review

Genetic variation associated with preterm birth: A HuGE review

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Preterm birth (PTB) is a major public health concern because of its high prevalence, associated mortality and morbidity, and expense from both short-term hospitalization and long-term disability. In 2002, 11.9% of U.S. births occurred before 37 weeks gestation. Epidemiologic studies have identified many demographic, behavioral, and medical characteristics associated with PTB risk. In addition, recent evidence indicates a role for genetic susceptibility. We reviewed 18 studies published before June 1, 2004, that examined associations between polymorphisms in the maternal or fetal genome and PTB risk. Studies of a polymorphism in tumor necrosis factor-α, a proinflammatory cytokine, showed the most consistent increase in the risk of PTB. Environmental factors such as infection, stress, and obesity, which activate inflammatory pathways, have been associated with PTB, suggesting that environmental and genetic risk factors might operate and interact through related pathways. This review highlights maternal and fetal genetic susceptibilities to PTB, the potential relationships with environmental risk factors, and the need for additional well-designed studies of this critical public health problem. ***Genet Med* 2005: 7(9):593–604.**

Key Words: *preterm birth, genetic polymorphisms, TNF-α, , IL6, IL4, IL1β, ILRA, toll-like receptor-4, MMP1, MMP9, β_2AR, VEGF*

Preterm birth (PTB) is a major public health concern because of its high prevalence, associated mortality and morbidity, and expense of hospitalization and long-term disability. In 2002, 11.9% of all births and 10.4% of singletons were preterm, a 7% increase since 1990.[1]

Pregnancy and parturition involve a complex molecular and biologic interplay of mother and fetus, which is not well understood. These processes have yet to be fully elucidated, hindering efforts to understand and prevent PTB. In the absence of complete knowledge of pregnancy maintenance biology and labor initiation, efforts to prevent PTB have focused on identifying risk factors.

The strongest risk factors for PTB suggest a maternal or fetal genetic predisposition toward PTB. Women born preterm are more likely to deliver preterm.[2] Approximately 20% of women who deliver preterm subsequently have another PTB with the same partner; changing partners reduces the risk by one third.[3,4] Twin studies of pregnancy outcome estimate the heritability of PTB as 17% to 36%.[5,6]

Epidemiologic studies have identified other risk factors: maternal age less than 18 years or more than 35 years,[7] underweight or overweight before pregnancy,[8] and short stature.[9] Black or African American women and women of low socioeconomic status consistently have higher rates of PTB.[1,7] Physical stress, such as standing for long periods, increases PTB risk,[5] and psychosocial stress has also been associated with higher preterm rates, although less consistently.[10,11]

Infections like chorioamnionitis may initiate preterm labor. Bacterial vaginosis (BV) is associated with increased PTB risk even without infection of the fetal membranes.[7] Increased inflammation occurs in normal parturition, and inflammatory cytokines are higher in women who deliver preterm.[12] Infection, stress, and obesity are all known to promote inflammation,[13,14] which suggests that these environmental exposures may promote an inflammation-mediated mechanism (Fig. 1) resulting in early parturition.

To establish and maintain a pregnancy, the immune response, which normally destroys foreign material, must make exception for the fetus. Promising research is elucidating some of the molecular mechanisms involved in this pregnancy-immune response. The immune system is composed of innate cellular responses; pregnancy affects both. The innate immune response involving monocytes, granulocytes, mast cells, natural killer cells, complement and acute phase proteins[15] is responsible for mounting an immediate response to pathogens. The cellular immune response, responsible for a slower but highly specific and sustained antiantigen response, is com-

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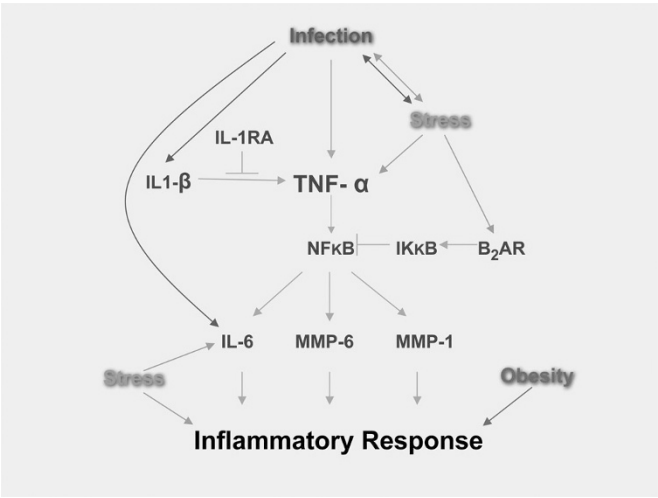

Fig. 1. Pathways to the inflammation response. Interactions of gene products and environmental exposures discussed in this review. Environmental exposures such as obesity, infection, and stress (hypothalamic-pituitary-adrenal axis activation from psychosocial and physiologic sources) induce inflammatory pathways. Many of these interact through tumor necrosis factor alpha (TNF-α). TNF-α induces activation of nuclear factor kappa-B (NFκB), a transcription factor that induces matrix metalloproteinase expression and extracellular matrix degradation. These pathway members are involved in various feedback loops not included in this figure, and their regulation can increase or decrease inflammatory response.

posed of T and B cells and antibody production.[15] In the adaptive immune response, T cells are inhibited. Some women with T-cell–mediated autoimmune diseases such as rheumatoid arthritis go into remission during pregnancy.[16,17] However, B-cell–mediated autoimmune diseases such as lupus worsen during pregnancy.[18] Normal pregnancy is characterized by a reduction in the proinflammatory cytokine, TNF-α, as well as type 1 cytokines, interferon-γ and interleukin (IL)2, and increases in type 2 cytokines, IL4, IL5, and IL10.[19] Some components of the innate immune response, including IL12 and IL1β, are activated.[20] PTB before 32 weeks of gestation is associated with infection or inflammation;[21–23] therefore, polymorphisms in genes regulating the adaptive or innate immune system might alter pregnancy-immune response and affect parturition timing.

