

Genetic Influences on Preterm Birth

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ABSTRACT

The high prevalence, increasing frequency, and adverse outcomes for mothers and infants of preterm birth have led to heightened awareness of this public health concern. The causes of preterm birth are likely to be multifactorial, with genetic, infectious, nutritional, behavioral, and other environmental contributors. Because of important differences in the physiology of human pregnancy and that of nonprimate mammals, extrapolation of mechanisms from animal model systems to humans has had limited impact on the understanding of human prematurity. This review summarizes work from many groups that implicates important genetic contributions to human preterm birth. These efforts use epidemiological, classical genetic, and more recently, genomic science approaches to determine pregnancies at risk for preterm delivery and to facilitate an understanding of the substantial racial disparity in preterm birth. Data revealing racial and familial predispositions to prematurity, along with genetic polymorphisms conferring increased preterm birth, promise new insights into the understanding and treatment of this critical problem.

KEYWORDS: Preterm birth, epidemiology, genetics, polymorphism

Preterm birth has come to the forefront as a major public health burden. It is currently the leading cause of neonatal morbidity and mortality in children without congenital anomalies,^{1,2} and has increased almost 30% rather than decreased in frequency during the last two decades, with a 10% increase during the last 10 years³ in the United States. In the United States, 12.3% of pregnancies end preterm (<http://www.march-of-dimes.com/peristats/>), defined as gestational age less than 37 weeks.

This increasing prevalence is particularly problematic for many reasons. First, infants born prematurely are at risk not only for death, but also for many severe acute and chronic medical disorders. These complications of prematurity include intraventricular hemorrhage with subsequent neurological dysfunction, respiratory

distress and mechanical ventilation resulting in chronic pulmonary disease, necrotizing enterocolitis, retinopathy of prematurity, growth failure, and other sequelae.^{4,5} The impact of these adverse outcomes is extremely large because the problems affect individuals at the earliest ages after birth. Indeed, perinatal disorders, of which preterm birth is the major contributor, are a prominent source of disability-adjusted life-years—far greater in impact than other chronic conditions such as major depression, ischemic heart disease, and cerebrovascular disease.⁶ Second, infants of low birthweight (whether growth was restricted for gestational age or the infant is the appropriate size but has a low birthweight because of preterm birth) are at increased risk for developing adult disorders such as hypertension, diabetes mellitus, and atherosclerotic disease.⁷ Third, preterm birth exhibits a

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major health disparity according to race. For example, the risk of preterm birth is approximately 2-fold higher in African Americans than whites even after correction for known confounding factors.^{8–10} Interestingly, the rate of preterm birth is far lower in other developed countries, with rates of ~5 to 6% in Scandinavia¹¹ and (<http://www.stakes.fi>). Lastly, the care for preterm infants results in great emotional toil and financial burden for families during both acute and chronic treatment. The net financial burden of the acute care of preterm infants has been estimated, as a lower limit, at approximately \$26.2 billion per year.⁴

An ideal therapeutic agent to treat preterm labor, which would delay parturition significantly and decrease the incidence of preterm birth, has not been identified. Many of the agents presently employed to control preterm myometrial contractions can cause substantial toxicity to the mother and developing fetus.¹² Clearly, novel therapeutic agents that can be instituted early in preterm labor with reduced maternal–fetal toxicity are needed. Genetic methods to identify new therapeutic targets or improve risk assessment and surveillance are actively being sought for these reasons.

CLINICAL INDICATORS OF PRETERM BIRTH AND THEIR UTILITY

Preterm birth often is preceded by spontaneous uterine contractions that gradually lead to softening, effacement, and dilatation of the cervix. These preterm uterine contractions frequently are symptomless, especially in primiparous women, making the diagnosis of preterm labor extremely difficult. Routine recording of uterine preterm contractions has not been helpful in assessing the risk of preterm birth.¹³ Obtaining a careful history of the nature of uterine contractions and the circumstances in which they occur combined with a digital cervical examination between 18 and 32 weeks of gestation may help clinicians to recognize women with increased risk of preterm birth. The correct diagnosis of true preterm labor is very unreliable, given that most women, even with clear clinical signs of preterm uterine contractions, deliver after 37 weeks of pregnancy.

Bacterial vaginosis (BV) is strongly associated with preterm birth. In a recent systematic review, Leitich et al¹⁴ showed that the earlier BV was diagnosed, the greater the risk of preterm birth. Although it is possible to eradicate the abnormal bacterial flora in BV with either local or systematic antimicrobial treatment, wide controversy still exists about possible benefits of such treatment in the prevention of preterm birth and its complications.^{15–17}

A large number of biochemical markers of imminent or true preterm labor in maternal serum, cervicovaginal excretion, or amniotic fluid have been studied, but only a few have been shown to be useful clinically.

