Preterm birth and single nucleotide polymorphisms in cytokine genes

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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)

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 homoeostasis 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-1β), 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-1β, 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-1β and IL-1Ra

Function and regulation

IL-1β is a typical proinflammatory cytokine and is regarded as one of the most influential inflammation mediators (24). IL-1β 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 chemotactic 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-1β, together with TNF-α, stimulates the amnion, decidua and myometrium to express prostaglandins (19,28). IL-1β promotes local progesterone metabolism (29), which is necessary for maintaining pregnancy (30). Allopregnanolone and endogenous opioids suppress the oxytocin neuron response to IL-1β in late pregnancy (31). IL-1β 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-1β pathway because IL-1Ra is a natural inhibitor of IL-1β proinflammatory effects, preventing IL-1β from sending signals to the cells (24). IL-1Ra, encoded by IL-1RN, is a member of the IL-1 family that inhibits IL-1α and IL-1β function and modulates the IL-1 family-related inflammatory responses. IL-1Ra is considered to be a marker of IL-1β production because IL-1Ra always accompanies IL-1β 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-1β/IL-1Ra and PTB

Several SNPs in IL-1β have been studied and are summarized in *Table 1*. IL-1β –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. Yılmaz *et al.* demonstrated that the frequency of IL-1β –511 TT genotype is statistically higher in the term birth group than in preterm neonates (19). Although the IL-1β –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-1β +3954 C>T	American	Mothers with a spontaneous PTB baby (20-36 weeks), a current singleton pregnancy case-control study	a cohort study rather than	TT vs. (CT+ TT)	1.07	0.76-1.49	No relationship
	TNF-α −308 G>A		AA vs. (AG+GG)		1.74	1.04-2.90	P=0.03, related to a shorter length of gestation	
	IL-6 −174 G>C		CC vs. GC/GG		1.71	0.36-8.22	P=0.15	
Jones 2010 (36)	IL-1β +3954 C>T	Non-Hispanic white	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=149	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=411	CC vs. (CT+TT)	1.0	0.8-1.5	No relationship between this SNP and PTB
		African American	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=81	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=366		0.8	0.4-1.4	
	TNF-α −308 G>A	Non-Hispanic white	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=149	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=411	GG vs. (GA+AA)	0.7	0.5-1.0	No relationship between this SNP and PTB
		African American	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=81	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=366		0.8	0.5-1.4	
	TNF-α −238 G>A	Non-Hispanic white	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=149	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=411	GG vs. (GA+AA)	1.0	0.5-1.9	No relationship between this SNP and PTB
		African American	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=81	Stratified analysis: maternal age, education level, Nugent score et al. in mother; n=366		1.6	0.7-3.4	
Bitner 2010 (37)	IL-1β +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
	TNF-α −308 G>A				--	1.74	0.66-4.64	No relationship
	IL-6 −174 G>C					0.77	0.27-2.13	
	TNF-α −308 G>A					0.72	0.26-1.90	
	IL-1β +3953 C>T					0.84	0.34-2.01	
	IL-1RN					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-1β +3954 C>T	Caucasian	Women had gestation <37 weeks n=62	Mother gave birth at term, n=63				No relationship
	IL-1RN VNTR				Women carried IL-1RN*2	2.75	1.02-4.13	Increased risk of PTB
	IL-6 −174 G>C and IL-1RN VNTR				GG+GC with IL-1RN*1/2, IL-1RN*1/3,IL-1RN*2/3	3.02	1.00-8.91	Manifold risk of PTB
Sugita 2012 (39)	IL-1RN +2018 T/C	Japanese	Mothers gave birth <37 weeks, n=51	Mother with term baby, n=71				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
	IL-10 −1087 G/A							P=0.525
	IL-10 −824 C/T							P=0.325
	IL-1β +3954 C>T							P=0.077
	IL-1β −31 C>T							P=0.701
Hollegaard 2008 (40)	IL-1β −511 C>T	Copenhagen	Fetal born <37 weeks, singleton, healthy n=62	Fetal >37 weeks n=55	C vs. T	6.36	1.3-60.5	P=0.010, they were linkage disequilibrium
	IL-1β −31 C>T							
	TNF-α −857 C>T				(CT+TT) vs. CC	3.09	1.04-10.33	P=0.035
Heinzmann 2009 (41)	TGFB rs1800471 rs1800469	Caucasian	Infants born before <32 weeks without chromosomal aberrations and/or congenital malformations n=121	Randomly chosen adults n=270				P=0.585
	IL-4 rs2243250							P=0.454
	IL-10 rs3024498 rs1800872							P=0.136
								P=0.469
								P=0.955
	IL-13/IL-4 rs1881457 rs1800925 rs20541 rs243250		Infants born before <32 weeks without chromosomal aberrations and/or congenital malformations n=164	Randomly chosen adults n=270				P=0.0001
Nuk M 2012 (42)	TNF-α −308 G>A		Mother/child pairs <37 weeks n=106	>37 weeks n=200				No relationship
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	4.22	1.197-14.8963	P<0.05
Lobat Jafarzadeh 2013 (44)	TNF-α −308 G>A		18-35 years Without pregnancy complications Gestational age <36+0weeks 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			Mother P=0.46 Infant P=0.47

