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Genetic Basis of Preterm Birth

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1. ABSTRACT

Epidemiologic data show that women who deliver prematurely often have a personal and/or family history of preterm birth (PTB) and that racial and ethnic differences influence the incidence of PTB. This may indicate genetic predisposition to PTB. However, since races and ethnic groups tend to share environmental factors (exposure to toxins, living conditions, diet, smoking), epidemiologic data may just confirm environmental influences on PTB. Alternatively, PTB may represent a consequence of gene-environment interactions. Infection and inflammation correlate with increased risk for preterm premature rupture of amniotic membranes (PPROM) and PTB. Immunomodulatory molecules and their receptors regulate these processes and many of them are products of polymorphic genes. Single nucleotide polymorphisms (SNPs) of a gene may lead to a differential expression of its product. So far, SNPs for several genes have been implicated in PTB. If it is confirmed that polymorphism(s) in particular gene(s) correlates with PTB, it may become possible to develop targeted diagnostic and therapeutic approaches tailored towards unique genetic characteristics of a mother/fetus pair.

2. INTRODUCTION

Despite progress in many aspects of obstetrical management the rates of PTB have been stable, or are even increasing. For example, in the US the rate of PTB was 12.1% in 2002: a 29% increase over the previous two decades (1,2). Premature birth accounts for 5-10% of annual births in developed countries and up to 25% in developing countries (3). The major reasons for this disparity are social-economic disadvantages in developing countries (maternal stress, malnutrition and heavy physical work) (4) complicated by infections (HIV (5), malaria, (6)). In developed countries, major causes of preterm birth are maternal medical indications (hypertension and pre-eclampsia, contribute to up to 25% of PTB) (7), intrauterine growth restriction (8), multiple pregnancies (9) and some of the same socio-economic and behavioral factors present in developing countries (smoking, inadequate prenatal care) (10).

Studies of epidemiology and pathophysiology of PTB suggested four major pathways that may lead to it (11):

1) Premature activation of hypothalamic-pituitary-adrenal axis (HPA). Fetus may initiate her/his own birth as a consequence of premature activation of the hypothalamic-pituitary-adrenal axis in a "hostile" intra-uterine environment. "Stressed" fetus may produce cortisol and stimulate placental prostaglandins, oxytocin, corticotropin-releasing hormone leading to PTB. Maternal stress may cause activation of her HPA, oxytocin release and regular uterine contractions (12).

2) Inflammation and Infection. Maternal genitourinary infections may stimulate cells of fetal innate immunity leading to disbalance between inflammatory and anti-inflammatory cytokines and their receptors. In experimental animals, PTB is initiated by pro-inflammatory cytokines: IL-1beta, which stimulates prostaglandin production leading to uterine contractions, while another pro-inflammatory cytokine, TNF-alpha acts on cervical smooth muscles leading to ripening of the cervix and rupture of membranes (13). Intraamniotic infections causing elevation of prostaglandins (14), or deficiency in chorio-decidual enzyme that metabolizes prostaglandin E_2 (15-hydroxyprostaglanin dehydrogenase) may lead to interrupted uterine quiescence as a consequence of stimulation of uterine muscle by prostaglandins (15). Inflammatory cytokines produced during infection may activate expression of matrix metalloproteinases leading to extracellular matrix degradation, premature rupture of amniotic membranes and cervical changes (16). Infection, inflammation and collagen degradation have been identified risk factors for PPROM (17).

3) Decidual hemorrhage. In placental abruption decidual hemorrhage and uterotonic activity of fibirin may stimulate uterine contractions (18)

4) Uterine overdistention. Epidemiologic data indicate that multiple pregnancies and pregnancies complicated by polyhydramnios result in earlier deliveries (9).

Activation of multiple pathways may result in PTB and some of these events may be genetically predetermined (19). Genetic factors that may play a role in PTB are explored in this review.