Researchers have begun to investigate the effects of candidate gene polymorphisms on PTB, focusing on polymorphisms related to inflammatory and immune response because of the evidence of the involvement of inflammation in pregnancy and parturition. We review studies published before June 1, 2004, that examine the association between PTB and polymorphisms in the maternal or fetal genome.

METHODS

PubMed and OVID were searched using the following keywords: preterm birth, preterm delivery, prematurity, premature birth, premature delivery, in combination with: polymorphism, genetic, allele, genetic polymorphism, TNF-α, IL6, IL4, IL1β, IL1-RA, IL-1RN, IL1RA, toll-like receptor-4, TLR4, MMP1, MMP9, β2AR, VEGF, and MTHFR. Publications were included if they examined maternal or fetal genetic variations having direct association with PTB or preterm premature rupture of the membranes (PPROM).

INFLAMMATORY CYTOKINES ASSOCIATED WITH PRETERM BIRTH

Cytokines are a large class of soluble molecules used in intercellular and intracellular communication that can increase and decrease the inflammatory response. The following section reviews studies of polymorphisms in inflammatory cytokines and PTB.

Tumor necrosis factor-α

Regulation and function

TNF-α is a proinflammatory cytokine with multiple roles in the immune response, including rapid defense infection. Regulation of this inflammatory response includes positive feedback loops, which can allow the response to escalate, resulting in the pathology of autoimmune disease. Anti-TNF-α drugs are used to control symptoms of autoimmune diseases such as Crohn disease, psoriasis, and rheumatoid arthritis by inhibiting this inflammation response.[24]

TNF-α is involved in remodeling the cervix and fetal membranes by promoting production of collagen-degrading matrix metalloproteinases (MMPs), including MMP1 and MMP9 (Fig. 1).[25] Monzon-Bordonaba et al. proposed that TNF-α functions as a mediator of pregnancy establishment and maintenance, and of normal and premature rupture of membranes (PROM) through its modulation of trophoblasts.[26] In this model, TNF-α under normal physiologic conditions induces trophoblast differentiation, invasion and adhesion, implantation, placental development, and fetal membrane growth and remodeling.[26] TNF-α secreted in early pregnancy encourages implantation; however, it can also trigger adverse events. Alterations in TNF-α levels can trigger endocrine function inhibition, protease activation, and extracellular matrix degradation resulting in pregnancy failure if misregulation occurs early in pregnancy, or PPROM and PTB if misregulation occurs later in pregnancy.[26]

TNF-α is produced in an insoluble pro-form. Once the pro-form is cleaved by TNF-α-converting enzyme, TNF-α is secreted and the systemic effects are observed.[27] Because of this type of protein regulation, the effects of TNF-α up-regulation might not be observed until after stimulation from infection or other environmental factors activating the cleavage mechanism.

Several gene polymorphisms within the *TNF* sequence are known. Herman et al.[28] identified five polymorphisms at positions −857, −851, −308, −232, and +691, and Kamizono et al.[29] identified three single nucleotide polymorphisms (SNPs) in which adenine is substituted for guanine in the upstream region of *TNF*, designated as −238G/−238A, −308G/−308A, and −376G/−376A. The *TNF(−308A)* allele (TNF2 or TNFA2) is located in the promoter region of *TNF*. Individuals

who have one or more copies of the *TNF(−308A)* allele produce slightly more TNF-α than those with two copies of the major alleles,[30,31] although actual production varies in tissues and individuals. It has been suggested that individuals with the *TNF(−308A)* allele may hyper-respond to infections with increased expression of TNF-α, and that these individuals have increased complications from infections such as sepsis, cerebral malaria, mucocutaneous leishmaniasis, and human papilloma virus.[32–35]

Tumor necrosis factor(−308) maternal genotype studies

Roberts et al. first reported an association between *TNF(−308A)* allele and PPROM in a case-control study of African American women[36]. Cases included 55 African American women who delivered before 37 weeks because of PPROM or idiopathic preterm labor. Exclusion criteria included multiple gestation and fetal anomalies. There were 110 control participants who delivered after 37 weeks with no history of PTB. No association with idiopathic PTB and the *TNF(−308A)* allele was found, but the power of the study may have been inadequate. There were significantly more carriers (homozygous and heterozygous) of the *TNF(−308A)* allele among preterm cases attributable to PPROM (N = 15/26, 58%) than among controls (N = 33/110, 30%) (odds ratio [OR] = 3.18, 95% confidence interval [CI] 1.33–7.83; P = .008). The carrier rate among controls was consistent with that reported in other studies.[37,38]

Another case-control study designed to look for an association between cytokine polymorphisms, periodontal disease, and PTB. The authors found that cytokine polymorphisms were not associated with periodontal disease, but the *TNF(−308A)* allele was positively associated with PTB.[39] Of the participants who delivered preterm, 48% (N = 23/48) possessed the −308A allele versus 29% (N = 24/82) of the controls (OR = 2.2, 95% CI 1.0–5.0, P = .026). The cases included women who delivered prematurely as the result of preterm labor or PPROM. The study population consisted of multiple racial groups.

An early study by Dizon-Townson et al. found no significant variation between the frequencies of *TNF(−308A)* allele in women who delivered preterm (N = 203) compared with controls (N = 41),[40] but the controls were unselected for medical or obstetric history. In a later article, the author stated that a reanalysis limited to infection-related PTBs found a trend toward significance.[41]

These studies support a possible correlation between maternal *TNF(−308A)* allele and PTB. Neither study that found an association between *TNF(−308A)* and PTB assessed the infant's genotype.[36,39]

TNF(−308) fetal genotype studies

Fetal tissues also produce TNF-α. Aidoo et al. found that infant genotype correlated with increased PTB risk in a cohort study of 1048 singleton infants in Western Kenya.[42] This study was designed to examine immunologic variation to malaria infection rather than PTB risk. The large study allowed analysis of both homozygotic (N = 19/1048, 1.8%) and heterozygotic infants (N = 160/1048, 15%). Both the *TNF(−308A)* homozygous genotype (relative risk = 7.3, 95% CI 2.85–18.9) and the heterozygous genotype (relative risk = 6.7, 95% CI 2.0–23) were significantly associated with PTB. Maternal *TNF(−308)* genotype was not ascertained.