(LPS) compared with untransfected cells (45).

SNP at +3954 of the gene encoding IL-1β contains a C to T substitution. This allele is associated with the enhanced production of IL-1β *in vitro* (46). There are no studies that have shown a significant association between IL-1β +3954 and PTB (35-39). Nonetheless, this doesn't mean that the IL-1β +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-1β 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 PPROM 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 PPROM 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 PPROM in a population of Polish women. In 2010, however, they conducted another case-control study on Polish women with PTB that resulted exclusively from PPROM. All the polymorphisms in their experimental genes had no impact on the risk of PTB. The PTB genetic factors resulting from PPROM seem to differ from those of sPTB without PPROM (50).

In preterm rats, IL-1β is triggered by LPS or hypoxia-ischemia (HI), but without any concomitant induction of counteracting anti-inflammatory cytokines (51). In addition, IL-1β 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 repture 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 PPROM, the allele is more common in women with PTB due to PPROM (OR=3.18, 95% CI, 1.33-7.83). Menon *et al.* suggested that PTB stimulated by PPROM and PTB not due to PPROM 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 PPROM in Polish women (37). These differences were 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. Yılmaz *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-1β, 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 macro phagocytes. 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-1β*, *IL-1RN*, *IL-2*, *IL-4*, *IL-6*, *IL-10*, *TNF-α*, *TNFRI*, *TNFRII*, *FcγRIIA*, *FcγRIIB*, *FcγRIIIA*, *FcγRIIIB* and *FcαR*. 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 repression (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-1β, 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-1β, 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, polyaminases 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

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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-β1, TGF-β2 and TGF-β3. It was also the original name of TGF-β1, 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-β2 prevents the cytokine-induced increase in premature delivery in the rabbit (116). In that study, 32% of fetuses were born prematurely in the IL1α-TNF-α group, whereas only 1.2% (P=0.0001) and 0.6% (P=0.0001) were born prematurely in the IL-1α-TNF-α-TGF-β2 group and in the control group, respectively. Furthermore, 23 rabbits were injected with IL-1α and TNF-α, and 88 fetuses with TGF-β2. 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-1α-TNF-α group than in the IL-1α-TNF-α-TGF-β2 group (P=0.05) or in the control group (P=0.02). Chegini *et al*. also found that TGF-β1 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 PPROM (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 PPROM 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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References

1. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. Acta Obstet Gynecol Scand 1977;56:247-53.

2. Ngoc NT, Merialdi M, Abdel-Aleem H, et al. Causes of stillbirths and early neonatal deaths: data from 7993 pregnancies in six developing countries. Bull World Health Organ 2006;84:699-705.

3. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012;379:2162-72.