3. EPIDEMIOLOGIC INFORMATION IMPLICATING GENETIC PREDISPOSITION TO PMB

3.1. Personal and family history

Personal obstetrical history shows the highest degree of correlation with PTB: women who previously delivered a premature, or small for gestational age (SGA) infant may experience preterm birth or have a growth-restricted fetus again, often at a similar gestational age (20). A daughter, or a sister, of women who delivered prematurely is at increased risk of delivering a premature infant. However, this predisposition does not apply to men who were born preterm (21). The specific risk varies and is increased if a previous infant was born before 32 weeks gestation, or a previous pregnancy was recent. In addition, women whose previous infant was SGA have an increased risk of stillbirth, particularly if the previous infant was premature (22). Treloar *et al.*(23) investigated genetic predisposition to PTB, using 905 parous twin pairs (579 monozygotic and 326 dizygotic) in Australia. They showed an additive effect of genetic influences and individual environmental effects. Heritability was 17% for preterm delivery in first pregnancy, and 27% for preterm delivery in any pregnancy.

3.2. Racial and ethnic differences

It is important to identify risk factors correlated to PTB in each ethnic and racial population. For example, Swedish women have a much lower incidence of PTB than elsewhere in Europe, likely because they have a lower incidence of genitourinary infections (14). However, microorganisms were detected in the amniotic fluid in 25% of women with PROM and in 16% of those progressed to PTB. Thus, infection-related PTB is not less frequent in northern Europe than elsewhere (14). In upper middle class Chinese population in Taiwan the main risk factors for PTB were more than two prior abortions, previous PTB, multiple gestations, and current obstetric conditions (fetal anomaly, abruption, placenta previa) (24). In contrast, large social disparities were noted in spontaneous preterm birth rates in transitional Russia: premature infants tend to be born to less educated women living in poverty (25).

In North Carolina the overall relative risk of preterm birth among African American women compared with Caucasian women was 2.6 (95% confidence interval (CI) 2.1-3.1). With adjustment for age, gravidity, marital status, education, and county of residence, the estimated relative risk for African American women compared with Caucasian women was 2.1 (95% CI 1.1-4.1) for medically indicated preterm delivery, 1.6 (95% CI 1.1-2.3) for preterm birth as a result of preterm labor, and 1.9 (95% CI 1.2-3.1) for preterm premature rupture of membranes (26). In a population-based birth certificate study, Zhang and Savitz (27) also showed that, when controlling for other factors, African-American women had 3.5 times the risk of Caucasian women of PTB and 3.3 times the risk of PPROM. However, higher incidence of prematurity does not correlate with an increase in mortality of African-American infants born prematurely, indicating that there may be accelerated maturation among African-American fetuses and neonates (3).

Studies with adequate sample size need to be performed to determine the extent to which gene polymorphisms contribute to the racial heterogeneity in PTB.

4. SINGLE NUCLEOTIDE POLYMORPHISM IMPLICATED IN PTB

Multiple genes in myometrium (28) and fetal membranes (29) are gestationally regulated and upregulated during premature labor or infection. Changes in the expression of these genes may account for individual differences in the risk for preterm birth (11). A mechanism for the proposed genetic differences may be related to the

single nucleotide polymorphisms (SNPs) of some of these genes.

Each individual carries two same (homozygous) or two different (heterozygous) genetic variants of each gene on his/her chromosomes. These variant are described as alleles. The difference between two alleles may be in one nucleotide: thus, single nucleotide polymorphisms (SNPs). SNPs may lead to differential expression of their products. For example, changes in a promotor region of a gene may provide differential binding of transcription factors, or lead to a preferential, or less frequent use of alternative promoters. Differences in the coding regions of the gene may alter its function, or stability as a protein/enzyme, or lead to post-translational modification. SNPs in the non-coding region of a gene may alter its splicing, post-transcriptional processing or transcript stability. *In vitro* and some *in vivo* studies provided experimental correlation between a particular allele and the expression of its gene product. These results were used as a basis for epidemiological correlations of prevalence of particular SNPs in a population and in different conditions and diseases.

