Oh no!" she exclaimed. "I would never want to risk losing my insurance!"

There is a pattern on the part of these mothers — they are concerned about their children and the risks they face in the absence of legislation that protects against genetic discrimination.

Other individuals gave equally powerful testimony. Tonia Phillips,

who worked for a small company of four persons including the owners, said that they were "a tight-knit family." They knew all that she had been through, including her mother dying of ovarian cancer, her own testing for *BRCA* mutations, her positive *BRCA1* mutation, and her prophylactic surgeries. She was in the midst of her reconstruction surgeries when "our group health insurance . . . [went] up $13,000 a year for four people. My boss got the bill and yelled it through the office. I knew that she was directing it towards me." Phillips was asked to change to her husband's health insurance and was even offered an increased hourly wage if she would remove herself from their policy and keep the cost from increasing. However, since she was in the process of undergoing reconstructive surgery, she was concerned about changing insurance.

Paula Funk has a strong positive family history of breast and ovarian cancer and a known *BRCA1* mutation in the family. She postponed testing for ten years "because doctors advised against testing because of discrimination. The doctors told us that there are no laws to protect against discrimination." She noted that she had paid for her test herself "to prevent insurance companies from learning about my genetic status." (Many individuals have related to us that they have paid for the testing themselves, often using a pseudonym. But everyone should know that paying for testing "out of pocket" does not protect against loss of insurance. The policies include a clause that requires disclosure of any information that is known, and if any information is determined to have been withheld, then the policy is nullified.) Eventually Funk was tested and learned that she did have the familial mutation. She went on to explain how her physicians had miscoded tests to avoid revealing her mutation status to her insurance company. She also had to await the decision of a "board of approval" for her prophylactic mastectomy, delaying this "life-saving surgery." She added, "Five years ago my father had bypass surgery to prevent a heart attack. His surgery did not have to go to a board of review to be approved. Both surgeries prevent life-threatening diseases."

Caroline Hinestrosa is a "10-year, two-time breast cancer survivor," "mother of a 13-year-old-daughter," and "executive vice president for programs and planning of the National Breast Cancer Coalition." Despite a family history of breast cancer, she said, "After carefully weighing the potential benefits and harms of genetic testing, I decided not to undergo testing for fear of potential consequences to my daughter." She was concerned that if she was positive, then her daughter might have to disclose this information and "she might suffer future discrimination in health insurance and employment as a consequence."

Phil Hardt noted that he has "two genetic diseases, hemophilia B, a bleeding disorder, which I inherited from my mother, and also Huntington disease, a degenerative brain disorder, which I inherited from my father." He was hired by Allied Signal Automotive in 1989 and was told by the human resources manager there "not to tell my boss about my hemophilia . . . [because] if he knew I might have a disability, I would never go anywhere in the company. Consequently, all future bleeding episodes had to be hidden from him." Hardt noted a series of experiences with genetic discrimination in his family. He was denied automobile credit insurance because he had seen a neurologist for his early Huntington disease. His daughter was denied mortgage life insurance by "every major insurance company" due to his Huntington disease, and each denial letter included two conditions: "they will not insure her until she has tested for Huntington disease, and . . . she is found to be negative." His grandson was denied health insurance because of his hemophilia. Hardt and a geneticist developed an anonymous Huntington disease testing program in Arizona "so that they can use a bogus name and social security number and address and all other information, and pay cash." He added that it was expensive at nine hundred dollars per test, "But it is completely concealed. But it's a shame that we have to do this."

These individuals' personal testimonies were so much more powerful in person than could ever be presented in print. We recommended to SACGHS that a DVD of the testimony and written comments that had been received by the committee documenting incredible and numerous examples of genetic discrimination be compiled into a phone-book-size volume that Congress would be unable to ignore. Excerpts were compiled into a DVD that was included in a book more than three hundred pages long, *Public Perspectives on Genetic Discrimination,* produced by the SACGHS. The weight of the evidence supporting the existence of genetic discrimination was intended to neutralize the charges that there was no need for legislation protecting against genetic discrimination.

Others found clear evidence of genetic discrimination as well. Paul Billings began to document the existence of genetic discrimination in 1992, when he was at the California Pacific Medical Center in San Francisco. Billings and his colleagues solicited examples of genetic discrimination in the New England region. Their definition was quite specific: "For the purposes of this study, genetic discrimination is defined as discrimination against an individual or against members of that individual's family solely because of real or perceived differences from the 'normal' genome of that individual. Genetic discrimination is distinguished from discrimination

based on disabilities caused by altered genes by excluding, from the former category, those instances of discrimination against an individual who at the time of the discriminatory act was affected by the genetic disease." Of the forty-one cases referred to the investigators, thirteen did not meet their strict definition, leaving twenty-nine, which included problems with insurance, employment, and adoptions.

Genetic discrimination often occurred even when the individual had been identified with the genetic disorder early, had instituted effective therapy to prevent symptoms, and was asymptomatic. Billings and colleagues referred to these individuals as "the asymptomatic ill." One such example was someone with hemochromatosis who was diagnosed early, had initiated regular blood removal from the veins to prevent iron overload, and still faced discrimination. Another was a heterozygous carrier of a biochemical genetic disorder, who was denied a government job because he was a "carrier, like a sickle cell." A third patient was a girl with phenylketonuria (PKU), who experienced discrimination that we have seen among our patients. Her genetic disorder was identified by newborn screening (chapter 15) at fourteen days of age, and effective dietary therapy was initiated. Despite development that was tested and measured consistently in the normal to above normal range, when her father changed employers, she was denied health insurance. Genetic testing that prevented severe mental retardation resulted in stigmatization. These individuals are healthy but are treated as if they are ill by those discriminating against them.

Others were discriminated against because phenotypic variability in the disorder, with which they were diagnosed, was not appreciated. Despite absent or minimal symptoms, individuals suffered discrimination, for example, in employment and auto insurance.

A third group involved those who were at risk for a genetic disorder. Two individuals, each with a parent who had Huntington disease, therefore having a 50 percent risk of developing the disorder, were refused to be considered as potential adoptive parents by adoption agencies. One agency wrote, "We feel that a fifty-fifty chance of getting a disease as serious as Huntington Disease is too great a risk, for our purposes and circumstances." The authors describe this as "the myth of genetic perfection . . . that the best possible family is the one least likely to face medical adversity — the 'perfect' family with a disease-free genome." This denies the fact that "all families are at risk."

Another family had a child with cystic fibrosis (a genetic disorder affecting lung function and ability to digest food and absorb nutrients, with life spans typically into the thirties) and a prenatal diagnosis of cystic fibrosis in

a second pregnancy. Despite identification of homozygosity for cystic fibrosis mutations, the couple decided to continue the pregnancy. When their HMO learned of the test results, the HMO threatened restrictions on coverage for the pregnancy and for the new baby, as well as for the child who was already covered, and only dropped these threats when the family discussed initiation of legal action. The investigators were extremely concerned about this attempt by the insurance provider to coerce the family into having an abortion. They stated: "These circumstances constitute a strong incentive for aborting a fetus based on its genotype, a practice that might be interpreted as a form of eugenics" (chapter 3).

Billings and his colleagues determined "that unfair and discriminatory uses of genetic data already occur" and current "state and federal laws are inadequate to prevent some forms of genetic discrimination." They concluded: "Without further changes in social attitudes, legal protection, and/or changes in the prevailing American health care system, many healthy and potentially productive members of our society will suffer genetic discrimination."

In 2005, Billings rebutted critiques of his work and that of others on genetic discrimination. He organized these criticisms into three categories. First, some critics asserted that the initial examples in the literature "did not constitute genetic discrimination, because genetic discrimination must arise solely from effects or perceptions directed by the genome, and . . . the reported cases were really a form of disability discrimination that was well known in U.S. society and jurisprudence." Billings felt that this criticism was "effectively silenced" by the description of a family with an electrical conduction defect in the heart that has no effect other than that it predisposes affected individuals to sudden death. This disorder, known as long QT syndrome, after the Q and T waves on the electrocardiogram (EKG), can be treated by drugs and avoidance of strenuous exercise. The family's "asymptomatic affected children could not qualify for health insurance coverage for their care, including preventive drug treatment." These children were not disabled and were being discriminated against solely based on their genotype.

The second criticism "held that the evidence for cases of genetic discrimination was anecdotal and that such cases were fleetingly rare if they occurred at all." Billings pointed out that formal investigation of this phenomenon would subject the victims to further abuse and would require the abusing organizations to participate and open themselves to potential liability. Therefore, such studies have not, and will not, be possible. The willingness of individuals to appear before the SACGHS and provide details of

the genetic discrimination to which they and their family members have been subjected demonstrates not only their commitment to changing policy but also their courage in coming forward with their stories.

The third area of criticism "centered on a 'right to discriminate.'" This criticism argues that an employer, school admissions committee, or other such agent has the right to use all information available in making a decision, including personal information. Billings said, "This argument has never been completely refuted, a sign of its strength, but polls and subsequent public policy determinations suggest that limitations of these business-based prerogatives are publicly acceptable and desirable." We argue that this position is refuted by the arbitrariness of these decisions, which do not recognize the incomplete state of the understanding of genetic diseases, the pervasive nature of genetic influences on diseases across the population, and considerable variability within genetic disorders.

States such as California have passed laws against genetic discrimination, but Billings felt that variation in the laws between different states was a reason for federal legislation. Billings noted: "Early strong opposition and lobbying by the insurance industry and chambers of commerce against legislation prohibiting genetic discrimination at the state level was evident." However, he also pointed out that major biomedical corporations, including large insurers and lab service providers, recognized the need to include genetic information in a nondiscriminatory manner to improve the practice of medicine. Concern has also been expressed that continued discrimination might hurt investment in genetic testing because of individuals' unwillingness to risk genetic discrimination.

Billings added that genetic discrimination extended beyond the more traditional venues of insurance and employment. He described a court case on which he had consulted involving termination of a marriage and child custody brought by a woman who had allegedly been assaulted repeatedly by her husband. Her husband filed a countersuit arguing that her family had a history of Huntington disease and that it was this genetic disorder that was causing her to make these claims against him and that made her an unsuitable caretaker of their children. She was evaluated clinically, and no evidence of Huntington disease was found. The husband then attempted to have the court order genetic testing for Huntington disease. Billings concluded from his experience with this case: "Nonconsensual, compulsory genetic testing carries the risks of genetic discrimination and can confuse the import of phenotypic effects and genotypic risks in civil and criminal evaluations."

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On February 8, 2000, at the American Association for the Advancement of Science building in Washington, D.C., President William Clinton signed Executive Order 13145, titled "To Prohibit Discrimination in Federal Employment Based on Genetic Information." Because of the size of the federal workforce, this order impacted large numbers of people throughout the country and set a standard independent of state laws, except for those states that had more rigorous prohibitions. The executive order amended Title VII of the 1964 Civil Rights Act, providing support for the concept that freedom from genetic discrimination is a civil right.

To support this executive order, the Office of the White House Press Secretary declared in a press release that "Americans fear that their genetic information will be misused." To support the assertion that "genetic discrimination is real," the release cited a 1996 article from *Science* that was based on 332 individuals who had at least one of 101 different genetic diseases in their family. Because of the presence of the genetic disorder, 25 percent of the participants, or affected members of their families, thought that they had been denied life insurance, 22 percent thought that they had been denied health insurance, and 13 percent thought that they had suffered employment discrimination. As a result of these perceptions, 18 percent of this group refused to reveal their genetic information to insurance companies, and 17 percent withheld this information from employers. To support the assertion that "fear of discrimination is widespread," the release cited a survey of a thousand individuals by the National Center for Genome Resources that found if their employers had access to genetic test results, 36 percent would probably not and 27 percent would definitely not have genetic testing. President Clinton's executive order protected the federal workforce but not all Americans from genetic discrimination. Broader coverage will require successful legislative efforts.

There have been numerous attempts to pass federal legislation against genetic discrimination. We will briefly review the efforts in the 108th (2003–2004), 109th (2005–2006), and 110th (2007–2008) Congresses. In the 108th Congress, Senate bill 1053 (S 1053) was introduced by Senator Olympia Snowe (R-ME) and passed unanimously with a vote of 95 aye and 0 nay. A similar bill was introduced in the House of Representatives and was never released from the desk, meaning that it was held at the desk of the presiding officer — it was not directed to a committee for a hearing by the Speaker of the House.

In the 109th Congress, Senator Snowe reintroduced this legislation as S 306 with 25 cosponsors, and it passed on February 17, 2005, with a vote of 98 aye and 0 nay. On March 10, 2005, the bill was introduced in the House of Representatives as HR 1227 with Representative Judy Biggert (R-IL) as sponsor and 126 cosponsors. The good news was that it was not held at the desk during this session, but the bad news was that it was referred to three different committees for hearings, which can be a lethally cumbersome process for legislation. A group has come together representing patient advocacy organizations, professional groups, companies, and other groups called the Coalition for Genetic Fairness, chaired by Sharon Terry. The group hired a lobbyist who was prominent in the Reagan administration, which should have been an advantage while there was a Republican majority in the House. However, HR1227 was not successful.

In the 110th Congress, the bills were reintroduced by Senator Snowe and thirty-three cosponsors as S 358 and by Representative Louise Slaughter (D-NY) and 224 cosponsors as HR 493. The House of Representatives passed HR 493 with a vote of 420 yeas and 3 nays on April 25, 2007. The Senate has yet to vote at the time of this writing. (June 23, 2007), but this legislation has moved further and more quickly than previously. Progress is being made on federal legislation against genetic discrimination, and with the Senate's unanimous passage of similar bills in the 108th and 109th Congresses, we are hopeful for success in the 110th Congress.

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Employment discrimination involves using genetic information in decisions about hiring and retaining individuals. It can involve an employer basing decisions on such information as a family history of, or a genetic test for, a genetic disease. We will see in some of the cases discussed here that individuals have had genetic testing performed without their knowledge. Some employers caught in this practice have said that they did not use the information in employment decisions, but it is unclear then why they would have been spending money on this testing.

The existence of employment discrimination using genetic information has been well documented at least back to 1989. In that year, the Congressional Office of Technology Assessment surveyed 330 companies and found that 12 of these (3.6 percent) used some sort of genetic screening at that time, which was limited to biochemical genetic testing because of the technology available. Northwest Life Insurance surveyed four hundred employers, also in 1989, and asked them if they would be using

genetic information in employment decisions in 2000. This survey found that 15 percent of these employers anticipated some form of genetic information evaluation on applicants and their dependents before they would make hiring decisions.

Paul Miller, an internationally recognized expert in disability law, was appointed commissioner of the Equal Employment Opportunity Commission (EEOC) by President Clinton in 1994 and served until 2004, when he moved to the University of Washington School of Law in Seattle. As EEOC commissioner, Miller argued cases that we will discuss below. Fundamental to these and other cases is the ADA and its reach. The ADA clearly provides protection against discrimination to individuals with a disability related to a genetic disease. The ADA also protects individuals who have had a disability, for example, cancer or stroke, from which they have recovered. If such a disability was due to a genetic disorder, then in that case the ADA would cover genetic discrimination. Miller has stated that the EEOC interprets the ADA as prohibiting genetic discrimination. Congress, recognizing that perception of impairment can be as serious as an actual or manifest disability, included in the language of the ADA those who are "regarded as" impaired. Miller stated: "Congress sought to address and combat the traditional myths, fears, and stereotypes about disabilities. Discrimination in the workplace based on genetic information is exactly the kind of behavior Congress intended to prohibit when it passed the ADA. Given this rationale, the EEOC issued policy guidelines on the definition of disability concluding that the ADA prohibits discrimination against workers based on their genetic makeup." He noted, however, that there was no court decision supporting the EEOC position, at least as of 2001.

There was, however, a U.S. Supreme Court case, *Bragdon v. Abbott,* that Miller felt was pertinent and suggested that the Court would agree with the interpretation of the ADA made by the EEOC. In this case, the majority opinion was that asymptomatic HIV infection qualified as a disability under the ADA, arguing that molecular or cellular changes, even in the absence of symptoms, qualified as a "physical impairment" under the ADA. Miller added: "However, in a foreboding dissent, Chief Justice Rehnquist wrote, 'Respondent's argument, taken to its logical extreme, would render every individual with a genetic marker for some debilitating disease 'disabled' here and now because of some future effects.' Justice Rehnquist's comments raise the specter that he might reject the ADA's protection of individuals with asymptomatic genetic conditions."

Miller noted that three subsequent decisions by the Supreme Court had

narrowed the reach of the ADA considerably and might have an impact on its application to genetic disease, when only a predisposition was present in a completely asymptomatic individual. We believe that individuals with disabilities need protection, as do those with genetic predisposition. If we were able to expand the ADA to cover genetic predisposition, then it would cover all of us. In doing this, we would be concerned that the disability community's protections might be diluted. Therefore, we need effective federal legislation against genetic discrimination.

Testing employees for sickle-cell disease without their knowledge became the foundation for the case *Norman Bloodsaw v. Lawrence Berkeley Laboratory.* Lawrence Berkeley Laboratory is a national laboratory of the U.S. Department of Energy (DOE) that is operated by the University of California under contract with the DOE. The plaintiffs, seven clerical and administrative employees at the Lawrence Berkeley Lab, brought suit when they learned that their intake medical examinations had involved blood and urine samples, including tests for sickle-cell trait (asymptomatic carrier state for sickle-cell disease; chapter 8), as well as syphilis and pregnancy. They claimed that this testing was inappropriately intrusive, particularly since they were uninformed and unaware of the specific tests that were performed. The Ninth Circuit Court of Appeals in San Francisco, on February 3, 1998, upheld the plaintiffs' claims regarding sickle-cell carrier testing on the basis of the ADA. Interestingly, the court maintained that while the statute of limitations for most plaintiffs had been exceeded, if one extended the claims to their hiring, the clock really only started ticking for the ADA time limits when the plaintiff became aware of the abuse.

Employment termination was at the center of another case, *Seargent v. Hanover Express and Surplus.* Terri Seargent worked for a small insurance company in Wilmington, North Carolina. Her brother died of progressive pulmonary disease caused by AAT deficiency, and when a physician recognized that she might have this, he ordered the genetic test for this disorder without warning her of possible risks such as genetic discrimination. Protein replacement therapy was available to slow the progression of the lung disease, but it cost Sergeant over $45,000 per year. She began treatment in October 1999 and had her annual personnel review in December. Prior reviews had all been quite good, and her 1999 review gave her an excellent rating and a 10 percent raise. However, on December 20, 1999, she was fired.

Seargent filed a claim of genetic discrimination with the EEOC, and on November 21, 2000, she received a determination supporting her allegation that she was a victim of genetic discrimination under the ADA. Seargent

now works as a support group coordinator for the company that makes the protein replacement, the cost of which is alleged to have led to her dismissal.

Christine Dent served on the front lines in the Gulf War and was planning a career in the U.S. Army. Her mother had died of Huntington disease, and Christine had a genetic test performed that came back positive. She alleges that when the army found out the results of her test, she was denied admission to officer candidate school. She left the army and subsequently was forced to leave another position with a municipal water department. Individual health insurance was extremely expensive because of her positive genetic test. Dent did not file a legal action in either case. In an article in 2000, it was noted that no one in Colorado, where Dent lived, had pursued genetic discrimination litigation, because state law actually permitted employers to access genetic information. Thus, in the absence of state protection, there is need for federal legislation.

The Burlington Northern Santa Fe Railway Company (BNSF) was carrying out a genetic test on its employees in an attempt to assess their risk for carpal tunnel syndrome (CTS) without their knowledge. CTS is caused by pressure on nerves going through the wrist, and is often caused by repetitive motion. The genetic test identified a marker for a relatively rare disorder, and at the time of the testing there was only one report of the association of this marker with CTS. Several BNSF employees filed a complaint with the EEOC about this testing, also alleging that if they refused to submit a blood specimen, they were threatened with disciplinary action.

On April 18, 2001, the EEOC announced a preliminary injunction against BNSF to cease the requirement for employees to have this test, not to analyze any specimens already drawn, not to consider any test results already in BNSF's possession, and not to retaliate against anyone involved. The press release describing this injunction stated that the EEOC "has settled its first court action challenging the use of genetic testing under the Americans with Disabilities Act of 1990." Commissioner Miller explained that "basing employment decisions on genetic testing is barred under the ADA's 'regarded as' wrong" for definition of a disability. He added: "Moreover, genetic testing, as conducted in this case, also violates the ADA as an unlawful medical exam."

On May 8, 2002, the EEOC announced settlement of its suit against BNSF for violation of the ADA. In the course of the EEOC investigation, it was learned that BNSF had tested "36 of its employees without their knowledge or consent." BNSF had required this test as part of a comprehensive evaluation for "certain employees who had filed claims or internal reports of work-related carpal tunnel syndrome injuries against the com-

pany." BNSF had agreed to voluntary mediation, and the mediated settlement was for $2.2 million for the employees who had the genetic testing and was part of a larger settlement involving federal legislation for on-the-job injuries on railroads. Miller said, "While the EEOC did not find that BNSF had used genetic tests to screen out employees, employers should be aware of the EEOC's position that the mere gathering of an employee's DNA may constitute a violation of the ADA."

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We will now consider genetic discrimination in the life insurance industry. William Nowlan is a physician and the medical director for a Vermont life insurance company. In a policy commentary in *Science* in 2002, he described this form of insurance as follows: "Life insurance in this country is not a societal right, although everyone is potentially eligible for limited survivorship benefits through social security. The private insurance system provides a financial safety net, but it is voluntary and unsubsidized. An individual life insurance policy is, in effect, a commercial transaction in which the insurer agrees to pay a death benefit in exchange for a premium proportional to the mortality risk assumed by the insurer. If this seems to resemble a grim lottery, its only 'winners' are those who are acutely in need."

The distinction expressed in Nowlan's statement between life insurance and, for example, health insurance or employment is one that is heard often in discussions of the role of genetic testing in life insurance underwriting. The contention is that life insurance is a luxury and "not a societal right." Phil Hardt, however, one of the individuals who had experienced genetic discrimination, included in his comments to the SACGHS the fact that his daughter had been refused mortgage life insurance unless she tested negative for Huntington disease. While owning one's own home is not a fundamental societal right either, certainly one of the basic tenets of our culture is that if one works hard, then one can aspire to be a home owner. Home ownership is an expression of our individuality and an important investment for many families. To limit individual aspirations because of genetic information, and to coerce individuals, like Hardt's daughter, into testing that they may not want, are abusive uses of that medical information.

Remember also that the arbitrary nature of available genetic predictions at this time represents a discriminatory action. In addition, life insurance underwriters should be aware of public opinion on this topic, because a short-term profit motive might lead to a longer-term vigorous negative

reaction against the industry. Mark Rothstein, a lawyer at the University of Louisville who chronicles the policy implications of genetics and genomics, with his colleague Carlton Hornung, carried out a telephone survey of more than 2,100 individuals in 2002 regarding their attitudes about the use of genetic information in the life insurance industry. Most of those surveyed opposed requirements for life insurance applicants to have a genetic test in order to purchase a policy. In fact, less than 25 percent of individuals across the four ethnocultural groups surveyed agreed with the statement that insurers should be "allowed to require all applicants to take a genetic test," and respondents identifying themselves as "white" agreed with this statement at a rate less than 15 percent. Rothstein and Hornung found that 69 percent of the respondents felt that "everyone needs life insurance," compared with 91 percent for the similar statement for health insurance. The investigators appeared surprised that 83 percent of those surveyed felt that "everyone has a right to life insurance," compared with 91 percent for the similar statement regarding health insurance.

Therefore, we contend that the public considers life insurance more of a right than some policy leaders might recognize, and members of the public are most definitely opposed to what they perceive as genetic discrimination in the life insurance industry. It will be important to follow trends in these public opinions over time. Experiences in the United States and the United Kingdom representing the powerful voice of these opinions will be informative.

In 1998, Vermont became the first state in the United States to place limits on the use of genetic information by insurers offering policies in the life, long-term care, and disability insurance markets. The law also prohibits insurers from using genetic test results on a relative or from ordering genetic tests to assess disease predisposition among those who are healthy at the time they apply. The Vermont legislation does, however, permit the sharing of genetic test results that existed before the new law was passed.

In 2000, Massachusetts passed legislation that regulates how life insurance underwriters are able to use existing genetic test results. The law requires a two-step consent process. If consent is given, then the underwriter is allowed to use the genetic test results, if this testing was determined to be "reliable," a decision that is made ultimately by the state's insurance commissioner.

Using selected genetic information that may be determined to be reliable by a panel of experts would seem appropriate superficially. However, we maintain that this is selective use of genetic discrimination. The individuals with a family history of a certain disease may consider themselves

fortunate when a reliable test becomes available. However, if the test is used for commercial rather than health-related decisions, then we wonder how those individuals would compare their fortune to that of another group with a genetic disorder that does not yet have a reliable test.

Selection of one disorder, Huntington disease, for mandatory testing to obtain life insurance was announced in the United Kingdom in 2000. The United Kingdom requires life insurance before one can obtain a mortgage. Therefore, this policy would have required individuals with a family history of Huntington disease to have the genetic testing, whether they wanted it or not, if they desired to own a home. It would be difficult to make the results anonymous to the life insurance-mortgage applicant. Lower life insurance rates would suggest that one did not have the disease expansion, while higher rates would indicate the presence of the expansion.

On October 13, 2000, John Durant, chair of the Genetics and Insurance Committee (GAIC) of the United Kingdom and a professor at Imperial College, London, announced the decision of the GAIC on the "reliability and relevance" of two genetic tests for Huntington disease. He stated that these tests were reliable and relevant and that normal test results would mean lower rates for life insurance for those who might pay more if family history was the only information available. No one would be required to have the test, but the GAIC acknowledged that in the absence of test results, insurance might be difficult to obtain for those with a positive family history.

There was an immediate and extremely vocal response to this decision. A moratorium on this policy was imposed by the government pending further consultation and consideration. On March 14, 2005, John Reid, the U.K. health secretary, announced that the government had reached a negotiated agreement with the Association of British Insurers that would prohibit the use of genetic disease predisposition testing to refuse life insurance for individuals. These restrictions would extend to November 2011. This was not an absolute prohibition, however. Use of GAIC-approved genetic tests would be permitted for life insurance policies greater than £500,000 and for critical illness and income protection insurance of more than £300,000, which represented only 3 percent of policies in the United Kingdom. This policy also did not require individuals to disclose to insurers genetic test results that were obtained as part of a research study, therefore removing a disincentive to participation in these studies. A breast cancer patient advocacy group worked very hard campaigning for this policy. It had found that approximately 30 percent

of women with a family history of breast cancer would not have genetic testing if insurers had access to the results. These women felt that they would have to choose between their health and their insurability.

We consider this a classic example of the human species' willingness to be seduced by new technology. The initial announcement of the GAIC was met with excitement that the United Kingdom would be the first to use genetic technology in this manner, and it would help those who were found not to have the Huntington disease expansion. Quickly, however, the unintended consequence of the GAIC decision became apparent, and eventually the policy was reversed with hard work by patients and their advocates.

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We have heard from individuals who presented to the SACGHS their stories about discrimination largely in the area of health insurance. The American College of Medical Genetics issued a position statement in 2001 that addressed many of the concerns raised in this chapter. This statement is clear: "Protection against genetic discrimination in health insurance is needed for individuals covered by all public and private programs, whether through group or individual plans, and regardless of the mechanism by which this program is regulated." The statement added that legislation should be cautious not to impede health care while ensuring privacy, including protection against genetic discrimination in access to health care and in the setting of individual rates.

Nearly 87,000 adults, enrolled in a screening program for hemochromatosis and iron overload (chapter 15), were surveyed to determine their level of concern about insurance discrimination. The investigators found that 40 percent of those surveyed agreed that "genetic testing is not a good idea because you might have trouble getting or keeping insurance." Among the reasons for concerns about genetic testing, insurance discrimination ranked the first.

Clearly genetic discrimination in health insurance is a major concern to the public. This has been demonstrated anecdotally with specific examples of discrimination, and the anecdotes are voluminous. The fears arising from these anecdotal abuses have clearly become accepted as a serious problem for the public. This is why federal legislation is required: so that the public can be reassured.


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the print version of this title.]

CHAPTER 11

Reproductive Technologies

On the Road to Designer Babies?

Opinions of the U.S. public about reproductive technologies
Treating infertility
Monitoring assisted reproductive technology
Diagnosis of genetic diseases in the embryo and fetus
Designer babies

Humans have entered an era in which many infertile couples are able to have babies. Those at risk for genetic diseases can have normal offspring. And some think that humans are not that far away from a time when scientists will be able to improve upon the human species by expanding options beyond those offered by evolution — evolving faster and better than will occur naturally — using technology to design the best babies who will grow to be better adults (chapter 3).

We will consider a variety of reproductive technologies, since they permit access to reproduction for those who would otherwise be infertile, and testing of pregnancies for individuals who desire specific pregnancy outcomes. If those who would develop designer babies move forward, then their efforts would bring these technologies — reproductive and genetic testing — together in the attempt to achieve their goals.

It is important to recognize the infusion of genetic determinism throughout the applications of these technologies. From our discussion of sex and gender (chapter 6), it should be evident that if there is to be

preimplantation or prenatal sex selection, then these efforts will be imperfect — the gender cannot be guaranteed by simply looking for the presence or absence of the Y chromosome or the *SRY* gene. A couple who undergoes prenatal diagnosis, for example, to determine whether there is an extra chromosome 21, and then has a child born with an unrelated disorder will generally be quite angry and will question why geneticists cannot test for all genetic disease. Even when it is possible to sequence the genome of the fetus, there will still be the influences of the environment (chapter 1). Therefore, while these reproductive technologies are both beneficial and desirable, their limitations must be recognized. These technologies cannot be assumed with absolute accuracy, since the biology is not deterministic.

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Before we consider the various reproductive technologies, however, let's first consider opinions of the U.S. public about these technologies — you might find them surprising. Researchers at the Genetics and Public Policy Center of Johns Hopkins University assessed the opinions of the U.S. public about reproductive technologies in 2002–4. Their methodology included more than 4,800 participants in focus groups, interviews, and town hall–style meetings around the country. The goal of these approaches was to obtain a broader range of opinions than is typically heard by policy makers.

Perhaps not so surprising, because of media popularity, was the respondents' high awareness of cloning (chapter 12), at 97 percent of those surveyed. Among those topics that are both assessed in the study and presented in this book, only preimplantation genetic diagnosis, which is one of the newer of these technologies, had an awareness level of less than 50 percent among respondents. When the investigators adjusted for demographic properties of the respondents, the two characteristics most significantly associated with higher awareness were female gender and higher education.

When these survey participants were asked to respond to the statement "Parents ought to do everything technologically possible to prevent their child from suffering, including using reproductive genetic technologies," 51.5 percent agreed or strongly agreed. When presented with the statement "Reproductive genetic technology will inevitably lead to genetic enhancement and designer babies," approximately 75 percent agreed, and only about 25 percent disagreed. Those respondents with higher education

were less likely to agree that technology would lead inevitably to designer babies. To the statement "Reproductive genetic technology is potentially the next step in human evolution," 53.6 percent agreed or strongly agreed. We believe that these responses derive at least in part from the scientific oversimplification and deterministic language that have spun off of the Human Genome Project.

The moral status of the human embryo and fetus was also considered in the center's 2004 survey. Those surveyed were asked to rank the moral worth of various stages, from an egg or sperm to a born child, on a five-point scale. As one might expect, the infant after birth had the highest moral worth. More than 50 percent of the respondents assigned the highest moral value to fetuses that have reached eight weeks of development. Only 62 percent assigned the highest moral value to a twenty-four-week fetus, the time that is considered the minimal age of viability.

The results of this survey are quite informative. The data on the fetus's moral status at various stages in development help to illuminate the public's opinions in ways that the usual rhetoric never could and were quite surprising for us.

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We will now discuss various approaches to assisted reproductive technology (ART) and some of the issues raised by these technologies. Louise Joy Brown was born on July 25, 1978, amid much fanfare and tight security. She was hailed as the "world's first 'test tube baby' " since she was conceived by in vitro fertilization (IVF). Her mother, Leslie, who was twenty-nine years old at the time, had blocked fallopian tubes leading from the ovaries to the uterus, which prevents passage of the eggs for fertilization by sperm and is still the most common reason for IVF. Her mother and father, John, then thirty-nine years old, had been told they could never have children. Louise Brown's family recognized that this would be a major news event and sold the rights to her story for £300,000.

In vitro fertilization involves hormonal treatment of the woman to increase the number of eggs released from the ovaries. The eggs are collected using a laparoscope, a fiber-optic device that permits the physician to see the ovaries containing the follicles from which the eggs will be released. The eggs are aspirated into a syringe for transfer to the laboratory, where they are mixed with sperm in a culture dish. After fertilization the embryos are cultured in the laboratory for approximately two days. The cultured embryos are then examined under a microscope, and the

healthiest among them are introduced into the woman's uterus, where the pregnancy proceeds.

Leslie Brown's pregnancy was not the first IVF conception. Patrick Steptoe, an Oldham hospital gynecologist, and Robert Edwards, a Cambridge University physiologist, had attempted this procedure approximately eighty times, but the women had not carried the pregnancies beyond the first few weeks. Previously they had cultured the embryos for approximately four to five days, until the cells had doubled six times and the embryos contained around sixty-four cells. However, with the Brown procedure they cultured the embryos for only 2.5 days to the eight-cell stage before transfer to the uterus.

The eggs could be donated from one woman to another woman — a surrogate — who would carry the pregnancy. This would permit a woman who was unable to produce eggs because of a disease or menopause to have a pregnancy. Donation would permit surrogacy if the biological mother could not or did not wish to carry the pregnancy. Similarly, the sperm could be from a woman's male partner, any other man willing to participate, or a sperm bank.

Investigators are carrying out research to attempt to obtain developing ova from late-stage fetuses to determine if donor eggs could be obtained from individuals who were never born (chapter 6). This would not be possible for sperm since they do not develop until puberty. However, if a man has had sperm frozen prior to his death, then those sperm could be used in IVF. It is also possible to collect sperm from a man when he is recently deceased, or neurologically dead but on life support, and freeze it for later use in ART. Questions have been raised about the appropriate indications for posthumous sperm use if the man did not have written instructions to guide decision making. There has been a growing interest in posthumous sperm procurement (PHSP) across the United States, but not all states honor the request for PHSP at this time.

While PHSP has been around since 1980, the first case with international visibility was in 1997 and involved Diane Blood, a widow in the United Kingdom. Blood requested that sperm be harvested from her neurologically dead husband. She obtained permission for PHSP and storage of the sperm. However, the U.K. Human Fertilization and Embryology Act of 1990 required review by an inquiry committee to protect the interests of the child that would be the product of PHSP and IVF. The committee actively discouraged but did not prohibit PHSP. Blood required approval by the British Court of Appeals for use of the stored sperm and its export to Belgium, where she was inseminated.

Embryos can be frozen in liquid nitrogen for long periods of time and preserved for later use. The status of frozen embryos in divorce proceedings frequently becomes an issue when the two parties do not agree on the disposition of the embryos. The outcomes of these cases are determined by state law, and the decisions appear to be quite variable across jurisdictions. One theme in these decisions is the IVF clinic's contract with the couple: when the language in such contracts is specific enough, then it may guide the decision of the court. Often the language does not provide adequate guidance and the decision seems to turn on which person's rights are more compelling. For example, in cases decided in the Supreme Courts of Tennessee and New Jersey, one parent wanted the embryos donated to an infertile couple and the other parent wanted them destroyed. The courts found that the right not to have children was more powerful. That right would be extinguished irrevocably if the embryos were allowed to be used to impregnate other women.

Intracytoplasmic sperm injection (ICSI, pronounced ik-see) is a process for dealing with infertility due to abnormalities of the sperm. In this process, sperm are injected directly into the egg's cytoplasm. ICSI does not rely on motility or penetration by the sperm. Sperm abnormalities may include extremely low numbers of motile sperm, sperm with abnormal shapes, defects that prevent the functional interaction of the sperm with the egg, and antibodies against the sperm that prevent normal fertilization. ICSI can also be used if frozen sperm are low in number or poor in quality. Other variations on ICSI have been developed, including microsurgical epididymal sperm aspiration, which permits sperm to be obtained even if there is a blockage in the epididymis that would prevent normal ejaculation, and testicular sperm aspiration, which permits direct collection of sperm from the testis.