4. Meis PJ, Michielutte R, Peters TJ, et al. Factors associated with preterm birth in Cardiff, Wales. II. Indicated and spontaneous preterm birth. Am J Obstet Gynecol 1995;173:597-602.

5. Goldenberg RL, Culhane JF, Iams JD, et al. Epidemiology and causes of preterm birth. Lancet 2008;371:75-84.

6. Pařízek A, Koucký M, Dušková M. Progesterone, inflammation and preterm labor. J Steroid Biochem Mol Biol 2014;139:159-65.

7. Shiozaki A, Yoneda S, Nakabayashi M, et al. Multiple pregnancy, short cervix, part-time worker, steroid use, low educational level and male fetus are risk factors for preterm birth in Japan: a multicenter, prospective study. J Obstet Gynaecol Res 2014;40:53-61.

8. Lutenbacher M, Gabbe PT, Karp SM, et al. Does Additional Prenatal Care in the Home Improve Birth Outcomes for Women with a Prior Preterm Delivery? A Randomized Clinical Trial. Matern Child Health J 2013. [Epub ahead of print].

9. Mogami H, Kishore AH, Shi H, et al. Fetal fibronectin signaling induces matrix metalloproteases and cyclooxygenase-2 (COX-2) in amnion cells and preterm birth in mice. J Biol Chem 2013;288:1953-66.

10. Petit E, Abergel A, Dedet B, et al. The role of infection in preterm birth. J Gynecol Obstet Biol Reprod (Paris) 2012;41:14-25.

11. Chen YH, Keller J, Wang IT, et al. Pneumonia and pregnancy outcomes: a nationwide population-based study. Am J Obstet Gynecol 2012;207:288.e1-7.

12. Horton AL, Boggess KA. Periodontal disease and preterm birth. Obstet Gynecol Clin North Am 2012;39:17-23, vii.

13. Azizia M, Lloyd J, Allen M, et al. Immune status in very preterm neonates. Pediatrics 2012;129:e967-74.

14. Sebire NJ. Choriodecidual inflammatory syndrome (CoDIS) is the leading, and under recognised, cause of early preterm delivery and second trimester miscarriage. Med Hypotheses 2001;56:497-500.

15. Romero R, Gómez R, Chaiworapongsa T, et al. The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol 2001;15:41-56.

16. Kumar D, Schatz F, Moore RM, et al. The effects of thrombin and cytokines upon the biomechanics and remodeling of isolated amnion membrane, in vitro. Placenta 2011;32:206-13.

17. Schoonmaker JN, Lawellin DW, Lunt B, et al. Bacteria and inflammatory cells reduce chorioamniotic membrane integrity and tensile strength. Obstet Gynecol 1989;74:590-6.

18. Kayaalt Z, Tekin D, Aliyev V, et al. Effects of the interleukin-6 (IL-6) polymorphism on toxic metal and trace element levels in placental tissues. Sci Total Environ 2011;409:4929-33.

19. Yılmaz Y, Verdi H, Taneri A, et al. Maternal-fetal proinflammatory cytokine gene polymorphism and preterm birth. DNA Cell Biol 2012;31:92-7.

20. Christian LM, Porter K, Karlsson E, et al. Serum proinflammatory cytokine responses to influenza virus vaccine among women during pregnancy versus non-pregnancy. Am J Reprod Immunol 2013;70:45-53.

21. Spaziani EP, Tsibris JC, Hunt LT, et al. The effect of interleukin-1 beta and interleukin-4 on the expression of prostaglandin receptors EP1 and EP3 in amnion WISH cells. Am J Reprod Immunol 1997;38:279-85.

22. Friebe-Hoffmann U, Baston DM, Hoffmann TK, et al. The influence of interleukin-1beta on oxytocin signalling in primary cells of human decidua. Regul Pept 2007;142:78-85.

23. Yazici AC, Atac FB, Verdi H, et al. Comparison of IL10 and IL2 genotypes of Turkish population with other populations. Int J Immunogenet 2009;36:97-101.

