

Preterm birth and single nucleotide polymorphisms in cytokine genes

Qin Zhu, Jian Sun, Ying Chen

Suzhou Hospital Affiliated to Nanjing Medical University, Suzhou 215002, China

Correspondence to: Ying Chen, Ph.D. No.26 Daoqian Street, Suzhou Maternal-Child Medical Center, Suzhou Hospital Affiliated to Nanjing Medical University, Suzhou 215002, China. Email: cyandzh@sohu.com.

Abstract: Preterm birth (PTB) is an important issue in neonates because of its complications as well as high morbidity and mortality. The prevalence of PTB is approximately 12-13% in USA and 5-9% in many other developed countries. China represents 7.8% (approximately one million) of 14.9 million babies born prematurely annually worldwide. The rate of PTB is still increasing. Both genetic susceptibility and environmental factors are the major causes of PTB. Inflammation is regarded as an enabling characteristic factor of PTB. The aim of this review is to summarize the current literatures to illustrate the role of single nucleotide polymorphisms (SNPs) of cytokine genes in PTB. These polymorphisms are different among different geographic regions and different races, thus different populations may have different risk factors of PTB. SNPs affect the ability to metabolize poisonous substances and determine inflammation susceptibility, which in turn has an influence on reproduction-related risks and on delivery outcomes after exposure to environmental toxicants and pathogenic organisms.

Keywords: Single nucleotide polymorphisms (SNPs); cytokine gene; preterm birth (PTB)

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Introduction

Preterm birth (PTB) is defined as any birth before 37 weeks of gestation, or less than 259 days of pregnancy, beginning the first day of a last menstrual period (1). Term birth is defined as a delivery no sooner than 37 weeks. PTB is one of the most complicated problems that contribute to prenatal morbidity and mortality, responsible for 75% of infant deaths and 62% of early neonatal deaths among pregnancies in six developing countries (2), and the rate of PTB is increasing every year. Worldwide, 7.8% of 14.9 million annual PTBs occur in China; this ranks second to India, which has the highest prevalence of PTB (3).

PTB can be categorized into two groups: induced PTB (such as preeclampsia and other severe complications during pregnancy) and spontaneous PTB [(sPTB); spontaneous onset of delivery or preterm premature rupture of membranes (PPROM)] (4). Approximately three-fourths of PTBs are sPTB including PPROM (5). In contrast to induced PTB, the etiology of most sPTB cases is still unknown because PTB is a complex syndrome with a variety of causes, involving a complex interaction between genetic and environmental factors. Clinical infection, a low progesterone level, multiple

pregnancy, a short cervix and placental aberrations are regarded as important risk factors (6-8).

Fetal fibronectin (fFN) in cervical and vaginal secretions has been used as a predictor of PTB. Mogami *et al.* reported that injection of fFN in pregnant mice results in PTB, which suggested that fFN may play an important role in the PTB pathogenesis (9). Inflammation or clinical infection is a main widely accepted factor and is studied by a large number of research institutions. Many systemic maternal infections such as intrauterine infection (10), pneumonia (11) and periodontal inflammation (12) that can trigger the onset of delivery have been widely studied. In addition, sepsis and organ dysfunction in the fetus can be induced by fetal exposure to inflammation (13). Although intrauterine infection can be present without clinical signs of maternal infection (14), a 12.8% mean rate of infection-positive amniotic fluid has been reported in a study of amniocentesis in women with PTB and intact membranes (15). Inflammation or infection-induced cytokine production has been associated with fetal membrane thinning and PPROM (16) because bacteria or bacteria-derived products initiate a signaling cascade in both immune and nonimmune

cells. This phenomenon strengthens the inflammatory response and is associated with a reduction in the integrity of chorioamniotic membrane and tensile strength (17). The inflammatory response pathway is complicated because of a variety of factors are involved in this pathway, including proinflammatory and anti-inflammatory cytokines.

Cytokines are a group of soluble proteins secreted by the cells that can increase or decrease the inflammation response. Cytokines maintain homeostasis during pregnancy and play crucial roles in regulating placentation (18). A delicate balance between proinflammatory and anti-inflammatory cytokines regulates the inflammatory response during pregnancy (19). Decreased inflammatory responses and increased anti-inflammatory responses were demonstrated in pregnant women (20). Interleukin 1 beta (IL-1 β), interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α) are common proinflammatory cytokines that are mainly produced by activated mast cells and macrophages. In contrast, interleukin 4 (IL-4), interleukin 10 (IL-10) and transforming growth factor β (TGF- β) are common anti-inflammatory cytokines mainly produced by T cells. The role of inflammation in the etiology of PTB is not clear, but elevated IL-1 β , TNF- α , IL-6 and IL-8 levels in amniotic fluid have been reported in women and rhesus monkeys with intra-amniotic infection followed by PTB. These data indicate that cytokines play a key role in the initiation of delivery. These cytokines can strongly induce prostaglandin E2 (PGE2) production in the amnion, decidua and chorion (21). PGE2 performs an important function in parturition because PGE2 induces and maintains uterine contractions during pregnancy (22) and cervix maturity and ultimately triggers labor and PTB. There is a large number of association studies on correlations between factors, especially some key single nucleotide polymorphisms (SNPs) in these cytokine genes, which can interfere with gene expression; these signs can serve as disease modifiers (23). The association between PTB and SNPs in these genes has also been analyzed. The purpose of this review was to assess the association between the SNPs of cytokine genes and PTB reported in recent years.

