Review Article

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Interplay of cytokines in preterm birth

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Preterm infants (*i.e.*, born before <37 wk of gestation) are at increased risk of morbidity and mortality and long-term disabilities. Global prevalence of preterm birth (PTB) varies from 5 to 18 per cent. There are multiple aetiological causes and factors associated with PTB. Intrapartum infections are conventionally associated with PTB. However, maternal genotype modulates response to these infections. This review highlights the association of cytokine gene polymorphisms and their levels with PTB. Varying PTB rates across the different ethnic groups may be as a result of genetically mediated varying cytokines response to infections. Studies on genetic variations in tumour necrosis factor-alpha, interleukin-1 alpha (*IL-1α*), *IL-1β, IL-6, IL-10* and toll-like receptor-4 genes and their association with PTB, have been reviewed. No single polymorphism of the studied genes was found to be associated with PTB. However, increased maternal levels of IL-1β and IL-6 and low levels of IL-10 have been found to be associated with PTB.

Key words Cytokines - inflammation - polymorphism - preterm birth - spontaneous preterm labour

About 27 per cent of neonatal mortality has been reported to be related to complications of preterm birth (PTB)[1] or delivery before 37 wk of gestation. In India, the incidence of PTB is about 21 per cent[2,3], which translates into 3.6 million births annually. This corresponds to 23.6 per cent of global annual PTB burden which is estimated to be 15 million[4].

PTB can be medically induced when there is an indication either related to the mother such as pre-eclampsia, eclampsia or foetus such as foetal distress. On the other hand, PTB can occur spontaneously due to multiple aetiologies such as uterine overdistension, as in multiple gestation, infection or inflammation[5,6]. Other risk factors for PTB are poor maternal nutritional status as evident by low maternal body mass index, periodontal disease and racial disparity (as reported higher risk is seen in African American than European American)[7]. Increased levels of inflammatory cytokines, such as toll-like receptor 4 (TLR4), tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6 have been reported in serum and/or amniotic fluid of women with spontaneous preterm labour (PTL)[8]. This review reports existing evidence on association of genetic variations in *TNF-α, IL-1α*, *IL-1β, IL-6, IL-10* and *TLR-4* with PTB.

Cytokines and preterm birth

PTB and spontaneous PTL (PTL is defined as 'regular contractions of the uterus resulting in changes in the cervix that start before 37 wk of pregnancy')[9] have been shown to be associated with infections such as bacterial vaginosis and chorioamniotis[6,10,11]. Infection leads to inflammation as evident by increased levels

of TLR4, TNF-α, IL-1 and IL-6 in the amniotic fluid. The release of pro-inflammatory cytokines is followed by leucocytosis which results in apoptosis, preterm premature rupture of membrane along with cervical ripening and onset of premature labour. Since specific genes regulate corresponding cytokines, genetic polymorphisms in mother have been investigated to assess their association with PTB[12,13].

Inflammatory signalling is a highly complex pathway (Figure). This pathway can be modulated by external as well as the internal signals. The balance between pro-inflammatory and anti-inflammatory cytokines is crucial for implantation of the foetus, preparation of placenta and pregnancy outcome. While the T-helper 1 (Th1) cytokine is responsible for inflammation, the Th2 cytokine manages the anti-inflammation counter-regulatory pathway. The dominance of Th2 cytokine expression plays an important role in reducing inflammation and prevents allograft dismissal of the foetus[14,15].

Genetic factors

Familial and twin studies have reported that PTB is sometimes heritable[16-19]. It has been observed that women with PTB have higher chances for recurrent PTB[7]. There seems to be a genetic predisposition to the PTB. Therefore, it seems plausible that polymorphisms in maternal genes regulating cytokine expression are related to PTB[17-19]. Tables I and II summarize the genes