Fetal fibronectin (FFN) and phosphorylated insulin-like growth factor binding protein-1 (phIGFBP-1) measurements in vaginal or cervical secretions have been shown to predict preterm birth better than the use of clinical history or symptoms as predictors. In a meta-analysis by Leitich et al,¹⁸ the mean sensitivity and specificity of FFN testing to predict delivery within 7 days after testing was 77% and 87%, respectively. Honest et al,¹⁹ in another meta-analysis study, concluded that FFN testing improved the probability in predicting spontaneous preterm birth within 10 days from 3% before testing to 14.4% after testing on average.

phIGFBP-1 is a protein produced in the decidua and secreted into the cervical fluid during preterm labor. Concentrations ≥ 10 $\mu\text{g/L}$ of phIGFBP-1 in the cervical secretion predicted preterm birth correctly (< 35 weeks of pregnancy) in 41% of women (seven of 17) with suspected preterm labor, whereas 7% (three of 46) with a negative phIGFBP-1 test had a preterm birth.²⁰ In predicting preterm birth < 35 weeks of gestation, the sensitivity and specificity of bedside phIGFBP-1 testing in symptomatic women was 72.7% and 83%, respectively.²¹ Other markers of preterm labor in cervical excretions or maternal serum (e.g., interleukin [IL] -6, IL-8, tumor necrosis factor alpha [TNF- α], relaxin, ferritin, and sialidase) have been shown to predict preterm labor, but the usefulness of these markers in predicting preterm uterine contractions and preterm birth in large low-risk populations requires additional evaluation.

Ultrasonographic evaluation of the cervical length and the width of the internal os in the second trimester for prediction of preterm birth have been used in several studies both in symptomatic and low-risk women. One problem with these studies has been the variable definitions of abnormal findings. The sensitivity and specificity of cervical length ranging from 18 to 30 mm in symptomatic women ranged from 68 to 100% and from 44 to 79%, respectively.²² In two large ultrasonographic studies in low-risk women, the sensitivity and specificity for preterm delivery with a shortened cervix were 54 and 19%, and 76 and 97%, respectively.^{23,24} When FFN testing and ultrasonographic findings (cervical length < 27 mm) were combined in symptomatic women, approximately 50% of the women delivered preterm.²⁵ When both findings were negative, only 5.6% had a preterm delivery.

Despite continued advances in the understanding of these clinical markers for preterm labor, an effective intervention to decrease the risk of preterm birth remains elusive. Perhaps the combination of one or more of these clinical or biochemical markers with genetic variations known to increase the risk of preterm birth will help us to better identify a population of women at the highest risk. Improving our ability to identify the group at highest risk may enhance the efficacy of treatments used for the prevention of preterm birth.

INDICATORS OF GENETIC INFLUENCE ON HUMAN PRETERM BIRTH

Racial Disparities and Population-Based Studies

Although preterm birth complicates 12% of pregnancies in the United States³ (<http://www.marchofdimes.com/peristats/>), ethnic groups within the population have dramatically different risks for this adverse outcome of pregnancy. For example, the risk of preterm birth for white women in the United States is 11.0%, whereas that for African American women is 17.7%. This increase in risk for preterm birth has been validated consistently across different geographical regions in the United States as well as across groups of differing socioeconomic status.^{26–28} This major health disparity is an area of active investigation. Although not an ideal index of genetic composition, race or self-reported ethnicity reflects geographical ancestry as implicated by genetic markers,²⁹ and allele frequencies of gene polymorphisms differ between various geographic isolates.³⁰ Differences in genetic makeup should then be present between individuals of different races. Clearly, significant non-genetic variables such as socioeconomic status, maternal education, and coexistent medical risk factors will also vary as a function of race.

How important are genetic aspects of race in determining the risk for preterm birth? Goldenberg et al,⁹ in a study of 1491 multiparous women, found an increased risk for preterm birth in African American women in Alabama even when controlling for medical, psychosocial, and behavioral risk factors. More recently, Kistka et al³¹ performed a population-based cohort study of all state-registered births between 1989 and 1997 using the Missouri Department of Health's maternally linked database for factors associated with recurrent preterm delivery. Again, the relative risk for preterm birth was increased in African American women, particularly at the earliest gestational ages. For example, the relative risk for African American women in comparison to white women for delivering at 32 to 34 weeks of gestation was 2.70 (95% confidence interval [CI], 2.59 to 2.82), whereas at 20 to 22 weeks, the relative risk was 4.51 (95% CI, 3.65 to 5.56).³¹ When correcting for other factors associated with socioeconomic status, maternal nutrition, maternal education, and coexisting medical conditions, the adjusted odds ratio (aOR) remained elevated for African American women for both isolated (aOR, 2.21; 95% CI, 2.11 to 2.31) and recurrent (aOR, 4.11; 95% CI, 3.78 to 4.47) preterm birth.³¹ In accord with these findings, Zhang and Savitz²⁸ found a greater increase in risk for extreme preterm births in African American women after adjustment for other factors. A study comparing African American and Mexican American women also found higher rates of prematurity in African Americans after adjustment for socioeconomic status and prenatal care (aOR, 1.5; 95% CI, 1.2 to 1.7).³²

Adams et al⁸ similarly found that African American women who have had a preterm infant are at greater risk for subsequent preterm delivery than white women with a similar history.