Interleukin-1 β and IL-1Ra

Function and regulation

IL-1 β is a typical proinflammatory cytokine and is regarded as one of the most influential inflammation mediators (24). IL-1 β participates in maintaining pregnancy in many ways. It regulates gene expression in the myometrial

smooth muscle cells (25). It also significantly increases the expression and secretion of IL-8, monocyte chemoattractant protein 1 (CCL2), granulocyte macrophage colony-stimulating factor (CSF2), TNF- α and IL-6 in the epithelial cells in the female reproductive tract (26,27). IL-1 β , together with TNF- α , stimulates the amnion, decidua and myometrium to express prostaglandins (19,28). IL-1 β promotes local progesterone metabolism (29), which is necessary for maintaining pregnancy (30). Allopregnanolone and endogenous opioids suppress the oxytocin neuron response to IL-1 β in late pregnancy (31). IL-1 β functions as a central regulator reacting with the receptor type I during an inflammatory response. The IL-1 receptor antagonist (IL-1Ra) can affect the IL-1 β pathway because IL-1Ra is a natural inhibitor of IL-1 β proinflammatory effects, preventing IL-1 β from sending signals to the cells (24). IL-1Ra, encoded by IL-1RN, is a member of the IL-1 family that inhibits IL-1 α and IL-1 β function and modulates the IL-1 family-related inflammatory responses. IL-1Ra is considered to be a marker of IL-1 β production because IL-1Ra always accompanies IL-1 β production (24,32). IL-1Ra has a polymorphic site in intron 2, containing an 86 bp variable number tandem repeat (VNTR). Polymorphisms in this gene together with the VNTR can modulate protein expression (33) and the response to inflammatory stimuli in the host. Except for genetic factors, environmental factors such as vitamin A can affect the preterm infants' immune function by inhibiting IL-1Ra secretion (34). Anti-inflammatory cytokines can also stimulate IL-1Ra secretion.

SNPs in IL-1 β /IL-1Ra and PTB

Several SNPs in IL-1 β have been studied and are summarized in *Table 1*. IL-1 β -511 C>T (located in the promoter region), -31 T>C and +3953 C>T (or +3954 C>T, located in exon 5) are frequently analyzed. Yilmaz *et al.* demonstrated that the frequency of IL-1 β -511 TT genotype is statistically higher in the term birth group than in preterm neonates (19). Although the IL-1 β -511 CT polymorphism was not associated with a maternal phenotype, mothers with the -511 TT genotype and their fetuses with the -511 CT genotype may result in PTB ($P < 0.01$). Hollegaard *et al.* reported that homozygous rare alleles -31 T>C and -511 C>T (C and T) are associated with an increased risk of PTB (OR 3.1, 95% CI, 1.0-10.3 and OR 6.4, 95% CI, 1.3-60.5, respectively) (40). The human monocytic cell line THP-1 transfected with both -511 T and -31 C alleles showed an increased transcription rate when stimulated by bacterial lipopolysaccharide

Table 1 Single nucleotide polymorphisms (SNPs) in genes and preterm birth (PTB) according to recent research