The American Society of Reproductive Medicine (ASRM) estimates that ICSI results in successful fertilization of 50 to 80 percent of injected eggs. Approximately 30 percent of ICSI cycles in the United States in 1998 resulted in a successful live birth, similar to rates with traditional IVF. The ASRM also notes that ICSI is a new technique, and therefore its safety has yet to be proved.

Concerns have been raised that males conceived through ICSI may have reproductive difficulties when they reach adulthood. Roger Gosden from McGill University in Montreal, Quebec, and his colleagues carried out mathematical modeling to look at the impact of male fertility on azoospermia, or low sperm numbers. They estimated that if 50 percent of males with low sperm counts underwent ICSI, then the incidence of this

problem would double in eight generations, or approximately two hundred years. If, however, 90 percent of affected males used ICSI, the incidence would nearly double in the first generation. They speculated: "Total male infertility could be reached, in theory, if 99% or more of the affected males undergo successful ICSI." They noted, however, that socioeconomic factors limiting access to this technology and lack of research progress "would prevent the occurrence of total male infertility." They concluded: "Although we must remain vigilant about the transgenerational effects of these new reproductive technologies, ICSI will probably not increase overall male infertility in the near future through the widespread propagation of genetic mutations."

One of the newer ART strategies is cytoplasmic transfer, or cytoplasmic donation, with the first pregnancy using this technique reported from China in 2003. This approach is employed when the infertility appears to be caused by cellular abnormalities in the egg. The technique involves ICSI using the egg and sperm from the parental couple. Immediately after fertilization and before the nuclei fuse, the nuclei contributed by the egg and the sperm are extracted from the egg by micromanipulation. Then these nuclei are transferred to an egg donated by another woman from which the egg's nucleus has been removed. The embryo undergoes early development in culture as it would for IVF. Usually the embryo is transferred to the uterus of the woman in the original couple from whom the maternal nucleus originated.

The concept behind this approach is that the environment of the donor's cytoplasm will be healthier for the early developmental processes in the embryo. These processes rely heavily on proteins already present in the cytoplasm before the nucleus begins to generate ribonucleic acid (RNA) for protein synthesis. In addition, there is the possibility that the mitochondrial problems in the original egg could be leading to infertility. Along with the donor's cytoplasm, the donor's mitochondria are included in the transfer.

Caution has been urged because of the unknown implications of "triparental inheritance" involving nuclear DNA from the mother and the father and mitochondrial DNA from the mother and the donor. The mitochondrial-mitochondrial interactions and mitochondrial-nuclear interactions are poorly understood. Concern has been expressed that cytoplasmic donation is close to cloning, but the processes really are quite different. In cytoplasmic transfer, there are contributions from the germ cells of the two parents, whereas cloning involves transfer of a nucleus from a somatic cell to the recipient egg.

University of Minnesota bioethicist Jeffrey Kahn expressed reservations regarding the move of this work on cytoplasmic transfer from the U.S. to China. The investigations had been initiated at New York University by James Grifo until the U.S. Food and Drug Administration imposed regulations requiring an Investigational New Drug Application that Grifo felt made it too difficult to proceed. Kahn said, "My concern is that people see this as an end run around oversight and restrictions in the United States." Interestingly, China has now banned this procedure as well, because officials there felt it was too close to cloning.

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The possibilities in reproductive technologies are quite impressive, but is anyone following this health sector for safety? Since 1996, as required by federal law — the Fertility Clinic Success Rate and Certification Act — the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, has been collecting data on ART. The CDC's 2005 report explained one of the recognized complications of this technology: "Patients who undergo ART treatments are more likely to deliver multiple-birth infants than women who conceive naturally. Multiple births are associated with increased risk for mothers and infants (e.g., pregnancy complications, premature delivery, low-birthweight infants, and long-term disability among infants)."

The 2005 report reviewed the data on ART from 2002, when that technology was conducted in 428 centers around the United States and accounted for 115,392 cycles of ovulation and attempted fertilization. ART in these centers was associated with 33,141 deliveries, but 45,751 infants, since a number of these deliveries included multiple births. This number means that in the United States in 2002, approximately 1 percent of all births were conceived through the use of ART.

The Fertility Clinic Success Rate and Certification Act was passed in 1992 in part to address the role of ART in the apparent "epidemic" of multiple births, meaning twins, triplets, and higher-order births per delivery. The obstetrics and pediatric communities had expressed concern because of the risks associated with multiple births, including higher rates of Cesarean section deliveries, premature birth, low birth weight (less than 2,500 grams, or less than 5.5 pounds), very low birth weight (less than 1,500 grams, or less than 3.3 pounds), neonatal death, and disability, including developmental delay and mental retardation.

The incentives for both the physician performing and the patient

undergoing ART is that the success rate for each transfer increases with the number of embryos in each of these transfers. The problem is that, as one might expect, the numbers of multiple births, and the associated negative health consequences, also increase the higher the number of embryos transferred. Whereas 3.3 percent of infants born in the United States are the products of multiple-birth deliveries, 53.3 percent of infants conceived by ART are in multiple-birth deliveries, a rate that is sixteenfold higher than the general birth population. Comparing the proportion of infants from twin versus higher-order deliveries among all U.S. infants, those from twin births are 3.1 percent and higher-order births are 0.2 percent, but among those conceived by ART, twin births represent 45.7 percent and higher-order births, with their even greater risks to mothers and infants, are 7.6 percent, representing a nearly fifteenfold increase in twin births and a thirty-eight-fold increase in higher-order births with ART.

The initial form of ART was IVF, and early reports of follow-up of infants conceived by IVF indicated that this procedure was safe. However, articles began to appear suggesting an increase in birth defects, in addition to low birth weight and prematurity, among individuals who had been conceived by ART. In 2003, Gosden and colleagues in Canada and the United Kingdom reviewed the literature on the increased rate of birth defects after ART. They noted conflicting reports on safety, some showing none or only minimal increase and others indicating an approximately twofold increase in the frequency of birth defects.

Others were also concerned, including Paul Merlob and his colleagues at the Rabin Medical Center in Israel, who examined the frequency of major congenital malformations using a registry established at their medical center and at the Israel Birth Defects Monitoring System, which is part of an international network. Birth defects registries like these collect data in a prospective and very precise manner and have been shown to be far more effective epidemiologically, compared with retrospective assessment of congenital malformations, because data from the latter are incomplete and inaccurate.

Merlob and his collaborators looked at two time periods: the first, 1986–94, included babies conceived only by IVF, and the second, 1995–2002, included IVF and additional ART such as ICSI. During the initial period, 0.9 percent of the neonates were conceived through IVF, and during the second period, the proportion conceived by ART increased to 3.1 percent, a 3.4-fold increase. The frequency of major malformations was 9.35 percent during 1986–94, which was a 2.3-fold increased compared with the control neonatal population of 4.05 percent. During 1995–

2002, the frequency of major malformations was 9.0 percent, or 1.75-fold higher than the rate of 5.18 percent in the control neonates. (You may be surprised by the frequencies of congenital malformations in the 4 to 5 percent range, but that range is in line with many other studies.) Organ systems most often affected in neonates conceived by ART were cardiac, renal, genital, and skeletal. These investigators observed an increased rate of prematurity, low birth weight, and multiple births among those babies who also had major malformations. They were unable to identify a cause for these congenital malformations, since this was an epidemiological study and therefore could only find associations and could not determine causation. These researchers concluded that there was a need for continued study of the relationship between congenital malformations and ART, and families who plan to use ART should be counseled about the potential risks.

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Diagnosis of genetic disorders can be performed on samples from the embryo and fetus and relies on reproductive technologies. In essence, any genetic test that can be performed in a laboratory on cells from a child or adult can be performed on cells from the fetus. Some of these prenatal diagnostic tests may require culturing the cells, but many can be performed directly on the fetal cells without culture.

Prenatal diagnosis began and still is used most often for evaluation of chromosome number in women thirty-five years of age or over, an age beyond which the typically insensitive medical terminology determines to be "advanced maternal age," or "AMA." This age was chosen because at the age of thirty-five, the risk of pregnancy loss secondary to an amniocentesis is roughly equal to the risk of trisomy 21 (47,+21), or Down syndrome, associated with mental retardation and characteristic physical findings. At twenty-five to twenty-nine years of age, a woman's risk of giving birth to a child with Down syndrome is approximately one out of a thousand, and that risk increases to approximately one in four hundred at thirty-five years, one in one hundred at forty years, and one in twenty-five at forty-five years and older. In the United States, at least 50 percent of women thirty-five years and older undergo amniocentesis, and courts have found physicians to be negligent if they do not offer a woman of thirty-five years or older the opportunity for prenatal diagnosis. With improvements in amniocentesis and fetal ultrasound, the American College of Obstetrics and Gynecology has recently recommended that all

pregnant women, regardless of age, have their fetuses screened for Down syndrome.

Amniocentesis is a sampling technique that permits testing of fetal cells in the amniotic fluid. Under ultrasound guidance, the sampling needle is directed away from the fetus in the amniotic sac containing the amniotic fluid. This technique is most often used in the fifteenth to the twentieth weeks of gestation, but it can occasionally be used as early as the twelfth week. The amniotic fluid is generated by the fetus. It is swallowed by the developing fetus and excreted as urine back into the amniotic fluid, and therefore it contains cells from the urinary tract. However, the larger cellular contributions are from the skin of the fetus and the fetal membranes surrounding the amniotic fluid.

Chorionic villus sampling (CVS) is an approach applied earlier in a pregnancy. Transcervical CVS involves ultrasound-guided passage of the sampling catheter through the vagina and cervix and next to the developing placenta containing the chorionic villi. CVS is typically performed in the tenth through twelfth week of gestation. There is a variation on this procedure called transabdominal CVS, in which the sampling needle is inserted through the abdominal wall to sample the chorionic villi. The chorionic villi contain maternal and fetal cells that can be separated from each other under a dissecting microscope. Some laboratories will use DNA forensic methods (chapter 7) to ensure that the cells used for diagnostic testing are fetal and are not contaminated by maternal cells.

CVS is considered somewhat more invasive than amniocentesis and has a slightly higher risk of pregnancy loss after the procedure. So why would a woman have CVS rather than amniocentesis? The answer is found in the timing of the procedure — CVS can be performed before the pregnancy is typically obvious, whereas by the time amniocentesis is possible, the pregnancy would usually be visible. In other words, any decision consequent to an amniocentesis is a public decision, whereas following CVS a decision would be private.

An even earlier diagnostic approach is preimplantation genetic diagnosis (PGD), which involves IVF and micromanipulation of the resulting early embryo. At approximately the eight-cell stage, or after three cell doublings, the embryo is held in place on the end of a pipette, and a second pipette is used to biopsy the embryo. One of the cells, or its nucleus, is removed. Since at this time all of the embryo's cells are "totipotent" — any cell could give rise to a complete and normally developed human — the embryo will not be affected by this procedure. The cell that is removed can be analyzed by amplifying the genes of interest using the

polymerase chain reaction (chapter 2) so that mutations can be identified. More recently, chromosome abnormalities have been analyzed by fluorescence in situ hybridization (FISH), using probes specific to individual chromosomes, or a marker on a chromosome, and are visualized with a special fluorescence microscope.

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When we introduced the topic of designer babies in the title for this chapter, you may have thought that we would discuss ethical issues that will arise only many years in the future. But that is not the case — these issues have already arrived with the availability of prenatal genetic diagnosis. Some would think we are well along the road to designer babies.

Let's first describe an example in which IVF and PGD were used to generate a pregnancy that would result in an unaffected stem cell donor to treat a genetic disease. Lisa and Jack Nash of Englewood, Colorado, had a daughter, Molly, who was diagnosed with Fanconi anemia, a disorder associated with decreased bone marrow production of red and white blood cells, and platelets, the clotting cells in the blood, along with other birth defects, often including missing thumbs. This genetic disease involves defective DNA repair, and the affected children often develop leukemia. Lisa and Jack Nash wanted another child. They also wanted one who would not have Fanconi anemia and would be a compatible hematopoietic stem cell transplant donor for their daughter.

The geneticist they approached initially refused to consider the family's request. With subsequent discussions, they convinced him that they had always planned for another child and they would love their second baby. They also loved Molly, and they did not want to lose her. If they carried out PGD and selected only on the basis of the Fanconi mutation, then they might require multiple pregnancies to have a baby that would be a transplant match for Molly, and if they were unlucky, then it might be too late for Molly. She might die from leukemia before a matching baby was born.

Yury Verlinsky and his group at the Reproductive Genetics Institute in Chicago examined thirty embryos generated by IVF with Molly's parents, and twenty-four were unaffected for the Fanconi mutation. Among these twenty-four embryos, five were also transplant-compatible and were transferred over four IVF cycles. In the last cycle, with only one embryo transferred, there was a successful pregnancy, and Adam Nash was born.

Fetuses and neonates have a large number of blood stem cells in their

circulation. Therefore, umbilical cord blood is a rich source of stem cells for populating bone marrow. Adam's cord blood was collected and infused into Molly, providing her with the equivalent of a bone marrow transplant. Survival of individuals with Fanconi anemia is 85 percent when they have a transplant from a sibling, but decreases to 31 percent with a transplant from an unrelated donor.

Selecting embryos by PGD that are not affected with a genetic disease is a relatively routine procedure, and in those cases PGD would benefit the individual being tested. However, in this case, there was also selection for a trait that did not benefit Adam but would benefit another individual, Molly. Lisa Nash said, "That's what we had to do for us, and I hope that people who felt this was inappropriate would feel it was inappropriate for them and not judge me unless they've been where I've been." She was saying that those not faced with this situation may not know what they would choose if they were in her place.

Let's take this another step. What would you think about selecting an embryo that did not have the Fanconi anemia mutation; was a compatible match for a sibling; *and* was a specific gender? As we discussed in chapter 6, even if there was a selection for the presence or absence of the Y chromosome or the *SRY* gene, there would be no guarantee of the gender, though there certainly would be an increased probability compared with 50 percent by random chance.

John Robertson at the University of Texas, Austin, addressed the issue of sex selection as chair of the Ethics Committee of the ASRM. He summarized the complex issues involved as "questions of gender stereotyping, procreative liberty, the misallocation of medical resources, and respect for the earliest stages of human life." In a previous report, the Ethics Committee recommended that couples who were not having IVF or PGD for genetic diagnostic reasons "should be discouraged from undergoing IVF with PGD solely to choose the gender of the offspring." Robertson and his committee wrote "that fertilized eggs and preimplantation embryos, although not persons or moral subjects in their own right, should not be treated like any other human tissue. Rather, because of the meanings associated with their potential to implant and bring forth a new person, they deserve 'special respect.' " Robertson concluded: "It has not yet been clearly established that a couple's desire for gender variety in offspring is sufficient to outweigh the need to show special respect to embryos."

Others, however, disagree with this position and argue for individual choice in sex selection. David McCarthy, from the University of Bristol

in England, has argued that reproduction decision making is a fundamental liberty. He added that those who would write laws to restrict sex selection would also constrain this basic liberty, and those who oppose such laws are not restricting anyone's liberty. McCarthy concluded, therefore, that there should not be laws prohibiting sex selection.

Bernard Dickens from the University of Toronto stated: "Prohibition on sex selection may well be unnecessary and oppressive as well as posing risks to women's lives." He presented data from the Haryana state of India, where for every 1,000 males there are 861 females, and for girls under six years of age there are only 820 females per 1,000 males. He maintained that the current social pressures for sons in places like India and China put the health of women at risk because of the numbers of pregnancies and deliveries some will face in order to have a son. The World Health Organization has noted that maternal mortality from complications of pregnancy in developing countries is four hundred times that in southern Europe. There are also risks of death to unwanted girls from infanticide, neglect, and poor nutrition. Dickens argued that application of technology to permit sex choice would prevent morbidity and mortality.

A total of 561 Boston women who were undergoing medical care for their infertility were surveyed regarding their interest in preimplantation sex selection. Their responses showed a significant demand for these services. If they could determine the sex of their next child at no added cost, 41 percent said they would use this technology. There is evidence that this demand is being addressed in the marketplace. The Genetics and IVF Institute in Virginia has advertised in the *New York Times* and promised the opportunity for sex selection, not through PGD but by sperm selection, at a cost of $2,300. In Australia, apparently six out of every ten women having PGD are doing so for sex selection, not for medical indication, and the cost is $2,400.

Some would consider Adam Nash to be a designer baby, but there was a medical reason why the Nash family made their odyssey. Others would consider a baby born after prenatal diagnosis for gender selection to be a designer baby. But can this technology be used to design the "best baby?" Open any major university's student newspaper in the United States and one may find ads recruiting women students to be egg donors. Frequently bonuses are paid for students with high SAT scores and other characteristics deemed to be beneficial. The Web site for the Genetics and IVF Institute even has a "Doctoral Donor" program with eggs harvested from women with "advanced degrees" and

"other especially accomplished women." The institute has extensive information on all of its donors, so that the recipient is given the impression that she, not the program, will determine her family's future.

This and similar Web sites demonstrate a pattern we have discussed previously. The institute is offering control to the prospective mother and therefore autonomous decision making regarding her family's future. There is also the implication of genetic determinism: if you obtain an egg from a woman with an advanced degree, then you will have a brighter child. This of course ignores the complex nature of intelligence and the equally complex environmental contributions to school and professional achievement.

Julian Savulescu at the Royal Children's Hospital in Melbourne, Australia, has argued that there is a fundamental principle rooted in legislation in Australia and the United Kingdom termed the "best interests of the child principle." For example, he quotes the Infertility Treatment Act of 1995 for the Australian state of Victoria: "the welfare and interests of any person born or to be born as a result of a treatment procedure are paramount." He determines that there is an ethical imperative that he terms "procreative beneficence." He also maintains that this is not eugenic (chapter 3), because it is not a population-based strategy. It is a private decision by a couple to have the "best child" they are capable to conceive. He describes the "best life" as "the life with the most well-being." He recognizes that there is a tension between the principle of procreative beneficence and commitment to equality and access to the technology, termed distributive justice.

In his book *Redesigning Humans: Our Inevitable Genetic Future* Greg Stock takes the position that humans have always found ways to improve themselves and that now we have the opportunity to do this more rapidly using genetic technology. He feels it is naive to think that we will not use this approach in the long term. Legal restraints may be enacted, but he argues these will be temporary. Stock concludes: "The crucial question is whether we will continue to embrace the possibilities of our biological future or pull back and relinquish their development to braver souls in more adventurous nations in the world. . . . As I see it, the coming opportunities in germinal choice technology far outweigh the risks. What is more, a free-market environment with real individual choice, modest oversight, and robust mechanisms to learn quickly from mistakes is the best way both to protect us from potential abuses and to channel resources toward the goals we value."

In his book *Enough: Staying Human in an Engineered Age,* journalist and author Bill McKibben takes a different position, arguing that we must at least begin to question whether our technological reach is sufficient at this time. He expresses concern that by being critical of technology, he may be labeled a Luddite. In part, he is reacting to the rhetoric of the Human Genome Project, but he has also been stimulated to consider these issues by the impending possibility of cloning (chapter 12). He introduces his subject as follows: "I think the stakes in this argument are absurdly high, nothing less than the meaning of being human. Must we forever grow in reach and power? Or can we, should we, ever say, 'Enough'?"

We argue that the genetic and biologic knowledge base does not exist at this time for anyone to develop or to claim that they would be able to develop a "best life" or a "best child." Those who feel that this will be possible with any reasonable time horizon have not yet recognized the complexity of the biological system (chapter 3). Moreover, we maintain that they have been seduced by the deterministic model of genetics. It is important that society begin to consider the ethical implications of developing designer babies, but everyone should recognize that these are only "thought experiments" and will remain so for quite some time. As research regarding complex genetic traits proceeds, it will be essential to be open and to express caution about what can be promised. In chapter 12 we will consider whether reproductive cloning provides any greater control over the outcome for one's offspring.

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Reproductive technologies have come a long way since 1978 and the birth of Louise Brown. We have become accustomed to increasingly intricate approaches to improving fertility and diagnosing genetic diseases earlier and earlier in pregnancy. Technological innovation will undoubtedly continue, and we will accept as familiar what today seems only fantasy. But we must be realistic in our expectations of what these new technologies will be able to provide. We will not be limited by technology so much as by biology.


[To view this image, refer to
the print version of this title.]

CHAPTER 12

Reproductive Cloning

From Farm Animals to Pets to Humans?

Normal sexual reproduction occurs when the sperm containing one set of twenty-three chromosomes fertilizes an egg containing another set of twenty-three chromosomes to form a zygote, or single-cell fertilized egg, containing two sets of the twenty-three, or forty-six chromosomes. Reproductive cloning occurs when the nucleus is removed from an egg and replaced with the nucleus of an adult somatic cell containing forty-six chromosomes, and then the clonal zygote is stimulated to divide and develop into an embryo. The process of cloning in this manner is therefore called somatic cell nuclear transfer (SCNT). The fetus has the same nuclear DNA as the adult who donated the nucleus. It is important to note that unless the egg was derived from the same woman who provided the nucleus, the mitochondrial DNA would be different, so the clone would not have identical mitochondrial DNA to that of the donor.

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Let's begin with a description of the most famous clone, Dolly the sheep. The announcement in February 1997 of Dolly's birth on July 5, 1996, made the front pages of newspapers and captured the imagination of people around the world. The report of her birth was delayed from July to February because of the filing of patent applications. The cloning process involved serum starving the donor cell so its nucleus became less active in terms of DNA synthesis and cell division. Speculation suggests that the donor nucleus in this more quiescent state can be effectively "reprogrammed" by the recipient egg to begin to develop into a viable embryo. In essence, this was a biological "syncing" process — synchronizing the states of the donor and recipient cells.

Dolly was not the first animal to be cloned. Tadpoles had been cloned in 1962, and cattle and other sheep had been cloned using nuclei from embryonic or fetal tissue. The article in the journal *Nature* in which Dolly's birth was reported related that seven other cloned animals had been born, three derived from the fibroblasts of a twenty-six-day fetus and four from cells of a nine-day embryo. Dolly's notoriety came from the fact that she was the first animal cloned using the nuclear genome of an adult somatic cell.

The process of cloning Dolly was a variation on SCNT that did not involve transferring only the donor nucleus, but a process of fusion of the entire donor cell containing its nucleus with the recipient cell from which the nucleus had been removed. The fusion was performed with an electric current, a routine process in cell and molecular biology laboratories called electrofusion — the two cells were joined as one.

To generate Dolly, a mammary gland cell from a six-year-old white-faced (Finn Dorset) ewe in her last trimester of pregnancy was fused to an enucleated egg from a sheep that had a black face and other markings (Scottish Blackface). The phenotype of the lamb would indicate whether it was the product of cloning or it was somehow the result of a normal pregnancy. Dolly showed the Finn Dorset white-faced phenotype and not the Scottish Blackface phenotype. Therefore, her nuclear genome derived from the udder cell's nucleus and not from the recipient egg. Additional genomic markers confirmed that Dolly was a clone — the first mammal produced using the nuclear genome from an adult cell.

Cloning is a very inefficient process, as shown by the numbers involved in the production of Dolly. In 277 attempts, 247 cloned zygotes were successfully generated. The zygotes were transferred to recipient Blackface ewes and placed in tied-off oviducts, or fallopian tubes, where they developed into the stage at which embryos are normally transferred to

surrogate mothers. In other words, they were "cultured" in living sheep rather than in a culture dish in the laboratory, and there were two surrogate mothers, one for early and a second for later development. The early embryos were harvested from the first surrogate mother; only 29 of the original 247 had survived to this stage. These 29 early embryos were implanted into the wombs of the second surrogate mothers, also Blackface ewes, and only one of these 29 developed normally to birth. This was Dolly.

Is Dolly truly a clone? No. Each of our cells has two genomes, nuclear and mitochondrial (chapter 2). Dolly's nuclear genome derived from the Finn Dorset ewe that provided the udder cell nucleus. However, her mitochondrial genome derived from both the Finn Dorset donor cell and the Scottish Blackface enucleated recipient egg, so she was not identical to the SCNT donor. As we discussed in chapter 11, the interactions between the nuclear and mitochondrial genomes are poorly understood, and just as for cytoplasmic transfer in ART, we do not know the implications of having nuclei from one individual in a cell in which the mitochondria derived from that and another individual.

Why did Dolly attract the world's attention? Dolly was a cute farm animal that we learned about in nursery rhymes, but that was not her real attraction. The implications of this experiment were what attracted so much attention. One could clone an organism without any invasive procedures, for example, from a sloughed skin cell or hair follicle. There were possibilities for using cloned farm animals to generate proteins for human therapeutic benefit, without the need to worry about the individual genetic backgrounds of different animals influencing production. Once a clonal line was established, eggs from that line could be used for the donor and recipient eggs, making true clones of the animals produced in this manner. The group that cloned Dolly talked about use of cloned animals to generate proteins, for example, to treat clotting disorders like hemophilia or emphysema such as that caused by alpha-1 antitrypsin deficiency (chapter 10), and perhaps eventually to generate human organs to deal with the scarcity of organs required for transplantation.

The real "hook" in the stories about Dolly, however, was the possibility of cloning those close to the interviewer or the audience. Everyone wanted to know if it would or should be possible for grieving parents to clone a child who had died, and they had a myriad of related questions. Should one be able to clone oneself? If laws were enacted in the United States and other developing countries to ban the cloning of humans, wouldn't that just drive the efforts "offshore"? When the questions about

human cloning were exhausted, then these questions were followed by those related to other "loved ones," the questioner's pets. Would it be possible to clone a dead pet and would that pet have the same characteristics of the one that had died? All of these questions usually related as enthusiastically to personality as they did to physical characteristics. Would the cloned human or pet be absolutely identical in appearance and behavior to the nucleus donor?

Why did Dolly die so young? Dolly was euthanized on February 14, 2003, at six years of age, because she was suffering from a severe lung infection. She had six lambs through sexual reproduction with a ram to show that although she did not derive from a fertilized ovum she was able to generate functional eggs. Reports of Dolly's health were somewhat difficult to interpret. We know that she was obese and had severe arthritis. There were also questions of whether she was showing evidence of premature aging and whether her arthritis could be attributable to this. Ian Wilmut, who was the lead author on the 1997 report of Dolly's cloning and her public spokesperson throughout her life, argued that features such as the lung infection and the length of her life were not unusual in a sheep maintained in a pen indoors much of time. In addition, he is reported to have commented that her arthritis was the result of all of the posing she had to do for photographers, who wanted her shown with her front legs raised up on the edge of her pen.

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The reasons for individuals' curiosity about evidence of Dolly's premature aging and Wilmut's apparent defensiveness about this issue relate to fundamental concerns about the biological implications of cloning and potential biological problems with cloned animals.

Telomeres are highly repetitive sequences in the DNA at the ends of chromosomes that are important for maintaining the integrity of chromosomal structure. They have been compared to the plastic tips at the ends of shoestrings. As cells divide, the telomeres shorten and eventually are not able to maintain chromosomal integrity. At that point, the cell senses this and ceases to continue to divide. Therefore, it has been hypothesized that telomere shortening is involved in cell senescence, or aging, and it has been suggested that telomere length is a kind of "mitotic clock." During development of germ cells, eggs have long telomeres, suggesting that the lengths are reset in eggs for the new generation. The telomere lengths in sperm are relatively short.

Concerns were expressed that cloned animals generated from somatic cells from older animals, particularly clones like Dolly, would have shorter telomeres because they were not reset during the development of the ova. Cloned sheep generated from embryonic or fetal cells had telomeres that were 10 to 15 percent shorter than those in age-matched controls. Dolly's telomeres when she was one year old were reported to be 19 percent shorter than age-matched controls. Therefore, there was concern that Dolly and other clones from adult somatic cells might be prone to aging more quickly or to suffer the biological consequences of aging earlier than animals generated by sexual reproduction.

More recently, however, data from cattle, mice, and pigs indicate that mammalian embryos go through a "reprogramming" and telomere lengthening at approximately the embryonic age when the protocol used to clone Dolly transferred embryos from the oviducts to the uterus. This reprogramming operated similarly in embryos cloned from adult somatic cells, and the telomere lengths in age-matched fetuses and newborns were identical in those derived by cloning and sexual reproduction. The telomeres were longer in embryos than in the donor cell lines from which the cloned embryos were generated.

These various groups of investigators concluded that the donor nuclei had telomere lengths that reflected the ages of the individuals from which they were obtained and possibly the culture conditions to which the cells were exposed. However, the telomeres were then lengthened during development in the cloned mammal in a process also found in animals generated by sexual reproduction. Therefore, it would appear that fears about early senescence due to shorter telomeres in cloned animals are not supported by the data.

Cloned cattle and sheep were observed to have the "large offspring syndrome," meaning that the neonatal animals were significantly larger than control animals produced by sexual reproduction. These increased birth weights were attributed to alterations of the normal process of genetic imprinting, the selective methylation and silencing of specific maternal or paternal genes, referred to as epigenetic as opposed to genetic effects (chapter 1). During development of eggs and sperm, this genomic imprinting is "erased," meaning that these genes are demethylated, and then imprinting is reestablished during embryonic development. If, however, a somatic cell is used in nuclear transfer, and this nucleus has its imprinting established and this imprinting is not erased, then there is concern that there might be dysregulation of imprinted genes in the cloned animals. The large-offspring phenotype is similar to the Beckwith-

Wiedemann syndrome, an imprinting disorder associated with very large neonatal length and weight, and therefore it was thought that the large-offspring syndrome might be due to imprinting abnormalities. It was unclear whether dysregulation of imprinting was due to the use of somatic cell nuclei or a consequence of embryo culture, since manipulation in culture was shown to alter imprinting.

Studies in mice have begun to clarify some of these issues. Kellie Tamashiro and colleagues have observed that adult mice generated by cloning were obese, compared to controls including mice that were exposed to the same manipulations and culture conditions as the cloned animals. Birth weights were comparable for clones and control mice exposed to similar conditions, and both were higher than untreated mice. However, the growth curves of the two former groups diverged at eight weeks of age, with the cloned mice growing increasingly obese.

There was an impression from the early experiences with cloned farm animals that there was an increased death rate in the clones. Investigations in mice by Narumi Ogonuki and colleagues confirmed this impression. Cloned mice died earlier than controls of two types, those generated by natural mating and those that were the products of spermatid injection (intracytoplasmic sperm injection, or ICSI). There was also evidence from cloned large animals of additional health problems, including an increased incidence of death from lung diseases associated with fluid leaking from the circulation into the air spaces of the lung. All six of the dead cloned mice that were examined post mortem had severe pneumonia with extensive accumulations of fluid and inflammatory cells in the lungs. Four of these six animals had areas of extensive tissue breakdown, or necrosis, in the liver, which the authors said they had never seen before in naturally mated mice except in liver tumors. There was also evidence of compromised immunity in cloned large animals, and the cloned mice showed significantly decreased levels of the immunoglobulins, or antibody proteins, compared with controls.

Examination of imprinting at the DNA level, by Rudolph Yaenisch from the Massachusetts Institute of Technology and his collaborators, showed that there was considerable variation in the methylation status of genomic regions in different cloned mice generated by using the nuclei from embryonic stem (ES) cells, the totipotent cells from early embryos, any one of which could give rise to an entire mouse. In fact, mice derived from the same ES cell subclone had very different methylation and gene expression patterns. The investigators referred to the extreme variations in methylation and expression patterns as "epigenetic instability." They

concluded that mammals have a greater tolerance for epigenetic variability than had been appreciated previously. Studies on cattle from France concluded similarly that abnormal methylation patterns in "preimplantation development of clones may significantly interfere with epigenetic reprogramming, contributing to the high incidence of physiological anomalies later during pregnancy or after clone birth."

We have discussed the fact that some cloning strategies result in zygotes with two populations of mitochondria, particularly when there is electrofusion of a somatic cell and a recipient egg. This situation also may arise with SCNT without fusion, since some cells have their mitochondria tightly networked around the nucleus, and the mitochondria might be physically connected to and transferred with the nucleus. Katsuro Inoue and colleagues in Japan used cloned mice generated by SCNT with direct injection of somatic nuclei or by electrofusion to examine the mitochondrial distribution from the donor and recipient cells. They measured the levels of donor and recipient mitochondrial DNA (mtDNA) in brain, kidney, liver, and tail tips. Eggs have the ability to recognize any sperm mitochondria by the way their membranes are marked biochemically during sperm development and then eliminate the paternal mtDNA. However, somatic cell mitochondria would not be marked in this way, and the authors hypothesized that the donor mtDNA would survive in the zygote. They found this to have occurred in twenty-four out of twenty-five, or 96 percent of their cloned mice, with only one mouse showing no evidence of donor mtDNA. There was also a consistent and statistically significant pattern of mtDNA distribution in liver and brain, with liver showing a higher representation of donor mtDNA (1.7 percent) compared with brain (0.6 percent). Although the reason for this "tissue specific selection" remains unknown, the investigators attribute it to interactions between the mitochondrial and nuclear genomes. The study of mtDNA in cloned animals remains an important area of investigation, particularly since the mitochondria generate the ATP that helps maintain cellular integrity, and the liver, with the highest proportion of donor mtDNA, is also so heavily prone to necrosis in cloned animals. It will be essential to include analyses of mtDNA distributions in the lungs of cloned animals as well.

These investigations and many others show that there are definite biological abnormalities in cloned animals. Although early speculation focused on telomere length and premature senescence, these concerns have not been confirmed by research on cloned animals. In fact, in refuting the role of telomere length in the increased morbidity and mortality

among cloned organisms, there has been an improvement in the fundamental understanding of mammalian development with recognition of the reprogramming of imprinting in early embryonic development. Imprinting abnormalities are common in cloned animals and may contribute to their problems. Investigators seem impressed by the developing organism's for the dysregulation of these epigenetic phenomena, but the robust nature of biological networks is a subject we discussed in chapter 3. Robust biological systems have an immense tolerance for variation. The interaction between the mitochondrial and nuclear genomes is not well understood, and perhaps cloned organisms will help to improve the knowledge in that area as well.

We feel that the conclusions about cloning currently are that it is a terribly inefficient process, the morbidity and mortality among cloned animals is very high for reasons that remain unknown, and even if it were to be considered morally acceptable in humans, the dangers to the birth mother from the large-offspring syndrome and the clone from a myriad of problems could not be justified at this time.

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On August 7, 2001, the Panel on Scientific and Medical Aspects of Human Cloning, established in response to claims regarding human cloning, met in Washington, D.C., and heard from an international group of experts and interested parties. The product of this panel was a report, entitled "Scientific and Medical Aspects of Human Reproductive Cloning," which was published in 2002. The report endorsed therapeutic cloning, but not reproductive cloning.

In 2001, at least three groups, based in the United States, Italy, and the Bahamas, announced that they were preparing to clone humans by adapting methods that had been used in cloning animals. Responding to these claims, the presidents of the U.S. National Academies (National Academy of Sciences, National Academy of Engineering, Institute of Medicine, and National Research Council) assembled the eleven-member panel chaired by Irving Weissman from Stanford University and charged the group "with assessing the scientific and medical issues surrounding the human reproductive cloning." The panel did not address the "fundamental ethical, religious, and societal questions," but focused on its charge to develop information and recommendations that could be used in the broader debates on this issue by society and the government.

The data from cloned animals compelled the panel to recommend "a

legally enforceable ban that carries substantial penalties . . . [since these would have] a much greater potential than a voluntary system or moratorium to deter any attempt to clone a human using these techniques." The strength of these recommendations was based on concerns for the well-being of both the cloned offspring and the mother. For example, the size of the human head is close to the limit that allows normal vaginal delivery, and children with larger heads often must be delivered by Cesarean section. Therefore, the large-offspring syndrome would place the mother at risk. The high rates of morbidity and mortality among cloned animals clearly would place the offspring at increased risk. Additional, more recent data, which have been presented in this chapter but were not available to the panel in deliberations, confirm its conclusions and recommendations. The panel recognized that progress would be made and therefore specifically stated that cloning should be reconsidered in five years. The committee also concluded that there was no scientific evidence supporting a similar ban on therapeutic cloning (chapter 13) and recommended that this area of research should be allowed.