Identification of genes and their polymorphisms predisposing to PTB would constitute a major breakthrough in its diagnosis and therapy (30). Such discoveries would enable individualized and potentially more successful approach to treatment of preterm labor.

In the following paragraphs mostly epidemiological studies are described. They examined SNPs of different genes and correlated prevalence of particular alleles in a population with the prevalence of PTB. In most studies, cells from buccal mucosa from mothers and their infants were examined for the presence of particular SNPs using oligonucleotides to generate specific PCR fragments. This non-invasive technique allowed for accumulation of substantial body of information. Since it is difficult to determine the timing and a compartment to measure the concentration of the gene products, most studies did not attempt to correlate SNPs with the gene expression. Thus, the primary end-point in most of the studies was the difference in the incidence of PTB. Since PTB is a multifactorial process, it is not surprising that the data available so far is non-conclusive and several "negative" results and multiple contradictory findings have been reported (Table 1). The other weakness of many studies is the number of subjects. Although investigators enrolled 100 or more subjects in most studies, the number of participants may not be sufficient for studies in population genetics, especially when studied population is not homogenous and prevalence differs among races or ethnicities. It is also clear that more than one gene product may be involved, or that environmental factors may modulate gene expression.

4.1. Polymorphism of genes involved in innate immunity

Microorganisms causing vaginal or intraamniotic infection interact with Toll-like receptors (TLRs). This interaction triggers production of one or more cytokines/chemokines and anti-microbial peptides leading to PTB or/and PPROM (14). Although infections are important triggering factors, an altered inflammatory cascade predetermined by polymorphisms in the genes of innate immune system, may influence progression to labor and impact frequency of PTB. Polymorphisms of innate immune system genes were examined in several studies comparing the prevalence of particular SNPs among preterm and term infants and their mothers (Table. 1).

4.1.1. Toll-like receptors (TLRs)

In women with Gram-negative genitourinary infections a bacterial cell wall component, lipopolysaccharide (LPS), initiates tissue responses leading to PPROM. LPS is recognized by two components of innate immunity: CARD15 and TLR4 genes. Mutation 2936insC in CARD15 and a polymorphism in TLR4 896 AinsG impair responses to LPS. Since African American women have a higher incidence of PPROM, Macones at al (17) investigated mutations in CARD15 and polymorphism in TLR4 among African American women and compared them to Caucasians. They found no difference in the frequencies of mutation 2936insC in CARD15 and a polymorphism in TLR4 896 AinsG between mothers or their fetuses. However, the CARD15 mutation was only detected in controls and not in PPROM cases.

In a Finnish population, the frequencies of TLR4 299Gly allele (Asp/Gly or Gly/Gly) were found to be higher in premature singleton infants than among term singleton infants ($p = 0.024$, $p = 0.028$, respectively) or in premature multiples ($p = 0.036$, $p = 0.044$, respectively) (31).

German Genetic Factors in Neonatology Study Group compared a large cohort of preterm (n=909) and term (n=491) infants and their mothers (n=747). They looked for frequencies of polymorphisms of the promotor region of TLR4-896G. Homozygosity or heterozygosity for TLR4-896G was not significantly different: 11.6 % preterm versus 10.5% term infants; and 8.1 versus 11.5% in their mothers (32).

Hartel *et al* (32) and Nguyen *et al* (33) investigated differences between TLR2 and other receptors involved in innate immunity (mannose binding protein, heat shock protein) and found no significant differences between term and preterm infants and their mothers.

4.1.2. Inflammatory cytokines

Intrauterine infection induces production of pro-inflammatory cytokines and chemokines that trigger preterm contractions, cervical ripening and rupture of membranes. Both maternal and fetal tissues seem to play a role in these processes. Although colonization and infection may lead to chorioamnionitis, fetal inflammatory syndrome and preterm birth, not all colonized women progress to deliver prematurely. In addition, clinical trials designed to prevent/abolish preterm birth using antibiotics showed mixed results (34). An alternative hypothesis suggests that common genetic variation in inflammatory cytokines genes may contribute to risk for PTB.