When evaluating the relative contribution of parental race on the timing of birth and risk of prematurity, one study suggested a much more robust effect of maternal race than paternal race.³³ A comparison of preterm birth to white mothers and fathers versus African American mothers and fathers demonstrated rates of 6.9 versus 15.3%, respectively. White mothers and African American fathers had a 9.1% rate of preterm birth, whereas African American mothers and white fathers had a 12.2% rate of preterm birth.

Familial Studies

Although the differences between ethnic groups provide evidence for a genetic predisposition in preterm birth, studies on families provide stronger evidence for the potential inherited predisposition to this adverse pregnancy outcome. The most significant risk factor for preterm labor is a prior history of preterm labor in the mother.^{8,34–37} The increase in risk for subsequent preterm birth has been confirmed across studies in women in the United States⁸ as well as Danish,³⁸ Scottish,³⁸ and Norwegian³⁹ women. A recurring finding in these studies is that the likelihood of a preterm birth increases with decreasing gestational age in the original preterm birth. This finding is consistent with recent studies demonstrating that subsequent preterm births to a mother occur most often within 1 to 2 weeks of her original preterm birth.³¹ The occurrence of preterm birth to the same mother at a similar time to her original preterm birth argues against acute environmental or infectious precipitants of spontaneous preterm birth, and further strengthens the notion of significant genetic contributors.

Even stronger evidence for a genetic contribution to preterm birth has emerged from cross-generational and sibling analyses. Porter et al⁴⁰ found for a mother who was herself born preterm, the risk for her delivering a child preterm increased. Moreover, the relative risk for her preterm birth increases as her own gestational age decreased (e.g., for mothers born at 30 weeks gestation, their OR for preterm birth was 2.38 (95% CI, 1.37 to 4.16)).⁴⁰ A Norwegian study examining the same topic failed to find an increased risk of preterm birth to mothers who were born preterm.⁴¹ However, this study excluded births before 28 weeks of gestation, the group likely to be at the greatest risk based upon the results of the Utah cohort.⁴⁰ A study of women in Scotland compared rates of preterm birth among sisters and sisters-in-law of women who had a preterm birth.⁴² An increased rate of preterm birth in sisters (16%) as compared with sisters-in-law (9%) again supports genetic contributors to birth timing.

Twin Studies

One classical method of assessing genetic versus environmental contributors to a defined phenotype involves determination of trait concordance in monozygotic and dizygotic twins. Two studies have analyzed rigorously the offspring of female monozygotic and dizygotic twin pairs for similarity in gestational length or risk of preterm birth. Treloar et al⁴³ evaluated 905 parous female Australian twin pairs to determine whether delivery had occurred more than 2 weeks preterm. Twin pair correlations were higher from monozygotic twin pairs than dizygotic twin pairs ($r = 0.3 \pm 0.08$ versus 0.03 ± 0.11 SE, respectively). Heritability was calculated at 17% for preterm delivery in the first pregnancy and 27% for preterm delivery in any pregnancy. The pregnancies evaluated in this study were not limited to spontaneous preterm birth in this study. A population-based twin study from Sweden evaluated 868 monozygotic and 1141 dizygotic female twin pairs that delivered single birth between 1973 and 1993.⁴⁴ Intraclass correlation for gestational length was higher for monozygotic compared with dizygotic twins. In accord with the Australian data, heritability estimates from model fitting were ~30% for gestational age and 36% for preterm birth. Genetic effects of the paternal genome in contributing to the timing of birth evaluated in twin studies have not been reported.

GENERAL CONTRIBUTORS TO PRETERM BIRTH

There are a multitude of causes of preterm delivery. In general, preterm deliveries can be classified as (1) spontaneous due to idiopathic preterm labor or preterm premature rupture of membranes (PPROM), or (2) medically indicated due to a significant maternal or fetal complication of pregnancy. Indicated preterm birth accounts for ~25% of preterm deliveries. Preterm labor and PPRM account for the remaining 75%, contributing 50% and 25%, respectively.⁴⁵ The mechanisms that lead to preterm labor and PPRM are often interrelated and occur as a cascade of a common inciting event. Many women develop PPRM during the course of preterm labor. Likewise, women presenting with PPRM usually go on to develop preterm labor. Hence, preterm births as a result of preterm labor and PPRM are often referred to collectively as spontaneous preterm births.