No ban has been enacted in the United States at the time of this writing. Bills have been introduced into Congress that would have outlawed reproductive cloning, but they have been quite variable in their scope. Some were so broad that they would have prohibited therapeutic cloning and specific areas of research (chapter 13), while others would have permitted these activities. A number of states have passed or are considering laws, and these are also quite variable in their scope of coverage.

The U.S. Food and Drug Administration (FDA) has placed a major roadblock for anyone trying to clone a human. The FDA has stated that it has jurisdiction over human cloning, and anyone desiring to undertake this effort in the United States must submit an application to the FDA. This agency would also be required to approve any applications of SCNT for human therapeutic purposes. Since the FDA is responsible for the safety and efficacy of human therapeutics, that agency would review all of the data, including the recommendations of the National Academies' panel and other bodies. At this time, approval by the FDA for human cloning seems very unlikely.

In March 2005, the United Nations General Assembly considered a nonbinding resolution that would encourage member nations to ban human cloning in all of its forms. Although the resolution was introduced by Honduras, many thought that Honduras was acting for the United States. The resolution passed with a vote of eighty-four for, thirty-four against, and thirty-seven abstaining. An anonymously authored editorial

in *Nature Cell Biology* following the vote stated: "Importantly, the declaration is ultimately a watered-down compromise that has no legal implications. The UN has again failed to institute formal hurdles to prevent what everyone agrees on in principle — a binding global ban of human reproductive cloning."

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Let's now consider the claims about human cloning. Clonaid is a private company founded by Raël, the spiritual leader of the Raëlian religious movement. Clonaid announced the birth of the first cloned baby, Eve, on December 26, 2002. According to the company's Web site, Clonaid has succeeded in cloning thirteen living children. Clonaid originally said that it would allow access to Eve by a third party who could carry out genetic testing to confirm that she was a product of cloning. However, the company revoked that access, and there has never been any independent genetic testing of these reportedly cloned human children. In a letter to *Science* in January 2003 entitled "Cloning Claim Is Science Fiction, Not Science," Wilmut and colleagues stated: "Fantastic claims of births of cloned babies have recently been made in the media. In the absence of any evidence for these claims, this destructive hoax must end."

In April and then November 2002, Severino Antinori, an Italian obstetrician-gynecologist specializing in infertility treatment, announced that he had helped three women become pregnant with human clones, and the first birth was anticipated in January 2003. No proof has been forthcoming of the birth of any of these cloned babies.

In June 2003, in a brief article in the journal *Reproductive BioMedicine Online,* Panos Zavos, president and CEO of Zavos Diagnostic Laboratories in Kentucky, claimed the creation of a cloned human embryo. The group used a process similar in its outline to that which generated Dolly, although the embryo was cultured for four days in vitro until it reached the eight- to ten-cell stage. The embryo was then frozen, awaiting genetic analysis, presumably by preimplantation genetic diagnosis, and subsequent implantation.

In a report in 2006, Zavos and Karl Illmensee reported SCNT from an infertile man's skin cells into oocytes from his spouse. From "three SCNT-reconstructed human oocytes" only one developed successfully. After transfer of the clone to the woman's uterus, her hormonal "levels showed a negative pregnancy result." They concluded: "Even though no pregnancy was established, human reproduction via SCNT may be pos-

sible and applicable in the future for patients with severe male or female infertility that have no other alternative options for procreating their own offspring." This statement ignores the inefficiency of, and health concerns associated with, reproductive cloning.

Other groups have cloned human embryos, but for therapeutic and not reproductive purposes. These therapeutic cloning efforts will be discussed in chapter 13.

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A veritable Noah's ark of animals have been cloned. We will discuss only a few examples. Dolly was not cloned as a curiosity, but as a part of the business strategy for the Edinburgh-based biotechnology company PPL Therapeutics. The company's goal was to generate farm animals that produced proteins for human therapeutics. Wilmut explained these possibilities in his publications and interviews. But cloning remained extremely inefficient and expensive, and the pioneer in cloning, PPL Therapeutics, was forced into bankruptcy. In the mid-1990s, this publicly held firm's shares were selling at more than four pounds each, but in June 2003 the price per share had fallen to six pence, losing 98.5 percent of its value. Transgenic sheep that produced alpha-1 antitrypsin in their milk had been developed by PPL, and clinical trials were planned in collaboration with a major pharmaceutical company, Bayer, to treat patients with alpha-1 antitrypsin deficiency and cystic fibrosis. However, PPL could not take on the debt required to build the production plant required for large-scale protein purification from the milk. Bayer canceled its clinical trials, and PPL culled its sheep. PPL had already sold its xenotransplantation (animal-to-human-organ transplantation) group, which involved the cloning of the first piglets, and PPL shut down its stem cell group. Its last remaining project involved a protein, fibrin, used as a natural surgical "glue," but this was a very competitive area. In 2005, PPL closed its doors after selling its intellectual property, including its recombinant protein production and fibrin patents to a Dutch biotechnology company, Pharming Group.

Pigs are interesting subjects for xenotransplantation because of their physiological similarities to humans and their ready availability, in contrast to nonhuman primates. There are concerns in any animal-to-human-organ transplantation because of the risk of activating animal viruses in the transplants that could be harmful to humans. While the infection risk cannot be dealt with by genetic manipulation, there is another problem,

cross-species rejection, that can be. Pigs have a system that modifies cell surface proteins with a sugar molecule that makes their cells appear extremely foreign to humans. In 2002, a University of Missouri research group, working with a company in Massachusetts, developed a pig that was missing the enzyme that catalyzed this protein-sugar modification, making the cells less prone to rejection by humans, a process referred to as "humanization." The researchers modified somatic cell nuclei and then used SCNT to move these altered nuclei into eggs to generate cloned piglets. Other companies are developing similar pigs to facilitate xenotransplantation. This genetic alteration in cloned pigs is another example of using genetically modified clones to facilitate human therapeutics.

Mules are crosses between horses and donkeys, and the hybrid mules are almost always sterile. Investigators at the University of Idaho in Moscow, funded by Donald Jacklin, president of the American Mule Racing Association, cloned a mule, Idaho Gem, which was born on May 4, 2003. Jacklin was interested in cloning his champion racing mule, Taz, and Idaho Gem was not a clone of Taz but of an unborn sibling. Taz's parents were mated, and a resulting embryo was allowed to develop for forty-five days and was then frozen. A cell from the frozen embryo was the source of the donor nucleus for SCNT to a mare's enucleated egg. The inefficiency of cloning was evidenced by the fact that even though an embryonic cell was used, it took more than three hundred attempts before the successful birth, and the investigators had to learn how to modify the in vitro medium to facilitate activation of the zygote's division after SCNT.

The cloning of a mule was announced during the Triple Crown thoroughbred racing season in 2003, after Funny Cide had won the first two legs of the three-race season. Funny Cide is a gelding — a neutered male horse — and therefore could not pass on his running prowess genetically. Reuters asked if cloning Funny Cide would be possible, allowing him to breed through his clone. There are other environmental factors such as nutrition and training that would differ, and therefore the clone might not be the racing equivalent of Funny Cide. All of this was purely academic, however, because the Jockey Club, which is the regulatory authority in thoroughbred racing, had already anticipated and blocked this possibility by prohibiting cloned animals from registration in the Jockey Club and therefore from racing. Thus, it might be difficult to assess the clone's running abilities, even if it was generated.

In August 2003, the birth of the first cloned horse was announced. This clone was the only one to survive to birth from 841 embryos that were ini-

tiated, 22 that survived seven days of culture, 17 that were transferred to surrogate mothers, and four that led to pregnancies.

Many other animals have been cloned, including cats, dogs, and monkeys. A California company, Genetic Savings & Clone, was charging clients $50,000 to clone their pet cats in 2004, and $32,000 in 2006. The company was hoping to have the price reduced in five years to $10,000 for a cat and $20,000 for a dog. While the company is careful to state that the cloned animal is a unique newborn animal and will have different personality traits from the SCNT donor, it does use terminology indicating that this is a genetic "resurrection" of the beloved pet. It is clear that the market strategy is based on the public's belief in genetic determinism.

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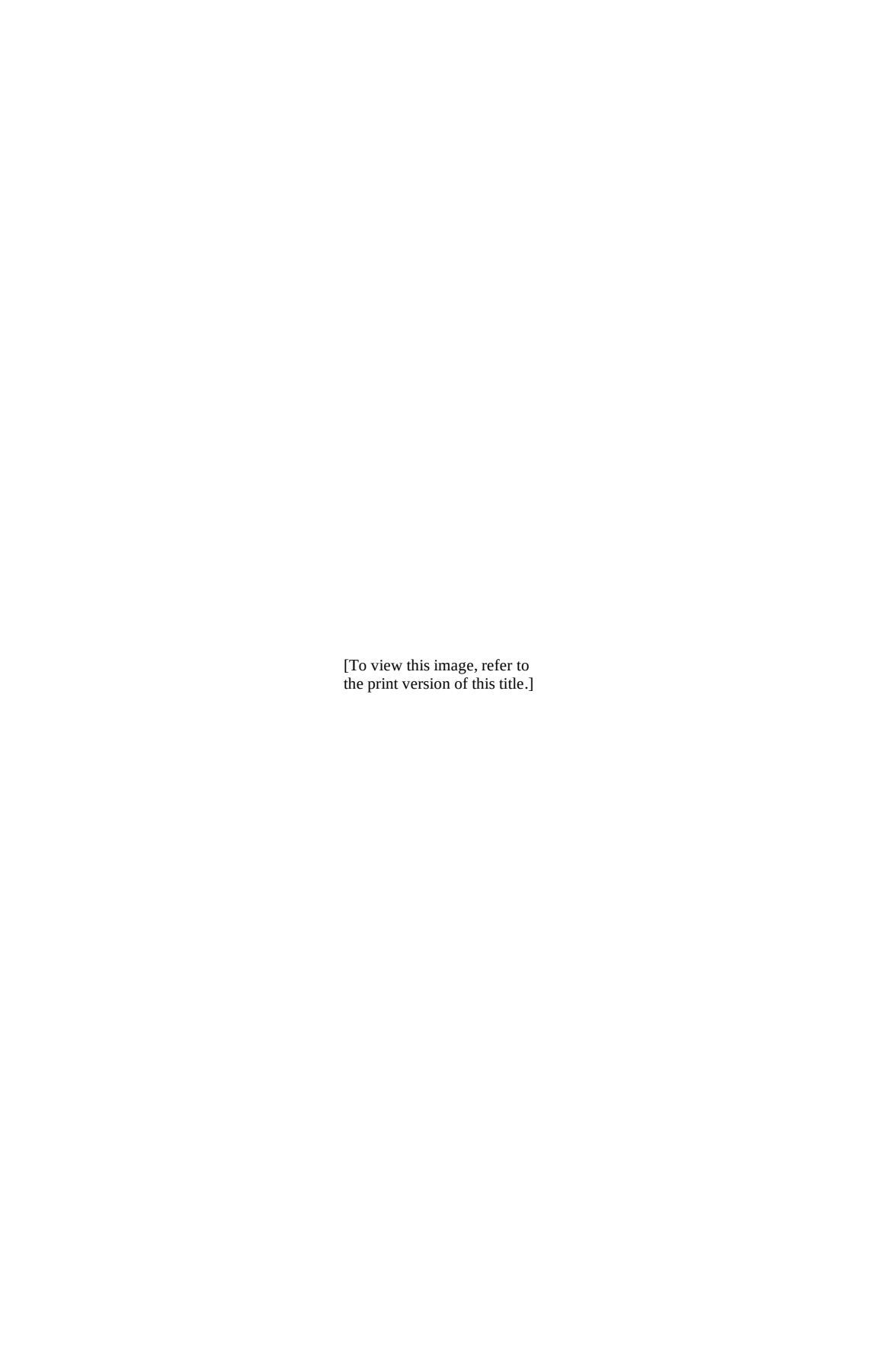
Reproductive cloning remains an extremely inefficient process, and to date the cloning of a human has not been scientifically confirmed. We would argue that the deep appeal of cloning is fundamentally deterministic, and clones will differ because of environmental influences and epigenetic changes.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CHAPTER 13

Therapeutic Cloning and Regenerative Medicine

Characteristics of stem cells
Adult stem cells
Embryonic stem cells
Combining therapeutic and reproductive cloning with gene therapy
Policy considerations related to stem cells

When we are born, our bodies are built to last a century or more. Do you know of any machine so durable that it would not require replacement parts in the course of one hundred years of operation? This means that our tissues must have mechanisms to deal with the wear and tear that they all experience. This is not simply the damage imposed by external trauma but also the tissue stress and strain of normal life. Our cells are in states of constant metabolism and are carrying out the functions for which they were designed. These functions lead to aging of cells over time, and the cells and tissues need to be rejuvenated or regenerated. Cells also undergo apoptosis, or programmed cell death. This means that the cells of a centenarian are not the same ones she was born with. The regenerative processes that maintain a tissue's integrity are mediated by stem cells that give rise to new cells specific for that tissue. Learning how to harness the proper-

ties of stem cells is the realm of a new area of clinical therapies, called regenerative medicine.

In addition to normal wear and tear, cells are damaged by disease and trauma. Replacement of these damaged cells may require a tissue transplant from another person. Even with tissue-type matching and other crude attempts to minimize immune responses to the transplant, the recipient often faces a lifetime of steroid or other drug treatments to prevent transplant rejection. The transplant may harbor infectious diseases, such as HIV or hepatitis, which complicate the medical care of the recipient. There simply is not enough tissue available for solid organ (including kidney, liver, heart, lung, and small bowel) transplants, and for these tissues as well as bone marrow transplants, there may be no donors that match the patient. Patients often die while waiting on a transplant list, and some organs, like the brain, cannot be transplanted.

One way to avoid the issue of infection from the tissue donor would be to use the patient's own cells or cells from uninfected embryos. To compensate for the scarcity of organs for transplantation, stem cells could be stimulated to grow to become a replacement tissue. Stem cells might also provide neural tissue for therapeutic benefit.

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What is a stem cell? Stem cells remain in an undifferentiated state, and when stimulated to do so, will divide and differentiate for the purpose of regenerating damaged or worn out cells. When stem cells divide, they undergo what is referred to as an asymmetric cell division. This means that the products of the division are not identical. If the stem cell divided into two cells that would both differentiate, then this stem cell would be lost to further utility in regeneration. If the stem cell divided into two stem cells, then the original stem cell would not contribute toward the immediate needs of the tissue. Therefore, it is thought that stem cells divide asymmetrically, yielding one cell that remains in an undifferentiated state of "stemness" and a second cell that differentiates to meet the regenerative need.

There are two general categories of stem cells, adult and embryonic. Although often confused with each other, these two types of stem cells are fundamentally quite different and have distinct roles.

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You have probably heard of bone marrow transplantation for treatment of cancer and other disorders, and you may even know someone who had this procedure. This is in fact an adult stem cell transplant that takes bone marrow from a donor, who may be a relative or a volunteer, and in some cases the bone marrow may come from the recipient before he or she undergoes another therapy. The bone marrow or cord blood is processed to concentrate the hematopoietic (blood-generating) stem cells; it may then be frozen, after which it is infused into the recipient to repopulate the bone marrow, which may have been lost or ablated as a consequence of a disease, such as a genetic anemia, as was the case for Molly Nash in chapter 11, or following chemotherapy, such as treatment for breast cancer or leukemia.

The hematopoietic stem cell is involved in the process of hematopoiesis, the process by which a single stem cell develops into the white blood cells involved in fighting infections, red blood cells involved in carrying oxygen, and platelets involved in blood clotting. Also present in the marrow, and occasionally used therapeutically, is the bone marrow stromal stem cell that can give rise to the other kinds of cells one finds in bone and bone marrow, like bone, cartilage, and fat cells. The stroma (supporting structure) of bone marrow is derived originally from the fetal connective tissue, also known as the mesenchyme, and therefore these are known as mesenchymal stem cells.

Adult stem cells are more plastic, meaning that they can differentiate into more types of tissue-specific cells, than had been initially thought. The following examples provide evidence of this plasticity. Hematopoietic stem cells can also differentiate into neurons and other cells found in the brain, liver, and skeletal and cardiac muscle cells. Bone marrow stromal cells can differentiate into cardiac and skeletal muscle cells. Stem cells from the brain can give rise not only to neurons and other cells in the brain but also to blood cells and skeletal muscle cells.

One of the more interesting sources of human adult stem cells is liposuction fat. Marc Hedrick, now at Cytori Therapeutics, developed the strategy to identify adult stem cells in the fat obtained at the time of cosmetic liposuction. He and his group developed approaches to manipulate the cells' environments in the laboratory with various growth factors and other cell culture conditions and to alter their tissue fates with the goal of using these cells for tissue regeneration. This approach would provide the opportunity for individuals to donate to a bank of tissue-typed cells

to be matched with recipients, as well as to become their own transplant donor.

Use of one's own cells for regenerative therapies would reduce the transmission of diseases from donors to recipients. In addition, since these cells would not be "foreign" to the recipient, they would not be rejected by the patient's immune system. Patients who receive transplanted cells or tissues from other donors, except when the donor is an identical twin, will almost always risk rejection and therefore must be on chronic immunosuppression therapy. The drugs used to suppress the immune system have their own side effects, which can include physical changes, such as the round face seen with steroid hormones, as well as malignant processes, such as lymphoma.

Liposuction involves the insertion of a cannula into the subcutaneous fat and applying suction that removes the fat cells and associated cellular elements, sort of like a "vacuum cleaner" for fat tissue. Adipose (fat) tissue, like bone marrow, is derived from mesenchyme (fetal connective tissue), and its stromal supporting structure can be easily aspirated by liposuction and separated from the fat cells.

In 2001, Hedrick and his colleagues demonstrated that human liposuction material contained cells with the characteristics of stem cells that could be induced in cell culture to differentiate into populations with the properties of fat, bone, cartilage, and muscle. They subsequently named these cells adipose tissue-derived stem cells (ADSCs). Hedrick's group and others have extended these observations to include differentiation of ADSCs into cells with properties of cardiac muscle and neurons. Important in the tissue-specific differentiation of these ADSCs are not only the cells and specific growth factors but also the scaffolds, or three-dimensional synthetic materials, on which the ADSCs are seeded for culture.

In 2004, there were two reports of the use of ADSCs in repair of bony defects of the skull, first in mice and then in a seven-year-old girl. Michael Longaker at Stanford University is also interested in regenerative applications of stem cells from fat, and his group used these cells from mice to repair skull defects that were large enough that they would not heal spontaneously. They seeded their murine ADSCs onto a three-dimensional scaffold material fabricated from a complex organic structure coated with apatite, a calcium-phosphate lattice which is a component of tooth enamel and bone. The cells were then cultured for twenty-four hours before implantation into the mouse skull defects. By two weeks after implantation, these scaffolds were associated with significant bone for-

mation, and by twelve weeks, bone bridging was observed. These experiments provided evidence that fat-derived stem cells can mediate new bone formation.

Working with colleagues in Germany, Hedrick carried out a clinical trial of bone formation by fat-derived stem cells. A seven-year-old girl suffered a significant fragmentation of her skull following a fall. She also had a part of her skull removed to prevent excessive pressure inside of the skull due to increased brain swelling from her injuries. Additional complications resulted in resorption of more of the bone in her skull, and unstable fragments of bone placed her at risk for brain injury if the fragments were displaced. A surgical procedure was carried out that involved resorbable scaffold sheets, to which were applied a limited amount of bone from her hip, and ADSCs, prepared in two hours from fat taken at the time of the hip bone fragment removal, as well as fibrin glue prepared two days prior to the procedure, to hold the ADSCs in place. The cells and the fibrin were prepared from the patient. Using hip bone fragments in such a situation is the standard of care, but children have such a limited amount of bone for these procedures that when there are major defects, as in this girl, the bone grafting is frequently ineffective. Three months following the procedure, imaging of the girl's skull showed impressive skull calcification and bone healing. The investigators were unable to determine the contributions of the bone grafting and the ADSC transplantation. However, this is always the nature of early clinical trials in which one offers the standard of care but supplements it with the new therapy. These results show that no harm was caused by the ADSC transplantation in this patient. This work will lead the way for additional clinical trials of fat-derived stem cells in tissue engineering.

In 2005, Hedrick and Brian Strem summarized the advantages of fat stem cells compared with bone marrow: "(i) minimal morbidity upon harvest; (ii) clinically relevant stem cell numbers extractable from tissue isolates, potentially removing the need for in vitro propagation; (iii) stem cell frequency is significantly higher in adipose tissue compared with bone marrow (2% vs 0.002%); and (iv) higher proliferation rates than BM-MSCs [bone marrow — mesenchymal stem cells]."

Hedrick and Strem also noted misconceptions about ADSCs, specifically that they could be derived only from obese individuals, and individuals with diabetes would not have normal ADSCs. Their studies had shown that normal-weight individuals "have an equal amount of stem cells as overweight or obese patients." They also investigated fat

stem cells from patients with adult-onset, or type 2, diabetes and found that "these show no differences with the mesenchymal stem cell assays" and they "are capable of multilineage differentiation (towards bone and fat)."

Therefore, stem cells from liposuction fat will be available from all individuals, lean as well as obese, and they may have amazing potential in regenerative medicine. The investigators must continue to identify the best ways to guide their differentiation for clinical application. These fat-derived stem cells represent only one of many adult stem cell types being studied at this time.

Fat stem cells have been shown to give rise to fat, and not all of the fat in an area of the body subjected to liposuction will be completely removed. The remaining fat contains approximately 2 percent stem cells. The fat stem cells that are embedded in adipose tissue do not give rise to bone, cartilage, muscle, or neurons, but they do give rise to fat. Therefore, despite claims to the contrary, after liposuction, fat may regrow in the area from which it was removed. After all, regeneration of tissue lost by trauma — in this case, liposuction — is the purpose of stem cells!

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When one's own stem cells will not provide the tissue needed, stem cells that can develop into any tissue type would be desirable. Embryonic stem (ES) cells are derived from embryos when the cells are still undifferentiated, and each cell could develop into a complete organism. In other words, ES cells are totipotent.

Embryos used in this research typically are derived from those that have been generated in an in vitro fertilization (IVF) clinic and frozen for future use (chapter 12). These embryos have been donated by the parent or parents of record in the clinic through a variety of approaches. The embryos may have been provided by active informed consent with the parent or parents signing a consent form at the time of the donation. Alternatively, the parents may have "abandoned" the embryos, meaning they have no plans to use them for a future pregnancy and the contract they signed at the time of the generation of the embryo indicated that abandoned embryos may be used for research purposes.

The human embryos used to generate ES cells are typically cultured from the stage called blastocysts. Blastocysts (derived from the Greek *blas-*

tos, for bud) are hollow spheres filled with fluid, approximately four to six days postfertilization and 0.1 to 0.2 millimeters in diameter, and represent the stage at which the embryo enters and implants into the uterus. A blastocyst contains a total of approximately one hundred to two hundred cells and includes two types of cells. The trophoblast is the layer of cells surrounding the embryo and enclosing the fluid-filled cystic cavity referred to as the blastocoel, and the trophoblast will give rise to the placenta. The inner cell mass is a collection of approximately thirty cells that mound up in the blastocoel cavity and beneath the trophoblast. The inner cell mass will differentiate and will give rise to the embryo proper and eventually to the human individual. The cells of the inner cell mass are totipotent, sometimes referred to as pluripotent.

To generate ES cells, the cells of the inner cell mass are removed from the blastocyst and maintained in an undifferentiated state in a culture in the laboratory. ES cells can be induced to differentiate into a variety of tissues. In fact, since these cells are totipotent, it is thought that ES cells could differentiate into any cell and tissue in the body, if we only understood the appropriate culture conditions needed.

When the culture conditions are altered, the ES cells begin to clump together and differentiate spontaneously into what are referred to as embryo bodies. These embryo bodies may contain a variety of cell types, including visible "blood islands" with red blood cells. Researchers can alter culture conditions to facilitate differentiation into specific cell and tissue types, a process referred to as "directed differentiation." In one example involving murine embryonic stem cells, the embryoid bodies were cultured in a medium containing the following growth factors: insulin, the hormone from pancreatic islets used for treatment of diabetes; transferrin, an iron-binding protein; fibronectin, a protein on the cell surface involved in cell-cell interactions; and selenium, an essential nonmetal element. The cells were then tested for the expression of the protein nestin, a marker of neural and pancreatic progenitor cells. Using defined growth factor media, or "recipes," the nestin-positive cells were induced to develop into specific cells and tissues, such as a particular neuronal type that secretes the neural transmitters dopamine and serotonin, or pancreatic islets that secrete insulin.

In November 1998, three independent groups announced findings involving isolation and characterization of human ES (hES) cells or related cells. James Thomson at the University of Wisconsin, Madison, and

colleagues reported in *Science* that they had isolated fourteen inner cell masses from donated embryos that they cultured to the blastocyst stage. From these, they generated five hES cell lines from five separate embryos, three with a normal 46,XY male karyotype, and two with a normal 46,XX female karyotype. They grew these hES cells on a mouse embryonic fibroblast feeder layer that had been irradiated so that the feeder layer cells could not divide but could still provide essential and incompletely understood growth factors and other critical components to the ES cells. This murine feeder layer is, however, one of the concerns for use of ES cells cultured in this manner: there is a risk that rodent viruses could have transferred to the hES cells and could pass to the human recipients of those cells. The hES cell lines expressed markers consistent with undifferentiated cells and showed the ability to differentiate into all three embryonic cell layers when injected into mice that had an abnormality in their immune system that prevented them from rejecting the human cells. They concluded: "Human ES cells should offer insights into developmental events that cannot be studied directly in the intact human embryo but that have important consequences in clinical areas, including birth defects, infertility, and pregnancy loss. . . . Screens based on the in vitro differentiation of human ES cells to specific lineages could identify gene targets for new drugs, genes that could be used for tissue regeneration therapies, and teratogenic or toxic compounds." The authors added that these hES cells could eventually facilitate cell-based therapies for disorders such as Parkinson disease and diabetes mellitus and could be genetically modified to prevent immune rejection, thus permitting therapies that would last a lifetime.

In 1998 John Gearhart, from Johns Hopkins University, and his colleagues published an article in the *Proceedings of the National Academies of Sciences, U.S.A.,* describing the production of a different kind of pluripotent human stem cell, called human embryonic germ (hEG) cells. These cells were derived from developing primordial germ cells retrieved from human embryos at five to nine weeks postfertilization, after therapeutic termination of pregnancies. In mice, it has been shown that ES and embryonic germ (EG) cells have very similar properties and pluripotency. From the thirty-eight hEG cell cultures that were initiated, thirty-six, or 95 percent, showed the characteristics of previously investigated pluripotent cell lines, including those from mice. The hEG cells could generate embryo bodies that included all three embryo tissue layers. They concluded that hEG cells would have applications very similar to those predicted by Thomson's group for hES cells.

The third group with a report in November 1998 was a biotechnology company in Worcester, Massachusetts, Advanced Cell Technology (ACT). The company's announcement was not in the scientific literature but in the *New York Times* and involved a very different strategy. ACT reported in its press release that it had fused the nucleus from an adult's differentiated cell to an enucleated bovine egg. The donor nucleus was from the somatic cell of a man from Texas who had been paralyzed in an accident that occurred when he was cycling. Researchers cultured the resulting zygote to an embryo made up of six cells. Their purpose in using bovine eggs was to make therapeutics more readily available than would be possible using human eggs. Robert Lanza, one of the ACT investigators involved in this research, countered the ethical concerns about their work: "Our intention is not to create cloned human beings, but rather to make lifesaving therapies for a wide range of human disease conditions, including diabetes, strokes, cancer, AIDS . . . Parkinson and Alzheimer disease." One of the issues with this approach is that the cells produced in this manner would have bovine, not human, mitochondria, and in addition to potential dysregulation of nuclear-mitochondrial genomic interactions, particularly with these interspecies genomes, there would be concerns about the rejection of such cells.

In August 2006, Lanza and his colleagues at ACT reported a new strategy for generating hES cells without destruction of embryos to obtain their inner cell masses. They extracted a single cell from an unused embryo using the same methods as for preimplantation genetic diagnosis (PGD, chapter 11). The cell was then cultured overnight and divided 58 percent of the time. From sixteen embryos, they extracted ninety-one individual cells and generated two hES cells. Lanza proposes that a woman who was submitting embryos for PGD would be given the option, rather than for the extracted cell to undergo genetic testing immediately, to have it cultured overnight and if it divided, then one cell would be used for testing and the other would be used for hES cell development. A technical issue that must be overcome is to ensure that the cell maintained in culture overnight would not degenerate and thereby not be available for genetic testing. ACT reported: "To date, over 1,500 healthy children have been born following the use of PGD." Many in the media responded that no study of children born after PGD, of the types of studies we described in chapter 11 for assisted reproductive technology, had been reported, and therefore there was no evidence of PGD's safety. We believe that such a study needs to be done

so that those desiring PGD can be fully informed. Those like the Nash family (chapter 11) may see no other choice than PGD, and ACT's approach would have no greater risk than PGD if the technical challenges are overcome.

A team led by Woo Suk Hwang at the Seoul National University reported in the journal *Science* in May 2005 that they had used somatic cell nuclear transfer (SCNT) to generate cloned hES cells. Hwang became a national hero, even hosting a visit to his laboratory by the Korean president and first lady. Hwang, however, responding to intense pressure to achieve success in this project, had fabricated the results for the 2005 paper. As a consequence, he was fired by the university and sued by egg donors, who claimed they were not informed of the risk. Hwang was indicted in May 2006 for embezzling research funds totaling $3 million U.S., committing fraud by applying for research funding with data he knew to be fabricated, and violating a statute that forbade purchasing eggs for use in research.

Another possible approach to generating hES cells is parthenogenesis. Parthenogenesis (from the Greek *partheno-*, virgin, and *-genesis*, birth) is a process by which an oocyte gives rise to a zygote without being fertilized, therefore generating hES cells without embryo destruction. It could involve an early enough stage in oocyte development before the chromosome number is reduced from forty-six to twenty-three, or another mechanism to get development of an egg with forty-six chromosomes. The cell is cultured in a way that development proceeds without chromosome reduction and then is stimulated in a manner that will simulate fertilization for the egg. This stimulus can involve chemicals, an electrical current, or some other mechanism. The oocyte then develops into a zygote and an early embryo.

Parthenogenesis has been carried out successfully in mice, nonhuman primates, and humans. In mice, a group from Japan and Korea used an innovative strategy; they took the nuclei from two eggs that had undergone chromosome reduction in preparation for fertilization. These two eggs were at different stages of development, and the parthenote was generated by nuclear transfer to restore the chromosome number to the normal number for a mouse somatic cell. Only two parthenotes survived, representing less than 0.6 percent of embryos transferred to surrogate mothers and 0.4 percent of eggs generated. The two surviving pups had body weights similar to the controls, but those delivered dead, as well as those born alive that then died, were all growth-retarded. One of the sur-

viving pups grew to maturity and was able to reproduce successfully. The investigators were able to demonstrate that the genes of the parthenotes were appropriately imprinted.

The group from ACT, working with colleagues in several academic institutions, reported the derivation of a stem cell line from parthenogenesis of eggs from macaques, which are nonhuman primates. They began with seventy-seven macaque eggs, twenty-eight of which achieved the stage of mature oocytes after chromosome reduction, and 4 or 5 percent of the original eggs developed to blastocysts. After the inner cell masses of the four blastocysts were removed, only one stable cell line, or 1.3 percent success from original eggs, was obtained. This cell line had morphological and expression characteristics similar to other primate ES cells. These cells were induced to differentiate in culture to cells with a neuronal appearance, expression of a protein that is involved in neurotransmitter synthesis, and synthesis of the neurotransmitters dopamine and serotonin. When injected into the abdominal cavities of immunodeficient mice, the cells differentiated into teratomas with the three embryonal cell layers, including those with characteristics of intestine, bone, and skin, the latter with the cellular follicle at the base of a hair shaft and a sebaceous gland that would normally secrete an oily substance called sebum.

Ian Wilmut and the group at the Roslin Institute, where Dolly was cloned, have been granted a license by the United Kingdom's Human Fertilization and Embryology Authority (HFEA) to carry out research on stem cells derived from human parthenotes. This is only the fourth license in the United Kingdom for hES cell research. Wilmut's group will obtain immature eggs that have not undergone reduction division from women who are having surgery for reasons not related to problems with fertility. Their goal is to determine the conditions that will permit these less mature oocytes to begin to develop into embryos by parthenogenesis. They will then determine whether these parthenogenically generated stem cells are similar or even identical to hES cells.

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The methods of therapeutic and reproductive cloning could be combined with gene therapy to generate a "designer baby" with a new gene incorporated into its genome. We include this approach for the sake of completeness so that you understand this strategy if you should hear about it.

However, please recognize that this approach would have all of the health problems for the mother and the offspring that are currently encountered in reproductive cloning (chapter 12). In addition, we are unable to see any advantage of this approach over PGD (chapter 11). The principal use that we can see for this strategy would be genetic augmentation — adding a gene that was not previously present in the individual's genome. This genetic enhancement would bring additional ethical issues on top of those introduced by human cloning.

This approach would involve in vitro fertilization and culture of the resulting embryo to the blastocyst stage. The inner cell mass would be removed and the ES cells cultured. A new gene could be added to the ES cells using a variety of methods, including a viral vector, a complex with lipids, proteins, or both to coat the DNA and facilitate its cellular uptake, or naked DNA directly taken up by the cells. This strategy is, in essence, gene therapy (chapter 14) of the ES cells. Colonies would be grown from individual stem cells so that the populations would be "clonal," or derived from a single cell, and therefore each population of cells would be genetically homogeneous.

The genetically modified ES cells would then be used in a process involving reproductive cloning. A new egg, not the one used in the therapeutic cloning approach, would have its nucleus removed. The nucleus of the modified ES cell or the entire ES cell would be injected into the enucleated egg. This embryo would be allowed to develop for several days and then would be implanted in the uterus of a woman who would carry the pregnancy.

There are several scenarios for this process. The same mother could provide the embryo for the ES cells, the egg that would be the recipient of the genetically modified nucleus, and the uterus for carriage of the pregnancy. Alternatively, any two of the three components of this approach could be provided by the same woman, or all three components could be provided by different women.

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Policies regarding stem cells vary dramatically in different countries, as well as states and provinces within countries. We will examine policies in other countries and then in the United States. Some countries have very formal approaches to cloning. The United Kingdom, which provided a license to the Roslin Institute for research on parthenogenesis,

has broad authority under a 1990 law to regulate any human IVF or embryo research. Legislation in 2001, related to the HFEA, prohibited all reproductive cloning in the United Kingdom and required destruction by fourteen days of development for any embryos generated artificially. Antiabortion groups challenged these rules and the HFEA, but in March 2003 the House of Lords, while confirming the prohibition of reproductive cloning, ruled that the HFEA has licensing authority for investigations related to generation of embryos through SCNT and parthenogenesis. In March 2005, the Science and Technology Committee of the House of Commons issued a report that recommended lifting the current ban on genetic human embryo modification and human-animal chimeric embryos, as well as reconsideration of human reproductive cloning. Five of the eleven members on this committee issued a statement disagreeing with the report and distancing themselves from it. They stated that the report was "unbalanced, light on ethics, goes too far in the direction of deregulation, and is dismissive of public opinion and much of the evidence."

The United Kingdom is among countries considered to have permissive policies toward therapeutic cloning, meaning that various hES cell derivation approaches are allowed, including SCNT. Also included among countries with these permissive policies are Belgium, China, India, Israel, Japan, Singapore, South Africa, South Korea, and Sweden. Other countries have flexible policies, meaning that SCNT derivations are permitted only from fertility clinics, and there are often additional restrictions. Countries with these flexible policies include Australia, Brazil, Canada, France, the Netherlands, Spain, and Taiwan.

The United States has more restrictive policies related to the use of a limited number of hES cell lines in federally funded research, and the U.S. Food and Drug Administration (FDA) exerts authority over any therapeutic uses of cloned cells. However, on the other hand, there are no laws restricting research using cloned embryos or prohibiting reproductive cloning, and therefore work on cloning in the United States is limited to private biotechnology firms and university investigators with nonfederal funds. In November 2004, California voters passed Proposition 71, which will provide $3 billion in bond funding for stem cell work. This state-based initiative, which is being replicated and considered by other states, raises significant legal questions in a federal system. For example, will the FDA have authority over thera-

peutic cloning if the treatment is developed and maintained within California?