Reference	Polymorphism	Study population	Case group No.	Control group No.	Comparison of genotypes	OR	95% CI	Outcome
Harper 2011 (35)	IL-1 β +3954 C>T	American	Mothers with a spontaneous PTB baby (20-36 weeks), a current singleton pregnancy. It's a cohort study rather than case-control study		TT vs. (CT+TT)	1.07	0.76-1.49	No relationship
	TNF- α -308 G>A					1.04-2.90	P=0.03, related to a shorter length of gestation	
Jones 2010 (36)	IL-6 -174 G>C	Non-Hispanic white African American	Stratified analysis: maternal age, education level, Nugent score <i>et al.</i> in mother; n=149	Stratified analysis: maternal age, education level, Nugent score <i>et al.</i> in mother; n=411	CC vs. (CT+TT)	0.36-8.22	P=0.15	No relationship between this SNP and PTB
						1.71	0.8-1.5	
Bitner 2010 (37)	IL-1 β +3954 C>T	Non-Hispanic white African American	Stratified analysis: maternal age, education level, Nugent score <i>et al.</i> in mother; n=81	Stratified analysis: maternal age, education level, Nugent score <i>et al.</i> in mother; n=149	GG vs. (GA+AA)	0.7	0.5-1.0	No relationship between this SNP and PTB
						0.8	0.5-1.4	
Bitner 2010 (37)	TNF- α -238 G>A	Non-Hispanic white African American	Stratified analysis: maternal age, education level, Nugent score <i>et al.</i> in mother; n=81	Stratified analysis: maternal age, education level, Nugent score <i>et al.</i> in mother; n=366	GG vs. (GA+AA)	1.0	0.5-1.9	No relationship between this SNP and PTB
						1.6	0.7-3.4	
Bitner 2010 (37)	IL-1 β +3954 C>T	Polish	Mother gave birth <37 weeks with PPROM n=32	Mother gave birth at term n=63	T vs. C	0.84	0.34-2.01	No relationship
						1.74	0.66-4.64	
Bitner 2010 (37)	TNF- α -308 G>A	Polish	Mother gave birth <37 weeks with PPROM n=32	Mother gave birth at term n=63	T vs. C	0.77	0.27-2.13	No relationship
						0.72	0.26-1.90	
Bitner 2010 (37)	IL-6 -174 G>C	Polish	Mother gave birth <37 weeks with PPROM n=32	Mother gave birth at term n=63	T vs. C	0.84	0.34-2.01	No relationship
						1.74	0.66-4.64	
Bitner 2010 (37)	TNF- α -308 G>A	Polish	Mother gave birth <37 weeks with PPROM n=32	Mother gave birth at term n=63	T vs. C	0.77	0.27-2.13	No relationship
						0.72	0.26-1.90	
Bitner 2010 (37)	IL-1 β +3954 C>T	Polish	Mother gave birth <37 weeks with PPROM n=32	Mother gave birth at term n=63	T vs. C	0.84	0.34-2.01	No relationship
						1.74	0.66-4.64	
Bitner 2010 (37)	IL-1RN	Polish	Mother gave birth <37 weeks with PPROM n=32	Mother gave birth at term n=63	T vs. C	0.84	0.34-2.01	No relationship
						1.74	0.66-4.64	

Table 1 (continued)

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Reference	Polymorphism	Study population	Case group No.	Control group No.	Comparison of genotypes	OR	95% CI	Outcome
Kalinka 2009 (38)	IL-1 β +3954 C>T	Caucasian	Women had gestation <37 weeks n=62	Mother gave birth at term, n=63	Women carried IL-1RN*2	2.75	1.02-4.13	No relationship
	IL-1RN VNTR							Increased risk of PTB
Sugita 2012 (39)	IL-6 -174 G>C and IL-1RN VNTR	Japanese	Mothers gave birth <37 weeks, n=51	Mother with term baby, n=71	GG+GC with IL-1RN*1/2, IL-1RN*1/3, IL-1RN*2/3	3.02	1.00-8.91	Manifest risk of PTB
	IL-1RN +2018 T/C							P=0.359
	IL-4 -590 C/T							P=0.885
	IL-4 -34 C/T							P=0.885
	IL-6 -572 G/C							P=0.407
Hollegaard 2008 (40)	IL-10 -1087 G/A	Copenhagen	Fetal born <37 weeks, singleton, healthy n=62	Fetal >37 weeks n=55	C vs. T	6.36	1.3-60.5	P=0.525
	IL-10 -824 C/T							P=0.325
	IL-1 β +3954 C>T							P=0.077
Heinzmann 2009 (41)	IL-1 β -31 C>T	Caucasian	Infants born before <32 weeks without chromosomal aberrations and/or congenital malformations n=121	Randomly chosen adults n=270	(CT+TT) vs. CC	3.09	1.04-10.33	P=0.701
	TNF- α -857 C>T							P=0.010, they were linkage disequilibrium
	TGFB rs1800471							P=0.035
	rs1800469							P=0.585
	IL-4 rs2243250							P=0.454
Nuk M 2012 (42)	IL-10 rs3024498	Caucasian	Infants born before <32 weeks without chromosomal aberrations and/or congenital malformations n=164	Randomly chosen adults n=270				P=0.136
	rs1800872							P=0.469
	TNF- α -308 G>A							P=0.955
	rs2243250							P=0.0001
Pu J 2009 (43)	TNF- α -308 G>A	21 with chorioamnionitis, 25 without chorioamnionitis <37 weeks n=46	50 cases of maternal serum, 20 cases of placental tissues >37 weeks n=70	GA or AA vs. GG	GA vs. GG	4.22	1.197-14.8963	No relationship
	TNF- α -308 G>A							P<0.05
Lobat Jafarzadeh 2013 (44)	TNF- α -308 G>A	18-35 years Without pregnancy complications Gestational age <36+0 weeks Peripheral and cord blood n=64	18-35 years Without pregnancy complications Gestational age >37+0 weeks Peripheral and cord blood n=71	GA vs. GG	GA vs. GG			Mother P=0.46 Infant P=0.47

(LPS) compared with untransfected cells (45).