Chen et al. examined the association between the *TNF(−308A)* allele and PTB risk in a Chinese population with a matched case-control study of familial triads (mother, father, and infant) of 54 case infants and 79 control infants.[43] Cases were live singleton births more than 28 but less than 37 weeks gestation without regard to birth weight. Control infants were matched for ethnicity, age, and delivery date. Multiple logistic regression models found that the OR for PTB was 1.77 (95% CI 0.75–4.2) among *TNF(−308A)* heterozygotes and 12.1 (95% CI 1.4–128.7) among homozygous infants. Matching was accounted for in the analysis. No association was found for the genotype of either parent.

The majority of studies showed a strong association between the *TNF(−308)* genotype and PTB, suggesting that genetic differences for inflammatory response might affect pregnancy length. One study found that the *TNF(−308A)* allele was not associated with PTB in women with cervical incompetence, a condition excluded from the case definition of most of the PTB studies in this review.[44] Although there are well-documented racial disparities in the rates of PTB, there was no variation in the *TNF(−308A)* allele frequency between whites and African Americans in North Carolina.[45] (The race and ethnicity classification/terminology used in the text of this review reflects the terminology used in the original referenced article.)

Interleukin-1 gene complex polymorphisms

Regulation and function

IL1β is another proinflammatory cytokine involved in pregnancy. In primate pregnancy, IL1β infuses into the amniotic cavity, resulting in TNF-α and prostaglandin production and increased uterine activity.[46] IL1β and IL1 receptor antagonist (IL1RA; gene name: IL1RN) compete to bind the IL1 receptor. Thus, IL1RA can inhibit IL1β-induced inflammation and labor (Fig. 1).[47–49] A genetic variation of IL1RN, IL1RA*2 (a penta-allelic 86-base pair tandem repeat-variable number tandem repeat (VNTR) in intron 2), reduces production of IL1RA, increasing the inflammatory response length and severity.[50] Patients with rheumatoid arthritis who have the IL1RA*2 allele combined with other variations in the inflammation pathway are less likely to respond to etanercept, a TNF-blocking agent.[51]

Interleukin-1β receptor antagonist and preterm birth

A case-control study of 52 singleton pregnancies delivered before 34 weeks and 197 control pregnancies delivered after 37 weeks was examined for an IL1β polymorphism and IL1RA*2.[52] Cases were excluded for human immunodeficiency virus infection, history of cervical incompetence, known uterine abnormalities, fetal anomalies, and abruption

of the placenta. Controls were excluded for previous PTB or cervical incompetence, fetal abnormalities, complicated antepartum course, and chorioamnionitis. Of the cases, 36.6% were Europeans, 36.5% were non-African Hispanics, and 26.9% were African Americans; the distribution of controls was 59.4%, 26.9%, and 13.7%, respectively. Among the Hispanic infants, the IL1RA*2 allele was associated with an increased risk for PROM and subsequent PTB (OR = 6.5, 95% CI 1.25–37.7; $P = .021$), and for PTB overall.[52] The carrier rate of IL1RA*2 allele was higher among Hispanic mothers who delivered preterm (57% vs. 26.4% $P = .028$). The IL1RA*2 allele frequency among the women who delivered at term differed by race: 7.4% in women of African descent versus 27.4% ($P = .002$) in women of European descent and 41.5% ($P = .004$) in women of Hispanic descent. The effect of this allele may have been found only among Hispanics because of the higher prevalence of polymorphism in that population or they may have other immune system polymorphisms that amplify the effects of the IL1RA*2 polymorphism.

The IL1RA*2 allele was also associated with PTB and neonatal mortality in a case-control study of multifetal pregnancies.[53] Multifetal pregnancies provide a natural experiment for observing the effect of multiple copies of a gene. In a study of 51 mothers (45 white, 3 Hispanic, and 3 Asian) and 104 neonates by Kalish et al., the median gestational age at delivery was 37 weeks (range 28–38 weeks). As expected, the IL1RA*1 allele (major allele) was more than twice as prevalent as IL1RA*2 allele.[53] Fifty percent (N = 12/24) of pregnancies in which both fetuses carried the IL1RA*2 allele, but only 11% (N = 3/27) in which only one or no fetus carried the IL1RA*2 allele, were complicated by PPROM (OR = 8.0, 95% CI 1.6–50.3 $P = .005$). A greater percentage of control twin pairs were both homozygous for the major allele IL1RN*1, 44.4% (N = 16/36) versus 6.7% (N = 1/15) of the PPROM cases (OR = 11.2, 95% CI 1.3–70.1; $P = .010$). However, infant genotype analysis may be confounded by zygosity, which was not determined. Also, no information on fertility treatments was given. Maternal genotype was not related to pregnancy outcome in this small study.

In a prospective cohort study of 291 consecutive singleton pregnancies undergoing amniocentesis at 15 to 17 weeks gestation, Witkin et al. found that fetal homozygosity for IL1RA*2 allele was significantly associated with birth before 37 weeks gestation ($P < .0001$),[54] although only 18 women delivered preterm. The study took place in Switzerland, and all participants were of European descent. The allelic distribution was in Hardy-Weinberg equilibrium. The population used for this study is problematic, however, because many indications for amniocentesis are also associated with an increased risk of PTB risk.

Interleukin-6

Regulation and function

IL6, generally considered a proinflammatory cytokine, can induce the acute phase inflammatory response by inducing T lymphocytes, C reactive protein, and B cell differentiation. IL6 concentrations are increased in the amniotic and cervical fluid and maternal serum of preterm deliveries.[55–57] A polymorphism in the *IL6* gene at position −174 (G to C) reduces IL6 production, the severity and onset of juvenile chronic arthritis, and kidney transplant rejection, and is associated with improved outcomes in high-risk patients with breast cancer.[58–60] One study investigated whether this polymorphism also reduces PTB risk.[61]

Interleukin-6 (−174) and preterm birth

Simhan et al. examined the polymorphism distribution among 156 women who delivered after 37 weeks of gestation (110 white, 46 African American) and 51 women who delivered before 34 weeks with spontaneous preterm labor and intact membranes (39 white, 12 African American).[61] Women with preeclampsia, diabetes, vaginal bleeding, collagen disease, and cervical cerclage were excluded. Overall, 19.2% of the controls and 2% of the cases (OR = 0.17, 95% CI 0.04–0.74) were homozygous for the *IL6 (−174*C/C) variant. Among whites, the C variant was found in 27.2% of the controls and 5.2% of cases (OR = 0.14, 95% CI 0.03–0.64). No African Americans carried the protective C/C genotype.