State laws that have been enacted and proposed vary considerably around the country according to a 2005 report in the *New England Journal of Medicine.* Only four states had legislation that explicitly permitted hES cell research: California, New Jersey, Connecticut, and Massachusetts, with California and New Jersey having state funding for this research. Eight states explicitly limited this research, including Arizona, North and South Dakota, Arkansas, Iowa, Indiana, Michigan, and Virginia. A number of states had bills pending that would permit, or prohibit, hES cell research. North and South Carolina had competing bills.

In 2005, the U.S. National Academies issued "Guidelines for Human Embryonic Stem Cell Research." These recommendations covered all hES cell lines and research using these cells derived from blastocysts generated for research using IVF or originally generated for reproductive purposes and subsequently obtained for research, as well as human SCNT into oocytes. The guidelines recommend an institutional oversight committee for this research; an institutional review board (IRB) to review procurement and "a full informed consent process before donation" with no waiver of informed consent permitted; adherence to standards of clinical care with no coercion of prospective donors to participate, no generation of excess oocytes, and no payment to clinics for procurement; compliance with all relevant regulations including those of the FDA; uniform guidelines for banking of hES cells approved by the IRB; and establishment of a national body to assess on a regular basis the guidelines and to permit continuing discussion of the issues surrounding human embryonic stem cells. These guidelines were intended to assist institutions, but they are also anticipated to assist state and national leaders in understanding the issues and setting policy.

Many individuals feel that guidelines need to be established to protect the donors as well as the researchers. For example, Douglas Melton, codirector of the Harvard Stem Cell Institute, noted that before beginning their SCNT research, he and his colleagues obtained permission not only from the appropriate institutions in which the research would be carried out, but also from the Middlesex County District Attorney, who would have jurisdictional authority over the venue in which the research would be performed. Presumably, this was obtained because individuals and groups opposing abortion have brought criminal charges against those whom they opposed, and some of these groups consider the zygote an

individual for the purposes of legal protection. Melton felt that the National Academies guidelines would have facilitated the process for their permissions.

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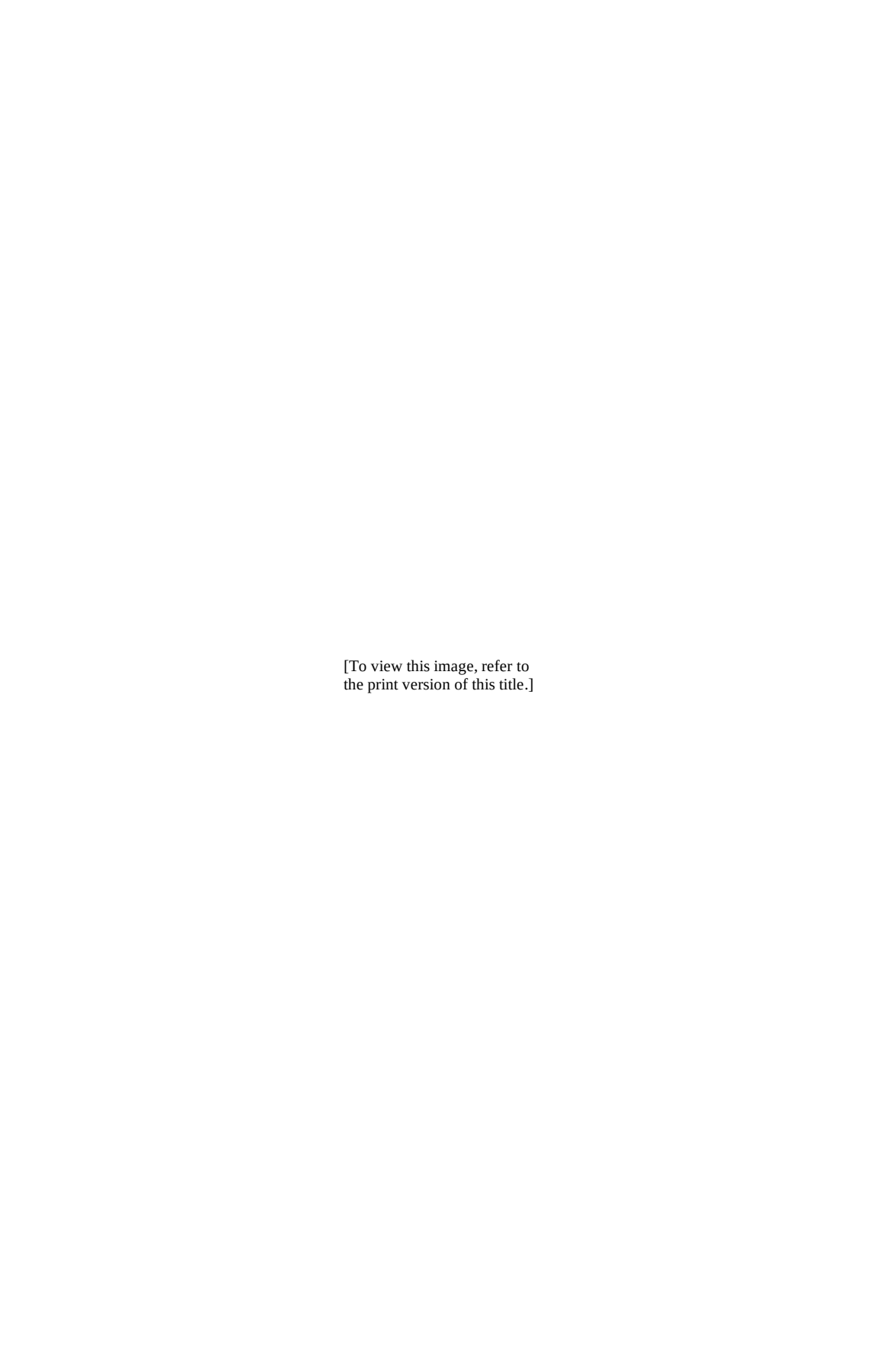
The area of stem cell research is highly contentious. The goal of this chapter is not to shape your opinion but to provide a factual basis on which to ground your opinion.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CHAPTER 14

Gene Therapy

Can the Promise Be Fulfilled?

Gene therapy involves the substitution of a normal genetic sequence in place of a disease-causing genetic sequence. A tremendous future promise for gene therapy has been suggested by many physicians and scientists over the last twenty to twenty-five years, and we were convinced by the early speculation. In the 1980s, when patients and their parents asked us how long it would be before gene therapy clinical trials would be available to help them with their metabolic disease, our response was that it would be five years. Then came the realization that we had been giving this five-year time frame for at least ten years. What is worse is that these were honest answers; we were as enthusiastic about this technology as the patients and their families.

Gene therapy clinical trials are ongoing, and there are definite benefits for certain disorders, as we will discuss. But gene therapy was described by high-level advisory committees in the 1990s as "more smoke than fire" and "more hype than substance."

Now patients and their families are told that there may be some individuals who will benefit from gene therapy, if the field can ever develop efficient and safe vectors. If that can be accomplished, then there may be benefits to specific groups of patients. We believe that stem cell therapies and regenerative medicine (chapter 13) will have broader clinical benefits than gene therapy, but perhaps our opinion is naive. Since regenerative medicine is a newer field, we do not yet know the frustrations that will face stem cell investigators. An examination of gene therapy and its history will assist all of us to understand regenerative medicine and other future therapeutic developments.

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Let's first consider the types of gene therapy. Gene therapy using a virus as the vector — the delivery vehicle — for the DNA involves insertion of the therapeutic gene into the genetic material of the virus, such that the viral genome now also includes and can express the therapeutic gene. This modified DNA is packaged into the virus, and an adequate dose of the virus is injected into the patient's circulation, from which it will home in on the tissue of interest. The virus will be taken up by the cells and deliver the therapeutic gene sequence to the nucleus. This therapeutic sequence will be used to synthesize ribonucleic acid and protein by the patient's cells.

Adenovirus is typically thought of as a respiratory disease virus, but it can enter and replicate in a wide range of cells and cause disease. This broad tissue tropism, or affinity, is one of the positive features of adenovirus as a gene therapy vector. The fundamental problem with viruses as vectors is that they contain proteins, for example, in their coats surrounding their genetic material, and these proteins are foreign to the recipient patients. Some patients will react violently to the viral proteins, as we will discuss in the death of Jesse Gelsinger later in this chapter. There are many types of viruses with unique properties, advantages, and disadvantages.

In addition to viruses, there are other approaches for the introduction of DNA into cells. DNA can be coated with lipids, proteins, or a mixture of the two. Signals can be added to the coating that cause the encased DNA to target a specific cell type and therefore a particular tissue. Another approach involves injecting naked DNA directly into tissues. This technique was originally used to inject the gene for dystrophin, which is mutated in boys with the X-linked progressive muscle disease,

Duchenne muscular dystrophy. When the DNA-encoding dystrophin was injected into dystrophic muscles, the DNA was taken up into the cells and the dystrophin protein was expressed in the cells. Unfortunately, the method was not sufficiently efficient to make a clinical difference in these patients.

Somatic cell gene therapy involves inserting DNA into the somatic cells, meaning cells that are differentiated, such as skin, bone marrow, and liver. Somatic cell gene therapy will only alter the genetic makeup of the individual receiving the therapy. This is in contrast to germline gene therapy, which would alter the genetic makeup of the individual's germ cells and therefore could be passed to future generations.

There are two other terms that are important in these discussions, *ex vivo* and *in vivo gene therapy*. Ex vivo gene therapy refers to genetic alterations of cells that are typically removed from a patient and manipulated in the laboratory to genetically alter the cells by introducing the new normal gene. Then the altered cells are returned to the patient. This is the approach used for gene therapy of hematopoietic—bone marrow—stem cells in patients with severe combined immunodeficiency (SCID; see chapter 8). In vivo gene therapy represents injection of the therapeutic vector directly into the patient, for example, into the circulation, and is the approach used in the clinical trial in which Jesse Gelsinger died.

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Let's look at early gene therapy in the United States. In 1974, the Recombinant DNA Advisory Committee (RAC) was established by the National Institutes of Health (NIH) to regulate all recombinant DNA (rDNA) research in the United States, and the RAC reported to the director of the NIH. Eventually the RAC would work with the U.S. Food and Drug Administration (FDA) to review all gene therapy protocols, with the FDA focusing on its mandate to review the safety and efficacy of the protocols. The RAC and FDA required preliminary approval from the investigator's institutional biosafety committee (IBC) and institutional review board (IRB), with the RAC then providing final review and approval before a gene therapy clinical trial could be initiated. All institutions with federal funding were required to adhere to these regulations, without regard to the source of funding or venue for the research.

The first reported human trial of gene therapy was carried out in 1980 by Martin Cline, a faculty member at the University of California, Los Angeles (UCLA), without the knowledge or approval of the UCLA

institutional review board. The trial involved attempted gene therapy for β-thalassemia, a hemoglobinopathy like sickle-cell disease (chapter 8) that provides some protection against malaria and is found at a substantial frequency in the Mediterranean basin. Cline tried to introduce a normal hemoglobin gene into the bone marrow of one patient with β-thalassemia in Italy and another in Israel. IRBs were not a part of the research infrastructure in Italy at the time, and the Israeli IRB was not fully informed of the proposed clinical trial. An article in the *Los Angeles Times* described this work, and Cline was censured by UCLA, the NIH, and Congress. The patients showed no evidence of normal hemoglobin expression.

Outcries regarding Cline's unauthorized trials, joined by national organizations representing Catholics, Jews, and Protestants, prompted the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research to consider the topic of gene therapy. Its report, "Splicing Life: A Report on the Social and Ethical Issues of Genetic Engineering with Human Beings," was released in 1982. This report concluded that responsible gene therapy research should continue, and reasonable individuals would be able to distinguish acceptable and unacceptable forms of gene therapy. They recommended, however, that the scope of RAC reviews should increase to encompass ethical and social issues involved in gene therapy trials.

In November 1982, timed to coincide with the release of "Splicing Life," a House of Representatives subcommittee, chaired by Representative Albert Gore Jr. (D-TN), held its first hearings on gene therapy. Gore later asked the congressional Office of Technology Assessment to develop a report on gene therapy. This report, "Human Gene Therapy: A Background Paper," was released in December 1984. The report concluded that somatic cell gene therapy was an extension of current therapeutic methods and had been accepted by all groups that had considered it. It was not a question of whether this form of gene therapy should be carried out, but when the safety and efficacy of this technology was sufficiently proved to move trials forward. The report also concluded that the popular opinion supportive of somatic cell gene therapy for genetic diseases did not extend to somatic gene therapy for genetic traits unrelated to disease or to germline gene therapy. It recommended that there be considerable public discussion before initiating germline gene therapy: "The risk to progeny, relative unreliability of the techniques for clinical use, and ethical questions about when to apply it remain unresolved."

Gore sponsored legislation to establish federal ethics advisory groups

that would give priority to discussions of topics in medical genetics and gene therapy. Although Congress provided a public forum to debate these issues, it did not pass any significant legislation in this area.

The first authorized gene therapy clinical trial was carried out in 1990 at the NIH, and the recipient was Ashanthi DeSilva, who had SCID (chapter 8). SCID is caused by a number of different genetic disorders, all of which result in profound abnormalities of the lymphocytes and cause patients to have frequent bacterial, fungal, and viral infections, resulting in pneumonia, diarrhea, and other life-threatening disorders. DeSilva had a specific form of SCID caused by mutations in the gene for an enzyme, adenosine deaminase (ADA), and the block in this enzyme-mediated chemical reaction causes a buildup of metabolites toxic to lymphocytes, which are infection-fighting white blood cells.

On September 14, 1990, Kenneth Culver injected DeSilva with 4 cubic centimeters, or less than a teaspoon, of lymphocytes that had been treated with a gene therapy vector containing the ADA gene. The crowd in the room at the NIH Clinical Center that day included the other principal collaborators on this project, R. Michael Blaese and W. French Anderson. The second patient to receive gene therapy from this team for SCID secondary to ADA deficiency was Cindy Cutshall, in January 1991. Both patients received "booster shots" of genetically modified lymphocytes. In an interview five years after DeSilva's first treatment, Culver and Blaese asserted their belief that the gene therapy trials had worked for both girls. DeSilva showed evidence of the ADA gene in 30 percent of her lymphocytes, and Cutshall had much less, but the investigators argued that there was enough to provide sufficient therapeutic effect.

The problem with interpretation of the SCID-ADA clinical trial of gene therapy was that these patients were already receiving treatment with a stabilized form of the ADA protein, called PEG-ADA, that appeared to be an effective treatment for their disease. These girls were kept on the protein therapy while they underwent gene therapy. Many investigators felt that it was impossible to sort out the effects of PEG-ADA and gene therapy. In fact, in an interview in 2000, after his move to the University of Southern California in Los Angeles, Anderson said, "There was initially a great enthusiasm [about gene therapy] that lasted three, four years where a couple of hundred trials got started all over the world. Then we came to realize that nothing was really working at the clinical level." Abbey Meyers, who was president of the National Organization for Rare Disorders, a federation of organizations advocating for rare or "orphan" diseases, was more indignant in 2000, when she said, "We haven't even

taken one baby step beyond that first clinical experiment. It has hardly gotten anywhere. Over the last 10 years, I have been very disappointed."

An ad hoc committee, appointed by then-director of the NIH Harold Varmus, the Panel to Assess the NIH Investment in Research on Gene Therapy, presaged the opinion of Meyers in its December 7, 1995, report and recommendations. The committee argued that there was too much "enthusiasm to proceed to clinical trials" with inadequate investigations of vector design and efficiency, or fundamental disease pathophysiology. In one particularly critical finding, the panel's report stated: "Overselling of the results of laboratory and clinical studies by investigators and their sponsors — be they academic, federal, or industrial — has led to the mistaken and widespread perception that gene therapy is further developed and more successful than it actually is. Such inaccurate portrayals threaten confidence in the integrity of the field and may ultimately hinder progress toward successful application of gene therapy to human disease." The panel had strong recommendations, including that the investigators and sponsors should "be more circumspect . . . regarding the aims and accomplishments of clinical protocols." Moreover, "the Panel insists on the adherence to rigorous standards of what constitutes appropriate and meaningful human experiments or clinical trials. Inadequacies of many clinical studies to date result from insufficient attention to research design, poorly defined molecular and clinical endpoints, and lack of rigor. . . . Relaxed standards are unacceptable and cannot be excused by unbridled enthusiasm for this treatment modality."

The panel's concerns about conflicts of interest, relaxed standards, overselling results, and unbridled enthusiasm would unfortunately prove to be prescient for Jesse Gelsinger.

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On September 17, 1999, Jesse Gelsinger, an eighteen-year-old with a rare metabolic disease, ornithine transcarbamylase (OTC) deficiency, died as a direct result of a gene therapy clinical trial. OTC deficiency is a metabolic disorder in which ammonia, the toxic product of protein metabolism, cannot be detoxified by the liver, and as the ammonia accumulates, it causes unconsciousness, coma, and death. OTC deficiency is an X-linked disease, meaning that it is seen almost exclusively in boys. In its classical and most severe form, the ammonia begins to accumulate when the umbilical cord is clamped and the ammonia can no longer be metabolized by the mother's liver after passage from the fetus to the mother across the

placenta. The accumulation becomes clinically evident in the first week of life, with vomiting, decreased responsiveness, coma, respiratory arrest, and death if untreated. Treatment consists of medications to help detoxify the ammonia and a diet with a carefully measured amount of protein. This regimen allows the child to grow but does not provide an excessive amount of protein, which is the source of the toxic ammonia.

There are milder forms of OTC deficiency, and Gelsinger's disease fell into this group. He was born on June 18, 1981, and was not diagnosed with OTC deficiency until March 1984. He was hospitalized at that time and on two other occasions with metabolic and central nervous system deterioration. These subsequent episodes included one in 1991, when he ingested more protein than usual, and another time, in 1998, when he was not compliant with his medication regimen, which required many pills, up to thirty-two every day. Following his 1998 episode, the metabolic geneticist who cared for him reorganized his medications, and he was symptom-free from his OTC deficiency, even during a severe case of influenza, which often triggers an episode in these patients.

Since Gelsinger had his disease under control with medications, his participation in the gene therapy clinical trial was for altruistic reasons. If successful, it might decrease his dependency on his medications and liberalize his diet, but he was really participating to help others. He had learned of the gene therapy work at the University of Pennsylvania when he was seventeen years old but also learned that he would have to wait until he was eighteen to determine whether he was even eligible. Gelsinger visited Steve Raper, the surgeon who would eventually administer the lethal adenovirus, four days after his eighteenth birthday and learned that he could participate. He told a friend in Tucson, right before he left on September 9 to participate in the University of Pennsylvania gene therapy trial, "What's the worst that can happen to me? I die, and it's for the babies."

The goal of the clinical trial in which Gelsinger was enrolled was to identify the maximum safe dose of the virus — the highest dose to optimize efficacy without major side effects. The principal investigators involved in this project, in addition to the surgeon, Raper, were James Wilson, director of the University of Pennsylvania's Institute for Human Gene Therapy, then the largest of the university-based gene therapy programs in the United States, and Mark Batshaw, a pediatrician who specialized in children with developmental disabilities and had worked with individuals who had OTC deficiency since he was a postdoctoral fellow.

The choice of who should be studied in this safety phase of the clini-

cal trial was debated in the University of Pennsylvania community. Typically — for example, in cancer chemotherapy clinical trials — the sickest individuals are the subjects in these safety trials, and Wilson proposed that the sick newborns participate. Batshaw noted that half of the affected babies would die within the first month, and half of those who survived the first month would be dead before they were five years old. However, Arthur Caplan, the University of Pennsylvania's chief bioethicist, disagreed, saying that informed consent was not possible with the parents of a dying child: "They are coerced by the disease of their child." Caplan recommended that subjects be limited to stable adults, including men like Gelsinger with milder disease, and carrier women.

When the protocol was reviewed by the RAC, the two primary reviewers, including Robert Erickson, ironically a faculty member at the University of Arizona in Gelsinger's hometown of Tucson, recommended that it be rejected. The experiments in mice with OTC deficiency were quite promising, but data on monkeys were extremely concerning. Three of the primates had died after severe inflammatory reactions in the liver and clotting problems, but the monkeys had received a "stronger" earlier adenovirus and doses twentyfold the highest dose in the human trials. The RAC was concerned that there was no prior human experience with direct injection of adenovirus into the circulation, and responses of human subjects were unknown. The investigators acknowledged the risks: bleeding as a consequence of the procedures involving the gene therapy injection; subsequent liver biopsy to determine efficacy that would be treated with surgery to stop the bleeding; and severe inflammation of the liver from the virus that would be treated with a liver transplant and could result in the subject's death. The protocol, however, was approved with at least one significant change. The original protocol involved injection of the virus via a catheter or a plastic tube directly into the right lobe of the liver, so that if there was a problem, the left lobe would presumably not be exposed to the virus. This approach, however, would expose the liver to a higher viral dose. The RAC required that the virus be injected into the bloodstream and not directly into the liver, but this was reversed by the FDA, and the RAC was never informed. The trial was approved for eighteen subjects, and nineteen individuals had volunteered. Gelsinger was the eighteenth and final subject in the trial.

The trial had escalating doses, with the earliest subjects getting the lowest doses. Gelsinger was the second of two subjects at the highest test dose. The seventeenth subject, a female carrier who went before him, had done well, though her virus and Gelsinger's were from different lots. On

September 13, the virus was infused by catheter into his liver, 30 cubic centimeters, or two tablespoons over two hours. The night after the infusion, Gelsinger had a fever, but prior subjects had also experienced fevers.

Approximately eighteen hours after the infusion, Gelsinger became disoriented and jaundiced—his skin turned yellow. The jaundice suggested either liver problems or excessive red blood cell breakdown as a consequence of an internal bleeding disorder. This problem had been seen in the monkeys and was quite concerning in an individual with OTC deficiency, since liver cell or red blood cell breakdown liberates protein from the patient's own cells. In essence, Gelsinger was getting a protein overload with his own protein, resulting in the risk of ammonia toxicity because of his OTC deficiency. Gelsinger's ammonia rose, and he was placed on a ventilator to breathe for him and on dialysis to reduce the amount of ammonia in his blood. His lungs began to stiffen and fail, so that he could not move oxygen across his lungs into his blood, and he was placed on equipment similar to a heart-lung machine used in open heart surgery to oxygenate his blood. By eighty-eight hours after the viral infusion, his liver and kidneys were clearly failing, and by ninety-four hours, testing showed that he had no evidence of brain activity—he was brain-dead. The physicians met with the family and recommended removal of life support. The family agreed, and Gelsinger was declared dead ninety-eight hours, or just over four days, after the infusion of the gene therapy vector. The family granted permission for an autopsy.

The autopsy showed evidence at the gross and microscopic levels of diffuse multiple organ–system failure. The lungs were filled with fluid and inflammatory cells. The liver showed hemorrhaging (bleeding) and necrosis (tissue breakdown) in all of the lobes, not just the lobe that received the infusion of the engineered adenovirus. Electron microscopy of the liver to look at extremely small structures showed no evidence of virus particles. The spleen showed necrosis affecting nearly the entire organ, with very little of the normal structure of the spleen remaining. The bone marrow showed an absence of the cells that become red blood cells, and there appeared to be a disturbance in white blood cell development as well. Other measures of inflammation showed that Gelsinger had an extreme inflammatory response. Review of markers of inflammation in other subjects in the trial indicated that the response was in proportion to the dose of viral particles, increasing with the escalating doses. The cause of death was attributed to systemic inflammatory response syndrome (SIRS), which is seen in many settings, including overwhelming infections, severe burns, and trauma.

The unexpected finding of SIRS was investigated further in animals after Gelsinger's death. It appeared that the protein coating, or capsid, of the viral particle activated the immune system. While there had been success in removing many of the native genes from the viral genome to attempt to reduce the immune response to the vector, it would be impossible to remove the capsid and have a functioning vector. It became clear that the experiments in animal models were comparable to humans in the transfection efficiency of the OTC gene, but the human subjects had much stronger immune and inflammatory reactions compared with the experimental animals. The investigators concluded that localized application of adenoviral vectors for gene therapy might be safe. They added: "One should proceed with caution, however, when diffuse dissemination of high dose of vector is anticipated."

A review of the other seventeen subjects involved in this clinical trial showed detectable expression of the OTC gene in liver biopsies of only seven of the seventeen subjects. Eleven of the subjects had OTC deficiency, and only three of these had "modest increases" in the metabolic activity of the ammonia detoxification pathway "that were not statistically significant." The investigators concluded: "The low levels of gene transfer detected in this trial suggest that at the doses tested, significant metabolic correction did not occur." Given the death of Gelsinger at the highest dose, evidence that the inflammatory response was dose-dependent, and the lack of therapeutic efficacy, it would appear that adenoviral gene therapy would not be a successful strategy for this metabolic disorder.

There ensued extensive investigations into the death of Jesse Gelsinger. The RAC met to discuss his death and the field of adenoviral gene therapy more broadly. Inder Verma, from the Salk Institute in La Jolla, California, cochaired the RAC. He said, "There really was no standard" in the field that was used to measure the strength or toxicity of vectors, or the desired end points of clinical trials. These same issues had been raised four years earlier by the NIH advisory committee but apparently went unheeded. Wilson reported duplicate sequences in the OTC vector not present in the original sequence. The RAC advised investigators to be more cautious in checking sequence integrity of their vectors — to be more sensitive to quality control — another point made by the NIH committee in 1995. The RAC also cautioned those infusing vector into specific organs to escalate the dose more cautiously because of the increased risk to the organs as the toxicity threshold was reached, a point made by Erickson in the original RAC review of this protocol and ignored by the FDA, the agency charged with concern for patient safety.

Additional investigations were carried out by the University of Pennsylvania, the NIH, and the FDA. In May 2000, after release of a report from an independent advisory committee to the university, the University of Pennsylvania shut down human investigations by Wilson's gene therapy institute. Conflict-of-interest concerns were raised, since Genovo, a company founded by Wilson, was heavily invested in developing liver gene therapy, reportedly having received $37 million in 1995 from Biogen for rights to market products in this area. The university moved its Center for Bioethics out of the Institute for Human Gene Therapy, questioning the wisdom of having its director, Caplan, report to an individual to whom the bioethicists were providing advice. The report also questioned the process by which bioethical decisions were made and called for a more formal process and review. The need for more formality in the process was based on the finding that Caplan's recommendations regarding the nature of the subjects, specifically stable adult male patients and healthy female carriers, for this safety phase trial was in an informal conversation rather than through any formal institutional review process.

Questions were raised about the adequacy of informed consent for subjects participating in the trial. The consent form, reported to have been reviewed and approved by the RAC, contained notification about the deaths of monkeys, one with problems similar to Gelsinger's. That information, however, was removed from the final consent form provided to the subjects. Adverse reactions in volunteers, including several with laboratory evidence of liver cell damage and one with more significant damage, were not added to the consent form, and the dosage escalation in the trial continued.

The FDA suspended all human gene therapy clinical trials in the Institute for Human Gene Therapy at the University of Pennsylvania. Adverse reactions had been suffered by four subjects in the OTC trial that met the criteria agreed to in advance and should have resulted in stopping the trial and notifying the FDA, but neither occurred. The FDA was also concerned about the elimination from the informed-consent document of the information about adverse events in the monkeys. Gelsinger's blood ammonia level before he began the study exceeded the limit set by the investigators, meaning that he should not have been given the virus. That blood ammonia limit had been raised by the investigators without informing the FDA.

In a letter to Wilson dated November 30, 2000, the FDA initiated procedures to have Wilson "disqualified" from clinical investigation because

he committed "repeatedly or deliberately violations governing the proper conduct of clinical studies." This action would prevent him from carrying out any research with human subjects in the United States that would be within the purview of the FDA. The FDA accused Wilson of not protecting the "rights, safety and welfare" of research subjects, and it said that he provided the FDA with "misleading and inaccurate" information. Batshaw and Raper were also sent letters of "warning" by the FDA, which alleged that they had also committed violations.

U.S. Attorney Patrick Meehan announced a settlement with the investigators and their institutions regarding a "civil fraud matter" on February 9, 2005. There were monetary penalties exceeding $1 million, as well as "restrictive controls on their clinical research activities" for the "three named investigators." Restrictions on Wilson were "more severe given his pivotal role as sponsor." The settlement agreement stated that the investigators and their institutions "do not admit to the government's allegations and contend that their conduct was at all times lawful and appropriate."

Gelsinger's family said that they initially trusted the physicians and did not consider them to have any blame in his death. But as information came forth from the government's investigations, and particularly regarding Wilson's apparent conflict of interest, Gelsinger's father, Paul, said, "This experiment was all about money, and it was never about money for Jesse." The family filed suit, and the University of Pennsylvania settled out of court.

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Now let's look at three disease types treated with gene therapy: SCID, chronic granulomatous disease, and hemophilia. Earlier in this chapter, we discussed the early gene therapy trials for SCID due to ADA deficiency in 1990 at the NIH. The efficiency of transfection and efficacy of gene therapy were quite poor at that time. Patients with SCID continued to have high morbidity and mortality, and "conventional" therapies had gained little control over the disease. PEG-ADA, prepared from the bovine protein, was considered to be incompletely effective and extremely expensive and was not an option for patients with the other numerous causes of SCID beyond ADA deficiency. Bone marrow transplantation was effective, but only if carried out early in the first year of life. Without a sensitive, specific, and affordable newborn screening program, which had not yet been achieved (chapter 15), very few infants with SCID were good candidates for bone marrow stem cell therapy (chapter 13).

For all of these reasons, there had been ongoing investigations to develop gene therapy for SCID, and it appeared that refinements in the preparation of the vector, the handling of the cells, and the management of the patients had improved this therapeutic approach. A number of reports appeared in the 2000–2005 period that showed efficacy of ex vivo retroviral gene therapy for X-linked SCID (SCID-X1), caused by a mutation in a lymphocyte (one type of white blood cell) surface protein that is critical for lymphocyte development, proliferation, and mobilization against infectious agents, and for SCID-ADA or ADA deficiency, like that in DeSilva and Cutshall. Lymphocyte counts and function were restored to normal, and clinical improvement was observed, including resolution of chronic diarrhea and skin infections, as well as the resumption of normal growth.

Just as it seemed that gene therapy would finally have a successful disease-example in the form of SCID, problems emerged in the clinical trials in France. On January 24, 2005, French authorities announced that, although seventeen children with SCID had been treated successfully, a third patient with SCID-X1, who had undergone gene therapy, had been diagnosed with a dramatic expansion of a lymphocyte population derived from a single cell, called a clonal lymphoproliferative disorder (CLPD), not unlike leukemia. In late 2002, two children in this clinical trial had developed CLPD, and one of these patients died in October 2004. It appeared that the retrotranscribed therapeutic DNA integrated in the patients' DNAs in regions near oncogenes, or genes that when activated cause cancer, and may have activated the clonal cell expansion in these patients. The trials were stopped after the initial two patients were diagnosed with CLPD but were reinitiated because of the efficacy of SCID gene therapy and pressures from the patients' families. After the third patient developed CLPD, the French authorities suspended all SCID gene therapy clinical trials for the second time.

None of the patients in British trials had developed CLPD, and their protocol used a different retroviral vector and culture conditions. All three of the patients who developed CLPD in France were patients of Alain Fischer at Necker Children's Hospital in Paris. He stated in an interview for the journal *Nature* that he would change vectors before he would treat any other patients. Fischer said, "The efficacy is there, but we have to improve on the safety." The FDA stopped three SCID gene therapy trials in the United States.

The second disease example is chronic granulomatous disease (CGD), a genetic disease in which a mutation results in the loss of a white blood

cell protein involved in the cellular production of hydrogen peroxide required to kill bacteria engulfed by these white cells. Individuals with CGD develop granulomas, or walled-off bacteria and fungi, that cannot be killed. Frequently the center of the granuloma is full of white cells and necrotic tissue and is described as suppurative, or full of pus. A granuloma can be thought of as a specific type of abscess. Patients with CGD frequently die of bacterial or fungal infections before they are thirty years old.

In 2006, Manuel Grez and collaborators reported the results of gene therapy in two men with CGD. They used retroviral vectors that provided treated cells with a proliferative advantage. Three weeks following infusion of the ex vivo manipulated hematopoietic stem cells, more than 20 percent of the patients' white blood cells expressed the therapeutic protein. At approximately four months after the infusion of cells, the treated cells underwent a dramatic expansion, so that the treated cells represented up to 60 percent of their circulating white blood cells. These cells also seemed to be effective in clearing the infections in these patients. One had a granulomatous lesion in his lung that healed, and the other had two liver lesions that resolved. One of the men was able to discontinue ongoing antibiotics used to prevent new infections.

Researchers in the United States expressed caution, however. They noted that the clones that had undergone expansion had the therapeutic gene inserted in one of three genomic locations near genes involved in cellular proliferation. If expansion became uncontrolled, then it could lead to a leukemia-like state, similar to that which led to the death of one of the French patients with SCID. Grez thinks that this will be unlikely, since the stem cell expansion has stopped. However, Donald Kohn, an investigator at the Children's Hospital, Los Angeles, said "it needs to be watched."

Our third example is hemophilia, a blood-clotting disorder caused by deficiencies of the proteins involved in the normal clotting cascade. In addition to external loss of blood from wounds and surgical sites, there can also be easy bruising and bleeding into muscles and joints. The joints most likely affected by bleeds, known as hemarthroses, are the major weight-bearing joints, including ankles, knees, and hips. Bleeding into the joints can result in not only significant swelling and pain but also decreased joint mobility and eventually degenerative arthritis. Hemorrhages into muscle can lead to tissue necrosis with the possibility of the released muscle proteins clogging the filtration system in the kidneys and causing kidney failure. Muscle bleeds can also cause contractures, from tis-

sue breakdown and scarring, and nerve damage, due to pressure on the nerves from the swelling of muscle tissue. The urine can become red from blood leaking from the kidneys into the urine, as well as from the released muscle protein myoglobin. Bleeding inside the skull, while relatively rare, can have dire consequences and may occur after very mild head trauma.

The two most common forms of hemophilia are both X-linked inherited disorders. The form seen most often is hemophilia A, caused by mutations in the gene encoding the blood-clotting protein, factor VIII (F8). The less common form is hemophilia B, caused by mutations in the gene for factor IX (F9). For hemophilia A, a relatively small increase in the residual F8 level has a major impact on the patient's phenotype. For example, an increase of circulating F8 level by 1 to 2 percent of normal can change the patient's clinical status from severe to moderate, and by 5 percent can change the status from severe to mild. Therefore, hemophilia was considered the ideal disorder for gene therapy: a relatively small increase in the expression of F8 or F9 would decrease the patient's need for exogenous protein replacement therapy; close regulation of protein production after gene therapy was not required; and the efficiency in terms of the number of cells transfected or the level of factor production in each cell could be low, as long as the total production was sufficient. Studies in mouse and dog models of hemophilia confirmed this speculation, and human gene therapy clinical trials for hemophilia A and B were begun. Pier Mannucci and Edward Tuddenham predicted in 2001: "Hemophilia is likely to be the first common severe genetic condition to be cured by gene therapy."

Strategies for gene therapy included in vivo and ex vivo approaches. In vivo gene therapy trials relied upon adenovirus, adeno-associated virus, and retrovirus. The size of the F8 messenger RNA is approximately 9 kilobases — 9,000 base pairs — which is quite large and exceeds the capacity of nearly all vectors except adenovirus. Animal experiments, however, showed that a truncated F8 could still be effective, and this shorter gene was accommodated more easily into the lower-capacity vectors. Injections into muscle were attempted, and while no side effects were observed, the production of the clotting factor was relatively low. Injections into a peripheral vein or directly into the major artery of the liver were included in the protocols. Ex vivo trials included nonviral and retroviral transfections of fibroblasts, the cells obtained from the patient's skin using a simple biopsy, and introduction of the treated cells into the omentum, the fat structure associated with the intestines in the abdomen. Unfortunately, the expression efficiency was insufficient for effective therapy.