As the overall rate of preterm birth is changing, so is the etiologic distribution of those births. It is difficult to delineate which factors exert the most important influence on the increasing rate of preterm birth, but the increase in women delaying childbearing until later in life and the increase in multiple gestations as a result of artificial reproductive technologies are both believed to contribute significantly.⁴⁶ In addition, the increasing rate of obesity, particularly in the United States, may contribute to an increase in indicated preterm births

because of the heightened risk for preeclampsia and gestational diabetes mellitus. One study emphasized the temporal changes in preterm birth subtypes and their impact on the overall rate of preterm birth.⁴⁷ In this study, Ananth et al⁴⁷ demonstrated that although the overall preterm birth rate is increasing, it increased in white women from 8.3% to 9.4% between 1989 and 2000 and decreased in black women from 18.5% to 16.2% over the same time period. They also reported that the proportion of preterm births attributable to PPRM decreased and those that were medically indicated increased over the same time period in both black and white women. These findings were recently corroborated by a similar study from a large population of Latin American women over a concurrent time period.⁴⁸ Advances in prenatal diagnosis, fetal surveillance, and care of the preterm infant are probable explanations for the contemporary increase in medically indicated preterm births and the concurrent decline in perinatal mortality.

Spontaneous Preterm Birth

PRETERM LABOR

The essential pathophysiologic mechanisms that lead to the onset of term or preterm labor are uncertain. In addition to mechanisms yet to be discovered, preterm labor may occur as a result of the concomitant activation or cascade of the following events: functional progesterone withdrawal, increase in corticotropin-releasing hormone, premature decidual activation, increased prostaglandin production, oxytocin initiation, and increased cytokine production due to infection/inflammation.^{1,49}

The roles of progesterone and estrogen in birth timing have been studied extensively in many mammalian species. An increase in estradiol and decrease in progesterone are associated with the onset of labor in most mammals except primates. Unlike most other mammals, a decline in serum progesterone has not been linked to the onset of labor in humans. It is hypothesized that rather than a decrease in serum progesterone, a change in the nature of the progesterone receptors may lead to the onset of labor in humans.⁵⁰ It has also been suggested that increased adrenal activity associated with the stress of labor causes an increase in cortisol, which blocks progesterone receptors.⁵¹ Several recent clinical trials have demonstrated the effectiveness of exogenous administration of progestins to decrease the incidence of recurrent spontaneous preterm birth in high-risk pregnancies.^{52,53} These studies support the notion that progesterone has a significant influence on uterine quiescence.

The progressive increase in estrogen levels throughout pregnancy also plays a role in the timing

of labor. As estrogen levels increase throughout pregnancy, oxytocin receptors increase in abundance, the myometrium becomes more responsive to oxytocin, and there is an increase in oxytocin release from the pituitary. The increase in estrogen is also involved in prostaglandin stimulation.⁴⁹ It is probable that all of these hormonal changes play a coordinated role in the cascade of events that lead to the onset of labor. Thus, events to which the fetomaternal unit are exposed, which impact on these hormonal regulatory pathways, such as infection, changes in nutrition, or toxins, could initiate a cascade of biochemical changes that results in preterm birth.

Inflammation and infection are important pathophysiologic processes in the etiology of preterm labor. Infection and/or inflammation are the mostly rigorously demonstrated pathologic processes that cause preterm labor, with identified molecular mechanisms.⁵⁴ The rate of positive bacterial culture from amniotic fluid of women who deliver as a result of preterm labor is 22%.⁵⁵ Microbial invasion of the amniotic cavity occurs in as many as 34 to 75% of women with PPROM, with increasing likelihood of infection in women with longer latency periods.⁵⁶ Proinflammatory cytokines (such as IL-1 and TNF- α) and other inflammatory regulators such as thrombin have been demonstrated in numerous studies to play important roles in the cascade of inflammatory events associated with the onset of preterm labor.⁵⁴ The onset of labor at term has also been found to be associated with these proinflammatory cytokines and chemokines.⁵⁷

Because the exact mechanism leading to the onset of spontaneous preterm labor has yet to be established with certainty, efforts to prevent it have focused primarily on the identification of risk factors. Several risk factor scoring systems have been proposed for the prediction of preterm labor. Unfortunately, these scoring systems have not proven to predict spontaneous preterm birth reliably, and most lack clinically important reference standards.⁵⁸ Many of the known risk factors for preterm labor are listed in Table 1.