24. Ruiz RJ, Jallo N, Murphey C, et al. Second trimester maternal plasma levels of cytokines IL-1Ra, Il-6 and IL-10 and preterm birth. J Perinatol 2012;32:483-90.

25. Bartlett SR, Sawdy R, Mann GE. Induction of cyclooxygenase-2 expression in human myometrial smooth muscle cells by interleukin-1beta: involvement of p38 mitogen-activated protein kinase. J Physiol 1999;520:399-406.

26. Wira CR, Fahey JV, Sentman CL, et al. Innate and adaptive immunity in female genital tract: cellular responses and interactions. Immunol Rev 2005;206:306-35.

27. Mosher AA, Rainey KJ, Giembycz MA, et al. Prostaglandin E2 represses interleukin 1 beta-induced inflammatory mediator output from pregnant human myometrial cells through the EP2 and EP4 receptors. Biol Reprod 2012;87:7, 1-10.

28. Blackburn S. Cytokines in the perinatal and neonatal periods: selected aspects. J Perinat Neonatal Nurs 2008;22:187-90.

29. Roberson AE, Hyatt K, Kenkel C, et al. Interleukin 1beta regulates progesterone metabolism in human cervical fibroblasts. Reprod Sci 2012;19:271-81.

30. Zachariades E, Mparmpakas D, Pang Y, et al. Changes in placental progesterone receptors in term and preterm labour. Placenta 2012;33:367-72.

31. Brunton PJ, Bales J, Russell JA. Allopregnanolone and induction of endogenous opioid inhibition of oxytocin responses to immune stress in pregnant rats. J Neuroendocrinol 2012;24:690-700.

32. Arend WP. The balance between IL-1 and IL-1Ra in disease. Cytokine Growth Factor Rev 2002;13:323-40.

33. Rafiei A, Hosseini SH, Taheri M, et al. Influence of IL-1RN Intron 2 Variable Number of Tandem Repeats (VNTR) Polymorphism on Bipolar Disorder. Neuropsychobiology 2013;67:116-21.

34. Bessler H, Wyshelesky G, Osovsky M, et al. A comparison of the effect of vitamin A on cytokine secretion by mononuclear cells of preterm newborns and adults. Neonatology 2007;91:196-202.

35. Harper M, Zheng SL, Thom E, et al. Cytokine gene polymorphisms and length of gestation. Obstet Gynecol 2011;117:125-30.

36. Jones NM, Holzman C, Friderici KH, et al. Interplay of cytokine polymorphisms and bacterial vaginosis in the etiology of preterm delivery. J Reprod Immunol 2010;87:82-9.

37. Bitner A, Kalinka J. IL-1β, IL-6 promoter, TNF-α promoter and IL-1RA gene polymorphisms and the risk of preterm delivery due to preterm premature rupture of membranes in a population of Polish women. Arch Med Sci 2010;6:552-7.

38. Kalinka J, Bitner A. Selected cytokine gene polymorphisms and the risk of preterm delivery in the population of Polish women. Ginekol Pol 2009;80:111-7.

39. Sugita N, Kobayashi T, Kikuchi A, et al. Immunoregulatory gene polymorphisms in Japanese women with preterm births and periodontitis. J Reprod

Immunol 2012;93:94-101.

40. Hollegaard MV, Grove J, Thorsen P, et al. Polymorphisms in the tumor necrosis factor alpha and interleukin 1-beta promoters with possible gene regulatory functions increase the risk of preterm birth. Acta Obstet Gynecol Scand 2008;87:1285-90.

41. Heinzmann A, Mailaparambil B, Mingirulli N, et al. Association of interleukin-13/-4 and toll-like receptor 10 with preterm births. Neonatology 2009;96:175-81.

42. Nuk M, Orendi K, Rosenberger S, et al. Genetic variations in fetal and maternal tumor necrosis factor-alpha and interleukin 10: is there an association with preterm birth or periventricular leucomalacia? J Perinatol 2012;32:27-32.