SNP at +3954 of the gene encoding IL-1 β contains a C to T substitution. This allele is associated with the enhanced production of IL-1 β *in vitro* (46). There are no studies that have shown a significant association between IL-1 β +3954 and PTB (35-39). Nonetheless, this doesn't mean that the IL-1 β +3954 polymorphism has no association with PTB. A larger sample size may result in statistical significance.

IL-1Ra synthesized by the gene *IL1RN* exerts an anti-inflammatory function, and genetic polymorphisms are associated with altered IL-1 β serum levels. In many studies, IL-1RN VNTR polymorphisms are significantly associated with PTB. IL-1RN is polymorphic because of the variable length of a repeat sequence in intron 2 (47). Five alleles of this polymorphism exist in humans: 2 repeats (IL1RN*2, 240 bp), 3 repeats (IL1RN*4, 325 bp), 4 repeats (IL1RN*1, 410 bp), 5 repeats (IL1RN*3, 500 bp) and 6 repeats (IL1RN*5, 595 bp). The presence of IL1RN*2 is associated with an increased rate of PTB. Polydorides *et al.* reported that although associations with IL1RN*2 did not reach statistical significance, there was a trend toward an increased incidence of PPRM with a homozygous IL1RN*2 neonatal genotype (48). Chaves *et al.* tested 116 mothers (59 samples were from term pregnancies and 57 were preterm) and 112 newborns (53 were of term birth and 59 were preterm) (49). A maternal ILRN*2 allele is associated with PTB in the current pregnancy, whether or not it was preceded by PPRM or with PTB in a previous pregnancy. This result was consistent with Kalinka's research in 2009 (38), but their data were from mothers with PTB not preceded by PPRM in a population of Polish women. In 2010, however, they conducted another case-control study on Polish women with PTB that resulted exclusively from PPRM. All the polymorphisms in their experimental genes had no impact on the risk of PTB. The PTB genetic factors resulting from PPRM seem to differ from those of sPTB without PPRM (50).

In preterm rats, IL-1 β is triggered by LPS or hypoxia-ischemia (HI), but without any concomitant induction of counteracting anti-inflammatory cytokines (51). In addition, IL-1 β expression is more prominent in the cerebral white matter than in the gray matter, thus leading to predominantly white matter damage (51,52).

Tumor necrosis factor α (TNF- α)

Function and regulation

TNF- α is a key proinflammatory cytokines with multiple

functions in the inflammatory network; its production is regulated both at the transcriptional and posttranscriptional levels (53,54). TNF- α can stimulate IL-1 and IL-6 release, and the latter enhances the response sensitivity of the tissues to TNF- α . TNF- α is regulated by many mediators. LPS can induce TNF- α production (55). CpG-driven innate immune activation may lead to upregulated TNF- α production and cause adverse pregnancy outcomes. Those authors proposed the notion that TNF- α mediates impairment of pregnancy induced by systemic CpG oligodeoxynucleotide (ODN) administration (56). Exogenous vascular endothelial growth factor (VEGF) upregulates TNF- α and IL-6 expression, whereas a VEGF blocker partially decreases the LPS-induced proinflammatory factors expression (57).

TNF- α is an important regulatory molecule during pregnancy, which mediates an inflammatory response and is also involved in labor activities such as membranes reupture and uterine contractions. Dysregulated TNF- α expression is linked to the pathological causes of various immune-mediated inflammatory diseases such as inflammatory bowel disease (58), arthritis and autoimmune hepatitis (59), as well as PTB; thus, TNF- α has drawn most attention in PTB research field. Burdet *et al.* demonstrated that increased TNF- α , which is induced by Stx2, predominant factor for PTB in rats (60). Several polymorphisms (-237 G>A, -308 G>A, -307 G>A, -857 C>T, -863 C>A, -1032 T>C, -238 G>A, -851, -323, +691 and -376 G>A) related to PTB have been previously reported. Both maternal and fetal genotypes can affect the risk of PTB. Morra *et al.* demonstrated by genotyping of 410 Brazilian ethnically matched women that the combination of TNF- α , IFN- γ and IL-6 maternal gene polymorphisms might contribute to the susceptibility to sPTB and may be regarded as possible genetic markers of the risk of sPTB (61). Women who were homozygous for the minor allele at the -308 position of the TNF- α gene had significantly shorter length of gestation than women who were either heterozygous or homozygous for the major allele [adjusted hazard ratio 1.74, 95% confidence interval (CI), 1.04-2.90, P=0.03]. Among women with this genotype, 20% (3/15) experience extreme spontaneous preterm delivery (less than 28 weeks of gestation; adjusted odds ratio 7.51, 95% CI, 1.84-30.72, P=0.005) (35).