PRETERM PREMATURE RUPTURE OF THE MEMBRANES

PPROM occurs in ~3% of all pregnancies and accounts for 25 to 30% of all preterm deliveries.^{45,59} PPROM can likely occur as a result of either sporadic influences or genetic contributors. The interval between membrane rupture and delivery is usually relatively short, but is inversely proportional to the gestational age at which it occurs (earlier gestational ages have longer latencies). When managed conservatively, more than half of women with PPROM prior to 34 weeks gestational age deliver within 1 week.^{59,60} Prolonging pregnancy decreases the incidence of neonatal morbidities associated with prematurity, yet longer latencies may increase the likelihood of developing complications of PPROM

Table 1 Maternal Risk Factors for Preterm Labor^{114–119}

Prior preterm delivery
African American race
Low socioeconomic status
Lack of prenatal care
High physical exertion or stress
Low body mass index
High body mass index
Extremes of maternal age (younger than 18 or older than 40 years)
Tobacco abuse
Substance abuse
Prior induced abortion
Prior cervical surgery
Periodontal disease
Uterine overdistension (i.e., multiple gestation or polyhydramnios)
Vaginal bleeding during pregnancy
Uterine anomaly
Anemia
Reproductive tract infections

(From Austin MP, Leader L. Maternal stress and obstetric and infant outcomes: epidemiological findings and neuroendocrine mechanisms. *Aust N Z J Obstet Gynaecol* 2000;40(3):331–337; Barnes DL, Adair LS, Popkin BM. Women's physical activity and pregnancy outcome: a longitudinal analysis from the Philippines. *Int J Epidemiol* 1991;20(1):162–172; Blackmore-Prince C, Kieke B Jr, Kugaraj KA, et al. Racial differences in the patterns of singleton preterm delivery in the 1988 National Maternal and Infant Health Survey. *Matern Child Health J* 1999;3(4):189–197; Cnattingius S, Lambe M. Trends in smoking and overweight during pregnancy: prevalence, risks of pregnancy complications, and adverse pregnancy outcomes. *Semin Perinatol* 2002;26(4):286–295; Iams JD. Preterm birth. In: Gabbe S, Niebyl JF, Simpson JL, ed. *Obstetrics: Normal and Problem Pregnancies*. New York: Churchill Livingstone; 1996:743–820; Pschirrer ER, Monga M. Risk factors for preterm labor. *Clin Obstet Gynecol* 2000;43(4):727–734.)

such as chorioamnionitis, placental abruption, and umbilical cord compression.

At term, membrane rupture is considered a normal part of parturition, which may occur prior to or after the onset of labor. It occurs due to a combination of physiologic processes that may be exacerbated by uterine contractions.⁶¹ PPROM is associated with a higher incidence of intrauterine infection than membrane rupture at term, especially at very early gestational ages. It is believed that ascending bacteria from the vagina lead to increased cytokine activation and resultant apoptosis in the amniotic membranes, making them more susceptible to rupture.⁶² PPROM can also occur as a complication of amniocentesis. This is fairly uncommon and occurs in less than 0.5% of amniocenteses.

Various risk factors for the development of PPROM have been identified, as they have been for spontaneous preterm labor. Many of the risk factors for PPROM, such as its racial disparity and likelihood of recurrence within the same mother, suggest an underlying genetic component. Some of the known risk factors for PPROM are listed in Table 2.

Table 2 Maternal risk factors for PPRM^{59,62,120–122}

Prior preterm birth
PPROM in a prior pregnancy
Low socioeconomic status
Low body mass index
Substance abuse including smoking
Infectious causes (amnionitis, cervicitis, and other vaginoses)
Increased uterine volume (polyhydramnios and multiple gestation)
Subchorionic hemorrhage
Cervical conization or cerclage
Fetal anomalies
Maternal trauma

PPROM, preterm premature rupture of membranes. (From Mercer BM. Preterm premature rupture of the membranes: current approaches to evaluation and management. *Obstet Gynecol Clin North Am* 2005;32(3):411–428; Naeye RL, Peters EC. Causes and consequences of premature rupture of fetal membranes. *Lancet* 1980;1(8161):192–194; Hadley CB, Main DM, Gabbe SG. Risk factors for preterm premature rupture of the fetal membranes. *Am J Perinatol* 1990;7(4):374–379; Harger JH, Hsing AW, Tuomala RE, et al. Risk factors for preterm premature rupture of fetal membranes: a multicenter case-control study. *Am J Obstet Gynecol* 1990;163(1 pt 1):130–137; Mercer BM, Goldenberg RL, Meis PJ, et al. The Preterm Prediction Study: prediction of preterm premature rupture of membranes through clinical findings and ancillary testing. The National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *Am J Obstet Gynecol* 2000;183(3):738–745.)