A number of studies have shown that the distribution of *IL6* polymorphisms differs among racial groups. African American and Asian populations have polymorphisms that increase IL6 expression levels, whereas the *IL6 (−174*C) allele, which reduces IL6 production, is more common among whites.[62] In a North Carolina population, the frequency of *IL6 (−174*C/C) homozygotes was significantly higher among whites (15%) than African Americans (2%).[45] Of whites, 35% were heterozygous or homozygous for the *IL6 (−174*C) allele, whereas 9% of African Americans were heterozygous and none were homozygous.[59]

Interleukin-4

Regulation and function

IL4 is a potent cytokine produced by TH_2 lymphocytes and involved in innate immunity. IL4 induces differentiation of B lymphocytes and blocks production of interferon-γ, thereby reducing proinflammatory cytokines such as TNF-α, IL6, and IL1. IL4 levels increase during the course of normal pregnancy.[63] A polymorphism in the promoter region of *IL4* at position −590 (C to T) is associated with increased severity of asthma and atopic dermatitis,[64,65] but with a decreased risk of Graves disease.[64] IL4 level variations seem to modulate the balance between innate and cellular immunity. Because cellular immunity is thought to be reduced and innate immunity increased during pregnancy,[15] any variation in this balance might affect pregnancy outcome.

Interleukin-4 and preterm birth

Kalish et al. investigated the relationship between PTB in multifetal pregnancies and maternal and fetal *IL4* and *IL10* genotypes.[66] The cohort study included 73 mother–infant

pairs, 71 twin sets, and 2 triplets. Mothers included 61 white, 5 Hispanic, 3 African American, and 4 Asian women. Spontaneous PTB was defined as delivery for any reason before 37 weeks gestation, with or without PPROM. Exclusion criteria included delivery before 24 weeks gestation and pregnancies complicated by intrauterine fetal death. No association between the *IL10 (−1082)* polymorphism and PTB was found, but the *IL4 (−590T)* allele in either the mother or infant was associated with increased PTB risk. The frequency of maternal heterozygosity for the *IL4 (−590T)* allele was significantly higher in women who delivered preterm than those who delivered at term, 36.2% versus 18.2% (OR = 2.6, 95% CI 1.1–5.9; $P = .02$). Although 20.7% of women who had a PTB were homozygous for the *IL4 (−590T)* allele, only 2.3% of women who did not have a PTB were homozygous (OR = 11.2, 95% CI 1.2–69.5; $P = .01$). When the fetal genotypes were examined, 55.2% of preterm deliveries had two fetuses with the *IL4 (−590T)* allele compared with only 29.5% of term deliveries (OR = 2.9, 95% CI 1.0–8.8; $P < .05$). The relationship between infant genotype and PTB may have been confounded by zygosity, which was not determined. This study suggested that the *IL4 (−590T)* allele may be a risk factor for PTB in multifetal pregnancies. The authors did not expect this finding because increased IL4 production occurs in healthy pregnancies,[63] and because it reduces proinflammatory cytokines associated with increased PTB. As previously discussed, this polymorphism is associated with an increased risk of asthma and other immunoglobulin-E–mediated conditions, leading Kalish et al. to speculate that there may be a relationship between allergic disease and PTB.[66] Alternatively, the authors suggest that the *IL4* polymorphism predisposes individuals to severe infection and PTB through an infection-mediated mechanism. Also, the result may be an artifact of small sample size and potentially confounded by zygosity or assisted reproductive technology, a frequent cause of multifetal pregnancies.

ADDITIONAL POLYMORPHISMS ASSOCIATED WITH PRETERM BIRTH

Matrix metalloproteinases

Regulation and function

Fetal membrane rupture involves MMP-mediated interstitial collagen degradation. MMP1 is the first enzyme to catabolize fibroblast collagen, which is further broken down by gelatinases, MMP2, and MMP9. MMP polymorphisms are associated with coronary heart disease (MMP1), poor prognosis in breast cancer (MMP1), severe chronic periodontitis (MMP1), abdominal aortic aneurysm (MMP9), atherosclerosis (MMP9), and emphysema (MMP9).[67–71]

Matrix metalloproteinase-1 and preterm birth

MMP1 concentrations increase with gestational age and at parturition in normal pregnancies and in cases of PPROM.[72–74] Fujimoto et al. examined the relationship between a G insertion (2G) allele in the promoter of *MMP1* at −1607 that had been shown to increase MMP1 expression[75] with PPROM and PTB.[76] They conducted a case-control study of African American singleton infants without induced labor or malformations. Pregnancies presenting with PPROM before 37 weeks were compared with term pregnancies with no history of PPROM and PTB. Rupture of membranes was diagnosed by vaginal fluid pooling and Nitrazine test. Infants from pregnancies complicated by PPROM had a significantly higher frequency of 1G/2G heterozygotes and 2G/2G homozygotes (N = 66/75; 88%) than did controls (N = 179/235; 76.2%) (OR = 2.29, 95% CI 1.09–4.82; $P = .028$). Conversely, the 1G/1G polymorphism, which reduces MMP1 expression, protected against PPROM. In this study, 1G/1G homozygotes frequencies were higher among whites than African Americans.[76]