Allele + 3953 T in exon 5 of IL-1beta results in an increased production of IL-1beta. While African-

Table 1. SNPs of some of the genes correlated with PTB

Gene	SNP region/allele or amino acid change	Expected Effect/*in vitro* confirmation	Effect in Population	Reference
IL-1ß	Exon 5 +3953 T allele	Increased expression	Increased PTB in African American women Not found in Caucasian women	35,19
TNF-a	Promoter -308 A allele -863 A allele (homozygocity)	Increased expression Increased expression	Increased PTB Increased : PTB/ chorioamnionitis/ neonatal morbidity	36,38
IL-6	-174 C (homozygocity) -174G (homozygocity)	Decreased concentration in amniotic fluid	Decreased PTB Increased PTB	32,39
IL-1ra	Intron 2 : IL1 RN*2 (homozygocity)	Increased IL-1ß in amniotic fluid	Increased PTB	41,42
IL-4	+590C (homozygocity) -509 C/C		Increased PTB Increased PTB	43,44
IL-10	-1082A/-819T/-592A -1082G/-819C/-592C (homozygocity)		Increased PTB Increased PPROM	44
TLR4	299asp/gly 299gly/gly 896AinsG	Impaired response to LPS	Increased PTB No correlation to PTB	17,31
TLR2	TLR2-Arg753Gln		No correlation to PTB	32
CARD15	mutation	Impaired response to LPS	Decreased PPROM	17
CD14	159T		No correlation to PTB	32
NOD	NOD2-3020insC		No correlation to PTB	32
MMP-1	–1607insertionG	Increased promoter activity in tissue culture	Increased PPROM (in fetal carriage of insertion)	49
MMP-8	-799C/T, -381A/G and +17C/G	Increased promoter activity in tissue culture	Increased PPROM	47
MMP-9	14 CA repeat allele	Increased promoter activity in tissue culture	Increased PPROM	48
$ß_2$-adrenergic receptor	Glu 27 allele Arg 16 homozygocity	Decreased expression *in vitro*	Increased PTB Decreased PTB	53-55
Factor VII	-121del/ins		Increased in PTB (infants and mothers)	56
Factor XIII	Val34Leu		Decreased in PTB	56
Factor V Leiden			No correlation to PTB	57
Prothrombin	G20210A		No correlation to PTB	57
MTHFR	C677T		No correlation to PTB in Caucasian women Increased in Mexican women	57-58
VEGF	-634 G/C + 936 C/T		Increased PTB	52
Paraoxonase	PON1 RR PON2 CC		Increased PTB	59
Dihydrofolate reductase	19-base pair deletion allele		Increased PTB	68
Microsomal Epoxide hydrolase	Tyr113/His in exon 3		Increased perinatal mortality	62
GlutathioneS-transferase T1	-1285G		Increased PTB	67

SNPs: single nucleotide polymorphisms; PTB: preterm birth; PPROM: preterm premature rupture of amniotic membranes. For others, see text.

merican women who delivered premature infants carried IL-1beta exon 5+3953 T allele more often than African-American women who delivered at term (35), African – American infants born prematurely were less likely to have IL-1beta exon 5+3953 T allele (7%) than term infants (41%) (15). This result was not found among Caucasian women (19). The differences between races could alternatively be explained by the increased infection rate among African-American women; thus by environmental, rather than genetic factors.

The A allele at –308 of the TNF-alpha promoter region results in increased production of TNF-alpha. Roberts *et al* (36) reported an association between the TNF-alpha promoter region polymorphism at –308 (–308 A allele) and PPROM and spontaneous PTB in African-American women. Moore *et al* (37) reported a similar finding among British women: 48% of women who delivered prematurely carried TNF-alpha-308 A allele, in contrast to 29% of women who delivered at term. In contrast, Amory *et al* (38) found that mothers homozygous for the TNF-alpha -863 A allele had significantly earlier deliveries ($p = .02$), more chorioamnionitis ($p = .03$), and greater neonatal morbidity ($p = .03$), but neither maternal nor fetal carriage of the TNF-alpha-308 polymorphism was associated with adverse outcome.