Indicated Preterm Birth

Medically indicated preterm births are brought about iatrogenically due to a significant maternal or fetal complication. These births may be preterm inductions of labor with vaginal delivery, or cesarean deliveries that either are planned or are a result of fetal intolerance to labor. As mentioned, the proportion of preterm births attributable to medical indications is increasing. This trend coincides with a decline in the perinatal mortality rate.⁴⁷

Maternal indications for preterm delivery include medical complications resulting from pregnancy (such as preeclampsia; hemolysis, elevated liver enzymes, and low platelet syndrome; or acute fatty liver of pregnancy). Preterm delivery may also be maternally indicated due to worsening disease status of a prepregnancy medical condition such as chronic hypertension, diabetes, or collagen vascular disease.

Fetal indications for iatrogenic preterm birth are increasing in number with advances in prenatal diagnosis and antepartum fetal surveillance. Some examples of fetal indications for preterm birth include a nonreassuring test of fetal surveillance by nonstress test or biophysical profile. Another example of a fetal indication for preterm birth is a significant abnormality in the fetal umbilical artery on Doppler examination (or Doppler of other fetal vessels) which may indicate fetal compromise. The finding of increased velocity in the fetal middle cerebral artery Doppler examination when used as a screen for fetal anemia may also lead the care provider

to deliver a patient prematurely rather than waiting for other evidence of fetal compromise that may occur at a later stage of the disease process.

Other maternal–fetal complications leading to iatrogenic preterm birth include evidence of significant uteroplacental insufficiency (intrauterine growth restriction, oligohydramnios, abnormal fetal Doppler studies), placental abruption, chorioamnionitis, hydrops fetalis, umbilical cord prolapse, and others.

APPROACHES TO IDENTIFYING GENES INVOLVED IN HUMAN PRETERM BIRTH

Genetically Altered Animal Models

Domestic ruminants, particularly sheep, have for many years been important model systems for analysis of endocrine pathways involved in normal parturition and preterm labor.^{63,64} As term gestation approaches, activation of the fetal hypothalamic–pituitary–adrenal axis in these species results in elevated fetal cortisol levels. Increased circulating cortisol leads to enzymatic alteration in placental function such that metabolism of pregnenolone away from progesterone and toward 17 α -hydroxypregnenolone and 17 β -estradiol occurs. This change in metabolism decreases maternal serum progesterone concentration and increases maternal estrone and 17 β -estradiol. In response to these changes of relative steroid abundance, increased prostaglandin F2 α (PGF2 α) production occurs, stimulating the uterine contractions of active labor. Clearly, precise extrapolation of this mechanism to humans is not possible because glucocorticoids do not precipitate labor, and progesterone levels do not decline with the onset of human labor.⁶⁵

To identify further specific gene isoforms and novel pathways involved in preterm and term labor, an animal model system that allows facile genetic modification would be extremely valuable. Although technically possible, gene knockout and transgenic methods are cumbersome in ruminants. In this light, mice provide a powerful experimental system to study fundamental timing mechanisms for birth.⁶⁶ Using gene knockout approaches in mice, essential components of the cascade of events that culminates in birth have been identified. Labor in mice is precipitated at day 19.5 of gestation by a decrease in serum progesterone, an event known as luteolysis.⁶⁷ This decrease in progesterone is required for induction of a series of myometrial contraction-associated proteins, such as the oxytocin receptor, cyclooxygenase-2, and connexin-43.⁶⁴ For luteolysis to occur, PGF2 α synthesis must be induced in the uterine epithelium and act upon specific ovarian PGF2 α (FP) receptors to cause a decrease in ovarian progesterone release. Mice deficient in PGF2 α receptor,⁶⁸ cytoplasmic phospholipase A2,⁶⁹ and cyclooxygenase-1⁷⁰ each

fail to initiate labor because of impaired luteolysis and persistent progesterone production, and have revealed the key regulatory isoforms in mice. Recently, mice with deficiency of 20- α hydroxysteroid dehydrogenase have also been found to delay parturition onset because of impaired progesterone catabolism associated with luteolysis.⁷¹ Although several models for eliciting preterm birth in mice have been developed, such as endotoxin administration,⁷² progesterone receptor antagonist treatment,⁷³ intrauterine heat-killed bacteria administration,⁷⁴ or intra-amniotic surfactant apoprotein-A injection,⁷⁵ no genetic model of spontaneous preterm birth in mice has been reported.