Matrix metalloproteinase-9 and preterm birth

MMP9 is involved in the degradation of the basement membrane and other extracellular matrix components and increases in humans at the time of parturition.[74] MMP9 expression is induced in epithelial cells, monocytes, and macrophages[77,78] by proinflammatory cytokines and bacterial endotoxins. Recent studies suggest that increased fetal levels of MMP9 are involved in PPROM and differentiate fetuses with PPROM from those undergoing premature labor with intact membranes.[79] *MMP9* promoter polymorphisms have been investigated as possible mechanisms of MMP9 overexpression and susceptibility to PPROM. Ferrand et al. designed a case-control study of African American women.[80] Cases (N = 74) were infants from pregnancies presenting before 37 weeks gestation with PPROM. Controls (N = 215) were full-term infants whose mothers had no history of PPROM or PTB. Individuals were excluded because of multiple gestation, fetal abnormalities, and medical complications requiring labor induction. Rupture of membranes was diagnosed by vaginal fluid pooling and Nitrazine test. Cases and controls were genotyped to determine the CA repeat sequence length in the *MMP9* promoter and for a polymorphism at −1562. No association was found for the −1562 polymorphism, but the 14 CA repeat was associated with a significantly increased PPROM risk compared with all other repeat sizes (OR = 3.06, 95% CI 1.77–5.27), and with two to threefold more MMP9 expression than the 20 CA repeat size. The frequency of the 14 CA repeat in MMP9 is lower in the African American population than in whites, 19% versus 50%.[80,81]

Toll-like receptor 4

Regulation and function

The toll-like receptor 4 (TLR4) is a transmembrane receptor involved in the innate immune response to Gram-negative bacteria.[82] There are many known polymorphisms in this critical receptor. One allele, *TLR4(Asp299Gly)*, has been associated with severe respiratory syncytial virus disease in infants and increased risk of Gram-negative bacterial infection in critically ill hospital patients.[83,84]

Toll-like receptor 4 and preterm birth

The relationship of the *TLR4(Asp299Gly)* allele with PTB was examined in a Finnish population.[85] The study compared the genotypes of 351 term infants (maternal history of PTB was not mentioned as an exclusion criterion) with those of 440 infants of less than 35 weeks gestation (282 singletons and 158 multiples). They also genotyped 94 unrelated women (74 premature, 62 premature singleton, and 12 premature multiparous deliveries).[85] The *TLR4(Asp299Gly)* and (*Thr399Ile*) polymorphisms exhibited linkage disequilibrium, so only one, (*Asp299Gly)*, was chosen for further examination. The frequency of the *TLR4(Asp299Gly)* allele did not vary among the women, but there was a significant difference in the allele frequency between singleton term and preterm infants (P = .024). The association was not significant when multiple births were included, which are not independent. The association of the allele was stronger with PPROM cases (P = .021) than other causes of PTB (P = .045).

Beta-2-adrenergic receptor

Regulation and function

Beta-2-adrenergic receptor (β_2AR) is a multifaceted receptor involved in linkage of the sympathetic nervous system and the immune system. β_2AR acts in the myometrium to relax muscle fibers at the time of birth,[86] and β_2AR agonists have been used for more than 30 years to inhibit uterine contraction during preterm labor.[87] Norepinephrine stimulation of β_2AR leads to antibody secretion, lymphocyte traffic, and cytokine production[88–90]. Norepinephrine activation of β_2AR receptor stabilizes IKκB (inhibitor of the TNF-α–induced transcription factor NFκB) and thus reduces expression of NFκB-induced genes such as *IL6* and *IL1*β [91] (Fig. 1). A few of the 19 known β_2AR gene polymorphisms have been associated with nocturnal asthma (*Gly-16*), obesity (*Gln-27*), and physical activity-dependent obesity risk (*Gln-27*).[92–96] In vitro studies suggest that the *Gln-27* allele is more resistant to down-regulation by adrenergic agonists than the major allele (*Glu-27*).[97] The *Arg16Gly (Gly-16)* allele increases the desensitization of the receptor when it is stimulated and thus reduces the response to stimuli over time. Given the suggested role of β_2AR in NFκB regulation[91], the *Gly-16* allele might decrease inhibition of the proinflammatory response.

Beta-2-adrenergic receptor and preterm birth

Landau et al. compared the genotype distribution of the *β_2AR* polymorphisms *Gln27Glu (Glu-27)* and *Arg16Gly (Gly-16)* among Hispanic women.[98] Cases (N = 28) were women with singleton pregnancies and without chorioamnionitis, uterine malformation, drug use, or fetal abnormalities who spontaneously delivered before 37 weeks of gestation. Controls (N = 251) had a singleton delivery after 37 weeks of gestation with no PTB history. *Arg16Arg* homozygosity was associated with a markedly reduced PTB risk; 4% of cases (N = 1/28) versus 31% of controls (N = 79/251) were homozygous for *Arg16* (OR = 0.08, 95% CI 0.01–0.58; P = .01). The *Arg16Arg* allele was significantly less frequent in the cases (29%) compared with controls (50%, P = .002). No association was found between the *Gln27Glu* allele and PTB. Being homozygous for the *Arg16Arg* allele (major allele) seemed protective for PTB.

A case-control study by Ozkur et al. also examined the association of *Gln27Glu* and *Arg16Gly* polymorphisms with PTB.[99] This small but well-designed study in a Turkish population examined 80 women who were admitted for preterm labor at 21 to 36 weeks gestation (determined by ultrasound), excluding women with PPROM, major bleeding, pre-existing hypertension, diabetes, kidney disease, obesity, serious maternal diseases, related parents, or uterine contractions before 20 weeks. Controls (N = 76) were women who delivered after 37 weeks of gestation with no PTB history or any other previously mentioned exclusion criteria. Because all women resided in the same district and none were referral patients, this study approximates a population-based sample, unlike the hospital based studies. The *Gln27Glu* allele frequency was higher among cases than controls, 0.42 versus 0.26 (OR = 2.14, 95% CI 1.32–3.46; P = .002). Unlike the Landau et al.[98] study, this study showed no significant associations for the *Arg16Gly* polymorphism. The conflict could be the result of different study populations (United States vs. Turkey), different exclusion criteria, or small sample size.