SNPs in TNF- α and PTB

SNPs in the promoter region of TNF- α gene have been analyzed most frequently. TNF- α -308 G>A polymorphism is associated increased TNF- α gene expression compared

to homozygous GG (62). Evidence from a large sample has demonstrated that the allelic and genotypic frequencies differ significantly between racial groups; the conclusions about the relationship between TNF- α -308 G>A with PTB were also different in each study (data are shown in *Table 1*) (63). Harper *et al.* used a cohort study to design and assess the association between genotype and length of gestation (64). Women enrolled in their study had a documented history of at least one previous singleton PTB (≤ 20 weeks and < 37 weeks). They reported that women with TNF- α -308AA in the promoter region had a significantly shorter duration of gestation than do women with -308 GG or GA (ORF): 1.74, 95% CI, 1.04-2.90, $P=0.03$, and they suggested that women with -308 AA were at an increased risk of sPTB. On the other hand, those authors observed that compared to women in whom PTB is not associated with PPRM, the allele is more common in women with PTB due to PPRM (OR=3.18, 95% CI, 1.33-7.83). Menon *et al.* suggested that PTB stimulated by PPRM and PTB not due to PPRM should be viewed as two distinct diseases because of different causes (65). Nonetheless, in another case-control study, Bitner and Kalinka showed that TNF- α -308 G>A had no impact on the risk of the PTB due to PPRM in Polish women (37). These differences may be caused by ethnicity. This is because different preterm rates and different effects on labor status in different ethnic groups. Jones conducted a case-control study of the gene-environment interaction: 777 term and 230 preterm deliveries were enrolled (36). Nugent's criteria were used for vaginal smears that were collected at 15-27 weeks of gestation. Women with a Nugent score of ≥ 4 and TNF- α -238 AG or AA were at an increased risk of PTB (OR 2.6, 95% CI, 1.2-5.8). The above is the correlation between the maternal genotype and PTB; furthermore, the fetal genotype was also being studied. Preterm infants ($n=121$) born before 32 weeks and 270 term-born babies as a control were enrolled in Andrea Heinzmann's study. Three SNPs (rs1799964, rs1799724 and rs1800629) in TNF- α were studied and no association with PTB was found (41).

Maternal-fetal TNF- α -238 heterozygosity is associated with term labor ($P<0.05$) (66). TNF- α -308 GA and AA genotypes were associated with term labor (mothers and neonates, respectively; $P<0.05$ and $P<0.001$). The incidence of term labor that was significantly increased in TNF- α -308 GA genotype. If a -308 GA carrier has a fetus with the GG genotype, the incidence of preterm labor increases ($P<0.01$). The 4845 T allele is significantly

higher in mothers and their preterm neonates ($P<0.001$ and $P<0.001$). The effect of maternal-fetal genotype on the pregnancy outcomes reveals that maternal 4845 GG and GT genotypes increase term labor incidence, whereas the fetal 4845 TT genotype is a significant independent risk factor of PTB. Yilmaz *et al.* found that the frequency of -238 G>A genotype is statistically high in 100 term neonates and their mothers compared to 101 preterm babies and their mothers, indicating a protective role of the -238 A allele (19). However, -238 AA genotype was not detected, which might indicate that the frequency of the allele A was low in their population. They also assessed -308 G>A and found that both maternal and fetal -308 GA and AA genotypes are associated with term birth ($P<0.05$ and $P<0.0001$, respectively). Maternal -308 GA combined with the fetal GG genotype induces PTB ($P<0.01$). Liang *et al.* conducted a study of case-parent triads and control parents; the study analyzed the -308 G/A polymorphism in Han Chinese (67). Two hundred fifty case families and 247 control families were enrolled. Those authors reported that there is a reduced risk of PTB when a mother's or child's genotype is G/A, but a relatively higher risk of PTB manifests itself when the genotype is A/A. Combined maternal-fetal genotype GA/GA shows the lowest PTB risk (68). Therefore it is necessary to consider maternal and fetal genotypes when studying the relationship between genetic factors and PTB.

Pu measured the maternal serum TNF- α levels and placental TNF- α mRNA expression using RT-PCR in preterm labor with or without chorioamnionitis (43). Significantly higher levels were found in mother who underwent preterm labors compared with mothers who had term labor, in chorioamnionitis compared with no chorioamnionitis, and in -308 GA/AA genotypes compared with GG genotypes. Thus, preterm labor can be predicted and diagnosed according to the level of TNF- α and its polymorphisms.

Interleukin-6 (IL-6)

Function and regulation

IL-6 is a multifunctional proinflammatory cytokine with a wide range of activities in the inflammatory response. IL-6 signaling is dependent on the transmembrane receptor (IL-6R) and homodimerization of gp130 (68). Soluble gp130 can inhibit the function of IL-6, which regulates cell growth and differentiation and stimulates acute phase

proteins that accompany inflammatory disease (18,28). IL-6 is regulated by progesterone in cultured term human uterine cervical fibroblasts. Vaginal progesterone might prevent sPTB by suppressing IL-6 production (69). IL-6 plays a crucial role in the inflammatory response to infectious factors. Similar to IL-1 β , the enzymes needed for prostaglandin synthesis are stimulated by IL-6 (28). IL-6 also participates in cell protection and anti-inflammatory functions because it can suppress *IL-1* and *TNF- α* expression in a macrophages. It has proinflammatory and anti-inflammatory characteristics, depending on its content in tissue: normal levels are advantageous and an excess is harmful for the body. IL-6 is generally considered a proinflammatory cytokine and is likely to show associations with PTB that are similar to those of *TNF- α* (70).