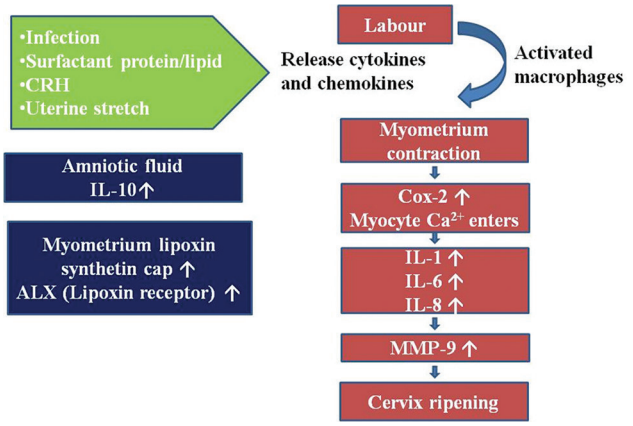

Figure. Factors initiating onset of labour. Schematic diagram showing that labour can be induced by various factors (*i*) infection, (*ii*) surfactant protein/lipid, (*iii*) corticotropin-releasing hormone (CRH), (*iv*) uterine stretch. These activate inflammatory cytokines (IL-1, 6, 8) and metalloproteinases (mainly MMP-9) cause cervical ripening. Increased Cox-2 levels in myometrium cause prostaglandin synthesis and initiate labour. These inflammatory cytokines are counter balanced by anti-inflammatory cytokine IL-10. Cox-2, cyclooxygenases-2; IL, interleukin; MMP, matrix metalloproteinases protein.

associated with inflammatory pathway and therefore, PTB[20-46]. It has been reported that altered production of pro-inflammatory cytokines mainly IL-1β, TNF-α and interferon (IFN)-lambda at the maternal-foetal interface results in PTB. On the contrary, IL-10 downregulates the secretion as well as expression of pro-inflammatory cytokines by other cells[47,48]. The present review focussed only on polymorphisms in the coding or promoter regions of genes listed in Tables I and II.

Toll-like receptor (TLR)

Location, function and regulation

TLR-4 gene is located on chromosome 9q33.1. Its alternative name is cluster of differentiation 284. TLR family has 13 distinctive proteins (TLR-1 to TLR-13). These are capable of recognizing microbial agents and initiating early immune response by activating various downstream pathways, such as transduction of nuclear-kappa β pathway which regulates expression of genes secreting pro-inflammatory cytokines[49].

TLR-2 and *TLR-4* genes have been extensively studied and their role has been identified in pathogen recognition and initiation of immune response. TLR4 regulates innate immune response during pregnancy and thus directly affects the duration of gestation. It is mainly expressed in human placenta[50].

TLR-4 pathway

It has been reported that most variations in TLR-4 are seen in the third exon[20]. *TLR-4* signal pathway includes enrolment of some signal transducer adapter proteins (MyD88, IRAK1/4 and TRAF6), rapid activation of intermediate kinases (RIP1, TAB2/3, TAK1 and IKK α/β) and phosphorylation/degradation of the chaperone protein (Iκβ)[51]. Activation of immune system by endogenous and exogenous ligands such as heat shock proteins and bacterial lipopolysaccharides (LPS) is mediated through *TLR-4*. *TLR-4* signalling activates the pro-inflammatory cytokines (IL-1, IL-6, IL-8) cascade which increases the level of prostaglandin (mostly PG-E and PG-F) and thus stimulates PTL causing PTB[50]. TLR-4 is expressed by macrophages located in placental villi and in intermediate trophoblast of the placenta. Increased expression of TLR-4 was found in placentas of patients with chorioamnionitis[50], an independent risk factor for PTL. Hence, it is extrapolated that increased levels of TLR-4 may be associated with PTB. However, TLR4 expression has been studied in the placenta *in vitro* only[50]. Corresponding serum levels have not been assessed.

Table I. Pooled data for the association of toll-like receptor-4 and tumour necrosis factor-alpha polymorphisms with preterm birth in different populations