Similar to outcomes from the ruminant studies, extrapolation of genetic targets in mice to humans will prove challenging because of the central role of progesterone withdrawal for labor onset in rodents. The onset on labor in humans has hypothesized to result from functional progesterone withdrawal that occurs without a decrease in plasma progesterone concentration. Alteration in progesterone receptor activation and functional progesterone withdrawal could result from induction of dominant negative isoforms of the progesterone receptor, alteration in abundance of progesterone receptor coactivators and corepressors, or local increases in progesterone metabolism in the myometrium with a subsequent decrease in intracellular progesterone abundance.^{76–78} To date, although evidence supporting each of these mechanisms has been obtained, none of these possibilities has been proven to confer progesterone resistance. In addition, microarray studies comparing regulation of mouse and human uterine mRNAs during pregnancy and just prior to labor demonstrate little conservation of genes induced or repressed.⁷⁹ If functional progesterone withdrawal is the mechanism for labor initiation in humans, the global myometrial gene expression profile at labor in humans and mice should be similar. A marked induction of inflammation-related genes characterizes human parturition, but not parturition in the mouse.⁷⁹

Human Polymorphism Association Studies

Racial disparities and familial and twin studies all support the possibility of an underlying genetic basis for preterm birth. Analogous to many other complex disease processes, such as hypertension and vascular disease, the intrauterine environment that is at increased risk for preterm labor is likely influenced by multiple coexistent genetic alterations and environmental stimuli to culminate in preterm birth. Several human genetic polymorphisms have been identified that are associated with an increased risk of preterm birth. Several of these are highlighted in this section.

Infection and inflammation are common pathways known to be associated with the onset of preterm

labor. Proinflammatory cytokines such as IL-1, TNF- α , and other inflammatory mediators have been shown to exhibit important roles in the cascade of inflammatory events associated with the onset of preterm labor. Most of the studies linking genetic polymorphisms to prematurity have demonstrated genetic alterations in these mediators responsible for regulating the inflammatory response. These gene alterations contribute to preterm birth by stimulation of proinflammatory mediators or inhibition of those with anti-inflammatory effects.

Genetic alterations in several proinflammatory cytokines (TNF- α , IL-1, IL-4, and IL-6) have been studied as possible etiologic mechanisms to preterm birth.⁸⁰ Of all cytokine polymorphisms, the strongest evidence of a causal link to preterm birth is in alterations of TNF- α . In pregnancy, TNF- α has several opposing functions regarding maintenance versus termination. Under normal conditions it contributes to pregnancy maintenance and prolongation. Some examples of this are its positive influences on trophoblast differentiation, placental implantation and development, and growth and remodeling of the amniotic membranes.⁸¹ During times of stress, particularly inflammatory stress, the actions of TNF- α may promote preterm birth. Genetic, environmental, and infectious stimuli may lead to dysregulation of TNF- α . Downstream pathways regulated by TNF- α include stimulation of prostaglandin production, matrix metalloproteinase (MMP)-mediated membrane degradation, and apoptosis, with resulting damage of the placenta and membranes.^{62,82–84} These changes ultimately can lead to early pregnancy failure, preterm labor, or PPROM.

Several studies have indicated a common polymorphism in the genetic sequence of TNF- α , TNF(-308A), that is associated with an increased risk of adverse pregnancy outcome. Although these studies were small, they demonstrated a relationship between a carrier state for the TNF(-308A) allele and an increased incidence of PPROM⁸⁵ and preterm birth.⁸⁶ A larger study of more than 1000 children in Africa found that those born prematurely were more likely to be homozygous for one of the two TNF(-308A) alleles, TNF2 (relative risk [RR], 7.3; 95% CI, 2.85 to 18.9), and that these infants were also at significantly increased risk of infant death (RR, 7.47; 95% CI, 2.36 to 23.6).⁸⁷ Polymorphisms in TNF(-488) have also been shown to increase the risk of spontaneous preterm birth.⁸⁸ Maccones et al⁸⁹ evaluated the genetic-environmental association of the TNF2 polymorphism and bacterial vaginosis as risk factors for preterm birth. In this study, the TNF allele again was identified as an independent risk factor for preterm birth (OR, 2.7; 95% CI, 1.7 to 4.5), but when present in the face of bacterial vaginosis, the risk was accentuated significantly (OR, 6.1; 95% CI, 1.9 to 21.0).

The presence of a polymorphism in the IL-1 receptor antagonist gene, an IL1 β receptor antagonist, IL1RA*2, has been shown to be associated with preterm birth if present either in the mother or preterm infant. The IL1RA*2 allele has been associated with an increase in preterm birth and neonatal mortality in twin gestations.⁹⁰ In this study, pregnancies in which both fetuses carried the IL1RA*2 allele were significantly more likely to develop PPROM than those in which only one fetus, or neither, carried the allele (OR, 8.9; 95% CI, 1.6 to 50.3). Another study evaluated fetal IL1RA*2 status via second-trimester amniocentesis.⁹¹ Although the incidence of preterm birth was only 6% in this study, the authors demonstrated that fetal homozygosity for IL1RA*2 allele was significantly associated with an increased risk of preterm birth. The IL1RA*2 carrier rate has been found to be higher in Hispanic women (41.5%) than those of European (27.4%) or African descent (7.4%) who deliver at term.⁹² Thus, it is suspected to be a stronger risk factor for preterm birth in white and Hispanic populations.