SNPs in IL-6 and PTB

The relationship between PTB and IL-6 has been well studied (61,71), shown in *Table 1*. IL-6 is a highly polymorphic gene in both the 5' and 3' flanking regions (72). Polymorphism in these regions can affect the serum IL-6 level. In PTB, IL-6 concentrations are increased in the amniotic fluid and maternal serum (73). Sugita and colleagues studied 51 patients with PTB and 71 cases of term birth in Japan (74). They evaluated 22 polymorphisms in various immunoregulatory genes: *IL-1A*, *IL-1 β* , *IL-1RN*, *IL-2*, *IL-4*, *IL-6*, *IL-10*, *TNF- α* , *TNFR1*, *TNFR2*, *Fc γ R1A*, *Fc γ R1B*, *Fc γ R1A3*, *Fc γ R1B3* and *Fc α R*. Periodontal parameters were also evaluated; chi-squared tests and multiple logistic regression analysis were used. In their study, there was no association between PTB and periodontitis, but the IL-6 -572 G>C polymorphism was related to PTB which was analyzed using multiple logistic regression (P=0.013, OR: 2.35, 95% CI, 1.20-4.64). Kalinka and Bitner showed that there is no relationship between IL-6 -174 G>C and PTB, but an increased risk of PTB was found when those authors combined IL-6 GG+GC, IL1RN*1/IL1RN*2, IL1RN*1/IL1RN*3 and IL1RN*2/IL1RN*3 (38).

Harper *et al.* enrolled 834 women with high risk of PTB into a study where they received ω -3 fatty acid to prevent recurrent PTB (35). They assessed IL-6 -174 polymorphisms, but the results were not exactly as expected: there was no difference in length of gestation or risk of PTB in patients with the IL-6 -174 polymorphism. Other studies have also recently reported that IL-6 polymorphisms are not related to PTB (38,50,61,64). On the other hand, IL-6 -174 G>C and -572 G>C polymorphisms

are linked to complications in preterm infants. The maternal IL-6 -174 G>C polymorphism is associated with chorioamnionitis, cerebral palsy and periventricular leukomalacia in preterm infants (75-77). Reiman reported that -174 CC and -572 GG genotypes are associated with reduced brain volume in one region (78).

Prediction of PTB

Many recent reports have suggested that IL-6 is still a predictive factor of PTB. And others have shown that amniotic fluid IL-6 may be involved. Elevation of amniotic fluid IL-6 levels associated with PTB has been recognized, and amniotic fluid IL-6 has been associated with PTB without apparent clinical infection. Most studies of PTB and IL-6 are cross-sectional (79). El-Bastawissi *et al.* showed that elevated amniotic fluid IL-6 is strongly associated with PTB, and it is used to predict PTB and guide patient care (79). Because an intrauterine infection could be responsible for 25% to 40% of PTB (10), effective preliminary tests for early inflammation are crucial. Although the presence of bacteria can be shown at an inflammatory site, an increased concentration of proinflammatory cytokines, especially IL-6, can serve as a reliable early diagnostic marker of infection and PTB onset (80-83). A low cervical IL-6 concentration can accurately identify symptomatic women with a low chance to progress to PTB within 2-7 days (81,82). At present, microarray analysis can be used successfully; for example, Brou *et al.* analyzed 36 biomarkers using a protein microarray (84). La Sala *et al.* reported analysis of amniotic fluids using protein microarrays shows significant differences in IL-1 β , IL-4, IL-6, and IL-8 levels between women with preterm and term delivery (85). A combination with other cytokines may prove more valuable. Variant genotypes of IL-6 express differently, and thus the IL-6 concentrations are associated with SNPs. Accordingly, the measurements of IL-6 in amniotic fluid could be combined with analysis of other SNPs in different populations.

Interleukin 10 (IL-10)

Function and regulation

IL-10 is considered an anti-inflammatory cytokine. Its biological functions are multifaceted and regulate almost all mononuclear macrophages. IL-10 represses the pro-inflammatory cytokines secretion in mononuclear macrophages leading to a decrease in *TNF- α* , IL-1 β , IL-6

levels and other factors. Simultaneously, IL-10 can enhance the release of anti-inflammatory cytokines, such as IL-1R antagonists and soluble TNF- α receptor. IL-10 is thought to inhibit IL-1 synthesis, thereby regulating the immune response (86). IL-10 levels are related to PTB, but the results are inconsistent: some have reported increased levels of IL-10 in PTB, and others showed that elevated IL-10 decreases the risk of PTB. Nevertheless, IL-10 is regarded as an important cytokine in the maintenance of pregnancy.