There were complications associated with both the adenoviral and adeno-associated viral vectors. Patients receiving adenovirus had problems that will sound familiar from the Gelsinger OTC trial: fever, liver cellular damage, and blood-clotting abnormalities. Problems with the adeno-associated virus trials included detection of vector sequence in the semen and evidence of liver cell damage. A recent trial reported by Mark Kay of Stanford University and his colleagues in 2006 with F9 and adeno-associated virus had similar results. Therapeutic levels, were maintained for eight weeks and the vector was detected in semen.

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Germline gene therapy would involve altering the DNA in eggs or sperm. This could be accomplished by manipulating the genetic material of the newly fertilized ovum or a very early embryo. To achieve germline gene therapy by manipulation of the embryo after the single-cell stage, the manipulation would have to be sufficiently early that there would be a high probability that the cells giving rise to germ cells would be altered in the chimeric individual. The individual would be made up of cells with two distinct genomes, those that were treated and those that remained untreated. The later in development that the embryo was treated, the less likely it would be that significant germ cell chimerism would be achieved.

In addition to intentional germline gene therapy, there would be the possibility of inadvertent integration of new genetic information into the germline. Since all of a woman's eggs develop before she is born, whereas a male's sperm develop throughout his adult life, such inadvertent gene therapy would be more likely in a male than in a female. This would be a problem for in vivo protocols, but not ex vivo gene therapy. Inadvertent gene therapy was a concern when adenoviral vectors were being used, because of the extremely broad tissue tropism of the adenovirus. If injected into the circulation, there was a risk that it might transfect cells involved in the early developmental stages of cells destined to become sperm. We noted previously, in an adeno-associated virus in vivo therapeutic trials for treatment of hemophilia, that the vector sequences were found in the semen. These sequences were observed in specimen samples from all patients in these trials. Although vector sequences were not found in the sperm, the vector was most definitely transfecting cells in the gonads. If developing sperm cells were even a rare target, then adeno-associated virus could give rise to inadvertent germline gene therapy.

The Johns Hopkins Genetics and Public Policy Center published a

report on Human Germline Genetic Modification in 2005. The report identified specific scenarios for this therapeutic approach and looked at the technical feasibility and consumer demand. The report included cytoplasmic donation to the egg (chapter 11) for treatment of mitochondrial disorders, since this will alter the mitochondrial genome in the germ cells of the woman's offspring. However, since mitochondrial diseases are so rare, the report writers thought that there would not be a great demand for this, even though it is already available.

Among the scenarios that the Genetics and Public Policy Center considered involving genetic diseases that would not be amenable to preimplantation genetic diagnosis (PGD; chapter 11), and therefore might be considered for germline gene therapy, were the following. An autosomal recessive disorder with two affected parents would mean that 100 percent of their offspring would be homozygous affected. The example the resport cited was the progressive pulmonary disease cystic fibrosis, in which two homozygous parents would have only affected children. Although the report estimated the technical feasibility to be moderate, it would depend on the availability of efficient methods for making the genetic modification. Such approaches are feasible in mice and other model organisms, but they involve manipulation of embryonic stem cells, and we question whether these approaches are sufficiently efficient for use in human medicine at this time or in the near future. Alternatives to germline manipulation for these unusual situations that are available at this time include adoption and the use of donor eggs, sperm, or embryos.

The other genetic scenario the Genetics and Public Policy Center's report cited involved a late-onset autosomal dominant disorder with one parent being homozygous, meaning that both copies of the gene responsible for the disorders would have mutations. In this situation, 100 percent of the offspring of the homozygous affected individuals would have the mutation for this dominant disease and therefore would be at risk of developing the disease. The example the report noted was one parent being homozygous for mutations in the *BRCA1* gene, and therefore all of the offspring of that parent would be at risk for breast cancer and other cancers. The issues around technical feasibility would be the same as the prior example, but the demand would be quite low because of the rarity of these situations. The reason is that for most dominant diseases, homozygosity for the dominant mutation is extremely detrimental, with earlier onset and more severe disease. Therefore, only rarely would the homozygous individual survive to an age and be healthy enough to be able to reproduce.

The report also discussed the use of germline genetic modification in a genetic "vaccine," such as modifying one of the coreceptors, CCR5, required by the human immunodeficiency virus (HIV) for cellular uptake and infection. A polymorphism in the gene encoding the CCR5 coreceptor protein is known to protect individuals from HIV infection, so germline alterations could be used to protect those who did not already have the polymorphism naturally. The technical feasibility would be the same as for the genetic situations described above, that is, judged to be moderate. This was the only scenario in the report for which the authors thought that consumer demand might achieve a "moderate" level. While some might consider this type of germline modification an "enhancement," providing medical protection in this manner might be more acceptable than physical, mental, or behavioral enhancements. The Johns Hopkins investigators thought, however, that there might be nongenetic opportunities that would be less drastic and without the risk of unintended genetic consequences. Pharmaceutical companies are developing drugs targeting the CCR5 coreceptor for treatment of HIV, called entry inhibitors.

When the Genetics and Public Policy Center surveyed public opinion in 2004 regarding human germline modification, 57 percent of respondents would approve of this approach to avoid fatal childhood disease, and 51 percent to avoid adult-onset disease, but only 19 percent would use germline alterations to improve intelligence or strength. Clearly, medical interventions would be favored over enhancements.

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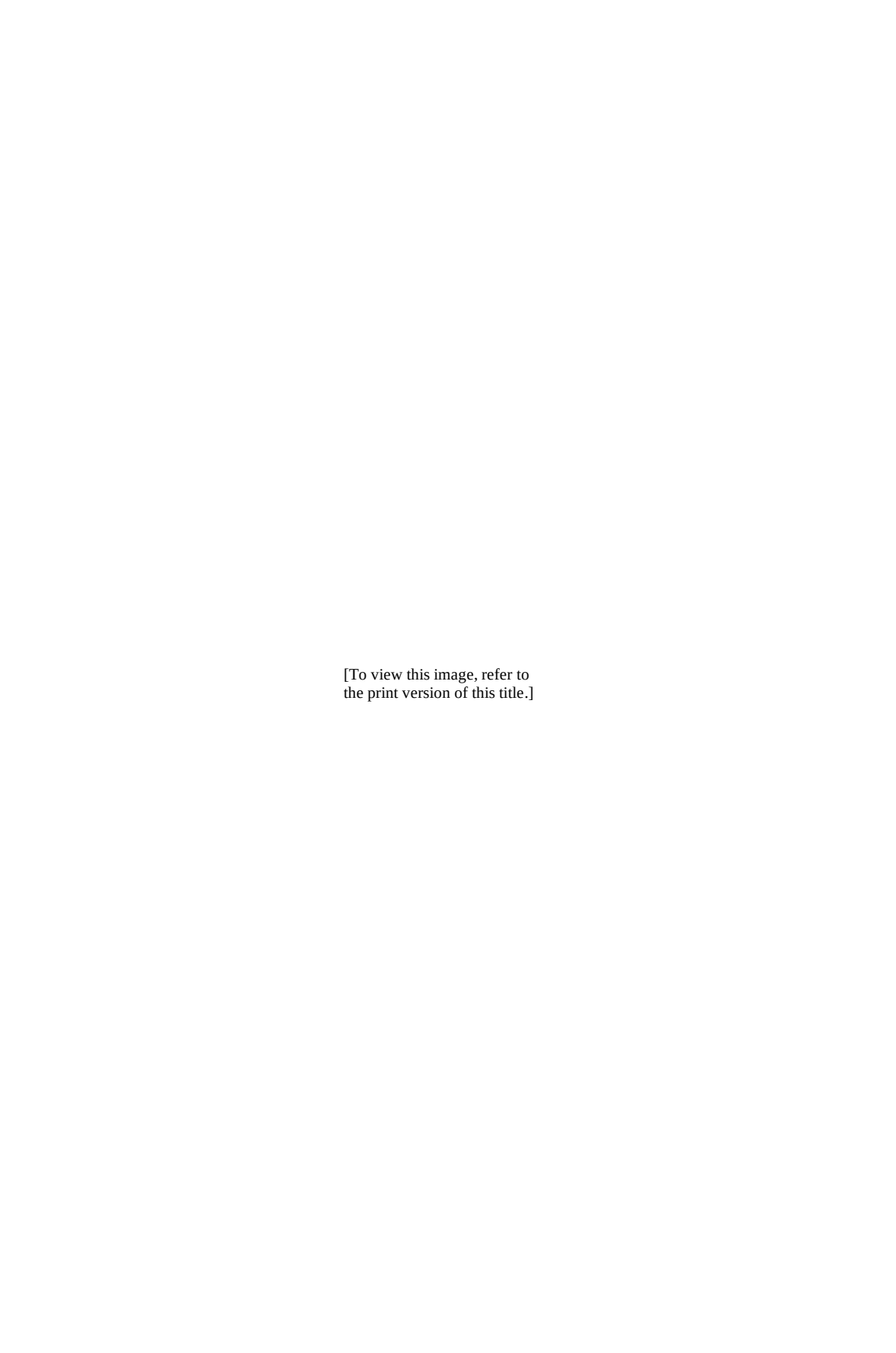
Somatic cell gene therapy is struggling and has yet to identify a completely safe strategy for transfection. However, investigators close to this technology still express remarkable enthusiasm for this approach. Success would have the potential for significant benefit, but our opinion is that such success will require a series of substantial incremental improvements or else a dramatically new conceptual approach.

Germline gene therapy has the same technical and far greater ethical concerns than somatic cell gene therapy, so it is not ready for clinical use at this time. What is really impressive about this topic is the opinion of the public, more than 50 percent of whom would accept germline modification for medical reasons, and nearly 20 percent would approve its use for enhancements. This is a dramatic change from ten years ago, when consideration of germline gene therapy was almost taboo. The rea-

sons for this shift are unclear, but it is tempting to consider as contributors the visibility of genetics through the Human Genome Project, the extraordinary enthusiasm of the investigators in this field that has been criticized in the past, and the public popularity of genetic determinism. It is the latter that creates the perception among the public that genetic treatments of diseases will be completely predictable and will have no possibility of unintended consequences, and enhancement of intelligence or strength is possible now or will be possible in the near term.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CHAPTER 15

Large Population Assessments

The Foundation for Genomic Medicine

Personalized genomic medicine
Newborn screening
Policy development in newborn screening
Population-based screening for genetic risk
Pharmacogenomics

The concepts that we discussed in this book are changing not only biology and law enforcement but also the practice of medicine. As we recognize the individuality of each one of us, we need to begin to tailor the medical management to the individual. In this chapter, we will explore how advances in genomic sciences are changing fundamental approaches to, and the very culture of, clinical medicine.

Medicine as practiced today is geared toward acute intervention when a clinical condition presents suddenly and unexpectedly, often with the patient in crisis. Patients with chronic illnesses, who would have succumbed to their disease in the past, are surviving, and the complications that they experience often are neither sudden nor unexpected. Yet these incidents are all too frequently addressed acutely, after they occur, and are not anticipated or addressed prospectively. It is time for a new paradigm in medicine.

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As a population, we are proud of the advances that have been made and celebrate these successes in film and television. Patients can be transported from the field to a hospital emergency department with care provided en route by well-trained paramedics who are in constant contact with physicians. A patient's vital signs and other data, such as an electrocardiogram, are communicated electronically. Transport vehicles include ambulances, helicopters, and fixed-wing aircraft, and the choice depends on the distance and urgency. The technological interventions available for patient management are extremely complex and effective and support survival of patients who would have died only a decade ago. Equipment is available and currently being tested that will permit robotic surgery, with the surgeon performing the procedure at a distant site from the patient. The culture of medicine is focused on acute intervention, and health care attracts many who are action-oriented and desire immediate gratification.

Genomic medicine, however, will be quite different — it will be predictive, preventive, and personalized. The ability to identify an individual's predisposition to disease will provide the opportunity to anticipate illness before its acute presentation. If the culture of medicine can adjust, then the possibility will exist to prevent the onset of disease, based on an individualization or personalization of medicine. This personalization will be sequence-based, but it will have to go beyond the individual's DNA sequence. Medications and doses will be selected based on the individual's genetic makeup. From previous discussions (chapter 1), it is clear that our unique experiences can be imprinted into our genomes and modify the expression of our genes. Therefore, genomic medicine will have to go beyond the individual's genomic sequence to interrogate the phenotypic consequences of the interaction between the genome and environment to personalize preventive medicine and medical care.

We are more than our genomic sequence, and we must recognize the limitations of using DNA sequencing to guide medical care. However, because the technology for sequencing DNA is so far ahead, this will guide the early phases of genomic medicine. This genomic approach to medicine will require the screening of populations to identify individual differences, to be able to predict disease predisposition, and to attempt to prevent the consequences. The developed world has had population-based screening since the 1960s, so let's explore the lessons learned from that experience.

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Nearly 100 percent of newborns in the developed world are screened for genetic diseases and other congenital abnormalities using relatively inexpensive laboratory methods. There are approximately 4 million births per year in the United States. All states and the District of Columbia screen for four disorders: congenital hypothyroidism, a deficiency of thyroid hormone associated with severe mental retardation and easily treated with thyroid hormone replacement; galactosemia, an inability to metabolize galactose, one of the subunits of the milk sugar lactose, which is associated with severe mental retardation, overwhelming bacterial infection, and liver disease if untreated and is treatable with a low-galactose diet; phenylketonuria (PKU), an inability to metabolize the amino acid phenylalanine, which is associated with severe mental retardation if untreated, but if treated with a diet restricted in phenylalanine and supplemented with tyrosine, the normal metabolic product of phenylalanine can result in essentially normal mental ability; and sickle-cell disease and related hemoglobinopathies (chapter 8). After these four disorders, the menu of diseases targeted by screening varies among the states. The number of genetic tests performed in newborn screening dwarfs all other genetic test volumes.

Despite the high volume of newborn screening tests, there is a lack of uniform policy across the United States. The March of Dimes Birth Defects Foundation publishes an annual Newborn Screening Report Card, which highlights the disparities. The American College of Medical Genetics recommends that every newborn be screened for twenty-nine diseases. This recommendation is supported enthusiastically by the March of Dimes and its president, Jennifer Howse, who stated: "All babies across America should receive the benefits of being screened for all of these 29 core conditions. Whether babies are screened and get the immediate treatment necessary to lead a healthy life should not depend on which state they are born in."

The Newborn Screening Report Card showed that in 2005 twenty-three states, representing 38 percent of U.S. newborns, screened for twenty of the core disorders, and only one, Mississippi, screened for all twenty-nine. There was a significant improvement in 2006, when thirty-one states, representing more than 64 percent of neonates, screened for more than twenty of these conditions. Five states — Iowa, Maryland, Mississippi, New Jersey, Virginia — plus the District of Columbia screened for all twenty-nine disorders in 2006, but this represented only 9 percent of U.S. births. The March of Dimes and others support a national standard for newborn screening. Howse reported: "Our advo-

cacy efforts are continuing at the state and federal levels. . . . For infants affected by these conditions, these screening tests can mean the difference between life and death, or health and lifelong disability."

We agree completely with the need for national uniformity in the newborn screening tests available to each baby. We would add that there should be standards for the testing technologies used in each state as well, because we have seen archaic methods used in some states, while others use the latest, highly accurate technology. We propose that these are critical policy issues that will extend more broadly as genetic and genomic screening programs expand in greater volumes beyond the neonatal period.

One individual had a major impact on newborn screening, developing the initial test and conceptualizing the program. Robert Guthrie at the State University of New York, Buffalo, had been measuring compounds in the blood of patients with cancer, using a technique he called the bacterial inhibition assay. This test involved a spot of blood on a filter paper disc that was placed on agar, a gelatin-like bacterial culture medium containing bacterial spores. The agar included a specific inhibitor for the compound of interest, so that the bacteria would not grow at normal concentrations of that chemical compound. But if the compound was elevated in the patient's blood sample, then the bacteria would grow in a halo around the blood spot. The size of the bacterial growth zone was proportional to the concentration of the compound.

Because one of his sons was mentally retarded, Guthrie became interested in PKU and other inherited metabolic diseases that caused serious developmental delays. He showed that his assay could be adapted to measure the elevated phenylalanine concentrations in blood samples from individuals with PKU. After overnight incubation of the agar plates and comparison of the growth zones around the blood spots with control paper discs containing known amounts of phenylalanine, elevated phenylalanine blood levels could be determined in a semiquantitative manner. The original purpose of the "Guthrie test" was to monitor the phenylalanine concentrations in blood samples from patients with PKU, who were on dietary therapy, to ascertain whether their dietary management was appropriate. If they were getting too much phenylalanine in their diets, then their blood levels would be elevated.

In 1961, two coincidences caused Guthrie to conceptualize newborn screening for PKU and other biochemical genetic diseases. He learned that his wife's fifteen-month-old niece with mental retardation had been diagnosed with PKU after a positive urine test for abnormal phenylala-

nine metabolites that was notorious for missing affected newborn babies. Guthrie tested her blood with his more direct test and it was positive. The second coincidence occurred when Guthrie traveled to visit members of his family in a small town in the province of Alberta, Canada. A physician in that community with whom Guthrie was talking about his work with PKU told him that he and a nurse tested every baby in his practice with the urine test. The College of Physicians sent every physician in Alberta the reagents for the urine testing.

Guthrie began to consider how one would screen all neonates for PKU using his test. It appeared that the earlier the restricted phenylalanine diet was initiated, the better the intellectual outcome, though in fact the data were only anecdotal at that time. Therefore, there was need for a test that was reliable earlier in life than the urine test permitted. All of Guthrie's work up to this time had been carried out with liquid blood specimens that were then soaked into discs of filter paper. He thought that drops of blood could be placed directly on the filter paper from a heel-stick with a lancet would spread by capillary action and could be dried. This would simplify sample collection, would foster stability of the phenylalanine in the dried blood specimen, and would permit samples to be mailed to centralized newborn screening laboratories. Using a quarter-inch-diameter office paper punch, he demonstrated that these dried blood-soaked spots gave quite accurate results. The dried blood specimens became known as "Guthrie spots." Guthrie told us that he was prouder of this form of sample collection than his test, since the test would be superseded, but dried blood collection would continue, as we saw with DNA forensic analyses (chapter 7).

Guthrie was interested in a screening program that would capture as close to 100 percent of the population as possible and would be early enough to initiate dietary management effectively. He determined that the blood phenylalanine concentration was sufficiently elevated in patients with PKU at the time of nursery discharge to be identified by the bacterial inhibition assay. Since nearly all babies were born in hospitals, he determined that taking specimens from all newborns at discharge from the nursery would be the optimal strategy.

Guthrie, with his extensive contacts in the mental retardation advocacy community, began to work locally and nationally, particularly with the National Association for Retarded Children — now the ARC — in what has been described as a "crusade" for newborn screening. What began as a program with two hospitals in Jamestown, New York, sending samples to his laboratory at Buffalo Children's Hospital in 1961, grew with state

ARC chapters and Guthrie campaigning for mandatory PKU newborn screening. Physicians and medical organizations generally opposed the legislation, concerned that there was not an evidence base to support the sensitivity or the measure of false negatives or misses with the test and specificity or measure of false positives or false alarms with the test. There were also concerns that some children would be harmed by unnecessarily restrictive diets. By 1966, however, the majority of states had mandatory newborn screening for PKU.

We will divide the history of newborn screening into three time periods: initiation, expansion, and consensus development, because these phases may be informative and accelerated in genomic medicine. The early phase lasted from 1961 to the mid-1970s and included the establishment of mandated screening for PKU throughout much of the United States. The other major accomplishment in this era was the consolidation of the newborn screening efforts in many states to centralized state or regional public health laboratories. PKU is a relatively rare condition, with a frequency of approximately one in 20,000 births and with all cases of increased blood phenylalanine, including benign conditions, totaling approximately one out of 12,000. The infrequency of actual cases in individual hospitals hindered quality assurance in a fragmented program involving hospital-based laboratories. In addition, it was recognized that newborn screening was not confined to laboratory testing, because without clinical follow-up there really was no point to the laboratory efforts. The integration of laboratory screening, or the analytic phase, with confirmatory diagnostic testing, clinical management and long-term follow-up, was considered to be more effective in consolidated programs based in state departments of health and academic medical centers.

The second discernible phase of newborn screening lasted from the mid-1970s until the early 1990s and involved expansion of the testing batteries. Newborn screening for congenital hypothyroidism was shown to be highly efficacious, and the costs for the analytical methods had been reduced sufficiently so that screening could be implemented on a large scale. Once state newborn screening advisory committees began to consider expanding testing panels from one disorder, PKU, to a second, congenital hypothyroidism, there was reason to consider further expansion. The automated equipment that punched and distributed discs from the dried blood specimens took four discs from a single blood spot. Therefore, programs frequently considered adding tests in multiples of four.

Newborn screening for the hemoglobinopathies was considered by a

number of states during this period, since preliminary evidence of benefit was available at the beginning of this period, the definitive trial was published in 1986, and the National Institutes of Health (NIH) consensus conference statement was issued in 1987 (chapter 8). The federal government stimulated states to initiate hemoglobinopathy screening with a $12 million infusion between 1987 and 1990. By the end of this second phase of newborn screening, the majority of states were screening for the hemoglobinopathies.

The expansion of screening panels that occurred during this period was carried out differently in each state. Some states used expert advisory committees and evidence-based decision making, while others relied on the political process and influential citizens and leaders who had specific agendas. As a consequence, the newborn screening experiences of babies born in different states varied considerably. There was no national consensus on what constituted an appropriate or even minimal disease panel, or what constituted appropriate testing technologies.

The third phase of newborn screening, from the early 1990s to the present, involved additional expansion in diseases and technologies and the beginning of consensus development regarding appropriate disease panels nationwide. At the beginning of this period the only uniformity was that all states tested for PKU and hypothyroidism, but some states screened for as few as three disorders and some for more than thirty. The technologies also differed dramatically. For example, to screen for PKU, some states used the Guthrie test developed in the early 1960s and others used state-of-the-art, technically sophisticated equipment.

Novel screening modalities were added during this period and included molecular genetic testing and tandem mass spectrometry, the latter based on fragmentation of molecules to determine their identities. Hearing screening was also added to identify deafness in neonates.

DNA confirmatory testing was an example of a two-tiered screening strategy. DNA analyses had been pilot tested in the late 1980s and early 1990s, and clinical trials, particularly of the value of DNA testing in hemoglobinopathy screening, had shown decreased time to diagnosis and more rapid initiation of penicillin prophylaxis for patients with sickle-cell disease (chapter 8). The repertoire of DNA tests expanded to include all of the common hemoglobinopathies detected by newborn screening in the United States.

These DNA tests are used as a second tier in newborn screening. For example, in the hemoglobinopathies, the first tier is a protein analytical method that determines the hemoglobin phenotype — the nature of the

abnormal hemoglobin protein. To confirm or to elucidate further the genetic abnormality, DNA testing can be carried out on the same original newborn screening blotter without having to take a second sample from the patient. Using an appropriate two-tiered strategy improves the performance of any screening program, reducing false positives without increasing the risk of false negatives.

Tandem mass spectrometry (MS/MS) is a technology that also was pilot tested beginning in the 1980s and, with its value established, began to be used more broadly in state and private newborn screening programs in the mid-1990s. MS/MS involves a series of two molecular fragmentation steps, with selected fragments of molecules from the first step being further fragmented in the second step. The unique weight and charge characteristics of the fragmented ions permit identification of "signature metabolites" specific to more than thirty individual genetic metabolic diseases that can be recognized in a single MS/MS run.

The disorder that provided the initial justification for MS/MS was medium chain acyl-CoA dehydrogenase (MCAD) deficiency, a disorder of fat metabolism. If unknown and untreated, children with this deficiency have episodes of low blood sugar, seizures, and coma associated with routine viral illnesses that lead to reduced oral intake and increased fat metabolism. The first episode typically occurs in the first or second year of life, and the risk of death with that initial episode is 20 percent. If a child with MCAD deficiency is detected by newborn screening, the family can be educated about what to do during these routine illnesses to protect their infant from dying. The treatment is quite straightforward and involves taking the child to the emergency department earlier than one usually would when a child refuses to eat and demanding that glucose be given intravenously. MS/MS is truly lifesaving for children with MCAD deficiency.

Newborn hearing screening involved moving to a new modality for testing by adding functional screening. Clicking sounds are delivered to each ear, and brain wave responses are measured by sensor electrodes on the baby's head. A child with a hearing problem would show an attenuation of one or more waves in the pattern. The strategy of testing neonates before nursery discharge was adopted from heel-stick screening as a mechanism for including as close to 100 percent of the neonatal population as possible.

The rationale for newborn hearing screening was based on pilot studies that showed earlier identification of hearing loss and improved language acquisition among those who were identified by screening, com-

pared with those who were identified by routine clinical assessment. For example, in a study of neonatal hearing screening in Colorado, 84 percent of those screened had their hearing loss identified by six months of age, whereas only 8 percent of those who were not screened were identified by that age. A review of ten years of experience in Austria also showed dramatic differences in identification and confirmation of hearing loss between the screened and unscreened populations. The age at which 50 percent were confirmed and intervention initiated showed these differences: by four and five months, respectively, for the screened group, and approximately thirty-seven and thirty-eight months, respectively, for the unscreened group. The age at identification was not influenced by the severity of the hearing loss for the screened population, but the degree of hearing loss was the best predictor of age at identification in the unscreened group. This is logical for the unscreened children, since one would anticipate that a more severely affected individual would be brought to a clinician's attention earlier. These data suggest that newborn hearing screening methods are able to identify those with mild hearing loss with the same facility that they can identify those with severe hearing loss.

The interest in neonatal hearing screening is not simply to ascertain and intervene, but like other forms of newborn screening, to improve the outcome of those babies who are identified early. The Colorado experience demonstrated better language outcomes for screened children in their study. The two groups, screened and unscreened, did not differ significantly in their cognition quotients or developmental quotients, which can be considered similar to the intelligence quotient (IQ), only for younger children. These groups did differ in their expressive language, or ability to generate words and combinations of words; receptive language, or ability to understand words and combinations of words; and total language quotients. The mean language quotients for the screened group were all above 80 and therefore were considered within the normal range. However, the means for the language quotients in the unscreened group with hearing loss were all less than 70 and were in the range for delayed language. If the distributions of these two populations are examined across language development categories, they also differ in a highly significant manner. Among those in the screened group, 56 percent had normal language development, but in the unscreened group, an even larger proportion, 68 percent, had delayed language development. These numbers do not tell the full story, however. The plasticity of the central nervous system means that if an individual is hearing-impaired and is not

using neurological pathways for receptive or expressive language, those pathways will be used for other purposes. Therefore, individuals identified late will be compromised permanently in their ability to understand and to generate language. They may never be able to obtain full language skills.

Cost-effectiveness studies have begun to assess the balance between costs for universal newborn hearing screening and the benefits from that screening program. Since these are new programs, the capital costs of equipment purchase are significant. Therefore, modeling showed the costs initially would exceed the benefits of a universal hearing screening program. However, the benefits would begin to exceed the costs after universal neonatal hearing screening had been operational for four years and could achieve an annual benefit of up to $7 billion after seventy-five years of operation. Another study that used a somewhat different modeling approach determined that a universal screening program's cost-effectiveness in the short term depended on the properties of the testing equipment, such as false positive and false negative rates. However, very importantly, the short-term cost-effectiveness also depended on the follow-up of those who failed the screening test, including maximizing the percentage of those infants with hearing loss who achieved language outcomes that were normal. Normal language outcomes would optimize the productivity of these individuals and increase the long-term benefits of the neonatal hearing screening program. Therefore, the reported problems involving delayed initiation of interventions after detection of hearing loss must be solved for the overall newborn hearing screening system to be improved. Long-term follow-up of babies identified by universal hearing screening needs to be carried out to determine the efficacy of early intervention and the extent of benefits to language and productivity long term. It would also be helpful to include second-tier DNA testing for genetic hearing disorders, since these results can determine appropriate interventions for specific disorders.

There are numerous other diseases that could be identified by newborn screening and for which modeling and experience suggest that testing will be cost-effective. A few among many examples include cystic fibrosis (CF), the progressive loss of lung function (chapter 14); congenital adrenal hyperplasia (CAH), an abnormality in response to stress due to a metabolic block in steroid hormone production, associated with masculinization of female genitalia (chapter 6); and severe combined immunodeficiency (SCID), resulting in an inability to respond to bacteria, fungi, and viruses and leading to death from overwhelming infections

(chapters 8 and 14). Each of these has the possibility of a two-tiered screening strategy, with the first tier involving evaluation of protein (CF and SCID) or metabolic (CAH) phenotype, and the second tier involving DNA analysis. However, a two-tiered approach for SCID is under consideration with DNA as the primary and a protein as the secondary screening test. As diseases are considered for addition to screening programs, the evidence base must be carefully evaluated to determine their appropriateness for newborn screening. Although panel expansion has typically occurred in increments of relatively small numbers, with MS/MS having one of the larger additions of thirty or more disorders, some authorities have suggested that future incremental additions may be in the hundreds.

Technology development will be required if newborn screening expansion is to occur at such a rate. One goal to consider would be a consolidation, and not expansion, of the technological platforms. With advances in biomedical engineering, it would seem possible to reduce the plethora of different methods currently used in newborn screening. The technologies being developed for high-throughput research in genomics and related disciplines should be applicable to newborn screening.

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Critical for disease panel expansion and technological innovation will be consideration of screening policies. We will review several relatively recent approaches to newborn screening policy development. Each builds upon the other, strengthening the recommendations that have gone before. These approaches should be informative as population-based screening expands beyond the newborn period.

The goal of the Newborn Screening Task Force, convened by the American Academy of Pediatrics and the federal government in 1999, was to consider the problems addressed previously in this chapter, specifically the lack of consistency in newborn screening disease panels and the test technologies used in the different states. The report of the task force called for a national agenda in newborn screening — that there should be a consensus on the diseases to be addressed and the technologies to be used nationally. The report also considered newborn screening as a system and not simply the test or testing laboratory. The report's authors recommended increased emphasis on education of professionals and the public prior to testing and follow-up of individuals who test positive, including long-term follow-up of management and outcomes.

The March of Dimes Birth Defects Foundation immediately expressed concern that the Newborn Screening Task Force had not gone far enough in its recommendations. Howse and her colleague Michael Katz argued that the task force "report would be more satisfactory if it examined systematically every available test, categorized these tests in terms of their quality, and then recommended steps toward assurance of quality control to provide national uniformity." They stated that the task force had been far too concerned about cost-benefit analysis of screening tests and should have focused more on what constituted "sound medical practice." They also took a stronger position on a national agenda in screening, calling for "mandatory uniformity among the states" and "an overarching authority that would ensure uniform quality of these tests."

The March of Dimes assembled a committee of experts to recommend a specific panel of newborn screening tests. This panel has grown over time, and the most recent list contains the twenty-nine disorders recommended by the Newborn Screening Expert Group. These included those that we have discussed previously: PKU, congenital hypothyroidism, sickle-cell disease and other hemoglobinopathies, MCAD deficiency, congenital adrenal hyperplasia, galactosemia, cystic fibrosis, and hearing loss. The March of Dimes is educating its local chapters and mobilizing them to demand this uniformity across states.

Under a contract with the federal government, the American College of Medical Genetics (ACMG), which is the education and policy arm of organized medical genetics, convened the Newborn Screening Expert Group to determine a uniform panel of tests to be adopted by all of the states. Members of the group asked individuals with expertise in newborn screening to rank conditions according to their priority for testing and then analyzed the evidence base to evaluate this prioritization. Those diseases that scored above a specific threshold in this process were designated the "core panel" and totaled twenty-nine disorders, twenty of which could be identified by MS/MS, such as PKU and MCAD deficiency, three hemoglobinopathies including sickle-cell disease, and six "others" such as congenital hypothyroidism, congenital adrenal hyperplasia, cystic fibrosis, galactosemia, and hearing loss. They also identified an additional twenty-five "secondary targets" that are variations on or related to the twenty-nine core conditions, and the Newborn Screening Expert Group felt these should be reported by the screening laboratory.

In addition to the recommended conditions, the Newborn Screening

Expert Group provided additional recommendations, including features such as quality improvement and quality assurance for the newborn screening system, standardization of reporting procedures and performance standards, national authority and oversight, and collection of follow-up data.

Newborn screening is the model for large-scale population-wide genetic screening programs. It is an initiative that was established as a completely new laboratory-based program in the 1960s and evolved fairly rapidly to be recognized as a system involving pretest education, screening, diagnostic confirmation, long-term clinical management, and quality assurance at all stages of the process. Newborn screening was recognized early in its history to be a public health initiative, and therefore, because the public health system in the United States is state-based, decisions about testing panels and technologies remained the responsibilities of individual states. It was not until the late 1990s that there began to be recognition of the need for national standards for newborn screening. This recognition was driven in part by the families of children in states with incomplete screening panels; those children suffered the consequences of diseases like MCAD deficiency, including death and severe mental retardation. These families learned that other states and private laboratories screened for these diseases, and they questioned why their state had not taken the initiative to protect their child. Mississippi was the first state to test for the core panel of twenty-nine disorders set by the ACMG Newborn Screening Expert Group and affirmed by the March of Dimes, and that achievement was directly due to the advocacy efforts of family members in Mississippi. Therefore, autonomy and activism are themes we see in newborn screening policy development, just as we have seen in other areas of genetics and genomics, and they will continue to play roles in determining disease targets for population-based screening programs.

Newborn screening had the nursery as an ideal venue in which to test nearly 100 percent of all babies. The venue for population-wide screening beyond the neonatal period is not so obvious. Perhaps this will be easier in countries with nationwide single-payer medical systems and centralized electronic medical records. Countries with those forms of health care may also have a medical culture more sympathetic to preventive medicine. Without changes in the economic drivers and fundamental culture of U.S. health care, inequities in access to genetic screening will remain.

An alternative to identifying a new venue would be to test for adult-onset diseases in the newborn screen. Policies currently recommend

screening in childhood only for those disorders in which intervention before adulthood could benefit the child. Therefore, neonatal testing for disorders with adult onset would be a significant change in the United States. Other timing opportunities for screening might also be possible. In Montreal, there has been a program in place to incorporate carrier screening for Tay-Sachs disease and β-thalassemia into the high school biology program. A twenty-year follow-up study showed that the students who were identified as carriers recalled and acted upon their results when it came time for their family planning. However, the legal, economic, and cultural differences between Canada and the United States would probably preclude testing in that venue. Minor children cannot consent to medical services in the United States, and there are concerns about social or economic stigmatization and genetic discrimination (chapters 8 and 10).

The sheer volume of newborn screening tests makes it a model that must be considered. With 4 million babies born in the United States each year and a core panel of twenty-nine tests recommended at this time, this would be a total of 116 million tests for genetic diseases annually. If the twenty-five secondary targets that are recommended for testing and reporting are included, then the total increases to 216 million tests per year. Therefore, for the near future at least, newborn screening is estimated to dwarf any other form of genetic testing.

Newborn screening was the pioneering system for predictive, preventive, and personalized medicine. The goal was to identify patients with laboratory testing before they manifested clinical signs and symptoms of their disease. By this predictive testing, therapeutic interventions could be initiated early to prevent the consequences of their disease. And these treatments had to be personalized to optimize the outcome for the patients. Newborn screening, therefore, is the model that must be carefully evaluated as health care begins to embrace genomic medicine.

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Principles of population-based screening have been developed by individuals and organizations. These principles consider the public health importance of the disorder, the quality of tests and interventions, and various features about policy development and implementation. The goal of population-based screening is to identify individuals with increased genetic susceptibility to a disease — those with higher levels of genetic risk.

Adult-onset diseases that meet the established public health criteria and

have significant genetic risks are the "single-gene" Mendelian disorders. Because these disorders have substantially lower frequencies than the common complex diseases like cancer, diabetes, and cardiovascular disease, research screening efforts for these relatively rare disorders will require the enrollment of sizable numbers of participants. Therefore, these investigations will be much larger than usually considered in pilot clinical trials and, with the high number of enrolled participants, will appear similar to a population-based clinical study.

Hemochromatosis is an example of an adult-onset disorder for which appropriate large population studies were carried out to investigate the relationship between genotype and phenotype. There was an interest in DNA-based screening to identify individuals at risk for this disease, but this interest assumed that genotype would predict phenotype. However, if DNA-based screening had been initiated without the appropriate large population studies, then significant numbers of individuals might have been treated unnecessarily. Fortunately, the studies were performed and genetic deterministic assumptions did not drive the course of care for this disorder.