43. Pu J, Zeng WY. Relationship among TNF-alpha gene promoter -308 site polymorphism, the levels of maternal serum TNF-alpha, and the mRNA expression placental TNF-alpha in preterm labor. Sichuan Da Xue Xue Bao Yi Xue Ban 2009;40:77-80.

44. Jafarzadeh L, Danesh A, Sadeghi M, et al. Analysis of Relationship between Tumor Necrosis Factor Alpha Gene (G308A Polymorphism) with Preterm Labor. Int J Prev Med 2013;4:896-901.

45. Chen H, Wilkins LM, Aziz N, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum Mol Genet 2006;15:519-29.

46. Pociot F, Mølvig J, Wogensen L, et al. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992;22:396-402.

47. Chaves JH, Babayan A, Bezerra Cde M, et al. Maternal and neonatal interleukin-1 receptor antagonist genotype and pregnancy outcome in a population with a high rate of pre-term birth. Am J Reprod Immunol 2008;60:312-7.

48. Polydorides AD, Kalish RB, Witkin SS, et al. Fetal polymorphisms in anti-inflammatory cytokine and beta-adrenergic receptor genes associated with placental pathological lesions. Int J Gynecol Pathol 2008;27:79-85.

49. Chaves JH, Babayan A, Bezerra Cde M, et al. Maternal and neonatal interleukin-1 receptor antagonist genotype and pregnancy outcome in a population with a high rate of pre-term birth. Am J Reprod Immunol 2008;60:312-7.

50. Bitner A, Kalinka J. IL-1β, IL-6 promoter, TNF-α promoter and IL-1RA gene polymorphisms and the risk of preterm delivery due to preterm premature rupture of membranes in a population of Polish women. Arch Med Sci 2010;6:552-7.

51. Brochu ME, Girard S, Lavoie K, et al. Developmental regulation of the neuroinflammatory responses to LPS and/or hypoxia-ischemia between preterm and term neonates: An experimental study. J Neuroinflammation 2011;8:55.

52. Girard S, Kadhim H, Beaudet N, et al. Developmental motor deficits induced by combined fetal exposure to lipopolysaccharide and early neonatal hypoxia/ischemia: a novel animal model for cerebral palsy in very premature infants. Neuroscience 2009;158:673-82.

53. Sariban E, Imamura K, Luebbers R, et al. Transcriptional and posttranscriptional regulation of tumor necrosis factor gene expression in human monocytes. J Clin Invest 1988;81:1506-10.

54. Garg PR, Saraswathy KN, Kalla AK, et al. Pro-inflammatory cytokine gene polymorphisms and threat for coronary heart disease in a North Indian Agrawal population. Gene 2013;514:69-74.

55. Shen CM, Lin SC, Niu DM, et al. Development of monocyte Toll-like receptor 2 and Toll-like receptor 4 in preterm newborns during the first few months of life. Pediatr Res 2013;73:685-91.

56. Sun Y, Qin X, Shan B, et al. Differential effects of the CpG-Toll-like receptor 9 axis on pregnancy outcome in nonobese diabetic mice and wild-type controls. Fertil Steril 2013;99:1759-67.

57. Nguyen BT, Minkiewicz V, McCabe E, et al. Vascular endothelial growth factor induces mRNA expression of pro-inflammatory factors in the uterine cervix of mice. Biomed Res 2012;33:363-72.

58. Zhang BB, Liu XZ, Sun J, et al. Association between TNF α gene polymorphisms and the risk of duodenal ulcer: a meta-analysis. PLoS One 2013;8:e57167.

59. Iwamoto S, Kido M, Aoki N, et al. TNF-alpha is essential in the induction of fatal autoimmune hepatitis in mice through upregulation of hepatic CCL20 expression. Clin Immunol 2013;146:15-25.

60. Burdet J, Sacerdoti F, Cella M, et al. Role of TNF-alpha in the mechanisms responsible for preterm delivery induced by Stx2 in rats. Br J Pharmacol 2013;168:946-53.