Unlike the IL-beta exon 5 + 3953 T allele and TNF-alpha promoter polymorphisms, the homozygosity for IL-6−174 C allele results in decreased cytokine production. Simhan *et al* (39) reported the association of homozygosity for IL-6−174 C allele with a decreased risk of preterm birth in a cohort of primarily Caucasian women. The C/C variant was present in 27% of controls and only 5% of Caucasian women with PTB. In contrast, no African-American women who gave birth preterm were found to

carry this polymorphism. Thus, the C/C variant in the IL-6-174 promoter region may play a 'protective role' in preterm birth. A similar result was reported among German women in whom homozygosity IL-6-174G was reported in 29 % if they delivered at term versus 38% if they delivered preterm (p = 0.018).

The interleukin-1 receptor antagonist (IL-1ra) inhibits the biologic effects of IL-1 by blocking its receptors. Reduction of IL-1-induced prostaglandin production by intrauterine tissues may postpone preterm labor associated with infection. (40). Witkin *et al* (41) showed that polymorphism in intron 2 of the fetal IL-1ra (IL-1RN * 2 carriage) was associated with 50% increase in midtrimester intra-amniotic IL-1beta levels above fetuses who were homozygous for IL1-RN*1. Bessler at al (42) reported a significantly higher frequency of IL1RN * 2 homozygosity among infants born prematurely. In addition, IL-1RN*2 homozygosity was related to PTB and increased vaginal colonization in African-American women (42).

4.1.3. Anti-inflammatory cytokines

Anti-inflammatory cytokines (IL-4, IL-10, IL-13) play a critical role in maintenance of pregnancy. Engel *et al* (43) showed that African-American women who were homozygous for IL-4 +590C had a greater risk of spontaneous preterm birth (OR=2.9, 95% CI=1.2-7.4). Haplotypes IL-10 -1082A/-819T/-592A (multivariable odds ratio, 2.1; p = .04), and IL-4 -509C/C (multivariable odds ratio, 3.4; p = .02) were associated independently with preterm birth at <29 weeks of gestation in white Australian women (44). Homozygosity for IL-10 -1082G/-819C/-592C haplotype (multivariable odds ratio, 1.9; p = .02) was more common in women with PPROM (44).

4.2. Matrix metalloproteinases

Intact amniotic fluid membranes serve as a physical and functional boundary for the fetus. Premature, pathological rupture of these structures is a major cause of spontaneous preterm labor and preterm birth. Activation of matrix metalloproteinases (the collagen metabolizing matrix metalloproteinases MMP-1, -3, -8, -9) lead to degradation of connective tissue, cervical changes and rupture of membranes. Bacterial products and/or the pro-inflammatory cytokines act on cervical smooth muscle cells to provoke the expression of MMPs (45).

Elevated mid-trimester concentrations of amniotic fluid matrix metalloproteinase-8 were found to correlate with early spontaneous preterm delivery (<32 weeks) (46). A case-control study of African-American neonates showed significant association between three alleles which display the highest MMP-8 promoter activity in trophoblast cells, with PPROM (47). Ferrend *et al* (48) studied functional significance of a variable number tandem repeats in the promoter region of MMP-9 gene on promoter activity and their association with PPROM. They found that the 14 CA-repeat allele was a stronger promoter than the 20 CA-repeat allele in amnion epithelial cells assay. A case-control study of African-American neonates revealed that the 14 CA-repeat allele was more common in newborns delivered to mothers who had PPROM than in those delivered at term.

Fugimoto *et al* (49) studied polymorphism of MMP-1 in amnion epithelial cells. An insertion of a guanine (G) at –1607 position of the promoter region of MMP-1 gene showed increased promoter activity. Fetal carriage of 2G alleles at –1607 of MMP-1 was associated with PPROM (49).