Gene	Reference cited	Study design	Population	dbSNP	Gene position	Main findings
TLR-4	Lorenz *et al*[20]	Case-control	Finish, 94 mothers 74 premature birth (of whom, 62 were singletons and 12 were multiples). 20 Term birth term neonates-351 Preterm neonates (<35 wk; 282 were singletons and 158 were multiples)	rs4986790	D299G	No significant differences among different groups of mothers. In premature infants, the frequency of TLR-4 Asp/Gly or Gly/Gly was higher than term singleton (P=0.02, 0.02, respectively) and preterm multiples (P=0.03, P=0.04, respectively).
	Härtel *et al*[21]	Retrospective study	Caucasian mothers=747 (of whom, 466 preterm and 181 term)	rs4986790	D299G	No association with *TLR4* polymorphism
	Bitner *et al*[22]	Case-control study	121 mothers with preterm delivery 152 mothers with term delivery	rs4986790	D299G	No association
TNF-α	Drews-Piasecka *et al*[23]	Case-control	Polish Preterm=150 Term=150	rs361525 rs1800629	Promoter Promoter	-238GA genotype (P=0.01) and -238A allele (P=0.002) were found significant in PTB group. Mothers belonging to 28-32 wk group were having high frequency of -238GA (P<0.01) and -238A allele.
	Pu and Zeng[24]	Random	Caucasian Term=46 Preterm=50	rs1800629	Promoter	In PTL, increased level of *TNF-α* in mRNA and maternal serum was seen in women carrying the GA and AA genotypes (P<0.05).
	Jones *et al*[25]	Cohort	Non-Hispanic African American, 777 term and 230 preterm	rs361525	Promoter	Increased risk of PTB was found in women with TNFα-238A/G or A/A genotype along with the nuget score ≥4 OR=2.6 (CI=1.2-5.8) P=0.02
				rs1800629	Promoter	No association was found in between *TNF-α* (-308G/A) polymorphism and PTB
	Liang *et al*[26]	Case-control, hybrid design	Han Chinese Preterm=250 Term=247	rs1800629	Promoter	Relatively higher risk of PTB was seen in mother and foetus with A/A genotype
	Yilmaz *et al*[27]	Case-control study	Turks Preterm=100 Term=101	rs1800629	Promoter	GA and AA genotypes were found associated in mother (P<0.05) and neonates (P<0.001) with term delivery; the incidence of PTL was increased in mother carrying GA genotype and foetus carrying the GG genotype (P<0.01).

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Gene	Reference cited	Study design	Population	dbSNP	Gene position	Main findings
	Bitner and Kalinka[28]	Case-control	Caucasian Preterm=32 Term=63	rs1800629	Promoter	No significant association was found between TNF-308G/A [OR=0.72 (CI=0.26-1.9)].
	Heinzmann *et al*[29]	Case-control	Caucasian, case (preterm)=121 Random control (term)=270	rs1800629	Promoter	No association was reported -308G/A; *P*=0.85
	Harper *et al*[30]	Cohort	American, total individuals=834	rs1800629	Promoter	Women with TNF-α-308AA genotype were at higher risk of PTB [*P*=0.03; hazard ratio=1.74 (CI=1.04-2.9)] than women with -308 GA or GG genotype
	Andalas *et al*[31]	Case-control	Acehnese Term=40 Preterm=40	rs1800629	Promoter	No association
	Kalinka and Bitner[32]	Case-control	Caucasian Preterm=63 Term=62	rs1800629	Promoter	No association
	Amory *et al*[33]	Cohort	American; mother-infant pair=118	rs1800629	Promoter	No association was reported between *TNF-α* (-308G/A) polymorphism and PTB
	Jafarzadeh *et al*[34]	Cross-sectional study	Caucasian, case-64 mothers and neonates with preterm delivery. Control-71 mothers and neonates with term delivery	rs1800629	Promoter	No significant association was found in both maternal and foetal genotypes. GA genotype frequency in mother (*P*=0.47) and in infant (*P*=0.40) was not increased in PTB.
	Speer *et al*[35]	Case-control	Caucasian, 88 preterm mother-infant pair; 88 term mother-infant pair	rs1800629	Promoter	Individually, no association was found between *TNF-α* (308G/A) with PTB.
	Nuk *et al*[36]	Case-control	European Preterm=106 Term=200	rs1800629	Promoter	Genotyping done in mother/child pair for *TNF-α*. No significant association was reported.
	Moura *et al*[37]	2 case-control sets	Brazilian, first set Preterm=122 Term=101 Second set Preterm=82 Term=105	rs1800629	Promoter	No association was found with PTB.
	Mattar *et al*[38]	Cohort	Mixed population (Caucasian, mixed race, African women) Term=119 Preterm=139	rs1800629	Promoter	No association was reported.
	Menon *et al*[39]	Meta-analysis	African-American, pooled data of 7 studies included 638 preterm and 1208 term individuals.	rs1800629	Promoter	No significant association was reported between *TNF-α* minor allele A and with increased expression of TNF-α OR=1.41 (CI=0.9-2.19).