SNPs in IL-10 and PTB

IL-10 -1082 G/A, -819 C/T and -592 C/A are common SNPs in PTB studies. Menon showed that an IL-10 SNP was significantly associated with PTB in Caucasian maternal samples (amniotic fluid, IL10, rs1800896, $P < 0.001$) (87). Moura *et al.* conducted two independent case-control sets of studies in Brazilian women and found different results (61). The difference between the two sets was the place where women delivered. They found no independent associations between 6 SNPs including IL10 -1082 G>A, IL10 -819 C>T, IL10 -592 C>A and PTB. This result was consistent with the research conducted by Sugita in Japanese women (39). There may also be the overabundance of proinflammatory cytokines in association with low levels of anti-inflammatory cytokines which can lead to PTB (88).

Interleukin 4 (IL-4)

Function and regulation

IL-4 is a cytokine with many biological roles that are similar to IL-13, including the stimulation of activated B-cell and T-cell proliferation and differentiation of B cells into plasma cells. IL-4 is a key regulator in humoral and adaptive immunity, interacting with the IL-4 receptor, which has been shown to drive mitogenesis, differentiation and metastasis in rhabdomyosarcoma (89). One study showed that overexpression of IL-4 is associated with allergies (90). IL-4 plays an important role in chronic inflammation and wound repair. The mechanism is as follows: IL-4 in extravascular matrix promotes alternative activation of macrophages into macrophages (M2) cells and inhibits classical activation of macrophages into M1 cells. An increase in repair M2 is coupled with secretion of IL-10 and TGF- β that result in a diminution of pathological

inflammation. Release of arginase, proline, polyamines and TGF- β by the activated M2 cell is linked to wound repair and fibrosis (91-93). Furthermore, IL-4 also skews tumor-associated macrophages (TAM) to M2 assisted by IL-13, which could be a tumor-suppressor cytokine. In this model, IL-4 from CD4+ T cells and IL-13 from NKT cells instruct TAM to assume M2-like polarization leading to tumor development. Conversely, blocking of IL-4R leads to diminished expression of M2-related genes and a switch to M1-associated gene expression, ultimately resulting in increased tumor surveillance. IL-4-induced M2 polarization of TAM has also been observed in a model of pancreatic cancer (93). IL-4 induces the activity of cathepsin in TAM, resulting in increased angiogenesis and tumor growth. The contribution of IL-13 to the M2 polarization of TAM has been demonstrated in the 4T1 mammary carcinoma model (94,95).

SNPs in IL-4 and PTB

Many studies have shown that successful pregnancy is associated with aberrant expression of IL-4 (96-99). Marzi *et al.* and Reinhard *et al.* isolated peripheral blood mononuclear cells, stimulated them with phytohaemagglutinin (PHA) and measured interleukin secretion by means of ELISA and flow cytometry (100,101). They saw a reduction in IFN- γ and IL-2 and an increase in IL-4 and IL-10 levels during pregnancy compared to nonpregnant controls. Tranchot-Diallo *et al.* demonstrated a Th1 to Th2 bias using PCR; this bias likely reflects a decreasing amount of messenger RNA (mRNA) of IFN- γ through pregnancy and a concurrent increase in IL-4 mRNA expression, which peaks in the 7th month compared with nonpregnant controls *in vivo* (102). Furthermore, El-Shazly *et al.* indicated that placentas from women with PPROM and preterm delivery have higher levels of Th1-inducing cytokines and placentas from women after preterm delivery compared with term delivery showed a bias towards the Th1 profile with significantly higher levels of IFN- γ and IL-2 as well as the Th1-inducing cytokine IL-12 (103). They also found that term placentas exhibited comparatively higher levels of the Th2 cytokines, IL-4 and IL-10, compared to the preterm placentas (102). Nonetheless, not all studies support the Th1 bias. Shimaoka *et al.* reported a reduction in PMA-stimulated IL-4 during pregnancy (104), whereas Matthiesen and colleagues presented data suggestive of an increase in both IL-4- and IFN- γ -secreting cells during pregnancy compared to nonpregnant

controls (105). Dudley *et al.* found that the IL-4 level in amniotic fluid is elevated in women with preterm labor and delivery, particularly in association with chorioamnionitis (106). Although some data are inconsistent, the important role of IL-4 in immunity can hardly be overestimated. Accordingly, there are a few studies exploring the association between SNPs in IL-4 and PTB. Harmon *et al.* screened 432 tag SNPs in 30 candidate genes to identify the genetic factors of PTB (107). The results indicated that several SNPs in the IL4 gene could be evaluated for the risk of PTB. Annells *et al.* found that the IL-4 590 C/C genotype is associated with PTB, but it is unclear how IL-4-590 SNP has been associated with both low and high IL-4 expression (108). Kalish *et al.* also found that a link exists between IL-4 promoter polymorphisms and PTB in multiple pregnancies (109); however, this polymorphism is actually associated with an increased level of IL-4. Although the mechanisms underlying the increased risk of PTB with SNPs in the IL4 gene have yet to be elucidated, a logical conclusion can be made that SNPs in IL4 are useful candidates for assessment of the risk of PTB.