61. Moura E, Mattar R, de Souza E, et al. Inflammatory cytokine gene polymorphisms and spontaneous preterm birth. J Reprod Immunol 2009;80:115-21.

62. Wilson AG, Symons JA, McDowell TL, et al. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997;94:3195-9.

63. Menon R, Velez DR, Morgan N, et al. Genetic regulation

of amniotic fluid TNF-alpha and soluble TNF receptor concentrations affected by race and preterm birth. Hum Genet 2008;124:243-53.

64. Harper M, Zheng SL, Thom E, et al. Cytokine gene polymorphisms and length of gestation. Obstet Gynecol 2011;117:125-30.

65. Menon R, Merialdi M, Betrán AP, et al. Analysis of association between maternal tumor necrosis factor-alpha promoter polymorphism (-308), tumor necrosis factor concentration, and preterm birth. Am J Obstet Gynecol 2006;195:1240-8.

66. Yılmaz Y, Verdi H, Taneri A, et al. Maternal-fetal proinflammatory cytokine gene polymorphism and preterm birth. DNA Cell Biol 2012;31:92-7.

67. Liang M, Wang X, Li J, et al. Association of combined maternal-fetal TNF-alpha gene G308A genotypes with preterm delivery: a gene-gene interaction study. J Biomed Biotechnol 2010;2010:396184.

68. Lee SY, Buhimschi IA, Dulay AT, et al. IL-6 trans-signaling system in intra-amniotic inflammation, preterm birth, and preterm premature rupture of the membranes. J Immunol 2011;186:3226-36.

69. Kim MG, Shim JY, Pak JH, et al. Progesterone modulates the expression of interleukin-6 in cultured term human uterine cervical fibroblasts. Am J Reprod Immunol 2012;67:369-75.

70. Romero R, Espinoza J, Gonçalves LF, et al. Inflammation in preterm and term labour and delivery. Semin Fetal Neonatal Med 2006;11:317-26.

71. Discacciati MG, Simoes JA, Silva MG, et al. Microbiological characteristics and inflammatory cytokines associated with preterm labor. Arch Gynecol Obstet 2011;283:501-8.

72. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369-76.

73. Weiyuan Z, Li W. Study of interleukin-6 and tumor necrosis factor-alpha levels in maternal serum and amniotic fluid of patients with premature rupture of membranes. J Perinat Med 1998;26:491-4.

74. Sugita N, Kobayashi T, Kikuchi A, et al. Immunoregulatory gene polymorphisms in Japanese women with preterm births and periodontitis. J Reprod Immunol 2012;93:94-101.

75. Resch B, Radinger A, Mannhalter C, et al. Maternal interleukin-6 (-174) C/C polymorphism is associated with chorioamnionitis and cystic periventricular leucomalacia of the preterm infant. J Perinatol 2010;30:712-6.

76. Djukic M, Gibson CS, Maclennan AH. Genetic susceptibility to viral exposure may increase the risk of cerebral palsy. Aust N Z J Obstet Gynaecol 2009;49:247-53.

77. Reiman M, Kujari H, Ekholm E, et al. Interleukin-6 polymorphism is associated with chorioamnionitis and neonatal infections in preterm infants. J Pediatr 2008;153:19-24.

78. Reiman M, Parkkola R, Lapinleimu H, et al. Interleukin-6 -174 and -572 genotypes and the volume of deep gray matter in preterm infants. Pediatr Res 2009;65:90-6.

79. El-Bastawissi AY, Williams MA, Riley DE, et al. Amniotic fluid interleukin-6 and preterm delivery: a review. Obstet Gynecol 2000;95:1056-64.

80. Brik M, Antonio P, Perales-Puchalt A, et al. Cervical interleukin-6 as a predictive test for preterm delivery in symptomatic women: preliminary results. Eur J Obstet Gynecol Reprod Biol 2011;155:14-8.