TLR, toll-like receptor; OR, odds ratio; CI, confidence interval; PTB, preterm birth; PTL, preterm labour; TNF-α, tumour necrosis factor-alpha; dbSNP, single nucleotide polymorphism database

Table II. Pooled data for the association of interleukin (IL)-1, interleukin-6 and interleukin-10 polymorphisms with preterm birth in different populations

Gene	Study design	Population	dbSNP	Gene position	Main findings	Reference cited
IL-6	Retrospective study	Caucasian Mothers=747 (out of whom, 466 preterm and 181 term)	rs1800795	Promoter	IL-6GG genotype frequency was high in mothers with preterm very low birth weight infants. Preterm mothers reported less expression of IL-6 -174C allele.	Härtel *et al*[21]
IL-1	Case-control study	Turks Preterm=100 Term=101	rs17561	Promoter	Minor allele of +4845T polymorphism increases the incidence of PTB in both mother (P<0.001) and foetus (P<0.001).	Yilmaz *et al*[27]
IL-6	Case-control	Caucasian Preterm=32 Term=63	rs1800795	Promoter	No significant association was reported between IL-6 -174G/C [OR=0.77 (CI=0.27-2.1)] and PTB.	Bitner and Kalinka[28]
IL-6	Cohort	American, total individuals=834	rs1800795	Promoter	No association was found with IL-6 -174G/C and PTB.	Harper *et al*[30]
IL-1 *IL-6*	Case-control	Caucasian Preterm=63 Term=62	rs1143634 IL-1RA rs1800795	Exon 5 Promoter Promoter	No association IL-1RN*2 was found to be associated with increased risk of PTB. IL-1RN*2 along with IL-6 -174G allele increased the risk of PTB.	Kalinka and Bitner[32]
IL-6 *IL-10*	Case-control	Caucasian, 88 preterm mother-infant pair; 88 term mother-infant pair	rs1800795 rs1800871 rs1800872 rs1800896	Promoter Promoter Promoter Promoter	Underlying infection and inflammation was reported to be associated with maternal and foetal IL-6 (-174G) and foetal TNF-α (-308GG) and foetal IL-10 (-1082A).	Speer *et al*[35]
IL-10	Case-control	European Preterm=106 Term=200	rs1800896 rs1800871	Promoter Promoter Promoter	Genotyping done in mother/child pair for -1082 and -819. No significant association was reported.	Nuk *et al*[36]
IL-6 *IL-10*	2 case-control sets	Brazilian, first set Preterm=122 Term= 101 Second set Preterm=82 Term=105	rs1800795 rs1800871 rs1800872 rs1800896	Promoter Promoter Promoter Promoter	No association was found with PTB No association was found with PTB.	Moura *et al*[37]
IL-6 *IL-10*	Cohort	Mixed population (Caucasian, mixed race, African women) Term=119 Preterm=139	rs1800795 rs1800896	Promoter Promoter	No association was reported. No association was reported.	Mattar *et al*[38]