Vascular endothelial growth factor

Regulation and function

Vascular endothelial growth factor (VEGF) is a critical signaling molecule that regulates the growth of vascular structures (angiogenesis). It is the only known mitogen that acts on endothelial cells.[100] There are a number of known polymorphisms in the VEGF gene including −1879 G/A, −1498 T/C, −1190 G/A, −1154 G/A, −634 C/G, −7 C/T, and 936 C/T.[101,102] A polymorphism at −634 (G to C) has been associated with increased serum levels of VEGF.[101] Diabetic patients with the −634 C allele are more likely to develop the vascular disease nonproliferative diabetic retinopathy than those with the −634 G major allele (OR 3.20; 95% CI 1.45–7.05; P = .0046).[101]

Vascular endothelial growth factor and preterm birth

Papazoglou et al. examined the association of two polymorphisms *VEGF(936C/T and −634G/C)* with spontaneous PTB in a case-control study of 54 women with preterm deliveries and 79 women attending a menopause clinic who had at least two term births and no history of preterm labor or pregnancy loss.[102] It should be noted that the control and case population may have been very different because the controls are taken from an entirely different population, menopausal women. All participants were of Greek ethnicity. The authors defined PTB in this study as cervical changes and regular contraction before 37 weeks gestation. The *VEGF(−634 G/C)* allele was not associated with PTB, but homozygotes or heterozygotes for the *936 C/T* polymorphism were more likely to have PTB (OR = 2.05, 95% CI 1.37–3.06).

Methylene tetrahydrofolate reductase

Methylene tetrahydrofolate reductase (MTHFR) is an enzyme involved in folate metabolism, which is critical to many cellular processes, including DNA replication and methylation. A *MTHFR* polymorphism at 677 (C to T) reduces activity of the protein and has been implicated in vascular disease, neural tube defects, and leukemia.[103–105]

Methylene tetrahydrofolate reductase and preterm birth

Lauszus et al. examined *MTHFR (677C/T)* and PTB in diabetic mothers.[106] In this Danish cohort, 233 insulin-dependent diabetic women were recruited from the maternity ward and genotyped. Sixty-one women delivered preterm (<37 weeks gestation). Heterozygosity at the *MTHFR* 677 locus was not associated with PTB. It is possible that these study results may not be readily generalized to nondiabetic women.

Factor 5

Factor 5 is a critical component of blood coagulation in which alterations can result in hemorrhagic or thrombotic diathesis. A common mutation in *Factor 5 (G1691A*) is associated with pregnancy complications and venous thromboembolism.[107–109]

Factor 5 and preterm birth

Hao et al. used high-throughput genotyping approaches to identify haplotype blocks containing multiple SNPs in 31 candidate genes potentially related to PTB.[110] The SNPs were in Hardy-Weinberg equilibrium. The study included 300 women who had PTB before 37 weeks, and 458 controls.[110] Study participants were racially and ethnically diverse: African American participants included 193 cases and 260 controls, Hispanic participants included 58 cases and 137 controls, and white participants included 50 cases and 61 controls. A Factor 5 haplotype was associated with PTB with a significance of $P = .025$, using haplotype-based association tests, 1000 permutation iterations, and Bonferroni correction. However, it is not clear how the haplotype affects coagulation. Some haplotypes that were not associated with PTB in the global model were associated with PTB among specific racial groups: ILR2 among African Americans ($P = .025$), NOS2A (inducible nitric oxide synthase) among whites ($P < .001$), and OPRM1 (mu-opioid receptor) among Hispanics ($P = .004$), but it is unclear whether these associations were significant after multiple test adjustment. Only maternal genotypes were examined, and interactions among genotypes and environmental factors were not considered.

CONCLUSIONS AND RECOMMENDATIONS

PTB likely involves multiple environmental and genetic risk factors. Many women delivering preterm have no known risk factors, and most interventions fail to substantially reduce risk. Therefore, expanding our knowledge of PTB risk and causality is critical to the discovery and implementation of effective intervention and treatment.

The studies reviewed here are the first steps in examining the association between genetic variation and PTB risk. However, the samples are generally small and are frequently convenience samples of high-risk populations at referral hospitals. The definition and measurement of the study outcomes are varied, and were generally not designed to examine the gene–gene and gene–environment interactions associated with PTB risk. Extensive replication along with information on racial/ethnic groups and subgroup analysis is needed before the impact of individual polymorphisms on PTB can be assessed, but results to date are intriguing.

Most studies described here found that maternal or fetal carriage of common polymorphisms in the inflammation pathway were associated with PTB risk. Generally, polymorphisms that increase the magnitude or duration of the inflammatory response (Table 1) were associated with increased PTB, and polymorphisms that decrease the response were associated with decreased PTB. This relationship concurs with epidemiologic evidence suggesting that environmental factors that increase inflammation also increase PTB risk.[13,14,111]

The *TNF(−308A)* allele was examined most frequently, and in most studies it was associated with increased PTB. Two studies suggest biologic mechanisms for this association. Hernandez-Guerrero et al.[112] demonstrated that in vitro amniochorion tissue with the TNF*2/*TNF(−308A)* allele responded similarly to lipopolysaccharide (surface lipopolysaccharide on Gram-negative bacteria, which triggers an immune reaction) as the major allele, except at higher lipopolysaccharide doses. The tissue with the *TNF(−308A)* allele produced significantly higher levels of TNF-α than the tissue with the major allele. This suggests that tissues expressing *TNF(−308A)* may hyperrespond to a uterine infection by releasing large amounts of TNF-α. Simhan et al. found that maternal *TNF(−308A)* allele in term pregnancies increased the risk of chorioamnionitis by 3.3-fold (95% CI; 1.3–7.1),[38] suggesting that the *TNF(−308A)* allele might also be involved in infection-mediated PTB.

Recently, Macones et al. found that the *TNF(−308A)* allele was associated with increased PTB (OR = 2.7, 95% CI 1.7–4.5),[113] and that the association was even stronger in the presence of BV (OR = 6.1, 95% CI 1.9–21.0). In an accompanying review, Romero and colleagues hypothesized that BV might predispose women with a certain combination of polymorphisms to PTB, and that many polymorphisms may be involved in promoting a hyperimmune or hypoimmune response.[114] In general, the studies reviewed here are consistent with this model, and there may be many additional multifactorial gene–gene and gene–environment interactions that produce a hyperimmune or hypoimmune responses that could lead to PTB (Fig. 1).