81. Perales-Puchalt A, Brik M, Diago VJ, et al. The negative predictive value of cervical interleukin-6 for the risk assessment of preterm birth. J Matern Fetal Neonatal Med 2013;26:1278-81.

82. Vousden N, Chandiramani M, Seed P, et al. Interleukin-6 bedside testing in women at high risk of preterm birth. J Matern Fetal Neonatal Med 2011;24:1301-4.

83. Masseva A, Dimitrov A, Nikolov A, et al. Role of IL-6 for the diagnosis of preterm birth. Akush Ginekol (Sofiia) 2012;51:9-14.

84. Brou L, Almli LM, Pearce BD, et al. Dysregulated biomarkers induce distinct pathways in preterm birth. BJOG 2012;119:458-73.

85. La Sala GB, Ardizzoni A, Capodanno F, et al. Protein microarrays on midtrimester amniotic fluids: a novel approach for the diagnosis of early intrauterine inflammation related to preterm delivery. Int J Immunopathol Pharmacol 2012;25:1029-40.

86. Corwin EJ. Understanding cytokines. Part I: Physiology and mechanism of action. Biol Res Nurs 2000;2:30-40.

87. Menon R, Fortunato SJ, Edwards DR, et al. Association of genetic variants, ethnicity and preterm birth with amniotic fluid cytokine concentrations. Ann Hum Genet 2010;74:165-83.

88. Makhseed M, Raghupathy R, El-Shazly S, et al. Pro-inflammatory maternal cytokine profile in preterm delivery. Am J Reprod Immunol 2003;49:308-18.

89. Hosoyama T, Aslam MI, Abraham J, et al. IL-4R drives dedifferentiation, mitogenesis, and metastasis in

rhabdomyosarcoma. Clin Cancer Res 2011;17:2757-66.

90. Hershey GK, Friedrich MF, Esswein LA, et al. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. N Engl J Med 1997;337:1720-5.

91. Stein M, Keshav S, Harris N, et al. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. J Exp Med 1992;176:287-92.

92. Mantovani A, Sica A, Sozzani S, et al. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004;25:677-86.

93. Gocheva V, Wang HW, Gadea BB, et al. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. Genes Dev 2010;24:241-55.

94. Sinha P, Clements VK, Ostrand-Rosenberg S. Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. Cancer Res 2005;65:11743-51.

95. Sinha P, Clements VK, Fulton AM, et al. Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. Cancer Res 2007;67:4507-13.

96. Piccinni MP. T cells in normal pregnancy and recurrent pregnancy loss. Reprod Biomed Online 2006;13:840-4.

97. Piccinni MP. T cells in normal pregnancy and recurrent pregnancy loss. Reprod Biomed Online 2007;14:95-9.

98. Jones CA, Finlay-Jones JJ, Hart PH. Type-1 and type-2 cytokines in human late-gestation decidual tissue. Biol Reprod 1997;57:303-11.

99. Piccinni MP. T cell tolerance towards the fetal allograft. J Reprod Immunol 2010;85:71-5.

100. Marzi M, Vigano A, Trabattoni D, et al. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. Clin Exp Immunol 1996;106:127-33.

101. Reinhard G, Noll A, Schlebusch H, et al. Shifts in the TH1/TH2 balance during human pregnancy correlate with apoptotic changes. Biochem Biophys Res Commun 1998;245:933-8.

102. Tranchot-Diallo J, Gras G, Parnet-Mathieu F, et al. Modulations of cytokine expression in pregnant women. Am J Reprod Immunol 1997;37:215-26.

103. El-Shazly S, Makhseed M, Azizieh F, et al. Increased expression of pro-inflammatory cytokines in placentas of women undergoing spontaneous preterm delivery or premature rupture of membranes. Am J Reprod Immunol 2004;52:45-52.

104. Shimaoka Y, Hidaka Y, Tada H, et al. Changes in cytokine production during and after normal pregnancy. Am J Reprod Immunol 2000;44:143-7.

105. Matthiesen L, Ekerfelt C, Berg G, et al. Increased numbers of circulating interferon-gamma- and interleukin-4-secreting cells during normal pregnancy. Am J Reprod Immunol 1998;39:362-7.