Hemochromatosis is an autosomal recessive disorder of iron overload that, if not treated by regular blood collections from the affected individual to remove the iron in the hemoglobin, will lead to disorders in multiple organ systems. These include liver disease from accumulation of iron in the liver cells, diabetes from iron deposits in the insulin-producing cells of the pancreas, heart disease from iron accumulation in the heart muscle cells, and bronzing of the skin from iron deposition close enough to the skin surface to be visible. Affected individuals also may have arthritis and fatigue. The frequency of hemochromatosis is stated to be approximately one in two hundred among those of northern European descent, but this often-quoted frequency is actually based on the frequency of genetic alterations, and as we will discuss, is probably a significant overestimate.

The gene mutated in hemochromatosis, *HFE,* was identified in 1996, and two sequence variants were observed at high frequency among those of northern European descent. These involved DNA alterations that resulted in the change of the amino acid cysteine (C) at position 262 in the HFE protein to a tyrosine (Y), which is written as C262Y; and the change of the amino acid histidine (H) at position 63 to an aspartic acid (D), written as H63D. Among northern Europeans previously diagnosed with hemochromatosis, 80 to 90 percent were C262Y homozygotes, and many of the remaining individuals were C282Y/H63D compound heterozygotes. The frequency of the C262Y allele was determined

to be 10 to 15 percent among Caucasians, which definitely exceeds the 1 percent threshold for a polymorphism. The C262Y/C262Y homozygous genotype is estimated at approximately one in 150 to one in 200 in northern European populations, which would make hemochromatosis, on a genotypic basis, the most common autosomal recessive disorder among individuals in this ethnocultural group.

The sizes of study populations for hemochromatosis were variable. Perhaps the largest was the NIH-funded Hemochromatosis and Iron Overload Screening (HEIRS) Study that was designed to enroll 100,000 individuals. The study was planned with a goal of including diverse ethnocultural groups from five sites in North America and establishing early phenotypic changes for individuals with hemochromatosis and iron overload, optimizing strategies for screening to identify affected individuals and examining effects of genotype, sex, age, and ethnocultural group on the prevalence of the key phenotypic changes. The HEIRS study was intended to "permit formulation of screening recommendations for hemochromatosis, iron overload, or susceptibility to develop iron overload applicable to heterogeneous populations and may be pertinent to testing for other heritable adult-onset disorders."

Results from the 99,711 participants, who were recruited over two years, showed that the C282Y and H63D alleles were most common in those described as whites in the study. The majority of C282Y/C282Y homozygotes in this study had elevations in measures of iron saturation in the blood. Among the men in this study, those who were C282Y/C282Y homozygotes were 3.3 times more likely to report liver disease, and those who were C282Y/H63D compound heterozygotes 1.7 times more likely, compared with participants without mutations. There was no increase in diabetes in those with *HFE* mutations. It should be noted that only the common northern European alleles — C282Y and H63D — were determined, so other sequence variations could be present that were not ascertained. This might be particularly true among those in the populations other than whites in whom testing indicated evidence of iron overload.

Other population-based studies have shown that the penetrance (the proportion of individuals with a sequence variation associated with an increased risk of disease who actually develop features of the disease) of the C282Y and H63D alleles is quite low. It is clear that these alleles may be associated with biochemical evidence of increased iron saturation, but the association of these alleles with clinical features of hemochromatosis is much less certain. The C282Y allele has been considered the more severe

of these two common northern European alleles, and yet a study in South Wales showed that only 1 percent of C282Y/C282Y homozygotes had clinical features of iron overload. An analysis of a group of previous clinical studies called a metanalysis concluded that "several large population-based studies have now shown that the penetrance of the C282Y/C282Y genotype is very low, indicating that C282Y homozygosity is a necessary but not sufficient factor in the causation of the disease." In addition to indications of genetic modifiers in the nuclear and possibly the mitochondrial genomes, there is also accumulating evidence for environmental modifiers, most prominently alcohol consumption.

Alcohol intake was known to be associated with increased iron saturation in the blood and iron concentrations in the liver. An association between "'excessive alcohol consumption' defined as > 60g/day" (translated from this weight of pure alcohol to more than four twelve-ounce bottles of beer, four glasses of wine, or six ounces of 80-proof spirits like whiskey or vodka) and significantly elevated levels of iron saturation was observed among C282Y/C282Y homozygotes, but there was no increase in clinical symptomatology in these individuals. However, among homozygotes for the C282Y allele who were identified clinically and had a liver biopsy, the rate of serious liver disease visible at a microscopic level was nine times higher among those who consumed more than 60 grams per day of alcohol: 66 percent of the higher-consumption group versus 7 percent among those who consumed less.

Therefore, for patients with hemochromatosis, it appears that the simple *HFE* genotype is only part of the story. There are additional genetic and environmental modifiers. For those individuals who are not of northern European descent, there may be other *HFE* alleles and other genes that may increase their risk of hemochromatosis. In addition, it is essential to recognize that the common northern European sequence variations in the *HFE* gene are extremely poor predictors of phenotype, because their penetrance is so low.

Large population studies are required before broad-based genetic screening should be recommended. Such screening should not be based solely on preliminary data from a population defined by the presence of clinical symptomatology in individuals who underwent DNA testing after clinical diagnosis, because such information can be extremely misleading. When a disorder is ascertained prospectively by a laboratory test, rather than by clinical evaluation after manifesting signs and symptoms of the disease, it is generally found that there is more clinical variation than had been recognized previously. Hemochromatosis demonstrates this clinical

variability and shows why carefully constructed, large-scale population-based studies are required to establish an evidence base for the practice of genomic medicine.

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Adverse drug reactions (ADRs) are a huge problem in the United States. In 2000, the Institute of Medicine estimated that ADRs cause 7,000 patient deaths each year. Other studies have placed this estimate substantially higher. For example, in nursing homes, 350,000 ADRs are estimated annually. Among hospitalized patients, more than 2 million serious ADRs occur each year, and 6.7 percent of patients will have a serious ADR. With a fatality rate estimated at 0.32 percent, this means that more than 100,000 deaths every year result from ADRs. The FDA states: "If true, then ADRs are the 4th leading cause of death — ahead of pulmonary disease, diabetes, AIDS, pneumonia, accidents, and automobile deaths."

Pharmacogenomics offers one way to reduce ADRs, because certain individuals are genetically predisposed to serious side effects. Pharmacogenomics applies genomic concepts, information, and technologies to improve the safety and efficacy of drugs. As with other uses of the term *genomics,* this is an umbrella that covers ribonucleic acid (RNA) and proteins as well. For example, the role of differential gene expression on drug activity or the influence of drugs on gene expression would involve measurements of RNA. The evaluation of proteins and the related discipline of structural biology, which determines the physical structures of proteins and the influence of drugs and other chemicals on these structures, have become critical to efforts in rational drug design and drug discovery. A more logical and intelligent approach to pharmaceutical development is a goal that would supplant the traditional approach with somewhat random testing of hundreds of thousands of molecules to identify the best candidates for further examination.

The genetic variations responsible for an ADR with a specific drug may be quite rare. Pharmacogenomics, therefore, requires large population studies to examine correlations between drug responses, including the relationship between toxic side effects and individuals' genetic variations. One example is the use of single-nucleotide polymorphisms (SNPs) to correlate with drug responses and toxicities. These SNPs may alter an amino acid that may affect activity or stability of a drug-metabolizing enzyme or a drug receptor and, consequently, alter responses to that drug or may simply be a marker for another genetic change of this type nearby

in the genome. Major drug companies have been collecting SNP data since at least the mid-1990s, and in 1999 a consortium of large pharmaceutical companies and other groups had collected and published on the Internet more than a million SNPs. Many clinical trials include collection of DNA samples for compilation of SNPs and other sequence data. It has been suggested that the FDA may someday require such DNA data and association analyses with every new drug application. Banked DNA specimens may also be valuable if problems arise with a drug after it is released for use in the community, a situation that appears to be occurring more often.

Side effects are not purely probabilistic, that is, not everyone has an equal probability of experiencing a side effect, but certain individuals are at increased risk of specific side effects based on their genomic sequences. If the pharmaceutical industry and the FDA could move toward a more personalized concept of medicine, then drugs that are extremely efficacious and safe in the vast majority of individuals, but have a low incidence of adverse drug reactions, might continue to be available for those in whom these agents are safe and effective. Those at risk could be tested before they were exposed to the agent and protected from exposure to that drug and the ADR. The FDA recalls very good drugs if there is any significant increase in adverse drug reactions. Wouldn't it be better to retain useful drugs for those who will benefit and shield those at risk after determining that they had a DNA sequence variation that would place them in danger?

Let's look at one example of pharmacogenomic testing and consider both the technology and the economics. Economic factors are critically important in the adoption of innovative technologies into clinical practice, particularly in the United States. Kathryn Phillips and Stephanie Van Bebber from the University of California, San Francisco, carried out an evaluation of the resource allocation frameworks, including cost-effectiveness and cost-of-illness analyses and the economic value of SNP testing for the drug-metabolizing enzyme CYP2D6.

The cytochrome P450 (CYP) enzyme superfamily is a group of enzymes involved in the metabolism of drugs and toxins, particularly the members of two of the families, termed CYP2 and CYP3, within the superfamily. In December 2004, the FDA approved the Roche AmpliChip, a DNA microarray that is designed to test for inherited SNP variations in two CYP enzymes: twenty-nine polymorphisms and mutations in the *CYP2D6* gene and two polymorphisms in the *CYP2C19* gene. The combination of the alleles (usually but not always two) determines the phenotype with

respect to drug metabolism for each of these enzymes: poor, intermediate, extensive, or ultrarapid metabolizers for the CYP2D6 enzyme; and poor or extensive metabolizers for the CYP2C19 enzyme.

The CYP2D6 ultrarapid metabolizers are interesting because they represent a phenomenon that is being seen more and more often. Those in the ultrarapid group show evidence of gene amplification with more than the two anticipated alleles, but instead three to thirteen copies of the normal alleles that produce normally functioning enzyme. Since, these individuals have more normal enzyme, they have excessive drug-metabolizing activities compared with those individuals who have only two normal alleles and are in the extensive metabolizer group. Examples of gene amplification, as well as gene deletion, are being recognized in the "normal" population and will undoubtedly be found throughout the genome as we examine more individuals' genomes. As we have discussed previously, new tools redefine the extent of human variation.

Knowledge of an individual's drug-metabolizing status has practical importance in the dose selected for that individual. Approximately one hundred drugs are known to be substrates for CYP2D6, and these include medications used to treat clinical depression, psychoses, heart arrhythmias, high blood pressure, and cough. Cough suppressants metabolized by CYP2D6, such as codeine and dextromethorphan, have serious central nervous system depression at higher blood levels. The number of functional alleles can have a dramatic effect on the metabolism of the antihypertensive drug debrisoquine and the antidepressant medication nortriptyline.

In their health economics analysis of CYP2D, Phillips and Van Bebber considered the prevalence in the population of the mutation, drug use, and the condition for which the drug is prescribed. They included the costs for the drug and the condition being treated and the relationship between genetic variation in CYP2D and metabolic rate, drug response, and clinical outcome. They noted that there were considerable limitations in the available data and that the package insert of only one drug then on the market noted the availability of testing for CYP2D variations. They concluded:

> In the case of CYP2D6 testing, our analyses suggest that such testing is potentially relevant to large populations that incur high costs. The most commonly used drugs metabolized by CYP2D6 account for 189 million prescriptions and U.S.$12.8 billion annually in expenditures in the U.S., which represent 5–10% of total utilization and expenditures for outpatient prescription drugs. Almost 75% of these drugs are for heart disease or mental health conditions, which are highly prevalent and expensive to treat, with each condition occurring in approximately

25% of the population at an approximate combined cost of U.S.$300 billion including indirect costs.

They identified areas in which more information would be required for future studies, including "association of genetic variation and drug metabolism, response, and clinical outcomes as well as data on adverse drug reactions."

Phillips and Van Bebber added, regarding pharmacogenomics: "Finally, many commentators have also noted that large, prospective and well-controlled clinical trials will be required to provide the evidence-base necessary to change clinical practice and to better understand the nature of genetic variation."

In a subsequent publication, these investigators noted an "economic disincentive" for the pharmaceutical industry in pharmacogenomics and stratification of a population into subgroups based on genetics. This disincentive "is the decreased likelihood for blockbuster drugs, which are used in widespread groups rather than in smaller select populations." We have heard others argue that such stratification may contribute to a change in the industry with an increasing number of "boutique" drug manufacturers that are more focused and can survive with a different business model than the large manufacturers.

We will conclude our discussion of pharmacogenomics and CYP variants with a recent recommendation from the FDA regarding an anticlotting drug. Warfarin is an anticoagulant, or "blood thinner," that is used to prevent life-threatening blood clots, for example, in the lungs and brain. Originally isolated from moldy hay as the fungal compound that caused cows to bleed to death, warfarin is an effective poison for mice and rats. Because the therapeutic dosing is so difficult, warfarin is underused, even though it is estimated that for every bleeding episode induced by the drug, twenty strokes are prevented by its use. Warfarin is metabolized by CYP2C9, and more than 30 percent of European and Caucasian individuals have one of two variant alleles, CYP2C9*2 and CYP2C9*3, which reduce warfarin metabolism by 30 to 50 percent and 90 percent, respectively. A meta-analysis by Simon Sanderson and colleagues from the United Kingdom and Australia showed patients with one of these two variants had daily warfarin doses that were up to 37 percent lower and a bleeding risk that was more than twofold higher compared to those who had the functionally normal CYP2C9* alleles.

Y. T. Chen from Taiwan and Duke University, working with a large international group of collaborators, investigated the interaction between

CYP2C9 variants and polymorphisms in the gene *VKOR,* encoding the enzyme inhibited by warfarin, vitamin K epoxide reductase complex 1. Missense mutations in *VKOR* had been identified previously in patients with two inherited disorders of blood clotting, one due to a deficiency of all of the vitamin K–dependent clotting factors, associated with life-threatening bleeding episodes, and treated with vitamin K, and the other due to resistance to the anticoagulant effects of warfarin.

Chinese patients were known to be more sensitive to warfarin, and Chen and his colleagues developed additional information to explain the interindividual differences in warfarin dosage as well as interethnic differences between Chinese and Caucasians. They identified CYP2C9*3 and two other sequence variants in their Chinese patients. These allelic variants were not present in any warfarin-resistant patients but more interestingly were present in some but not all warfarin-sensitive patients. They sequenced the *VKOR* gene and found allelic variants that correlated with warfarin resistance or sensitivity and explained that those sensitive patients that had only the normal CYP2C9*1 allele.

Based on data of this type, the FDA is recommending that when a patient is placed on warfarin, he or she should have a test that will identify sequence variants in the *CYP2C9* and *VKOR* genes. After a two-year period of this testing in a large population study, the data will be analyzed to determine if this testing should become a requirement rather than a recommendation.

As the work of Chen and colleagues shows, it will also be important to identify additional sequence variants in diverse ethnocultural groups. With the cost of DNA sequencing diminishing, the possibility of sequence-based pharmacogenomics will lead the way toward a more comprehensive genomic medicine that will include every individual and her or his unique sequence variants. Only when this is possible can it be assured that genomic medicine will not be exclusive but truly inclusive. In the area of pharmacogenomics, this will personalize drug therapies and save lives.

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The consequence of human genetic and genomic variation is that the large datasets are required to tease apart and understand this variation. Relatively rare sequence variations can cause severe problems for those who harbor them in their genomes. These investigations will demand large populations of clinical subjects and incredible volumes of data. If

you will accept the fact that each of us is a unique individual, with even identical twins having differences in gene expression profiles based on their individual experiences, then you might have to follow that logic to its natural conclusion. All of us may have to enroll in ongoing population-based genomic studies. If any of us decide not to participate, then those individuals will need to recognize that they may be excluding themselves from the benefits of genomic medicine.


[To view this image, refer to
the print version of this title.]

CHAPTER 16

Hidden Destiny

Unbounded by Your DNA

A synthetic view of the individual
The Human Genome Project provided information, not knowledge
Genetic determinism as paradigm
Your sense of identity is unbounded by genomics

In this book, we have attempted to refute the concept of genetic determinism — that individuals are the products of their individual genomes. We argue that the expression of your genome is altered by your experiences, and therefore even if the DNA in your genome could be sequenced, your future would not be known. Your destiny would remain hidden.

We have shown that genetic determinism infuses all of the areas touched by genetics and genomics. Yet there is absolutely no biological basis for such deterministic views. Identical genomes exposed to differing environments — and environments always will differ in some manner — will give different patterns of gene expression. These epigenetic changes can be long-lasting and are passed from cell to cell in that individual.

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Experiences can leave indelible memories with each of us throughout our entire lives. But our biological experiences can be just as indelible,

imprinted in our genomes. The Dutch Hunger Winter and the Barker hypothesis showed that experiences in the womb can alter an individuals' physiology throughout their lifetime. Subsequent molecular genetic investigations have shown that these influences become literally written in the genome, changing which genes are turned on and off. The imprinting phenomenon chemically modifies specific individual genes, crossing out or highlighting those genes, sometimes with indelible ink. Surely, there are more subtle effects of experiences and environment than the stress of starvation in the womb, and these also undoubtedly influence the reading of the genes of one's genome. Thus, it is not only philosophical or religious thought that tells all of us that we are more than the genome with which we were born. Science also shows that the genome is plastic and changeable by our experiences in ways that may be transient or permanent.

The consequence of epigenetic effects like imprinting is that all individuals are biologically unique. Identical twins in the present day and reproductively cloned individuals in the future are identical, or nearly identical, at the genomic level, but they are still unique. Identical twins experience gestation in the same mother at the same time, so one might anticipate that they would be exposed to the same nutritional environment. But, despite being in the same womb, very often there are differences in the birth weights between the two twins, sometimes quite significant. This indicates that identical twins experience discrepancies in access to nutrients and other factors important in their growth that are delivered by the maternal circulation across the placenta. These obvious disparities in size, as well as many other differences that are not so obvious but just as real and substantive undoubtedly result in dissimilarities in imprinting and therefore lifelong distinctions in gene expression.

If the scientific explanation for individuality of identical twins was not sufficient, then we should rely upon the reports of twins themselves. They consistently report that sometime in the middle of the first decade of their lives they begin to realize that they have an identity distinct from that of their twin. Interviews with unseparated conjoined twins who are genetically identical and share a common circulation, and perhaps even share certain organs, have very different personalities and interests — they recognize their uniqueness and individuality despite passing through life permanently attached to each other. These differences are not limited to their central nervous systems, which result in these concepts of uniqueness, but are present epigenetically throughout their individual cells and organs.

We propose that genetic determinism has the potential for serious consequences for personal liberty that we will explore briefly for human clones. If conjoined identical twins recognize their unique and separate self-identities, then an individual produced by reproductive cloning would be anticipated to have a unique identity clearly distinct from the person from whom he or she was cloned. Even if the process of reproductive cloning for that individual uses the same maternal womb as the original donor, the mother and her womb will be a different age, and her environment and experiences during the gestation of the clone will surely vary from those of the original pregnancy. In addition, the cloned individual, even if raised by the same parents, will grow up at a different time in the life course of the parents, will have a different position in the family birth order, and will have different historical experiences.

We have no knowledge of cloned humans, but we do have experience with cloned animals. They have their own personalities, and often they even have physical features different from those of the original animal from which they were cloned. It would be unlikely that human clones would have less independence of spirit than other cloned animals. We assert that any culture that would attempt to enforce conceptual identity upon, and deny the uniqueness of, two distinct individuals would have the intent to enslave the clone, and such action would be unacceptable. We argue that although the dominant and powerful members of such a culture might reinforce the conceptual identity of the original and the clone, it is doubtful that the clone would agree. The clone might be afraid to express an opinion, but would most assuredly recognize her or his independent identity. Those who enslave others always attempt to dehumanize the slaves, and cloning with the intent to enslave would be no different, though the vocabulary might be couched in scientific language, just as was the "progressive" language of eugenics. A synthetic view of the individual as a product of genome and experience represents a fundamental denial of genetic determinism.

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Genetics is a new discipline. Although the science of genetics and the practice of agricultural genetics have been around longer, human and medical genetics are the products of the second half of the twentieth century. New disciplines frequently begin with less nuanced concepts and more absolutes. This was clearly the situation among human molecular geneticists, including ourselves, who did not give up the concept of

genetic determinism until the late 1990s. We really believed — and we must consider it a belief, since there was ample evidence to the contrary among model organisms — that genotype was an absolute determinant of phenotype. This concept even seduced us into "going molecular" in our research.

Perhaps the problem was compounded by the erroneous concept of human exceptionalism, that is, model organisms may have modifier genes, but humans are special and our biology will not be complicated by modifiers. If one thinks even slightly more deeply about these issues, then this would argue that humans are simpler than "simple" model organisms. This "logical" extension is quite different from the usual conceptual consequence of human exceptionalism, which argues that humans are far more complex than other biological organisms.

As is typical among new disciplines, genetics has proceeded from the simple to the more complex. The science of genetics did not change, but the concepts evolved. We must always remember that science, though we may wish it to be absolute, is filtered by human thought. Science, including genetics, changes over time because it is a product of human concept and perception, and our concepts and perceptions evolve with culture. Science, as we can know it, is a construct of the human mind and as such has an enormous social component.

The Human Genome Project provided information, not knowledge. It is imperative that all of us understand how little we really know about genetics and genomics. The reason we have made this statement so strongly is that there is the significant risk that individuals will mistake information for knowledge and speculation for conclusion, with the very real possibility that false statements will be made as if they are facts.

Genetics and genomics are new disciplines, and the concepts are still nascent, and, in fact, as we have discussed, some of these concepts are incredibly naive and some will prove to be wrong. Some individuals have even said that the knowledge of genetics is so superficial at this time that they fear that the actions we consider to be based on science and firmly objective will be looked upon one or two hundred years from now as the products of superstitious behavior. We encourage any readers tempted to scoff at this idea to look at the medical practices of a hundred to two hundred years ago. What do you think about these practices and the concepts on which they were based? The "magical thinking" from one to two centuries ago appears truly incredible today. For example, one might consider

the mechanisms used to protect individuals from infectious diseases in the time before the germ theory was understood, or in the even less remote era before the availability of antibiotics. We hope that our concepts of genetics will fare better over time but fear that many may not.

The Human Genome Project and the sequencing projects for so many other organisms have given us a phenomenal amount of information. However, this sequence information is simply raw data; it is not knowledge. Knowledge involves the processing of data to generate an accurate understanding. We do not mean to belittle the remarkable accomplishments of the genome-sequencing projects. Considerable knowledge is already beginning to be gleaned from these projects. But we consider that an accurate understanding of genetics and genomics is still in a relatively rudimentary stage. Therefore, all of us must be cautious in the use of this relatively early knowledge.

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The examples discussed above, and throughout this book, indicate a commitment to the concept of genetic determinism by the genetics and genomics communities. This has been such a firmly held, staunchly supported, and broadly reaching idea that we might even consider it a paradigm, or a construct to which a field of inquiry is firmly committed and around which it is organized. Yet a paradigm may not be grounded in fact. A paradigm can often constrain a field because individuals refuse to consider alternative explanations. In his book *The Structure of Scientific Revolutions,* Thomas Kuhn argued that science is driven by paradigms, or structured ways of thinking that are shared broadly in the community. These paradigms provide the boundaries for that discipline and eventually are challenged when it is recognized that they do not account for all of the phenomena and become limiting factors blocking the advance of the discipline. Fortunately, however, paradigms do not last forever, and the absolutism of genetic determinism is beginning to crumble.

Genetic determinism can erroneously alter individuals' concept of their own identity. Equating or simply overidentifying individuals with their disease is an example of genetic determinism. This deterministic thinking is too often implied by the shorthand medical description of a patient: "a fifty-year-old male with Huntington disease" becomes "a fifty-year-old Huntington male"; or on daily rounds, when the patients are well known, "the Huntington patient" or "the Huntington disease." The

individual has been stripped of his identity beyond the disease, with no malevolence intended, but nevertheless the disease has been substituted for his humanity. Similarly, the individual with a disease, especially one as overwhelming as Huntington disease, may see her whole life, even in the presymptomatic phase, only through the lens of the disorder. Losing sight of her additional roles, for example, friend, child, parent, and coworker, is a form of genetic determinism — "I am my disease and only my disease." Some health care professionals have exceptional skill in avoiding these impressions and helping their patients see those qualities in themselves that transcend their disease.

• • •

We are products of all that happens to us. Our components include, but are certainly not limited by, our genomes. Science tells us that our genomes are incredibly plastic — literally molded by our environment and our experiences. We already know that one aspect of our biology, our nervous system, is also remarkably mutable — changing and learning — adding and subtracting connections, and even cells, as a consequence of stimuli and insults ranging from external forces such as trauma, to the inevitable processes of aging. Should we have expected less from our genetics than from our brain?

The concept of coevolution tells each of us that our interactions with our genomes, mitochondrial and nuclear, and the various components of our environment and experiences are dynamic, multidimensional, and extremely intricate. Therefore, the concept of coevolution gives us some initial, if still superficial, realization of our complexity.

Some have said that genomics provides humans with a way to begin to conceptualize our biological predestination. We hope we have convinced you that this is far from true. But please do not mistake these statements as our refutation of the importance of genetics and genomics in our futures. Genetics and genomics will change the very culture involved in the practice of medicine, not only by technological innovation, but also by changing medicine to be more predictive, preventive, and personalized.

Our goal has been to refute genetic determinism and all of its corollaries. Another goal, unspoken until now, has been to present this overview of genetics and genomics in order to expand your boundaries. By understanding the opportunities that are afforded us in this genomic

or postgenomic age, we hope that your concept of your own identity can truly be liberated. We hope that you will find your boundaries unbounded, while recognizing that your destiny remains hidden. As you consider the powerful tools of genetic and genomic sciences, we ask you to recognize the peril and celebrate the promise in this age of DNA.

Bibliography

Web sites cited were active as of March 1, 2007. Many Web sites used in the research for this book were no longer maintained at the time of editing and therefore have been deleted from this bibliography.

Chapter 1

BOOKS AND ARTICLES

Barker, D. J. P. The fetal origins of adult disease. Wellcome Foundation Lecture, 1994. In *Proceedings of the Royal Society of London* Series B, 262 (1995): 37–43.

Barker, D. J. Fetal origins of coronary heart disease. *British Medical Journal* 311 (1995): 171–174.

Barker, D. The midwife, the coincidence, and the hypothesis. *British Medical Journal* 327 (2003): 1428–1430.

Buchanan, M. *Nexus: Small Worlds and the Groundbreaking Science of Networks.* New York: Norton, 2002.

Dipple, K. M., and E. R. McCabe. Phenotypes of patients with "simple" Mendelian disorders are complex traits: Thresholds, modifiers, and systems dynamics. *American Journal of Human Genetics* 66 (2000): 1777–1786.

Eriksson, J. G. The fetal origins hypothesis — 10 years on. *British Medical Journal* 330 (2005): 1096–1097.

Hood, L., J. R. Heath, M. E. Phelps, and B. Lin. Systems biology and new technologies enable predictive and preventive medicine. *Science* 306 (2004): 640–643.

Kitano, H. Systems biology: A brief overview. *Science* 295 (2002): 1662–1664.

Roseboom, T. J., J. H. P. van der Meulen, A. C. J. Ravelli, C. Osmond, D. J. P. Barker, and O. P. Bleker. Effects of prenatal exposure to the Dutch famine on adult disease in later life: An overview. *Molecular and Cellular Endocrinology* 185 (2001): 93–98.

Sriram, G., J. A. Martinez, E. R., McCabe, J. C. Liao, and K. M. Dipple. Single-gene disorders: What role could moonlighting enzymes play? *American Journal of Human Genetics* 76 (2005): 911–924.

Weston, A. D., and L. Hood. Systems biology, proteomics, and the future of health care: Toward predictive, preventative, and personalized medicine. *Journal of Proteome Research* 3 (2004): 179–196.

WEB SITES

Human Genome Project

http://www.iitk.ac.in/infocell/Archive/dirnov3/science.html
http://www.cnn.com/SPECIALS/2000/genome/story/overview/
http://www.accessexcellence.org/RC/CC/watson.html

Chapter 2

BOOKS AND ARTICLES

Adams, M. D., G. G. Sutton, H. O. Smith, E. W. Myers, and J. C. Venter. Authors' reply to Waterston et al. *Proceedings of the National Academy of Sciences of the USA* 100 (2003): 3025–3026.

Collins, F. S., M. Morgan, and A. Patrinos. The Human Genome Project: Lessons from large-scale biology. *Science* 300 (2003): 286–290.

Cozzarelli, N. R. Revisiting the independence of the publicly and privately funded drafts of the human genome. *Proceedings of the National Academy of Sciences of the USA* 100 (2003): 3021.

de Chadarevian, S. The making of an icon. *Science* 300 (2003): 255–257.

DeLisi, C. The human genome project. *American Scientist* 76 (1988): 488–493.

Dulbecco, R. A turning point in cancer research: Sequencing the human genome. *Science* 231 (1986): 1055–1056.

Garrod, A. The incidence of alkaptonuria: A study in chemical individuality. *Lancet* 2 (1902): 1616–1620.

Gibson, G., and S. V. Muse. *A Primer of Genome Science.* Sunderland, MA: Sinauer, 2002.

Grady, D. 50 years later, Rosalind Franklin's X-ray fuels debate. *New York Times,* February 28, 2003.

Grossman, L. Rosalind Franklin — Mystery woman: The dark lady of DNA. *Time,* February 14, 2003.

International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 409 (2001): 860–921.

Judson, H. F. *The Great Betrayal: Fraud in Science.* Orlando, FL: Harcourt, 2004.

Khoury, M., L. L. McCabe, and E. R. B. McCabe. Population screening in the age of genomic medicine. *New England Journal of Medicine* 348 (2003): 50–58.

Maddox, B. The double helix and the "wronged heroine." *Nature* 421 (2003): 407–408.

———. *Rosalind Franklin: The Dark Lady of DNA.* New York: Harper Collins, 2002.

McCabe, E. R. B. Translational genomics in medical genetics. *Genetics in Medicine* 4 (2002): 468–471.

McKusick, V. A. A 60-year tale of spots, maps and genes. *Annual Review of Genomics and Human Genetics* 7 (2006): 1–27.

Olby, R. *The Path to the Double Helix: The Discovery of DNA.* New York: Dover, 1994.

———. Quiet debut for the double helix. *Nature* 421 (2003): 402–405.

Ridley, M. *Genome: The Autobiography of a Species in 23 Chapters.* New York: HarperCollins, 1999; with a new foreword in 2000.

Roberts, L. Private pact ends the DNA data war. *Science* 299 (2003): 487–489.

Shendure, J., R. D. Mitra, C. Varma, and G. M. Church. Advanced sequencing technologies: Methods and goals. *Nature Reviews Genetics* 5 (2004): 335–344.

Shendure, J., G. J. Porreca, N. B. Reppas, X. Lin, J. P. McCutcheon, A. M. Rosenbaum, M. D. Wang, K. Zhang, R. D. Mitra, and G. M. Church. Accurate multiplex polony sequencing of and evolved bacterial genome. *Science* 309 (2005): 1728–1732.

Shreeve, J. *The Genome War: How Craig Venter Tried to Capture the Code of Life and Save the World.* New York: Knopf, 2004.

Sulston, J., and G. Ferry. *The Common Thread: A Story of Science, Politics, Ethics, and the Human Genome.* Washington, DC: Joseph Henry Press, 2002.

Waterston, R. H., E. S. Lander, and J. E. Sulston. More on the sequencing of the human genome. *Proceedings of the National Academy of Sciences of the USA* 100 (2003): 3022–3024.

Watson, J. D. *DNA: The Secret of Life.* New York: Knopf, 2003.

———. *The Double Helix: A Personal Account of the Discovery of the Structure of DNA.* New York: Atheneum, 1968.

Watson, J. D., and F. H. C. Crick. A structure for deoxyribonucleic acid. *Nature* 171 (1953): 737–738.

WEB SITES

Century of Biology

http://www.epa.gov/owow/wetlands/restore/
http://www.hhmi.org/bulletin/dec2001/gleevec/

Human Genome Project

http://www.genome.gov/Pages/EducationKit/download.html
http://www.the-scientist.com/news/20031024/04
http://web.mit.edu/invent/n-pressreleases/n-press-03LMP.html
http://www.fplc.edu/risk/vol5/spring/cookdeeg.htm
http://genomesymposium.ucsc.edu/fs-1985SCW.html
http://genomesymposium.ucsc.edu/bio-sinsheimer.html
http://www.krellinst.org/csgf/deixis/2004/genome_1.cgi
http://www.genome.gov/page.cfm?pageID=10001772
http://www.genome.gov/10001356
http://www.bio-itworld.com/issues/2005/June/document.2005–06–13.9758503149
http://www.bio-itworld.com/archive/111202/horizons_venter.html
http://celera.com/

Kurzweil/Moore Law of Computing

http://www.kurzweilai.net/meme/frame.html?main=memelist.html?m=17%23593

Chapter 3

BOOKS AND ARTICLES

Allen, G. E. The social and economic origins of genetic determinism: A case history of the American eugenics movement, 1900–1940, and its lessons for today. *Genetica* 99 (1997): 77–88.

Barabasi, A.-L., and Z. N. Oltvai. Network biology: Understanding the cell's functional organization. *Nature Reviews Genetics* 5 (2004): 101–113.

Bates, B. R., A. Templeton, P. J. Achter, T. M. Harris, and C. M. Condit. What does "a gene for heart disease" mean? A focus group study of public understandings of genetic risk factors. *American Journal of Medical Genetics* 119A (2003): 156–161.

Bouchard, T. J., and M. McGue. Genetic and environmental influences on human psychological differences. *Journal of Neurobiology* 54 (2003):4–45.

Chen, S. Negotiating a policy of prudent science and proactive law in the brave new world of genetic information. *Hastings Law Journal* 53 (2001): 243–263.

Condit, C. M. How the public understands genetics: Non-deterministic and non-discriminatory interpretations of the "blueprint" metaphor. *Public Understanding of Science* 8 (1999): 169–180.

Condit, C. M., T. Dubriwny, J. Lynch, and R. Parrott. Lay people's under-

standing of and preference against the word "mutation." *American Journal of Medical Genetics* 130A (2004): 245–250.

Condit, C. M., A. Ferguson, R. Kassel, C. Thadhani, H. C. Gooding, and R. Parrott. An exploratory study of the impact of news headlines on genetic determinism. *Science Communication* 22 (2001): 379–395.

Condit, C. M., N. Ofulue, and K. M. Sheedy. Determinism and mass media portrayals of genetics. *American Journal of Human Genetics* 62 (1998): 979–984.

Crick, F. Looking backwards: A birthday card for the double helix. *Gene* 135 (1993): 15–18.

Dipple, K. M., and E. R. B. McCabe. Modifier genes convert "simple" Mendelian disorders to complex traits. *Molecular Genetics and Metabolism* 71 (2000): 43–50.

——. Phenotypes of patients with "simple" Mendelian disorders are complex traits: Thresholds, modifiers, and systems dynamics. *American Journal of Human Genetics* 66 (2000): 1729–1735.

Dipple, K. M., J. K. Phelan, and E. R. B. McCabe. Consequences of complexity within biological networks: Robustness and health, or vulnerability and disease. *Molecular Genetics and Metabolism* 74 (2001): 45–50.

Dipple, K. M., Y. H. Zhang, B. L. Huang, L. L. McCabe, J. Dallongeville, T. Inokuchi, M. Kimura, et al. Glycerol kinase deficiency: Evidence for complexity in a single gene disorder. *Human Genetics* 109 (2001): 55–62.

Epstein, C. Is modern genetics the new eugenics? *Genetics in Medicine* 5 (2003): 469–475.

Fukuyama, F. Second thoughts: The last man in a bottle. *The National Interest* (Summer 1999): 16–33.

Gilchrist, D., D. M. Glerum, and R. Wevrick. Deconstructing Mendel: New paradigms for genetic mechanisms. *Clinical Investigational Medicine* 23 (2000): 188–198.

Holtzman, N. A. Eugenics and genetic testing. *Science in Context* 11 (1998): 397–417.