4.3. Vascular endothelial growth factor (VEGF)

VEGF an angiogenic factor expressed in fetal membranes and deciduas that modulates placental perfusion and amniotic membranes permeability. Daneshmand *et al.* (50) reported that in response to hypoxia and chorioamnionitis expression of VEGF and its receptors (VEGF-R1 and -R2) were reduced in comparison with controls. Since VEGF is essential for fetal and placental angiogenesis (51), its absence may lead to placental dysfunction and PTB.

Parazoglou *et al* (52) linked two common functional VEGF gene polymorphisms (-634G/C and 936C/T) with altered VEGF gene responsiveness, and spontaneous preterm delivery.

4.4. Beta2-adrenergic receptor (beta2-AR) polymorphism

Beta2-adrenergic receptors have been identified in the uterine blood vessels and in the placenta (53). Beta agonists have been used to inhibit uterine contractions and shown to prolong pregnancy for at least 48 hours. Two biologically relevant SNPs were identified: at codon 16 and codon 27 of the beta2-AR gene. Homozygosity for allele A at codon 16 (Arg-16) has been associated with a down-regulation of beta2-AR gene expression *in vitro* (54) and thus, was predicted to have a "protective" effect on PTB.

Several investigators (53-55) studied association between polymorphisms at these two loci of beta2-AR gene and pregnancy outcome. Landau *et al* (54) compared the distribution of beta2-AR genotype between 251 Hispanic women who delivered at term and 28 Hispanic women who delivered preterm. Only one woman who delivered preterm was homozygous for Arg16 versus 79 (31%) in the control group (p =.01, odds ratio: 0.08). They did not find any association of preterm labor with genotype at position 27. In contrast, Ozgur *et al* (53) showed a correlation between Gln 27Glu allele and PTB in population of Turkish women (80 preterm and 76 term) with odds ratio for the occurrence of preterm labor 2.14 (95% CI, 1.32-3.46; P=0.002) for the Glu 27 allele.

Doh *et al* (55) studied 159 mother-infant pairs after a preterm or term birth. Homozygosity for allele A at codon 16 (Arg-16) occurred in 20% mothers with a term birth and in none of the mothers who had a spontaneous preterm birth (p=0.002). Conversely, 75% mothers with a spontaneous preterm birth, as compared to 46% mothers with term births, were Arg-16/allele G (Gly-16) heterozygotes (p=0.003) (55).

4.5. Polymorphism of thrombotic and anti thrombotic markers

Epidemiologic data indicate association between thrombosis and spontaneous abortions and preterm birth (56). The explanation for this association may be related to the placental vascular disease (hyperfibrinogenemia is associated with arterial and venous thromboembolism) or uterine contractions induced by thrombin (a potent uterotonic agent) (12).

Polymorphisms in several thrombotic and anti thrombotic markers have been associated with obstetrical complications and placental vascular disease. However, conflicting results were reported when polymorphisms in these genes were studied in correlation with PTB. Hartel at al (56) studied the impact of V Leiden and the prothrombin G20210A mutation, and polymorphisms in the factor VII (121del/ins) and the factor XIII (Val34Leu) in preterm very-low-birth-weight (VLBW, n=593) and term-born-infants (n=278) and their mothers (n=785). The maternal factor VII-121del/ins polymorphism (26.2 vs. 17.6 %; p=0.009) and the infant's factor VII-121del/ins polymorphism (29.0 vs. 20.0 %; p=0.009) were more frequent in VLBW and their mothers compared to term infants and their mothers. Furthermore, the frequency of the factor XIII-Val34Leu polymorphism was significantly lower in VLBW than in term infant controls (5.1 vs. 9.6%, p=0.025). In a multivariate regression analysis, previous preterm delivery (OR=3.8, 95% CI: 1.7-8.4), the maternal carrier status of the factor-VII-121del/ins polymorphism (OR=1.7, 95% CI: 1.12-2.5, p=0.007) and the lower frequency of infant's factor-XIII-Val34Leu polymorphism (OR=0.53; 95% CI: 0.29-0.96; p=0.038) were found to be independently associated with preterm delivery.