If *TNF(−308A)* allele is found to promote PTB by oversecretion of TNF-α, high-risk women could be treated with anti–TNF-α drugs. There is no evidence these drugs are embryotoxic or teratogenic,[115] but thorough study would be necessary before they could be used for a pregnant population.

Table 1.
Studies of genetic polymorphisms and risk of preterm birth published before June 1, 2004

	Cases		Comparison Group							
Study Area, Population	Type, Gestational Age, Dating Method	No.	Type, Gestational Age, Dating Method	No.	Polymorphism	Genotype Associated with Outcome	Comparison (minor vs. major)	Degree of Risk	95% CI and/ orp-value	Reference
US, African-American	Mothers in hospital for delivery, < 37 weeks, unspecified	PPROM=26 IPTB= 29	Mothers with no history of PTB, ≥37 weeks, unspecified	110	*TNF(-308)*	Maternal, yes Infant, N/A	A_vs. GG	OR 3.18	1.33 - 7.83 p=0.008	Roberts 1999[36]
UK, Multiple racial groups	Mothers in hospital for delivery, < 37 weeks, unspecified	PPROM and IPTB = 48	Mothers with no history of PTB, ≥37 weeks, unspecified	82	*TNF(-308)*	Maternal, yes Infant, N/A	A_vs. GG	OR 2.2	1.0 - 5.0 p=0.026	Moore 2004[39]
					IL1 β (+3953)	Maternal, no Infant, N/A	T_ vs. CC	None	N/A	
W. Kenya, Luo ethnic group, African	Infants born in the region, <37 weeks, Determined by Dubowitz score	IPTB = 33	Infants born in the region, ≥37 weeks	926	*TNF(-308)*	Maternal, N/A Infant, yes	AG vs. GG	RR 6.7	2.0 - 23 p=0.009	Aidoo 2001[42]
							AA vs. GG	RR 7.3	2.85 - 18.9 p=0.002	
Anqing, China, Han Chinese	Mother-Father-Infant Triad, <37 weeks, unspecified	54	Mother-Father-Infant Triad matched for age and delivery date, ≥37 weeks, unspecified	79	*TNF(-308)*	Maternal, no Infant, yes Paternal, no	AG vs. GG	OR 1.77	0.75 - 4.2	Chen 2003[43]
							AA vs. GG	OR 12.1	1.4 - 128.7	
US (Utah), Multiple racial groups	Mothers and Infants,<37 weeks, unspecified	Mothers IPTB and PPROM = 203 Infants IPTB and PPROM =43	Unselected mothers, ≥37 weeks, unspecified	41	*TNF(-308)*	Maternal, no Infant, no	A_ vs. GG	None	N/A	Dizon-Townson 1997[40]
US (NY), Multiple racial groups	Mothers and infants,<34 weeks, unspecified in the NICU	IPTB and PPROM = 52	Mothers and infants, ≥37 week, unspecified	197	*IL1 β (+3953)*	Maternal, no Infant, no	T_ vs. CC	None	N/A	Genc 2002[52]
					*IL1RN*2 (VNTR)*	Maternal, no Infant, yes	2,_, vs. 1,1	OR 6.5 (among Hispanics only)	1.25 - 37.7 p=0.021	
US (NY), Multiple racial groups	Mothers of multiples with PPROM, > 24 weeks, unspecified	PPROM = 15	Cohort total Sample Mothers=51 Infants=104 (49 twins, 2 triplets)	No PPROM = 36	*IL1 β (+3953)*	Maternal, no Infants, no (multiple gestations)	T_ vs. CC	None	N/A	Kalish 2003[53]
					*IL1RN*2 (VNTR)*	Maternal, no Infants, yes (multiple gestations)	2,2 in 2 infants vs. 1,1	OR 8.0	1.6 - 50.3 p=0.005	
Switzerland, Non-Hispanic Whites	Mothers w/ amniotic fluid sample, IPTB < 37 weeks, unspecified	18	Mothers,≥37 weeks, unspecified	245	*IL1RN*2 (VNTR)*	Maternal, no Infant, yes	2,2 vs. 1,1	Not given	P < 0.0001	Witkin 2003[54]
US (PA), Multiple racial groups	Mothers, IPTB < 34 weeks, unspecified	51	Mother's with spontaneous labor,≥ 37 wks, unspecified	156	*IL6 (-174)*	Maternal, yes Infant, N/A	CC vs G_	OR 0.17	0.04 - 0.74	Simhan 2003[61]
US (NY), Multiple racial groups	Mothers and infants from multi-fetal pregnancies with or without PPROM and their infants, > 24 weeks and <37 weeks, unspecified	29	Mothers and infants from multi-fetal pregnancies, ≥37 weeks, unspecified Cohort total Sample Mothers=73 Infants=148 (71 twins, 2 triplets)	44	*IL4 (-590)*	Maternal, yes Infant, yes	TT vs C_	OR 11.2	1.2 – 69.5 p=0.01	Kalish 2004[66]
					IL4 (-590)	Maternal, yes Infant, yes	2 infants T_ vs < 2	OR 2.9	1.0 - 8.8 p<0.05	
					IL10 (-1082)	Maternal, no Infant, no	GG vs A_	None	N/A	

Table 1. Continued

Studies of genetic polymorphisms and risk of preterm birth published before June 1, 2004