Jaroff, L. The gene hunt. *Time,* March 10, 1989.

Kevles, D. J.. *In the Name of Eugenics: Genetics and the Uses of Human Heredity.* New York, Knopf, 1985.

Kirby, D. A. Extrapolating race in *GATTACA:* Genetic passing, identity, and the science of race. *Literature and Medicine* 23 (2004): 184–200.

——. The new eugenics in cinema: Genetic determinism and gene therapy in GATTACA. *Science Fiction Studies* 27 (2000): 193–215.

Looren de Jong, H. Genetic determinism: How not to interpret behavioral genetics. *Theory and Psychology* 10 (2000): 615–637.

Manek, N. J., D. Hart, T. D. Spector, and A. J. MacGregor. The association of body mass index and osteoarthritis of the knee joint. *Arthritis and Rheumatism* 48 (2003):1024–1029.

Marenberg, M. E., N. Risch, L. F. Berkman, B. Floderus, and U. de Faire.

Genetic susceptibility to death from coronary heart disease in a study of twins. *New England Journal of Medicine* 330 (1994): 1041–1046.

McGuffin, P., and M. Neilson. Behaviour and genes. *British Medical Journal* 319 (1999): 37–40.

Nerlich, B., R. Dingwall, and D. D. Clarke. The book of life: How the completion of the Human Genome Project was revealed to the public. *Health* 6 (2002): 445–469.

Pence, G. *Who's Afraid of Human Cloning?* New York: Rowman & Littlefield, 1998, esp. p. 168.

Ridley, M. *Nature via Nurture: Genes, Experience, and What Makes Us Human.* New York: HarperCollins, 2003.

Rose, S. Moving on from old dichotomies: Beyond nature-nurture towards a lifeline perspective. *British Journal of Psychiatry* 178 (2001): s3–s7.

———. *Lifelines: Biology Beyond Determinism.* Oxford: Oxford University Press, 1997.

Simoneau, J.-A., and C. Bouchard. Genetic determinism of fiber type proportion in human skeletal muscle. *FASEB Journal* 9 (1995): 1091–1095.

Sriram, G., J. A. Martinez, E. R. B. McCabe, J. C. Liao, and K. M. Dipple. Single-gene disorders: What role could moonlighting enzymes play? *American Journal of Human Genetics* 76 (2005): 911–924.

Stock, G. *Metaman: The Merging of Humans and Machines into a Global Superorganism.* New York: Simon and Schuster, 1993, esp. pp. 165, 168.

Stock, G., and J. Campbell, eds. *Engineering the Human Germline.* New York: Oxford University Press, 2000, esp. pp. 79, 85.

Zdravkovic, S., A. Wienke, N. L. Pedersen, M. E. Marenberg, A. I. Yashin, and U. De Faire. Heritability and death from coronary heart disease: A 36-year follow-up of 20,966 Swedish twins. *Journal of Internal Medicine* 252 (2002): 247–254.

Zierath, J. R., and J. A. Hawley. Skeletal muscle fiber type: Influence on contractile and metabolic properties. *PLoS Biology* 2, no. 10 (2004): e348.

WEB SITES

Systems Biology and Networks

http://www.systemsbiology.org/
http://www.nd.edu/~networks/
http://www.opte.org/

Twin Studies

http://www.apa.org/monitor/apr04/second.html

Behavioral Genetics

http://www.ornl.gov/sci/techresources/Human_Genome/elsi/behavior.shtml

Eugenics

http://www.csu.edu.au/learning/ncgr/gpi/odyssey/dolly-cloning/eugenics.html

Genetic Testing and Eugenics

http://www.jrn.columbia.edu/studentwork/race/2002/gene-shimo.shtml

The Media

http://news.bbc.co.uk/1/hi/sci/tech/805803.stm
http://archives.cnn.com/2000/HEALTH/06/26/human.genome.04/

Chapter 4

BOOKS AND ARTICLES

Amores, A., A. Force, Y. Yi-Lin, L. Joly, C. Amemiya, A. Fritz, R. K. Ho, et al. Zebrafish hox clusters and vertebrate genome evolution. *Science* 27 (1998): 1711–1714.

Angelini, D. R., and T. C. Kaufman. Comparative developmental genetics and the evolution of arthropod body plans. *Annual Reviews of Genetics* 39 (2005): 95–119.

Beja-Pereira, A., G. Luikart, P. R. England, D. G. Bradley, O. C. Jann, G. Bertorelle, A. T. Chamberlain, et al. Gene-culture coevolution between cattle milk protein genes and human lactase genes. *Nature Genetics* 35 (2003): 311–313.

Belloch, C., A. Querol, M. D. García, and E. Barrio. Phylogeny of the genus *Kluyveromyces* inferred from the mitochondrial cytochrome-c oxidase II gene. *International Journal of Systematic and Evolutionary Microbiology* 50 (2000): 405–416.

Bersaglieri, T., P. Sabeti, N. Patterson, T. Vanderploeg, S. F. Schaffner, J. A. Drake, M. Rhodes, D. E. Reich, and J. N. Hirschhorn. Genetic signatures of strong recent positive selection at the lactase gene. *American Journal of Human Genetics* 74 (2004): 1111–1120.

De la Cruz, N., S. Bromberg, D. Pasko, M. Shimoyama, S. Twigger, J. Chen, C. F. Chen, et al.. The Rat Genome Database (RGD): Developments toward a phenome database. *Nucleic Acids Research* 33 (2005): D485–D491.

Deschamps, J., and J. van Nes. Developmental regulation of the Hox genes during axial morphogenesis in the mouse. *Development* 132 (2005): 2931–2942.

Goodman, M., L. I. Grossman, and D. E. Wildman. Moving primate genomics beyond the chimpanzee genome. *Trends in Genetics* 21 (2005): 511–517.

Grand, R. J., R. K. Montgomery, D. K. Chitkara, and J. N. Hirschhorn. Changing genes; losing lactase. *Gut* 52 (2003): 617–619.

Harbison, C. T., D. B. Gordon, T. I. Lee, N. J. Rinaldi, K. D. Macisaac, T. W. Danford, N. M. Hannett, et al. Transcriptional regulatory code of a eukaryotic genome. *Nature* 431 (2004): 99–104.

Innis, J. Priming the search for *HOX* mutations. *Teratology* 65 (2002): 47–49.

Jaaro, H., G. Beck, S. G. Conticello, and M. Fainzilber. Evolving better brains: A need for neurotrophins? *Trends in Neurosciences* 24 (2001): 79–85.

Kellis, M., B. W. Birren, and E. S. Lander. Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae. Nature* 428 (2004): 617–624.

Kellis, M., N. Patterson, B. Birren, B. Berger, and E. S. Lander. Methods in comparative genomics: Genome correspondence, gene identification and regulatory motif discovery. *Journal of Computational Biology* 11 (2004): 319–355.

Kellis, M., N. Patterson, M. Endrizi, B. Birren, and E. S. Lander. Sequencing and comparison of yeast species to identify genes and regulatory elements. *Nature* 423 (2003): 241–254.

Lehoczky, J. A., M. E. Williams, and J. W. Innis. Conserved expression domains for genes upstream and within the *HOXA* and *HOXD* clusters suggests a long-range enhancer existed before cluster duplication. *Evolution & Development* 6 (2004): 423–430.

Liu, G., S. Zhao, J. A. Bailey, S. C. Sahinalp, C. Alkan, E. Tuzun, E. D. Green, and E. E. Eichler. Analysis of primate genomic variation reveals a repeat-driven expansion of the human genome. *Genome Research* 13 (2003): 358–368.

Mark, M., F. M. Rijli, and P. Chambon. Homeobox genes in embryogenesis and pathogenesis. *Pediatric Research* 42 (1997): 421–429.

McCabe, E. R. Clinical genetics: Compassion, access, science, and advocacy. *Genetics in Medicine* 3 (2001): 426–429.

McConkey, E. H., and A. Varki. A primate genome project deserves high priority. *Science* 289 (2000): 1295.

Modrek, B., and C. Lee. Alternative splicing in the human, mouse and rat genomes is associated with an increased frequency of exon creation and/or loss. *Nature Genetics* 34 (2003): 177–180.

Mouse Genome Sequencing Consortium. Initial sequencing and comparative analysis of the mouse genome. *Nature* 420 (2002): 520–562.

Nekrutenko, A. Identification of novel exons from rat mouse comparisons. *Journal of Molecular Evolution* 59 (2004): 703–708.

Olson, M. V., and A. Varki. Sequencing the chimpanzee genome: Insights into human evolution and disease. *Nature Reviews Genetics* 4 (2003): 20–28.

Rat Genome Sequencing Project Consortium. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* 428 (2004): 493–520.

Spitz, F., C. Herkenne, M. A. Morris, and D. Duboule. Inversion-induced disruption of the *Hoxd* cluster leads to the partition of regulatory landscapes. *Nature Genetics* 37 (2005): 889–893.

Wazshietl, S., I. L. Hofacker, and P. F. Stadler. Fast and reliable prediction of noncoding RNAs. *Proceedings of the National Academy of Science USA* 102 (2005): 2454–2459.

WEB SITES

Evolution

http://sci.waikato.ac.nz/evolution/EvolutionOfLife.shtml

The Copernican Revolution

http://www-groups.dcs.stand.ac.uk/~history/Mathematicians/Aristarchus.html
http://phyun5.ucr.edu/~wudka/Physics7/Notes_www/node41.html
http://www.phys-astro.sonoma.edu/people/faculty/tenn/CopernicanRevolution.html
http://www.phy.hr/~dpaar/fizicari/xcopern.html
http://www.ronaldbrucemeyer.com/archive/galileo2.htm

Comparative Genomics

http://www.bio-itworld.com/archive/040702/boston_it_secret.html
http://www.nature.com/nature/mousegenome/timeline/mouse_start.html
http://rgd.mcw.edu

Genomes to Genes

http://animaldiversity.ummz.umich.edu/site/accounts/information/Arthropoda.html
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=142989
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=140000
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=605432

Chapter 5

BOOKS AND ARTICLES

Aldhous, P. Geneticist fears "race-neutral" studies will fail ethnic groups. *Nature* 418 (2002): 355–356.

Bamshad, M., S. Wooding, B. A. Salisbury, and J. C. Stephens. Deconstructing the relationship between genetics and race. *Nature Reviews Genetics* 5 (2004): 598–609.

Behar, D. M., M. C. Thomas, K. Skorecki, M. F. Hammer, E. Bulygina, D. Rosengarten, A. L. Jones, et al. Multiple origins of Ashkenazi Levites: Y

chromosome evidence for both Near Eastern and European ancestries. *American Journal of Human Genetics* 73 (2003): 768–779.

Cavalli-Sforza, L. L., P. Menozzi, and A. Piazza. *The History and Geography of Human Genes.* Princeton, NJ: Princeton University Press, 1994.

Cooper, R. S., J. S. Kaufman, and R. Ward. Race and genomics. *New England Journal of Medicine* 348 (2003): 1166–1170.

Duster, T. Race and reification in science. *Science* 307 (2005): 1050–1051.

Editorial. Illuminating BiDil. *Nature Biotechnology* 23 (2005): 903.

Edwards, A. W. F. Human genetic diversity: Lewontin's fallacy. *BioEssays* 25 (2003): 798–801.

González Burchard, E., E. Ziv, N. Coyle, S. L. Gomez, H. Tang, A. J. Karter, J. L. Mountain, E. J. Pérez-Stable, D. Sheppard, and N. Risch. The importance of race and ethnic background in biomedical research and clinical practice. *New England Journal of Medicine* 348 (2003): 1170–1175.

Kahn, J. D. Pharmacogenetics and ethnically targeted therapies. *British Medical Journal* 330 (2005): 1508.

Kittles, R., and K. M. Weiss. Race, ancestry, and genes: Implications for defining disease risk. *Annual Review of Genomics and Human Genetics* 4 (2003): 33–67.

Lewontin, R. C. The apportionment of human diversity. In *Evolutionary Biology,* 6th ed., ed. T. Dobzhansky, M. K. Hecht, and W. C. Steere, 381–398. New York: Appleton-Century-Crofts, 1972.

Macaulay, V., C. Hill, A. Achilli, C. Rengo, D. Clarke, W. Meehan, J. Blackburn, et al. Single, rapid coastal settlement of Asia revealed by analysis of complete mitochondrial genomes. *Science* 308 (2005): 1034–1036.

McElreavey K, and L. Quintana-Murci. A population genetics perspective of the Indus Valley through uniparentally-inherited markers. *Annals of Human Biology* 32 (2005): 154–162.

Mishmar, D., E. Ruiz-Pesini, P. Golik, V. Macaulay, A. G. Clarke, S. Hosseini, M. Brandon, et al. Natural selection shaped regional mtDNA variation in humans. *Proceedings of the National Academy of Science* 100 (2003): 171–176.

Motulsky, A. G. Jewish diseases and origins. *Nature Genetics* 9 (1995): 99–101.

Pääbo, S. The Y chromosome and the origin of all of us (men). *Science* 268 (1995): 1141–1142.

Phimister, E. G. Medicine and the racial divide. *New England Journal of Medicine* 348 (2003): 1081–1082.

Qamar, R., Q. Ayub, A. Mohyuddin, A. Helgason, K. Mazhar, A. Mansoor, T. Zerjal, C. Tyler-Smith, and S. Q. Mehdi. Y-chromosome DNA variation in Pakistan. *American Journal of Human Genetics* 70 (2002): 1107–1124.

Quintana-Murci, L., R. Chaix, R. S. Wells, D. M. Behar, H. Sayar, R. Scozzari, C. Rengo, et al. Where west meets east: The complex mtDNA landscape of the southwest and central Asian corridor. *American Journal of Human Genetics* 74 (2004): 827–845.

Rosenberg, N. A., J. K. Pritchard, J. L. Weber, H. M. Cann, K. K. Kidd, L. A.

Zhivotovsky, and M. W. Feldman. Genetic structure of human populations. *Science* 298 (2002): 2381–2385.

Skorecki, K., S. Selig, S. Blazer, R. Bradman, N. Bradman, P. J. Warburton, M. Ismajlowicz, and M. F. Hammer. Y chromosomes of Jewish priests. *Nature* 385 (1997): 32.

Tan, H., T. Quertermous, B. Rodriguez, S. L. R. Kardia, X. Zhu, A. Brown, J. S. Pankow, et al. Genetic structure, self-identified race/ethnicity, and confounding in case-control association studies. *American Journal of Human Genetics* 76 (2005): 268–275.

Thomas, M. G., K. Skorecki, H. Ben-Ami, T. Parfitt, N. Bradman, and D. B. Goldstein. Origins of Old Testament priests. *Nature* 394 (1998): 138–140.

Zhivotovsky, L. A., N. A. Rosenberg, and M. W. Feldman. Features of evolution and expansion of modern humans, inferred from genomewide microsatellite markers. *American Journal of Human Genetics* 72 (2003): 1171–1186.

WEB SITES

Race and Ethnicity

http://www.economicexpert.com/a/Race.html
http://raceandgenomics.ssrc.org/Lewontin/
http://www.stanfordalumni.org/news/magazine/1999/mayjun/articles/cavalli_sforza.html

Population Predictions from Genetic Analyses

http://www.stanford.edu/dept/news/report/news/2003/january8/genetics-18.html

Melungeons

http://www.melungeon.org/
http://www.wired.com/news/technology/0,1282,53428,00.html
http://www.continuitypress.com/melmyssolved7.html

Mitochondrial Eve

http://www.nzedge.com/heroes/wilson.html

Paternity

http://www.swinke.com/2005/shorts/jul-aug-research.html

Chapter 6

BOOKS AND ARTICLES

Bailey, J. M., K. T. Bechtold, and S. A. Berenbaum. Who are tomboys and why should we study them? *Archives of Sexual Behavior* 31 (2002): 333–341.

Baron, D., F. Batista, S. Chaffaux, J. Cocquet, C. Cotinot, E. Cribiu, E. De Baeree, et al. Foxl2 gene and the development of the ovary: A story about goat, mouse, fish and woman. *Reproduction Nutrition Development* 45 (2005): 377–382.

Blizzard, R. M. Intersex issues: A series of continuing conundrums. Pediatrics 110 (2002): 616–621.

Chang, K. Women in physics match men in success. *New York Times,* February 22, 2005, p. D2.

Colapinto, J. *As Nature Made Him: The Boy Who Was Raised as a Girl.* New York: Harper Collins, 2000.

Dennis, C. The most important sexual organ. *Nature* 427 (2004): 390–392.

Dewing, P., C. W. K. Chiang, K. Sinchak, H. Sim, P.-O. Fernagut, S. Kelly, M.-F. Chesselet, et al. Direct regulation of adult brain function by the male-specific factor SRY. *Current Biology* 16 (2006): 415–420.

Dewing, P., T. Shi, S. Horvath, and E. Vilain. Sexually dimorphic gene expression in mouse precedes gonadal differentiation. *Molecular Brain Research* 118 (2003): 82–90.

Dickinson, B. D., M. Genel, C. B. Robinowitz, P. L. Turner, and G. L. Woods. Gender verification of female Olympic athletes. *Medicine and Science in Sports and Exercise* 34 (2002): 1539–1542.

Elsas, L. J., A. Ljungqvist, M. A. Ferguson-Smith, J. L. Simpson, M. Genel, A. S. Carlson, E. Ferris, A. de la Chapelle, and A. A. Ehrhardt. Gender verification of female athletes. *Genetics in Medicine* 2 (2000): 249–254.

Eugenides, J. *Middlesex.* New York: Farrar, Straus and Giroux, 2002.

Gallagher, R. No sex research please, we're American. *Scientist* 17 (2003): 6.

Houk, C. P., I. A. Hughes, S. F. Ahmed, and P. A. Lee. Summary of consensus statement on intersex disorders and their management. *Pediatrics* 118 (2006): 753–757.

Hrabovszky, Z., and J. M. Hutson. Androgen imprinting of the brain in animal models and humans with intersex disorders: Review and recommendations. *Journal of Urology* 168 (2002): 2142–2148.

Imperato-McGinley, J., R. E. Peterson, T. Gautier, and E. Sturla. Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5alpha-reductase deficiency. *New England Journal of Medicine* 300 (1979): 1233–1237.

McElreavey, K., E. Vilain, N. Abbas, I. Herskowitz, and M. Fellous. A regulatory cascade hypothesis for mammalian sex determination: SRY represses a negative regulator of male development. *Proceedings of the National Academy of Sciences USA* 90 (1993): 368–372.

Miller, W. L. Disorders of androgen biosynthesis. *Seminars of Reproductive Medicine* 20 (2002): 205–215.
New, M. I. Diagnosis and management of congenital adrenal hypoplasia. *Annual Review of Medicine* 49 (1998): 311–328.
Nussbaum, R. L., R. R. McInnes, and H. F. Willard. *Thompson and Thompson, Genetics in Medicine,* 6th ed. Philadelphia: Saunders, 2001.
Pinsky, L., R. P. Erickson, and R. N. Schimke. *Genetic Disorders of Human Sexual Development.* New York: Oxford University Press, 1999.
Roy, W. *Making Societies.* Thousand Oaks, CA: Pine Forge Press, 2001, esp. pp. 110–155.
Vilain, E. Genetics of sexual development. *Annual Review of Sex Research* 11 (2000): 1–25.
Vilain, E., and E. R. McCabe. Mammalian sex determination: From gonads to brain. *Molecular Genetics and Metabolism* 65 (1998): 74–84.
Wade, J., and A. Arnold. Sexual differentiation of the zebra finch song system. *Annals of the New York Academy of Sciences* 1015 (2004): 540–559.
Zucker, K. J. Intersexuality and gender identity differentiation. *Journal of Pediatric and Adolescent Gynecology* 15 (2002): 3–13.

WEB SITES

Intersex Frequency

http://www.isna.org/faq/frequency/

Urogenital Development

http://www.med.unc.edu/embryo_images/unit-genital/genital_htms/genitaltoc.htm

Testing Athletes

http://ai.eecs.umich.edu/people/conway/TS/OlympicGenderTesting.html
http://bb.gogreece.com/ultimatebb.cgi?ubb=get_topic;f=1;t=002623
http://news.bbc.co.uk/sport2/hi/front_page/3270591.stm

María Patiño

http://www.medhelp.org/www/ais/articles/MARIA.HTM

David Reimer

http://www.cbc.ca/news/background/reimer/

Intersex Society of North America

http://www.isna.org/

Berdache

http://www.laurenhasten.com/berdache.htm

Chapter 7

BOOKS AND ARTICLES

Baker, L. E., W. F. McCormick, and K. J. Matteson. A silica-based mitochondrial DNA extraction method applied to forensic hair shafts and teeth. *Journal of Forensic Science* 46 (2001): 126–130.

Beauval, C., B. Maureille, F. Lacrampe-Cuyaubere, D. Serre, D. Peressinotto, J. G. Bordes, D. Cochard, et al. A late Neandertal femur from Les Rochers-de-Villeneuve, France. *Proceedings of the National Academy of Sciences USA* 102 (2005): 7085–7090.

Budowle, B., M. W. Allard, M. R. Wilson, and R. Chakraborty. Forensics and mitochondrial DNA: Applications, debates, and foundations. *Annual Review of Genomics and Human Genetics* 4 (2003): 119–141.

Chui, G. New genetic analysis helps to ID old remains. Houston Chronicle, August 18, 1991, p. 10A.

Descartes, M., Y. Huang, Y. H. Zhang, L. L. McCabe, R. Gibbs, B. L. Therrell, and E. R. B. McCabe. Genotypic confirmation from the original dried blood specimens in a neonatal hemoglobinopathy screening program. *Pediatric Research* 31 (1991): 217–221.

Enserink, M. FBI's top scientist takes the lead in forensic biology. *Science* 300 (2003): 41–43.

Hickey, G. The search for the South Side Strangler. *Readers Digest,* April 1991, pp. 73–78.

Gill, P., A. J. Jeffreys, and D. J. Werrett. Forensic applications of DNA "fingerprints." *Nature* 318 (1985): 577–579.

Jeffreys, A. J., J. F. Y. Brookfield, and R. Semeonoff. Positive identification of an immigration test-case using human DNA fingerprints. *Nature* 317 (1985): 818–819.

Jeffreys, A. J., V. Wilson, and S. L. Thein. Individual-specific "fingerprints" of human DNA. *Nature* 316 (1985): 76–79.

Jinks, D. C., M. Minter, D. A. Tarver, M. Vanderford, J. F. Hejtmancik, and E. R. B. McCabe. Molecular genetic diagnosis of sickle cell disease using dried blood specimens on blotters used for newborn screening. *Human Genetics* 82 (1989): 134–136.

McCabe, E. R. B. Applications of DNA fingerprinting in pediatric practice. *Journal of Pediatrics* 120 (1992): 499–509.

McCabe, E. R. B, S. Z. Huang, W. K. Seltzer, and M. L. Law. DNA microextraction from dried blood spots on filter paper blotters: Potential applications to newborn screening. *Human Genetics* 75 (1987): 213–216.

Redd, A. J., A. B. Agellon, V. A. Kearney, V. A. Contreras, T. Karafet, H. Park, P. de Knijff, J. M. Butler, and M. F. Hammer. Forensic value of 14 novel STRs on the human Y chromosome. *Forensic Science International* 130 (2002): 97–111.

Seiden, S. C., K. Morin, Council on Ethical and Judicial Affairs, and American Medical Association. The physician as gatekeeper to the use of genetic information in the criminal justice system. *Journal of Law, Medicine and Ethics* 30 (2002): 88–94.

Ubell, E. Whodunit? Quick, check the genes! *Parade Magazine*, March 31, 1991, pp. 10–13.

Wambaugh, J. *The Blooding*. New York: Bantam Books, 1989.

Williams, C., L. Weber, R. Williamson, and M. Hjelm. Guthrie spots for DNA-boased carrier testing in cystic fibrosis. *Lancet* 2 (1988): 693.

Wilson, M. R., M. W. Allard, K. Monson, K. W. P. Miller, and B. Budowle. Recommendations for consistent treatment of length variants in the human mitochondrial DNA control region. *Forensic Science International* 129 (2002): 35–42.

WEB SITES

Paternity Testing

http://www.pbs.org/wgbh/aso/resources/guide/earthappenact3.html

Identification by Relative's DNA

http://www.newscientist.com/news/print.jsp?id=ns99994908

Sir Alec Jeffries

http://www.sciencewatch.com/interviews/sir_alec_jeffreys.htm

Florida Rapist

http://www.pbs.org/wgbh/pages/frontline/shows/case/revolution/databases.html
http://www.txtwriter.com/Backgrounders/Genetech/GEpage14.html
http://www.crimelibrary.com/criminal_mind/forensics/dna/6.html?sect=21

People v. Wesley

http://194.88.95.39/article.asp?articleid=15237&searchresults=1
http://www.brooklaw.edu/students/journals/bjlp/abstracts/filocoma.shtml

South Side Strangler

http://scholar.lib.vt.edu/VA-news/VAPilot/issues/1995/vp950820/08180586.htm

Military DNA Database

http://www.findarticles.com/p/articles/mi_m6052/is_2003_July-August/ai_111027996
http://www.newyorker.com/talk/content/articles/011001ta_talk_in_the_rubble

DNA Databases

http://www.usatoday.com/news/washington/2003–04–15-dna-usat_x.htm

Interpol

http://www.interpol.int/Public/Forensic/dna/inquiry/default.asp

Exoneration by DNA

http://www.fadp.org/news/washingtonpost-20030921.htm
http://www.cjcj.org/press/dna_tests.html

Indicting DNA

http://slate.msn.com/id/2086729/
http://www.the-scientist.com/news/20030821/02
http://www.da.saccounty.net/pr/030626_robinson.htm

Adoption Databases

http://www.usatoday.com/news/science/2004–08–16-dnaadopt_x.htm

Chapter 8

BOOKS AND ARTICLES

Abbott, A. Icelandic database shelved as court judges privacy in peril. *Nature* 429 (2004): 118.

Anonymous. Navajo Nation IRB. Protecting Human Subjects, U.S. Department of Energy, no. 8 (Spring 2003): 1–2.

Ashley-Koch, A., Q. Yang, and R. S. Olney. Sickle hemoglobin (*Hb S*) allele and sickle cell disease: A HuGE review. *American Journal of Epidemiology* 151 (2000): 839–845.

Billings, P. R., M. A. Kohn, M. de Cuevas, J. Beckwith, J. S. Alper, and M. R.

Natowicz. Discrimination as a consequence of genetic testing. *American Journal of Human Genetics* 50 (1992): 476–482.

Breithaupt, H. Health care and privacy—an interview with Kári Stefánsson, founder and CEO of deCODE Genetics in Reykjavik, Iceland. *EMBO Reports* 21 (2001): 964–967.

Consensus Conference. Newborn screening for sickle cell disease and other hemoglobinopathies. *JAMA* 258 (1987): 1205–1209.

Dyson, S. M. "Race," ethnicity and haemoglobin disorders. *Social Science and Medicine* 47 (1998):121–131.

Gaston, M. H., J. I. Verter, G. Woods, C. Pegelow, J. Kelleher, G. Presbury, et al. Prophylaxis with oral penicillin in children with sickle cell anemia: A randomized trial. *New England Journal of Medicine* 314 (1986): 1593–1599.

Githens, J. H., P. A. Lane, R. S. McCurdy, M. L. Houston, J. D. McKinna, and D. M. Cole. Newborn screening for the hemoglobinopathies in Colorado: The first 10 years. *Archives of Pediatrics and Adolescent Medicine* 144 (1990): 466–470.

Gregory, R. S., and T. A. Satterfield. Beyond perception: The experience of risk and stigma in community contexts. *Risk Analysis* 22 (2002): 347–358.

Hall, A. What the Navajo culture teaches about informed consent. *HEC Forum* 14 (2002): 241–246.

Holve, S., D. Hu, and S. E. McCandless. Metachromatic leukodystrophy in the Navajo: Fallout of the American-Indian Wars of the nineteenth century. *American Journal of Medical Genetics* 101 (2001): 203–208.

Li, L., Y. Zhou, J. Wang, D. Hu, and M. J. Cowan. Prenatal diagnosis and carrier detection for Athabascan severe combined immunodeficiency disease. *Prenatal Diagnosis* 22 (2002): 763–768.

Markel, H. The stigma of disease: Implications of genetic screening. *American Journal of Medicine* 93 (1992): 209–215.

McDowell, G. A., E. H. Mules, P. Fabacher, E. Shapira, and M. G. Blitzer. The presence of two different infantile Tay-Sachs disease mutations in a Cajun population. *American Journal of Human Genetics* 51 (1992): 1071–1077.

McInnis, M. G. The assent of a nation: Genethics and Iceland. *Clinical Genetics* 55 (1999): 234–239.

O'Marcaigh, A. S., K. DeSantes, D. Hu, H. Pabst, B. Horn, L. Li, and M. J. Cowan. Bone marrow transplantation for $T^{-}B^{-}$ severe combined immunodeficiency disease in Athabascan-speaking Native Americans. *Bone Marrow Transplantation* 27 (2001): 703–709.

Pálsson, G. Iceland—The case of a national human genome project. *Anthropology Today* 15 (1999): 14–18.

Rabinow, P. *French DNA: Trouble in Purgatory.* Chicago: University of Chicago Press, 1999.

Wadman, M. Jewish leaders meet NIH chiefs on genetic stigmatization fears. *Nature* 392 (1998): 851.

WEB SITES

Cajun Tay-Sachs Disease

http://www.acadian-cajun.com/index.htm
http://www.angelfire.com/tx/cajundon/baby.html
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=606869

Sickle-Cell Disease and Carriers

http://www.emedicine.com/ped/topic2096.htm
http://genes-r-us.uthscsa.edu/

Iceland's deCODE Genetics

http://www.decode.com/
http://sunsite3.berkeley.edu/biotech/iceland/iceland.html
http://www.mannvernd.is/english/lawsuit.html

Navajo Genetics

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
http://www.smithsonianmag.si.edu/smithsonian/issues97/dec97/bosque.html
http://www.pbs.org/weta/thewest/people/a_c/carson.htm
http://www.southernnewmexico.com/Articles/Southeast/De_Baca/FortSumner/BosqueRedondo-destination.html

Chapter 9

BOOKS AND ARTICLES

Andrews, L. Genes and patent policy: Rethinking intellectual property rights. *Nature Reviews Genetics* 3 (2002):803–808.

Arnold, B. E., and E. Ogielska-Zei. Patenting genes and genetic research tools: Good or bad for innovation? *Annual Review of Genomics and Human Genetics* 3 (2002): 415–432.

Bovenberg, J. Whose tissue is it anyway? *Nature Biotechnology* 23 (2005): 929–933.

Butler, D., and S. Goodman. French researchers take a stand against cancer gene. *Nature* 413 (2001): 95.

Dalton, R. When two tribes go to war. *Nature* 430 (2004): 500–502.

Dennis, C. Geneticists question fees for use of patented "junk" DNA. *Nature* 423 (2003):105.

Gold, E. R., and T. A. Caulfield. The moral tollbooth: A method that makes use of the patent system to address ethical concerns in biotechnology. *Lancet* 359 (2002): 2268–2270.

Grizzle, W., W. Grody, W. N. Noll, M. E. Sobel, S. A. Stass, T. Trainer, H. Travers, V. Weedn, and K. Woodruff. Recommended policies for the uses of human tissue in research, education, and quality control. *Archives of Pathology and Laboratory Medicine* 123 (1999): 296–300.

Harris, R. F. Patenting genes: Is it necessary and is it evil? *Current Biology* 10 (2000): 174–175.

Henry, M. R., M. K. Cho, M. A. Weaver, and J. F. Merz. DNA patenting and licensing. *Science* 297 (2002): 1279.

Institute of Medicine. *Saving Women's Lives: Strategies for Improving Breast Cancer Detection and Diagnosis.* Washington, DC: National Academies Press, 2005.

Le Saux, O., Z. Urban, C. Tschuch, K. Csiszar, B. Bacchelli, D. Quaglino, I. Pasquali-Ronchetti, et al. Mutations in a gene encoding an ABC transporter cause pseudoexanthoma elasticum. *Nature Genetics* 25 (2000): 223–227.

Malakoff, D. NIH roils academe with advice on licensing DNA patents. *Science* 303 (2004): 1757–1758.

Marshall, E. Patient advocate named co-inventor on patent for the PXE disease gene. *Science* 305 (2004): 1226.

Merz, J. F. Disease gene patents: Overcoming unethical constraints on clinical laboratory medicine. *Clinical Chemistry* 45 (1999): 324–330.

Schissel, A., J. F. Merz, and M. K. Cho. Survey confirms fears about licensing of genetic tests. *Nature* 402 (1999): 118.

Sylvester-Jackson, D. A., S. L. Page, J. M. White, H. M. McCabe, Y. H. Zhang, B. L. Therrell Jr., and E. R. McCabe. Unbiased analysis of the frequency of β-thalassemia point mutations in a population of African-American newborns. *Archives of Pathology and Laboratory Medicine* 117 (1993): 1110–1114.

Terry, S., and C. D. Boyd. Researching the biology of PXE: Partnering in the process. *American Journal of Medical Genetics* 106 (2001): 177–184.

WEB SITES

Donna Mac Lean Applies to Patent Her Genome

http://www.guardian.co.uk/genes/article/0,2763,191908,00.html
http://www.cs.cornell.edu/home/llee/i.html

Cell Line Patent: Moore v. Regents of the University of California

http://www.richmond.edu/~wolf/moore.htm

Canavan Disease Gene Patent: Greenberg et al. v Miami Children's Hospital

http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=271900
http://www.bioethics.upenn.edu/prog/benefit/canavan.shtml

http://online.sfsu.edu/~rone/GEessays/WhoOwnsYourGenes.html
http://www.canavanfoundation.org/news/09–03_miami.php

Havasupai

http://www.havasupaitribe.com/
http://www.cpluhna.nau.edu/People/pais.htm

HeLa Cells and Henrietta Lacks

http://www.jhu.edu/~jhumag/0400web/01.html
http://www.lrb.co.uk/v22/n08/enri01_.html
http://www.news.harvard.edu/gazette/2001/07.19/04-filmmaker.html

Patents

http://www.uspto.gov/index.html
http://inventors.about.com/library/weekly/aa061499.htm
http://caselaw.lp.findlaw.com/data/constitution/article01/39.html

Monopolization through Exclusive Licensing

http://www.acmg.net/resources/policies/pol-015.asp

Pseudoexanthoma elasticum (PXE)

http://www.pxe.org/
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=264800
http://www.tht.com/News/news_tht_news_press_release_genetic.htm

Challenges to Myriad Breast Cancer Gene Patents

http://www.nap.edu/books/0309092132/html/257.html
http://www.gene-watch.org/genewatch/articles/17–3lowy.html

Chapter 10

BOOKS AND ARTICLES

Billings, P. R. Genetic nondiscrimination. *Nature Genetics* 37 (2005): 559–560.
Billings, P. R., M. A. Kohn, M. de Cuevas, J. Beckwith, J. S. Alper, and M. R. Natowicz. Discrimination as a consequence of genetic testing. *American Journal of Human Genetics* 50 (1992): 476–482.
Hall, M. A., J. E. McEwen, J. C. Barton, A. P. Walker, E. G. Howe, J. A.

Reiss, T. E. Power, et al. Concerns in a primary care population about genetic discrimination by insurers. *Genetics in Medicine* 7 (2005): 311–316.
Joly, Y., B. M. Knoppers, and B. Godard. Genetic information and life insurance: A "real" risk? *European Journal of Human Genetics* 11 (2003): 561–564.
Lapham, E. V., C. Kozma, and J. O. Weiss. Genetic discrimination: Perspectives of consumers. *Science* 274 (1996): 621–624.
Miller, P. Genetic discrimination in the workplace. *Genetics in Medicine* 3 (2001): 165–166.
Nowlan, W. A rational view of insurance and genetic discrimination. *Science* 297 (2002): 195–196.
Rothstein, M. A., ed. *Genetics and Life Insurance: Medical Underwriting and Social Policy.* Cambridge, MA: MIT Press, 2004.
Watson, M., and C. L. Greene. Points to consider in preventing unfair discrimination based on genetic disease risk: A position statement of the American College of Medical Genetics. *Genetics in Medicine* 3 (2001): 436–437.