A study by Resch *et al* (57) showed no correlation between polymorphisms in factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T mutations, and preterm birth in Caucasian women. However, Valdez at al (58) showed significant increase in the frequency of polymorphisms for MTHFR C677T and the angiotensin-converting enzyme insertion/deletion polymorphism (deletion) among Mexican women who had a history of preterm delivery compared with controls.

Human paraoxonase (PON) is an enzyme involved in vasodilatation and thrombosis and thus, potentially in redistribution of blood flow through the placenta. In one study, polymorphisms in two paraoxonase genes, genotypes PON1RR and PON2 CC, were found more commonly among Chinese infants born prematurely (59).

A potential explanation of the diverse findings among the ethnic and racial groups may be related, like in the case of SNPs for other genes, to the sample size, underlying disorders and effects of environmental factors.

4.6. Genes involved in xenobiotic metabolism

Genes involved in xenobiotic metabolism regulate response to oxidant challenges. Oxidant-antioxidant balance may modulate the likelihood of PTB. When components of the antioxidant defense system in placental tissues were studied in women with normal pregnancy and term delivery and women with late spontaneous abortions it was found that glutathione-S-transferase activity was decreased in placentas that resulted in spontaneous abortions (60). Similarly anti-oxidant enzymes: glutathione S-transferase, selenium dependent glutathione peroxidase, catalase activities were depleted in pregnancies complicated by diabetes and preeclampsia (61).

Genetic polymorphism in microsomal epoxide hydrolase (EPHX His113/His113 genotype,) and glutathione S-transferase P1 (GSTP1) was found in women who experienced perinatal mortality caused by placental pathology, congenital disorders and complications of preterm birth (62).

4.7. Uterine distention and PTB

Majority of multiple pregnancies are delivered preterm. Thus, uterine distention may play a role in PTB. Kalish *et al* (63-65) investigated different polymorphic genes in multifetal pregnancies and reported an association of premature rupture of membranes and carriage of TNF-alpha –308 and/or heat shock protein-70 allele 2 by the first-born fetus; maternal and fetal carriage of the IL-4 T allele an increased risk of spontaneous preterm birth; PPROM and a polymorphism at position -670 in the Fas gene.

5. INTERACTIONS BETWEEN GENETICS AND ENVIRONMENT

Environmental influences modify genetic predisposition. An example is the association between maternal cigarette smoking and infant's birth weigh. The greatest reduction in birth weight was found among smoking mothers with the CYP1A1 Aa/aa and GSTT1 absent genotype -1285 G. Glutathione S-transferase, (GSTT1) is one of the enzymes responsible for the metabolism of tobacco-related mutagens and carcinogens. Nukui *et al* (67) have shown that women and their babies homozygous in the glutathione S-transferase T1 "null" genotype, are at increased risk of PTB.

Folate is critical for cell division, and a normal fetal development. Dihydrofolate reductase (DHFR) is essential enzyme in folate metabolism. In one study (68) women with a deletion allele in DHFR had a significantly greater risk of PTB (OR=3.0; 95% CI: 1.0-8.8) than did those without a deletion allele. If they also had a low folate intake then they had a significantly greater risk of having an infant with a low birth weight (OR=8.3; 95% CI: 1.8-38.6) (68).

Another example of interaction between genetics and environment is the association between TNF-alpha polymorphism and PTB that is modified by the presence of bacterial vaginosis (69). Mothers with a "susceptible" genotype and bacterial vaginosis had increased risk of PTB (OR 6.1, 95% CI 1.9-21.0) (69).

Labor is regulated by multiple overleaping and redundant mechanisms. Thus, it is likely that multiple gene products (and their polymorphisms) may be involved in the control of PTB and that combinations of genes and their products together with obstetric family history and distribution of the alleles in the population, will be required for the diagnosis and treatment of PTB. Several studies support this hypothesis. Engel *et al* (43) studied polymorphism of six cytokine genes and Annells *et al* (44) studied 22 SNPs in immune response genes previously correlated with PTB. Both groups found that not one, but several haplotypes increase risk for PTB. Hao *et al* (70) conducted a large study in which the authors explored 426 SNPs in 300 mothers who had PTB and compared them to 458 mothers who delivered at term. Twenty-five candidate genes were included in the final haplotype analysis.