Transforming growth factor β

Function and regulation

TGF- β is a type of cytokine that plays a role in immunity, cancer, bronchial asthma, heart disease, diabetes, Marfan syndrome and Vascular Ehlers-Danlos syndrome (110-115). TGF- β is a secreted protein that exists in at least three isoforms called TGF- β 1, TGF- β 2 and TGF- β 3. It was also the original name of TGF- β 1, which was the founding member of this family. The TGF- β family is a part of a superfamily of proteins known as the transforming growth factor beta super-family, which includes inhibins, activin, anti-mullerian hormone, bone morphogenetic protein, decapentaplegic and Vg-1. Here, we focus on the effects of TGF- β on the immune system. TGF- β is believed to be a major participant in regulation of the immune system via Foxp3+ regulatory T cells and the differentiation of both Foxp3+ regulatory T cells and of Th17 cells from CD4+ T cells. TGF- β appears to block the activation of lymphocytes and monocyte-derived phagocytes. Additionally, increased TGF- β expression assists in regulating progesterone, a hormone involved in the maintenance of pregnancy (28). This way, like other anti-inflammatory cytokines, TGF- β may perform an important function in pregnancy.

SNPs in TGF- β and PTB

Many studies have shown that TGF- β can decrease the risk of PTB. Bry *et al.* presented some evidence that TGF- β 2 prevents the cytokine-induced increase in premature delivery in the rabbit (116). In that study, 32% of fetuses were born prematurely in the IL1 α -TNF- α group, whereas only 1.2% (P=0.0001) and 0.6% (P=0.0001) were born prematurely in the IL-1 α -TNF- α -TGF- β 2 group and in the control group, respectively. Furthermore, 23 rabbits were injected with IL-1 α and TNF- α , and 88 fetuses with TGF- β 2. They found that 6 in 23 delivered all of their fetuses prematurely versus 0 in 88. The PGE2 concentrations in the amniotic fluid were higher in the IL-1 α -TNF- α group than in the IL-1 α -TNF- α -TGF- β 2 group (P=0.05) or in the control group (P=0.02). Chegini *et al.* also found that TGF- β 1 and TGF- β type II receptors are expressed differently in myometrium of women who had unsuccessful labor induction compared to those without labor or with preterm labor complicated by chorioamnionitis (117). Although some studies have searched for an association between SNPs in TGF- β and diseases including abdominal aortic aneurysm, pancreatic carcinoma and ovarian cancer, several SNPs studied have no association with PTB (107). Due to the significant role of TGF- β in decreasing the risk of PTB, further research is needed of the association between SNPs in TGF- β and PTB.

Summary

The mechanism that couples infection-induced inflammation with PTB is not clear at present; but several studies have uncovered cytokine genes polymorphisms are related to PTB. Many investigators have explored the association between selected SNPs in candidate cytokine genes and rates of preterm delivery (35). So, systematic review and meta-analysis are necessary in a large number of articles, such as meta-analysis of -308 SNP in TNF- α by Menon *et al.* (118) and IL-6 by Wu W *et al.* (119). The former indicated no relationship, but negative results may not indicate a lack of a physiologic role for this variant, but only the lack of statistic association. Moreover, online genetic association data on PTB database are available which can provide the latest data in this field (120,121). The notion that such cytokines (implicated in the initiation of delivery) stimulate prostaglandin production has been widely accepted (122). In this study, we summarized recent

information on cytokines SNPs and PTB. Nevertheless, the results produced by associative studies in different population groups and with different analytical methods are often inconsistent (123). The genetic predisposition of different ethnics or racial is different: black or African women have higher rates of PTB. Population structure is an important confounding factor which should be controlled. In addition, it is unlikely that a polymorphism in a single gene can have a pivotal impact on PTB; thus, numerous genes with polymorphic alleles might have a significant collective influence on pregnancy. The combined haplotype of both the mother and the fetus as well as specific populations should be considered during validation of the genes responsible for PTB. This concept will be essential for effective diagnosis and prevention of PTB. At present, prevention of PTB is a significant challenge; cytokines can be regarded as biomarkers of PTB in amniotic fluid. Proinflammatory cytokines could be measured for an early diagnosis of intra-amniotic infection or inflammation. Elevation of the protein level of these markers could also help to identify the patients at high risk of PTD and PPRM (124). Because cytokines genotypes are different among different populations, amniotic fluid has both pro- and anti-inflammatory properties that vary among patients and pathogens. Interventions to prevent PTB and PPRM may need to be customized based on a patient's ethnic origin, type of bacterial infection, and indicators in her amniotic fluid (125).

In conclusion, although no conclusive evidences has been obtained for the role of SNPs of cytokine genes in PTB, future research should focus more on the SNPs in cytokine genes during pregnancy, to identify women who are at risk of PTB. Functional research is welcome too.

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Footnote

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