WEB SITES

Genetic Discrimination

http://www.medterms.com/script/main/art.asp?articlekey=26385

Secretary's Advisory Committee on Genetic Testing (SACGT)

http://www4.od.nih.gov/oba/sacgt.htm

Secretary's Advisory Committee on Genetics, Health and Society

http://www4.od.nih.gov/oba/sacghs.htm

Assessment of Genetic Discrimination in the US

http://www.nature.com/news/2004/040216/full/nj6976–762c.html

Executive Order 13145

http://www.eeoc.gov/abouteeoc/35th/thelaw/13145.html
http://clinton4.nara.gov/WH/New/html/20000208.html
http://www.genome.gov/10002328
http://www.genome.gov/10001390

109th Congress: U.S. Senate Bill 306 (S.306) and Predecessors

http://thomas.loc.gov/
http://www.genome.gov/12513982
http://geneticfairness.org/act.html

Genetic Discrimination in Employment

http://www.genome.gov/10001390
http://www.life.umd.edu/classroom/BSCI410/Genes-WashPost.html
http://www.genelaw.info/pages/casedetail.asp?record=51
http://www.paralysisproject.org/newmobility/genetic_crystal_ball.html
http://www.geocities.com/hdsarmc/fall_00.htm
http://www.eeoc.gov/press/4–18–01.html
http://www.eeoc.gov/press/5–8–02.html
http://bmwe.org/News/2001/07JUL/13.htm

Genetic Discrimination in Life Insurance

http://www.uvm.edu/~cgep/News/Nov1999/NowlanQ&A.html
http://www.medicalnewstoday.com/medicalnews.php?newsid=21198
http://news.bbc.co.uk/1/hi/health/968443.stm

Chapter 11

BOOKS AND ARTICLES

Aittomäki, K., C. Bergh, J. Hazekamp, K. Nygren, A. Selbing, V. Söderström-Antilla, and B. Wennerholm. Genetics and assisted reproduction technology. *Acta Obstetricia et Gynecologica Scandinavica* 84 (2005): 463–473.

Barritt, J. A., S. Willadsen, C. Brenner, and J. Cohen. Epigenetic and experimental modifications in early mammalian development: Part II — cytoplasmic transfer in assisted reproduction. *Human Reproduction Update* 7 (2001):428–435.

Batzer, F. R., J. M. Hurwitz, and A. Caplan. Postmortem parenthood and the need for a protocol with posthumous sperm procurement. *Fertility and Sterility* 79 (2003): 1263–1269.

Boyle, R. J., and J. Savulescu. Ethics of using preimplantation genetic diagnosis to select a stem cell donor for an existing person. *British Medical Journal* 323 (2001): 1240–1243.

Dickens, B. M. Can sex selection be ethically tolerated? *Journal of Medical Ethics* 28 (2002): 335–336.

Ethics Committee, American Society of Reproductive Medicine. Sex selection and preimplantation genetic diagnosis. *Fertility and Sterility* 82 (2004): S245–S248.

Faddy, M. J., S. J. Silber, and R. G. Gosden. Intra-cytoplasmic sperm injection and infertility. *Nature Genetics* 29 (2001): 131.

Gosden, R., J. Trasler, D. Lucifero, and M. Faddy. Rare congenital disorders, imprinted genes, and assisted reproductive technology. *Lancet* 361 (2003): 1975–1977.

Grady, D. Pregnancy created using egg nucleus of infertile woman. *New York Times,* October 14, 2003, pp. A1, A18.

Jain, T., S. A. Missmer, R. S. Gupta, and M. D. Hornstein. Preimplantation sex selection demand and preferences in an infertility population. *Fertility and Sterility* 83 (2005): 649–658.

McCarthy, D. Why sex selection should be legal. *Journal of Medical Ethics* 27 (2001): 302–307.

McKibben, B. *Enough: Staying Human in an Engineered Age.* New York: Holt, 2003.

Merlob, P., O. Sapir, J. Sulkes, and B. Fisch. The prevalence of major congenital malformations during two periods of time, 1986–1994 and 1995–2002, in newborns conceived by assisted reproduction technology. *European Journal of Medical Genetics* 48 (2005): 5–11.

Nussbaum, R. L., R. R. McInnes, and H. F. Willard. *Thompson and Thompson, Genetics in Medicine,* 6th ed. Philadelphia: Saunders, 2001.

Ogilvie, C. M., P. R. Braude, and P. N. Scriven. Preimplantation genetic diagnosis — an overview. *Journal of Histochemistry & Cytochemistry* 53 (2005): 255–260.

Robertson, J. A. Extending preimplantation genetic diagnosis: The ethical debate — ethical issues in new uses of preimplantation genetic diagnosis. *Human Reproduction* 18 (2003): 465–471.

Robertson, J. A. Sex selection for gender variety by preimplantation genetic diagnosis. *Fertility and Sterility* 78 (2002): 463.

Savulescu, J. Procreative beneficence: Why we should select the best children. *Bioethics* 15 (2001): 413–426.

Sheldon, S., and S. Wilkinson. Should selecting saviour siblings be banned? *Journal of Medical Ethics* 30 (2004): 533–537.

St. John, J. C. Ooplasm donation in humans — the need to investigate the transmission of mitochondrial DNA following cytoplasmic transfer. *Human Reproduction* 17 (2002): 1954–1958.

Stock, G. *Redesigning Humans: Our Inevitable Genetic Future.* Boston: Houghton Mifflin, 2002.

Verlinsky, Y., S. Rechitsky, W. Schoolcraft, C. Strom, and A. Kuliev. Preimplantation genetic diagnosis for Fanconi anemia combined with HLA matching. *JAMA* 285 (2001): 3130–3133.

Wright, V. C., L. A. Schieve, M. A. Reynolds, and G. Jeng. Assisted reproductive technology surveillance — United States, 2002. *MMWR Surveillance Summaries* 54, no. SS02 (2005): 1–24.

WEB SITES

Johns Hopkins University Genetics and Public Policy Center

http://www.DNApolicy.org/

Imprinting, and Angelman and Prader-Willi syndromes

http://www-ermm.cbcu.cam.ac.uk/02004611h.htm

ART

http://www.emedicine.com/med/topic3288.htm
http://www.ncsl.org/programs/health/genetics/art.htm
http://www.bioethics.umn.edu/resources/topics/reproductive_tech.shtml

IVF

http://news.bbc.co.uk/1/hi/health/3091241.stm
http://news.bbc.co.uk/onthisday/hi/dates/stories/july/25/newsid_2499000/2499411.stm
http://history1900s.about.com/od/medicaladvancesissues/a/testtubebaby.htm
http://www.guardian.co.uk/medicine/story/0,11381,988615,00.html

Amniocentesis and Chorionic Villus Sampling

http://www.lpch.org/DiseaseHealthInfo/HealthLibrary/pregnant/tests.html

Preimplantation Genetic Diagnosis (PGD)

http://www.DNApolicy.org
http://bmj.bmjjournals.com/cgi/content/full/330/7502/1228?ecoll

Selecting Embryos for Transplant Donation

http://archives.cnn.com/2000/HEALTH/10/03/testube.brother/
http://news.bbc.co.uk/1/hi/health/955644.stm

Sex Selection

http://newsmax.com/archives/articles/2003/10/8/23812.shtml

Designing the "Best Babies"

http://www.theage.com.au/news/National/New-meaning-to-being-the-best-you-can-be/2004/11/15/1100384495240.html

Chapter 12

BOOKS AND ARTICLES

Agres, T. NAS endorses therapeutic cloning: Now, the science and ethics go up for debate. *Scientist* 16 (2002): 9.

Betts, D. H., V. Bordignon, J. R. Hill, Q. Winger, M. E. Westhusin, L. C. Smith, and W. A. King. Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle. *Proceedings of the National Academies of Science USA* 98 (2001): 1077–1082.

Bour'chis, D., D. Le Bourhis, D. Patin, A. Niveleau, P. Comizzoli, J. P. Renard, and E. Viegas-Pequiqnot. Delayed and incomplete reprogramming of chromosome methylation patterns in bovine cloned embryos. *Current Biology* 11 (2001): 1542–1546.

Brock, D. Human cloning and our sense of self. *Science* 296 (2002): 314–316.

Cameron, C., and R. Williamson. In the world of Dolly, when does a human embryo acquire respect? *Journal of Medical Ethics* 31 (2005): 215–220.

Caulfield, T. Cloning and genetic determinism — a call for consistency. *Nature Biotechnology* 19 (2001): 403.

Dobson, R. Claims of clone pregnancy could threaten US stem cell research. *British Medical Journal* 324 (2002): 868.

——. Cloning of pigs bring xenotransplants closer. *British Medical Journal* 320 (2000): 826.

Hogan, J. Dolly firm in trouble after transgenic milk fails to flow. *Nature* 423 (2003): 907.

Humphreys, D., K. Eggan, H. Akutsu, A. Friedman, K. Hochedlinger, R. Yanagimachi, E. S. Lander, T. R. Golub, and R. Jaenisch. Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. *Proceedings of the National Academies of Sciences USA* 99 (2002): 12889–12894.

Humphreys, D., K. Eggan, H. Akutsu, K. Hochedlinger, W. M. Rideout, D. Biniszkiewicz, R. Yanagimachi, and R. Jaenisch. Epigenetic instability in ES cells and cloned mice. *Science* 293 (2001): 95–97.

Inoue, K., N. Ogonuki, Y. Yamamoto, K. Takano, H. Miki, K. Mochida, and A. Ogura. Tissue-specific distribution of donor mitochondrial DNA in cloned mice produced by somatic cell nuclear transfer. *Genesis* 39 (2004): 79–83.

Jaenisch, R., K. Eggan, D. Humpherys, W. Rideout, and K. Hochedlinger. Nuclear cloning, stem cells, and genomic reprogramming. *Cloning and Stem Cells* 4 (2002): 389–396.

Jiang, L., D. B. Carter, J. Xu, X. Yang, R. S. Prather, and X. C. Tian. Telomere lengths in cloned transgenic pigs. *Biology of Reproduction* 70 (2004): 1589–1593.

Kaiser, J. Cloned piglets may help overcome rejection. *Science* 295 (2002): 25–26.

Lee, J., K. Inoue, R. Ono, N. Ogonuki, T. Kohda, T. Kaneko-Ishino, A. Ogura, and F. Ishino. Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells. *Development* 129 (2002): 1807–1817.

Meerdo, L. N., W. A. Reed, and K. L. White. Telomere-to-centromere ratio of bovine clones, embryos, gametes, fetal cells, and adult cells. *Cloning and Stem Cells* 7 (2005): 62–73.

Ogonuki, N., K. Inoue, Y. Yamamoto, Y. Noguchi, K. Tanemura, O. Suzuki, H. Nakayama, K. Doi, Y. Ohtomo, M. Satoh, A. Nishida, and A. Ogura. Early death of mice cloned from somatic cells. *Nature Genetics* 30 (2002): 253–254.

Rhind, S. M., J. E. Taylor, P. A. De Sousa, T. J. King, M. McGarry, and I. Wilmut. Human cloning: Can it be made safe? *Nature Reviews Genetics* 4 (2003): 855–864.

Schaetzlein, S., A. Lucas-Hahn, E. Lemme, W. A. Kues, M. Dorsch, M. P. Manns, H. Niemann, and K. L. Rudolph. Telomere length is reset during early mammalian embryognesis. *Proceedings of the National Academy of Sciences USA* 101 (2004): 8034–8038.

Schatten, G., R. Prather, and I. Wilmut. Cloning claim is science fiction, not science. *Science* 299 (2003): 344.

Shiels, P. G., and A. G. Jardine. Dolly, no longer the exception: Telomeres and implications for transplantation. *Cloning and Stem Cells* 5 (2003): 157–160.

Shiels, P. G., A. J. Kind, K. H. S. Campbell, D. Waddington, I. Wilmut, A. Colman, and A. E. Schnieke. Analysis of telomere lengths in cloned sheep. *Nature* 399 (1999): 316–317.

Tamashiro, K. L. K., T. Wakayama, H. Akutsu, H. Yamazaki, J. L. Lachey, M. D. Wortman, R. J. Seeley, D. A. D' Alessio, S. C. Woods, R. Yanagimachi, and R. R. Sakai. Cloned mice have an obese phenotype not transmitted to their offspring. *Nature Medicine* 8 (2002): 262–267.

Verma I. Good-bye Dolly. Molecular Therapy 7:565, 2003

Wertz, D. C. Embryo and stem cell research in the United States: History and politics. *Gene Therapy* 9 (2002): 674–678.

Wilmut, I., A. E. Schnieke, J. McWhir, A. J. Kind, and K. H. Campbell. Viable offspring derived from fetal and adult mammalian cells. *Nature* 385 (1997): 810–813.

Yanagimachi, R. Cloning humans? *Journal of Assisted Reproduction and Genetics* 18 (2001): 476.

Zavos, P. M., and K. Illmensee. Possible therapy of male infertility by reproductive cloning: One cloned human 4-cell embryo. *Archives of Andrology* 52 (2006): 243–254.

WEB SITES

Introduction to Cloning

http://www.arhp.org/patienteducation/onlinebrochures/cloning/index.cfm?ID=282
http://www.ornl.gov/sci/techresources/Human_Genome/elsi/cloning.shtml
http://www.nap.edu/catalog/10285.html?onpi_topnews_011802

Dolly the Sheep

http://www.sciencemuseum.org.uk/antenna/dolly/
http://www.nature.com/nature/dolly/index.html
http://news.bbc.co.uk/2/hi/science/nature/2764039.stm
http://news.bbc.co.uk/1/hi/sci/tech/1741559.stm

National Academies Panel Report

http://www.nap.edu/books/0309076374/html/R1.html

Cloning Legislation and Policy

http://www.dnapolicy.org/genetics/cloning.html;$sessionid$2VAUZOQAAABCAQBICNWBNWQ

Cloning Humans

http://www.channel4.com/science/microsites/S/science/medicine/humanclone.html
http://www.reproductivecloning.net/zavos/

Cloning Other Animals

http://www.wired.com/news/medtech/0,1286,59032,00.html
http://www.uidaho.edu/cloning/
http://www.newscientist.com/article.ns?id=dn4026
http://news.bbc.co.uk/1/hi/sci/tech/3663277.stm

Chapter 13

BOOKS AND ARTICLES

Check, E. Back in the race. *Nature* 435 (2005): 544–545.
Cibelli, J. B., K. A. Grant, K. B. Chapman, K. Cunniff, T. Worst, H. L. Green,

S. J. Walker, et al. Parthenogenetic stem cells in nonhuman primates. *Science* 295 (2002): 819.

Cowan, C. M., Y.-Y. Shi, O. O. Aalami, Y.-F. Chou, C. Mari, R. Thomas, N. Quarto, C. H. Contag, B. Wu, and M. T. Longaker. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. *Nature Biotechnology* 22 (2004): 560–567.

Fraser, J. K., R. E. Schreiber, P. A. Zuk, and M. H. Hedrick. Adult stem cell therapy for heart. *International Journal of Biochemistry and Cell Biology* 36 (2004): 658–666.

Hwang, W. S., Y. J. Ryu, J. H. Park, E. S. Park, E. G. Lee, J. M. Koo, H. Y. Jeon, et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science* 303 (2004): 1669–1674.

Inman, M. Divided committee urges less restriction on embryo research. *Science* 308 (2005):30.

Kiessling, A. Eggs alone — human parthenotes: An ethical source of stem cells for therapies? *Nature* 434 (2005): 145.

Kono, T., Y. Obata, Q. Wu, K. Niwa, Y. Ono, Y. Yamamoto, E. S. Park, J.-S. Seo, and H. Ogawa. Birth of parthenogenetic mice that can develop to adulthood. *Nature* 428 (2004): 860–864.

Lendeckel, S., A. Jödicke, P. Christophis, K. Heidinger, J. Wolff, J. K. Fraser, M. Hedrick, L. Berthold, and H.-P. Howaldt. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: Case report. *Journal of Cranio-Maxillofacial Surgery* 32 (2004): 370–373.

Mandavilli, A. Profile: Woo-Suk Hwang. *Nature Medicine* 11 (2005): 464.

Niwa, K., R. Takano, Y. Obata, H. Hiura, J. Komiyama, H. Ogawa, and T. Kono. Nuclei of oocytes derived from mouse parthenogenetic embryos are competent to support development to term. *Biology of Reproduction* 71 (2004): 1560–1567.

Okie, S. Stem cell research — signposts and roadblocks. *New England Journal of Medicine* 353 (2005): 1–5.

Shamblott, M. J., J. Axelman, S. Wang, E. M. Bugg, J. W. Littlefield, P. J. Donovan, P. D. Blumenthal, G. R. Huggins, and J. D. Gearhart. Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proceedings of the National Academy of Sciences USA* 95 (1998): 13726–13731.

Strem, B. M., and M. H. Hedrick. The growing importance of fat in regenerative medicine. *Trends in Biotechnology* 23 (2005): 64–66.

Thomson, J. A., J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, and J. M. Jones. Embryonic stem cell lines derived from human blastocysts. *Science* 282 (1998): 1145–1147.

Vastag, B. UK licenses human embryo creation — "Dolly" cloner to create embryos for research. *JAMA* 290 (2005): 449–450.

Vogel, G. Scientists take step toward therapeutic cloning. *Science* 303 (2004): 937–938.

Wakayama, T. On the road to therapeutic cloning. *Nature Biotechnology* 22 (2004): 399–400.

Weiss, R. The power to divide. *National Geographic,* July 2005, 3–27.
Zuk, P. A., M. Zhu, P. Ashjian, D. A. De Ugarte, J. I. Huang, H. Mizuno, Z. C. Alfonso, J. K. Fraser, P. Benhaim, and M. H. Hedrick. Human adipose tissue is a source of multipotent stem cells. *Molecular Biology of the Cells* 13 (2002): 4279–4295.
Zuk, P. A., M. Zhu, H. Mizuno, J. Huang, J. W. Futrell, A. J. Katz, P. Benhaim, H. P. Lorenz, and M. H. Hedrick. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Engineering* 7 (2001): 211–228.

WEB SITES

Stem Cells

http://en.wikipedia.org/wiki/Stem_cell
http://stemcells.nih.gov/info/basics/basics4.asp
http://www.abc.net.au/pm/content/2006/s1723442.htm
http://www.boston.com/news/local/articles/2006/08/24/stem_cell_method_preserves_embryo/

Stem Cells from Fat: Adult Stem Cells

http://med.stanford.edu/research/centers/archive/childrensurglab.html
http://surgery.org/press/news-release.php?iid=206&section=news-lipoplasty

Human Embryonic Stem Cells

http://www.advancedcell.com/press-release/advanced-cell-technology-announces-technique-to-generate-human-embryonic-stem-cells-that-maintains-developmental-potential-of-embryo
http://www.isscr.org/public/escells.htm
http://www.nap.edu/books/0309096537/html/
http://www.nature.com/news/2005/051219/full/051219-3.html
http://www.nature.com/news/2006/060109/full/439122a.html
http://www.nature.com/news/specials/hwang/index.html
http://stemcells.nih.gov/info/basics/basics3.asp

Human ES Cells

http://www.advancedcell.com/press-release/advanced-cell-technology-announces-use-of-nuclear-transfer-technology-for-successful-generation-of-human-embryonic-stem-cells

Human ES Cells Derived from Cloned Embryos

http://news.bbc.co.uk/1/hi/sci/tech/3480921.stm

http://www.cnn.com/2004/HEALTH/02/13/science.clone/

Designer Babies by Combining Therapeutic and Reproductive Cloning

http://www.arhp.org/patienteducation/onlinebrochures/cloning/index.cfm?ID=282

International Stem Cell Policy

http://mbbnet.umn.edu/scmap.html
http://mbbnet.umn.edu/scmap/scresearchmap.html

U.S. National Academies' Guidelines on Use of ES Cells

http://www.nap.edu/openbook/0309076307/html/
http://books.nap.edu/catalog/11278.html

Chapter 14

BOOKS AND ARTICLES

Aiuti, A., S. Slavin, M. Aker, F. Ficara, S. Deola, A. Mortellaro, S. Morecki, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* 296 (2002): 2410–2413.

Antonarakis, S. E., H. H. Kazazian, and E. G. D. Yuddenham. Molecular etiology of factor VIII deficiency in hemophilia A. *Human Mutation* 5 (1995): 1–22.

Berns, A. Good news for gene therapy. *New England Journal of Medicine* 350 (2004): 1679–1680.

Beutler, E. The Cline affair. *Molecular Therapy* 4 (2001): 396–397.

Blaese, R. M., K. W. Culver, A. D. Miller, C. S. Carter, T. Fleisher, M. Clerici, G. Shearer, et al. T lymphocyte-directed gene therapy for ADA-SCID: Initial trial results after 4 years. *Science* 270 (1995): 475–480.

Cavazzana-Calvo, M., and A. Fischer. Efficacy of gene therapy for SCID is being confirmed. *Lancet* 364 (2004): 2155–2156.

Cavazzana-Calvo, M., S. Hacein-Bey, G. de Saint Basile, F. Gross, E. Yvon, P. Nusbaum, F. Celz, C. Hue, S. Certain, J.-L. Casanova, P. Bousso, F. Le Deist, and A. Fischer. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 288 (2000): 669–672.

Check, E. Gene therapy put on hold as third child develops cancer. *Nature* 433 (2005): 561.

Chinen, J., and J. M. Puck. Perspectives of gene therapy for primary immune deficiencies. *Current Opinion in Allergy and Clinical Immunology* 4 (2004): 523–527.

Cook-Deegan, R. M. Human gene therapy and Congress. *Human Gene Therapy* 1 (1990): 163–170.

Couzin, J., and J. Kaiser. As Gelsinger case ends, gene therapy suffers another blow. *Science* 307 (2005): 1028.

Culver, K. W., W. F. Anderson, and R. M. Blaese. Lymphocyte gene therapy. *Human Gene Therapy* 2 (1991): 107–109.

Culver, K., K. Cornetta, R. Morgan, S. Morecki, P. Aebersold, A. Kasid, M. Lotze, S. A. Rosenberg, W. F. Anderson, and R. M. Blaese. Lymphocytes as cellular vehicles for gene therapy in mouse and man. *Proceedings of the National Academy of Sciences USA* 88 (1991): 3155–3159.

Gaspar, H. B., K. L. Parsley, S. Howe, D. King, K. C. Gilmour, J. Sinclair, G. Brouns, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. *Lancet* 364 (2004): 2181–2187.

Graw, J., H.-H. Brackmann, J. Oldenberg, R. Schneppenheim, M. Spannagl, R. Schwaab. Haemophilia A: From mutation analysis to new therapies. *Nature Reviews Genetics* 6 (2005): 488–501.

Hacein-Bey-Abina, S., F. Le Deist, F. Carlier, C. Bouneaud, C. Hue, J.-P. De Villartay, A. J. Thrasher, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *New England Journal of Medicine* 346 (2002): 1185–1193.

Kaiser, J. Retroviral vectors: A double-edged sword. *Science* 308 (2005): 1735–1736.

———. Side effects sideline hemophilia trial. *Science* 304 (2004): 1423–1424.

Manno, C. S., G. F. Pierce, V. R. Arruda, B. Glader, M. Ragni, J. J. Rasko, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nature Medicine* 12 (2006): 342–347.

Manucci, P. M., and E. G. D. Tuddenham. The hemophiliac — from royal genes to gene therapy. *New England Journal of Medicine* 344 (2001): 1773–1779.

McCabe, E. R. Clinical application of gene therapy: Emerging opportunities and current limitations. *Biochemical Medicine and Metabolic Biology* 50, no. 3 (1993): 241–253.

McCormack, M. P., and T. H. Rabbitts. Activation of the T-cell oncogene *LMO2* after gene therapy for X-linked severe combined immunodeficiency. *New England Journal of Medicine* 350 (2004): 913–922.

Nathwani, A. C., A. M. Davidoff, and D. C. Linch. A review of gene therapy for haematological disorders. *British Journal of Haematology* 128 (2004): 3–17.

Nussbaum, R. L., R. R. McInnes, and H. F. Willard. *Thompson and Thompson, Genetics in Medicine,* 6th ed. Philadelphia: Saunders, 2001.

Office of Technology Assessment. *Human Gene Therapy—A Background Paper.* Washington, DC: U.S. Congress, OTA-BP-BA-32, December 1984.

Ott, M. G., M. Schmitt, K. Schwarzwaelder, S. Stein, U. Siler, U. Koehl, et al. Correction of X-linked chronic granulomatous disease by gene therapy,

augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nature Medicine* 12, no. 4 (2006): 401–409.

Raper, S. E., N. Chirmule, F. S. Lee, N. A. Wivel, A. Bagg, G. Gao, J. N, Wilson, and M. L. Batshaw. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral transfer. *Molecular Genetics and Metabolism* 80 (2003): 148–158.

Raper, S. E., M. Yudkoff, N. Chirmule, G. P. Gao, F. Nunes, Z. J. Haskal, E. E. Furth, et al. A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. *Human Gene Therapy* 13 (2002): 163–175.

Smaglik, P. Tighter watch urged on adenoviral vectors . . . *Nature* 402 (1999): 707.

Sun, M. Another round in Rifkin versus gene splicing. *Science* 226 (1984): 1404–1405.

Thrasher, A. J. Gene therapy: Great expectations. *Medical Journal of Australia* 182 (2005): 440–441.

WEB SITES

Introduction to Gene Therapy

http://ghr.nlm.nih.gov/info=gene_therapy/show/gene_therapy
http://www.fda.gov/fdac/features/2000/500_gene.html

Early Gene Therapy in the United States

http://www.georgetown.edu/research/nrcbl/publications/scopenotes/sn24.htm
http://www.nih.gov/news/panelrep.html
http://www.fda.gov/fdac/features/2000/500_gene.html
http://www.chron.com/content/houston/interactive/special/gene/gfirst.html

The Death of Jesse Gelsinger

http://www.washingtonpost.com/wp-dyn/health/specials/genetherapy/gelsingercase/
http://www.gene.ch/gentech/1999/Dec/msg00005.html
http://www.uvm.edu/~cgep/Education/Expert.html
http://www.usdoj.gov/usao/pae/News/Pr/2005/feb/UofPSettlement%20release.html

Severe Combined Immunodeficiency (SCID)

http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=601457
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=102700
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=300400

Chronic Granulomatous Disease (CGD)

http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=306400

Hemophilia

http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=306700
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=306900

Chapter 15

BOOKS AND ARTICLES

Adams, P. C., D. M. Reboussin, J. C. Barton, C. E. McLaren, J. H. Eckfeldt, G. D. McLaren, F. W. Dawkins, et al. for the Hemochromatosis and Iron Overload Screening (HEIRS) Study Research Investigators. Hemochromatosis and iron-overload screening in a racially diverse population. *New England Journal of Medicine* 352 (2005): 1769–1778.

Bertilsson, L., M.-L. Dahl, P. Dalén, and A. Al-Shurbaji. Molecular genetics of CYP2D6: Clinical relevance with a focus on psychotropic drugs. *British Journal of Clinical Pharmacology* 53 (2002): 111–122.

Bhardwaj, U., Y.-H. Zhang, D. S. Jackson, G. R. Buchanan, B. L. Therrell, L. L. McCabe, and E. R. B. McCabe. DNA diagnosis confirms hemoglobin deletion in newborn screen follow-up. *Journal of Pediatrics* 142 (2003): 346–348.

Bhardwaj, U., Y.-H. Zhang, F. Lorey, L. L. McCabe, and E. R. B. McCabe. Molecular genetic confirmatory testing from newborn screening samples for the common African-American, Asian Indian, Southeast Asian, and Chinese β-thalassemia mutations. *American Journal of Hematology* 78 (2005): 249–255.

Bhardwaj, U., Y.-H. Zhang, and E. R. McCabe. Neonatal hemoglobinopathy screening: Molecular genetic technologies. *Molecular Genetics and Metabolism* 80 (2003): 129–137.

Collins, F. S. Shattuck lecture — medical and societal consequences of the human genome project. *New England Journal of Medicine* 341 (1999): 28–37.

Descartes, M., Y. Huang, Y. H. Zhang, L. L. McCabe, R. Gibbs, B. L. Therrell, and E. R. McCabe. Genotypic confirmation from the original dried blood specimens in a neonatal hemoglobinopathy screening program. *Pediatric Research* 31 (1992): 217–221.

Gorgas, M. P., and S. T. Neely. Cost-effectiveness and test-performance factors in relation to universal newborn hearing screening. *Mental Retardation and Developmental Disabilities Research Reviews* 9 (2003): 103–108.

Guttmacher, A. E., and F. S. Collins. Genomic medicine — a primer. *New England Journal of Medicine* 347 (2002): 1512–1520.

——. Welcome to the genomic era. *New England Journal of Medicine* 349 (2003): 996–998.

Hanson, E. H., G. Imperatore, and W. Burke. *HFE* gene and hereditary hemochromatosis: A HuGE review. *American Journal of Epidemiology* 154 (2001): 193–206.

Howse, J. L., and M. Katz. The importance of newborn screening. *Pediatrics* 106 (2000): 595.

Keren, R., M. Helfand, C. Homer, H. McPhillips, and T. A. Lieu. Projected cost-effectiveness of statewide universal newborn hearing screening. *Pediatrics* 110 (2002): 855–864.

Khoury, M. J., L. L. McCabe, and E. R. B. McCabe. Population screening in the age of genomic medicine. *New England Journal of Medicine* 348 (2003): 50–58.

Koch, J. H. *Robert Guthrie: The PKU Story: A Crusade against Mental Retardation*. Pasadena, CA: Hope Publishing House, 1997.

Ledesma, M. C., J. A. G. Agúndez. Identification of subtypes of *CYP2D* gene rearrangements among carriers of *CYP2D* gene deletion and duplication. *Clinical Chemistry* 51 (2005): 939–943.

Li, T., C. Y. Chang, D. Y. Jin, P. J. Lin, A. Khvorova, and D. W. Stafford. Identification of the gene for vitamin K epoxide reductase. *Nature* 427 (2004): 541–544.

McCabe, L. Efficacy of targeted genetic screening programs for adolescents. *American Journal of Human Genetics* 59 (1996): 762–763.

McCabe, L. L., and E. R. B. McCabe. Newborn screening as a model for population screening. *Molecular Genetics and Metabolism* 75 (2002): 299–307.

——. Postgenomic medicine — presymptomatic testing for prediction and prevention. *Clinics in Perinatology* 28 (2001): 425–434.

McCabe, L. L., B. L. Therrell, and E. R. B. McCabe. Newborn screening: Rationale for a comprehensive, fully integrated public health system. *Molecular Genetics and Metabolism* 77 (2002): 267–273.

McCune, C. A., L. N. Al-Jader, A. May, S. L. Hayes, H. A. Jackson, and M. Worwood. Hereditary hemochromatosis: Only 1% of adult HFEC282Y homozygotes in South Wales have a clinical diagnosis of iron overload. *Human Genetics* 111 (2002): 538–543.

McGhee, S. A., E. R. Stiehm, M. Cowan, P. Krogstad, and E. R. B. McCabe. Two-tiered universal newborn screening strategy for severe combined immunodeficiency (SCID). *Molecular Genetics and Metabolism* 86, no. 4 (2005): 427–430.

McLaren, C. E., J. C. Barton, P. C. Adams, E. L. Harris, R. R. Acton, N. Press, D. M. Reboussin, et al. for the Hemochromatosis and Iron Overload Study Research Investigators. Hemochromatosis and iron overload screening (HEIRS) study design for an evaluation of 100,000 primary care–based adults. *American Journal of the Medical Sciences* 325 (2003): 53–62.

Mitchell, J. J., A. Capua, C. Clow, and C. R. Scriver. Twenty-year outcome analysis of genetic screening programs for Tay-Sachs and β-thalassemia dis-

ease carriers in high school. *American Journal of Human Genetics* 59 (1996): 793–798.

Natowicz, M. Newborn screening — setting evidence-based policy for protection. *New England Journal of Medicine* 353 (2005): 867–870.

Newborn Screening Task Force. Serving the family from birth to the medical home — newborn screening: A blueprint for the future — a call for a national agenda on state newborn screening programs. *Pediatrics* 106 (2000): 389–422.

Nussbaum, R. L., R. R. McInnes, and H. F. Willard. *Thompson and Thompson, Genetics in Medicine,* 6th ed. Philadelphia: Saunders, 2001.

Phillips, K. A., and S. L. Van Bebber. Measuring the value of pharmacogenomics. *Nature Reviews Drug Discovery* 4 (2005): 500–509.

——. Regulatory perspectives on pharmacogenomics: A review of the literature on key issues faced by the United States Food and Drug Administration. *Medical Care Research and Review* 63 (2006): 301–326.

Rost, S., A. Fregin, V. Ivaskevicius, E. Conzelmann, K. Hortnagel, H. J. Pelz, K. Lappegard, E. Seifried, I. Scharrer, E. G. Tuddenham, C. R. Muller, T. M. Strom, and J. Oldenburg. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* 427 (2004): 537–541.

Sadler, J. E. K is for koagulation. *Nature* 427 (2004): 493–494.

Sanderson, S., J. Emery, and J. Higgins. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: A HuGEnet systematic review and meta-analysis. *Genetics in Medicine* 7, no. 2 (2005): 97–104.

Varmus, H. Getting ready for gene-based medicine. *New England Journal of Medicine* 347 (2002): 1526–1527.

Waalen, J., B. G. Nordestgaard, and E. Beutler. The penetrance of hereditary hemochromatosis. *Best Practice & Research Clinical Hematology* 18 (2005): 203–220.

Weichbold, V., D. Nekahm-Heis, and K. Welzl-Mueller. Ten-year outcome of newborn hearing screening in Austria. *International Journal of Pediatric Otorhinolaryngology* 70, no. 2 (2006): 235–240.

Worwood, M. HFE mutations as risk factors in disease. *Best Practice & Research Clinical Hematology* 15 (2002): 295–314.

Yoshinaga-Itano, C., D. Coulter, V. Thomson. Developmental outcomes of children with hearing loss born in Colorado hospitals with and without universal newborn hearing screening programs. *Seminars in Neonatology* 6 (2001): 521–529.

Yuan, H. Y., J. J. Chen, M. T. Lee, J. C. Wung, Y. F. Chen, M. J. Charng, M. J. Lu, et al. A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. *Human and Molecular Genetics* 14, no. 13 (2005): 1745–1751.

Zhang, Y. H., and E. R. McCabe. RNA analysis from newborn screening dried blood specimens. *Human Genetics* 89 (1992): 311–314.

Zhang, Y. H., L. L. McCabe, M. Wilborn, B. L. Therrell, and E. R. McCabe.

Application of molecular genetics in public health: Improved follow-up in a neonatal hemoglobinopathy screening program. *Biochemical Medicine and Metabolic Biology* 52 (1994): 27–35.

WEB SITES

Genomic Medicine

http://www.boston.com/news/globe/editorial_opinion/oped/articles/2005/07/17/personalized_medicine?mode=PF

Newborn Screening

http://genes-r-us.uthscsa.edu/
http://www.marchofdimes.com/aboutus/15796_20475.asp

Tandem Mass Spectrometry

http://www.sciencemag.org/cgi/content/full/294/5550/2272

Hearing Screening

http://www.glensfallshospital.org/news%20releases/2003/newsrelease_10.08.03.htm

Policy

http://www.marchofdimes.com/professionals/14332_1200.asp
http://www.marchofdimes.com/professionals/14332_15455.asp
http://mchb.hrsa.gov/screening/summary.htm

Pharmacogenomics

http://www.fda.gov/cder/drug/drugreactions
http://www.findarticles.com/p/articles/mi_m1200/is_11_162/ai_92203129/print
http://www.bio-itworld.com/archive/090902/pharma.html

Chapter 16

BOOKS AND ARTICLES

Bhardwaj, U., Y.-H. Zhang, D. S. Jackson, G. R. Buchanan, B. L. Therrell, L. L. McCabe, and E. R. B. McCabe. DNA diagnosis confirms hemoglobin deletion in newborn screen follow-up. *Journal of Pediatrics* 142 (2003): 346–348.

Kuhn, T. *The Structure of Scientific Revolutions,* 2nd ed. Chicago: University of Chicago Press, 1970.

WEB SITES

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http://plato.stanford.edu/entries/thomas-kuhn/
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