6. FUTURE DIRECTIONS: GENOMICS AND PROTEOMICS

Genomics technologies like transcriptional profiling and proteomics allow researchers to simultaneously study expression of thousands of genes even without knowledge of their function and determine their potential relationship to PTB. Several groups have recently used a genomic approach to evaluate changes in myometrial gene expression during labor in an attempt to identify potential markers of labor. Aguan *et al* (71) performed microarrays to compare expression of 588 genes in the myometrium from three laboring and three non-laboring women and found 21 genes that were two fold up- or down-regulated during labor. Using differential hybridization, Chan *et al* (72) found thirty differentially expressed genes in labor.

Proteomics analysis using mass spectrometry (MS) is able to identify virtually all peptides and proteins present in biological specimens and provide data about timing, amounts and posttranslational modifications of genes identified by analysis of genotype and mRNAs (by microarrays). This technique may be used to identify prognostic markers of PTB, to block them when present, or to follow the progression of PTB in a single women over time. The limitation of methods used for proteomic analyses (surfaced-enhanced laser desorption-ionization-time of flight SELDI-mass spectroscopy MS) of serum is that although it can separate thousands of proteins it cannot identify them without a purification (73). Arrays of specific antibodies may become available in future and facilitate identification of proteins. At present, identification of all peptides and proteins in biological specimens is only theoretical. In practice, certain techniques identify preferentially certain proteins and peptides (74). However, these techniques appear more sensitive that standard immunologic tests. Buhimschi *et al* (75) analyzed four biomarkers (human neutrophil peptide 1, defensins, calgranulin C [Cal-C], and Cal-A) using proteomic technology (SELDI-TOF MS) and showed that it is more sensitive than enzyme-linked immunosorbent assay (ELISA) for detection of these biomarkers in intra-amniotic infection. Gravett *et al* (76), also, used proteomic profiling to diagnose intra-amniotic infection. Two specific peptides, calgranulin B and a unique fragment of insulin-like growth factor binding protein-1, were characterized and subsequently used to detect sub-clinical intra-amniotic infection. This information would not be available if the study of genome was performed since a fragment of insulin-like growth factor 1 in women with sub clinical infection may reflect proteolytic activity of an enzyme that is unique to labor.

Pharmacogenomics determines drug responses (potential for therapeutic effect and toxicity) based, in part, on the genetics of the subject. In contrast to "traditional" drugs in which the same medication is used to treat all patients, in this case therapy will be tailored towards patient's specific genomic profile. This approach may allow for the identification a group of patients that would respond to therapy, optimize therapeutic window and minimize toxicity. One could speculate that blocking receptors for inflammatory cytokines, or providing soluble receptors to compete for them, could prevent a progression of preterm labor to delivery.

These rapidly evolving techniques are able to identify and measure multiple markers in various biological specimens. They may be able to identify specific biomarkers of pretem labor/PTB and propose novel therapeutic target molecules that would block them. It may become possible to develop a patient-specific treatment (based on her pharmacogenetics). However, the amount of data generated and difficulty in interpreting their relevance represents an obstacle that needs to be overcome before application of these technologies become applicable to patient care.

In conclusion, available evidence that genetic factors (especially factors modulating inflammatory response in fetus and mother) play an important role in PTB and delivery was reviewed. It was emphasized that environmental factors act in concert with genetic predispositions modifying the risk of an individual during a particular pregnancy. New technologies (genomics and proteomics) will likely be able to identify women that are at increased risk for PTB and provide targeted treatments based on individual risk. However, these are evolving fields, and while identification of genetic predisposition is important and it may evaluate the risk it is a long way before these new techniques could be used for diagnosis or treatment.

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