106. Dudley DJ, Hunter C, Varner MW, et al. Elevation of amniotic fluid interleukin-4 concentrations in women with preterm labor and chorioamnionitis. Am J Perinatol 1996;13:443-7.

107. Harmon QE, Engel SM, Olshan AF, et al. Association of Polymorphisms in Natural Killer Cell-Related Genes With Preterm Birth. Am J Epidemiol 2013;178:1208-18.

108. Annells MF, Hart PH, Mulligan CG, et al. Interleukins-1, -4, -6, -10, tumor necrosis factor, transforming growth factor-beta, FAS, and mannose-binding protein C gene polymorphisms in Australian women: Risk of preterm birth. Am J Obstet Gynecol 2004;191:2056-67.

109. Kalish RB, Vardhana S, Gupta M, et al. Interleukin-4 and -10 gene polymorphisms and spontaneous preterm birth in multifetal gestations. Am J Obstet Gynecol 2004;190:702-6.

110. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 2000;14:163-76.

111. Taipale J, Miyazono K, Heldin CH, et al. Latent transforming growth factor-beta 1 associates to fibroblast extracellular matrix via latent TGF-beta binding protein. J Cell Biol 1994;124:171-81.

112. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. N Engl J Med 2000;342:1350-8.

113. Xu JB, Bao Y, Liu X, et al. Defective expression of transforming growth factor beta type II receptor (TGFBR2) in the large cell variant of non-small cell lung carcinoma. Lung Cancer 2007;58:36-43.

114. Habashi JP, Judge DP, Holm TM, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 2006;312:117-21.

115. Robinson PN, Arteaga-Solis E, Baldock C, et al. The molecular genetics of Marfan syndrome and related disorders. J Med Genet 2006;43:769-87.

116. Bry K, Hallman M. Transforming growth factor-beta 2 prevents preterm delivery induced by interleukin-1 alpha and tumor necrosis factor-alpha in the rabbit. Am J Obstet Gynecol 1993;168:1318-22.

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117. Chegini N, Ma C, Davis J, et al. Differential expression of transforming growth factor-beta 1 and transforming growth factor-beta receptors in myometrium of women with failed induction of labor, no labor, and preterm labor. J Soc Gynecol Investig 1999;6:258-63.

118. Menon R, Merialdi M, Betrán AP, et al. Analysis of association between maternal tumor necrosis factor-alpha promoter polymorphism (-308), tumor necrosis factor concentration, and preterm birth. Am J Obstet Gynecol 2006;195:1240-8.

119. Wu W, Clark EA, Stoddard GJ, et al. Effect of interleukin-6 polymorphism on risk of preterm birth within population strata: a meta-analysis. BMC Genet 2013;14:30.

120. Uzun A, Laliberte A, Parker J, et al. dbPTB: a database for preterm birth. Database (Oxford) 2012;2012:bar069.

121. Dolan SM, Hollegaard MV, Merialdi M, et al. Synopsis of preterm birth genetic association studies: the preterm birth genetics knowledge base (PTBGene). Public Health Genomics 2010;13:514-23.

122. Gomez-Lopez N, Vega-Sanchez R, Castillo-Castrejon M, et al. Evidence for a role for the adaptive immune response in human term parturition. Am J Reprod Immunol 2013;69:212-30.

123. Cetin I, Pileri P, Villa A, et al. Pathogenic mechanisms linking periodontal diseases with adverse pregnancy outcomes. Reprod Sci 2012;19:633-41.

124. Flídrová E, Krejsek J. Innate immunity in pathogenesis of intraamniotic inflammation in pregnancies complicated by preterm premature rupture of membranes. Ceska Gynekol 2011;76:46-50.

125. Peltier MR, Drobek CO, Bhat G, et al. Amniotic fluid and maternal race influence responsiveness of fetal membranes to bacteria. J Reprod Immunol 2012;96:68-78.