	Cases		Comparison Group							
Study Area, Population	Type, Gestational Age, Dating Method	No.	Type, Gestational Age, Dating Method	No.	Polymorphism	Genotype Associated with Outcome	Comparison (minor vs. major)	Degree of Risk	95% CI and/ orp-value	Reference
US (PA, MI), African-American	Infants from pregnancies complicated by PPROM, <37 weeks, unspecified	74	Infants, Term pregnancies, unspecified	215	*MMP9 (CA repeat)*	Maternal, N/A Infant, yes	14 vs 15 – 24 CA repeats	OR 3.06	1.77 - 5.27	Ferrand 2002[80]
				207	*MMP9 (-1562)*	Maternal, N/A Infant, no	T_ vs CC	None	N/A	
US (PA, MI) African-American	Infants from pregnancies complicated by PPROM, <37 weeks, unspecified	75	Infants, Term pregnancies, unspecified	235	*MMP1 (-1607)*	Maternal, N/A Infant, yes	_G2G vs 1G1G	OR 2.29	1.09 - 4.82	Fujimoto 2002[76]
Finland, Unspecified	Infants, <35 weeks, unspecified	440	Infants, Term, unspecified	351	TLR4 (Asp299Gly)	Infant, yes	Gly_ vs Asp/ Asp	OR 1.64 (calculated)	p=0.028	Lorenz 2002[85]
	Mothers, <35 week, unspecified	74	Mothers, Term, unspecified	20	TLR4 (Asp299Gly)	Maternal, no	Gly_ vs Asp_	None	N/A	
US (NY), Hispanic	Mothers IPTB, < 37 weeks, unspecified	28	Mothers with uncomplicated pregnancies, ≥37 weeks, unspecified	251	*B2AR (codon 16)*	Maternal, yes Infant, N/A	Gly_ vs Arg/ Arg	OR 0.08	0.01 – 0.58 p=0.01	Landau 2002[98]
					B2AR (codon 27)	Maternal, yes Infant, N/A	Glu_ vs Gln/ Gln	None	N/A	
Turkey, Caucasian	Mothers hospitalized for labor without PPROM, >21 and <36 weeks, ultrasound	80	Mothers with no history of PTL or PPROM, ≥37 weeks, ultrasound	76	*B2AR (codon 27)*	Maternal, no Infant, N/A	Glu_ vs Gln/ Gln	OR 2.14	1.32 – 3.46	Ozkur 2002[99]
					B2AR (codon 16)	Maternal, no Infant, N/A	Gly_ vs. Arg/ Arg	None	N/A	
Greece, Caucasian	Mothers, <37 weeks, ultrasound	54	Menopausal women, ≥2 term pregnancies,unspecified	79	*VEGF (-634)*	Maternal, no Infant, N/A	CC vs G_	None	N/A	Papazoglou 2004[102]
					VEGF (936)	Maternal, yes Infant, N/A	TT vs C_	OR 2.05	1.37 – 3.06 p=0.0009	
Denmark, Unspecified	Mothers with IDDM, < 37 weeks gestation, unspecified	61	Mothers with IDDM, > 37 weeks, unspecified	172	*MTHFR (677)*	Maternal, no Infant, N/A	T_ vs CC	None	N/A	Lauszus 2001[106]
US (Boston), Multiple racial groups	Mothers, <37 weeks, unspecified	300	Mothers with infant >2500g, ≥37 weeks, unspecified	458	*F5 a*	Maternal, yes Infant, N/A	Haplotype block that included rs6019, rs6022 and rs2213869	Not given	p=0.025	Hao 2004[110]

[a] Multiple haplotype blocks were tested; however, only F5 had information sufficient enough to be included in the table.IPTB, idiopathic preterm birth; PPROM, preterm premature rupture of the membranes; PTL, preterm labor; VNTR, variable number tandem repeat; IDDM, insulin-dependent diabetes mellitus; NICU, neonatal intensive care unit; OR, odds ratio; RR, relative risk; CI, confidence interval; N/A, not available.

Future studies

Multiple interactions exist between the genes examined in this review (Fig. 1), suggesting that gene–gene interactions may affect PTB risk. For example, women who carry both the *TNF(−308A)* allele, which increases inflammation, and the *IL6(−174C)* allele, which reduces inflammation, may not be at increased PTB risk. Conversely, women who have both the *TNF(−308A)* allele and the *IL1RA*2* allele might have a compounded risk of PTB. Therefore, a study of polymorphism combinations, in conjunction with modeling and analyses of maternal/fetal genotypes and phenotypes, would be valuable. Polymorphisms can be racially and ethnically distributed, so stratification is recommended.

Because many of the existing inflammation studies were based on small study populations not designed to examine gene–gene and gene–environment interactions in PTB risk, a large well-designed study would be invaluable. This study will need to model and analyze maternal/fetal genotypes and polymorphism combinations, phenotypes, sibling and parental health, and environmental exposures (infection, obesity, and stress). Such a study could easily be expanded to examine polymorphisms in other biologic systems that are important in pregnancy and parturition, such as placental vasculature development and hypothalamic-pituitary-adrenal axis regulation.

Researchers have focused on candidate genes in the inflammation pathway. In addition, pathways not yet discovered could be critical in PTB cause. Future studies could use whole genome scans to look for previously unidentified risk loci that confer an increased risk of PTB. Proteomic serum analysis could elucidate particular protein profiles that predict increased risk for PTB, and microarray-based expression studies could identify changes in gene expression between women who deliver preterm or at term. Successful use of any of these techniques requires consistent and specific case definitions, large sample size, and analysis of interactions between multiple factors.

Preclinical and predictive impact

In the future, genetic polymorphisms alone or in combination with information on environmental exposures may be useful in screening women for PTB susceptibility. To date, however, interventions for preventing PTB or treating preterm labor, such as antibiotics to treat infection or social support to offset the effects of stress, have been largely unsuccessful.[7] Tocolytic agents do not consistently stop labor,[116] and antibiotic administration has had conflicting results, and in some cases may worsen prognosis.[117,118] However, recent progesterone prophylaxis trials have had promising results.[119,120] These and other emerging interventions and treatments might improve outcomes in the future.

CONCLUSION

The studies reviewed here suggest that polymorphisms in inflammatory pathway genes may modulate a woman's PTB risk. Additional large and well-designed studies may show the critical genetic and environmental factors necessary to predict PTB risk. Genetic screening may someday be used to locate women at highest risk for preterm delivery, and to develop tailored interventions based on that woman's particular inflammatory tendencies, stress susceptibilities, vascular insufficiencies, or tissue-remodeling predisposition. Future research is needed to develop the tools to enable PTB risk prediction and assist in the development of targeted treatments to alleviate this major public health problem.

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