Gene polymorphisms of two other interleukins, IL-4 and IL-6, also influence preterm birth risk. IL-4 modulates the balance between innate and cellular immunity. Patients with either heterozygosity or homozygosity for the IL-4(-590T) allele have a significant risk factor for preterm birth.⁹³ The IL-6 polymorphism, IL6(-174C), reduces IL-6 production and has been shown subsequently to reduce the risk of preterm birth when present in the homozygous state (OR, 0.14; 95% CI, 0.03 to 0.64).⁹⁴

Several noncytokine gene polymorphisms have also been identified that confer an increased risk of preterm birth. These include genetic alterations in vascular endothelial growth factor⁹⁵; the methylenetetrahydrofolate reductase enzyme⁹⁶; factors V, VII, or VIII⁹⁶⁻⁹⁸; Toll-like receptor 4⁹⁹; β_2 -adrenergic receptor^{100,101}; and the human paraoxonase enzyme.¹⁰²

MMPs are mediators of interstitial collagen degradation and are associated with rupture of the amniotic membranes.^{103,104} Polymorphisms of several MMPs (MMP-1, MMP-8, and MMP-9) have been associated with an increased risk of PPROM.¹⁰⁵⁻¹⁰⁷ One study evaluated levels of MMPs in the amniochorionic membranes of African American versus white women when exposed to endotoxin stimuli. The membranes of African American women produced significantly more MMP-9.¹⁰⁸ Underlying polymorphisms in MMP-9 that may be more common in African American than white women could explain this overexpression and explain further why African American women have a higher incidence of PPROM.

Although we have learned a great deal about the genetic influence of these gene polymorphisms on the risk of preterm birth, there is still little known about their interactions with other candidate genes or environmental

factors. Recently it has been demonstrated that many of these polymorphisms, such as TNF- α , confer a higher risk of preterm birth than the most common environmental risk factors listed in Tables 1 and 2. Therefore, it will be intriguing to evaluate further whether the risk of preterm birth is compounded when two or more polymorphisms (gene-gene interactions) are present simultaneously, such as has been done in one recent study.¹⁰⁹ Likewise, the affects of environmental risk factors coinciding with gene polymorphisms (gene-environment interactions) may also augment preterm birth risk further. Wang et al¹¹⁰ have taken this approach to evaluate the susceptibility to preterm birth stratified by maternal carriage of different alleles of the toxifying enzymes cytochrome P450 *CYP1A1* and glutathione-S-transferase *GSTT1* exposed to low-level benzene or cigarette smoke.¹¹¹ Alteration in risk for preterm birth as a function of variants in both genes was found in the context of these environmental toxins, with the greatest effects for smoking occurring for mothers with variant genotypes at both the *CYP1A1* and *GSTT1* loci.

FUTURE DIRECTIONS

The future of application of genomic and bioinformatics advances to shed light on pathways involved in term and preterm labor is bright. Preterm birth initiatives by the National Institutes of Health, March of Dimes, Burroughs Wellcome Fund, National Academy of Sciences, and others, have highlighted the importance of this area of investigation and generated the funding resources to move the science forward. As with the study of most complex disease processes, several areas in experimental design will be of utmost importance.¹¹² Given that many candidate genes logically can be put forward for evaluation, careful consideration should be given to nonbiased, genome-wide screening methods. With the advent of single-nucleotide polymorphism DNA chips capable of simultaneously screening 500,000 variants covering the genome, these genome-wide methods will be accomplished readily. Perhaps the greatest hope of gene identification comes from similar studies in obesity that have recently proven fruitful.¹¹³ Given the likely multigenic contribution to preterm birth (each gene with perhaps a modest increase in risk conferred), the necessity will be to assimilate large numbers of individuals for analysis. To make the greatest impact most efficiently, collaborative efforts across centers must be encouraged and coordinated with methods to measure gene expression at the mRNA and/or protein level systematically in gestationally relevant tissues. Most importantly, careful phenotyping and attention to rigorous validation of ethnicity or race will be essential to avoid introduction of variance that will mask relevant genes to subpopulations with differing etiologies. With this investment of funding and effort, the return for maternal and child

health should be enormous. Genetic screening tools, identification of metabolic pathways conferring increased risk for prophylaxis—analogue to folate supplementation during pregnancy to prevent neural tube deficits—and new targets for therapy will emerge.

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