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Gene	Study design	Population	dbSNP	Gene position	Main findings	Reference cited
IL-1	Cohort	Caucasian Term=74 Preterm=341	rs1800587	Promoter	Increased risk was reported in women carrying IL-1α -889T rare/minor allele P=0.01, OR=2.5 (CI=1.3-4.6).	Sata *et al*[40]
			rs17561	Promoter	Increased risk was reported in women carrying IL-1α +4845T allele P=0.01, OR=2.4 (CI=1.2-4.2).	
					Haplotypic association showed that IL-1αTT (P<0.001) was significantly higher in mother with PTB and IL-1αCG was higher in term mothers (P<0.001).	
IL-1	Case-control	Caucasian Preterm=100 Term=100	rs16944	Promoter	No association was found between IL-1β-511C/T P=0.47; OR=1.3 (CI=0.7-2.3).	Schmid *et al*[41]
			rs1143634	Exon 5	IL-1β (+3953C/T was found associated P=0.04; OR=0.6 (CI=0.3-1.0)	
IL-1	Case-control	Danish Caucasian, 117 singleton pregnant women; 62 with PTB and 55 control	rs16944 rs1143627	5'UTR Promoter Promoter	Increased risk was found in women carrying the -857C>T (rare allele T) along with IL-1β CC [OR=3.1 (1.0-10.3)] and IL-1βTT [OR=6.4 (CI=1.3-60.5)] of IL-1β-31T/C and IL-1β-511 C/T, respectively.	Hollegaard *et al*[42]
IL-1	Case-control	Japanese Term=71 Preterm=57	rs1143634 rs17561 rs1143627 rs16944	Promoter Promoter Promoter Promoter	No association was found.	Sugita *et al*[43]
IL-6			rs1800796	Promoter	No association was found.	
IL-1	Case-control	559 preterm and 559 term mothers	rs17561 rs1143627 rs16944 rs1800587	Promoter Promoter Promoter Promoter	Increased risk was found in women with CT genotype of IL-1α-889C/T [P=0.002; OR=1.7 (1.24-2.46)] and IL-1 +4845C/T [P=0.003; OR=1.5 (1.1-1.9)].	Pandey and Awasthi[44]
IL-6	Meta-analysis	European-descent and non-European-admixed population, 1165 PTB 3830 term	rs1800795	Promoter	Meta-analysis revealed that only for European population, CC genotype was found to be protective [P<0.05; OR=0.68 (CI=0.51-0.91)] against PTB, no significant association was found in between foetal genotype and PTB [OR=0.98 (CI=0.72-1.33)].	Wu *et al*[45]
IL-10	Prospective cohort	European, total individuals=1616 pregnant women	rs1800896	Promoter	No association was reported.	Stonek *et al*[46]

OR, odds ratio; CI, confidence interval; PTB, preterm birth; dbSNP, single nucleotide polymorphism database; TNF-α, tumour necrosis factor-alpha

Polymorphism of TLR-4 gene

TLR-4 is located on long arm of chromosome 9. The polymorphic site rs4986790 is present on position 896. This A/G transition causes substitution of amino acid aspartic acid by glycine at position of 299 (*i.e.* Asp299Gly). This polymorphism has also been found to be associated with increased risk of severe disease due to respiratory syncytial virus and Gram-negative bacterial infection in children[52]. Thus, it can be hypothesized that substitution of aspartic acid by glycine in *TLR-4* gene at position 299 can exaggerate the chances of infection and thus inflammation during pregnancy leading to PTB.

Many studies were conducted to determine the association of *TLR-4* and PTB. Table I summarizes the studies of *TLR4* and PTB. Lorenz *et al*[20] reported significant association of PTB with *TLR4*Asp299Gly in infants but not in mothers and this was supported by other studies also[21,22,53]. On the contrary, other groups[50,52-54] reported increased expression of TLR-4 in chorioamniotic membranes of patients with histologic chorioamnionitis regardless of their gestational status and in mothers with PTL, respectively. Equivocal results have been found for the association of polymorphism of *TLR-4* gene and PTB.

Tumour necrosis factor-alpha (TNF-α)

Location, function and regulation

TNF-α is located on chromosome 6p21.3. It is a pro-inflammatory cytokine, which promotes the production of collagen-degrading matrix metalloproteinases, and suppresses biosynthesis of tissue inhibitors of metalloproteinases[55,56]. The metalloproteinases act on foetal membrane collagen resulting in loss of tensile strength. It also impairs the progesterone stimulating receptor B thus blocking the progesterone release. Both these actions promote onset of PTL[56].

Polymorphism of TNF-α

Increased level of TNF-α was linked with various reproductive diseases such as frequent spontaneous abortions, pre-eclampsia, infections or endometriosis[57]. Elevated levels of TNF-α can change the delicate equilibrium between the anti-inflammatory and pro-inflammatory cytokines and thus induce PTB. Till date, two polymorphisms, -238G/A and -308G/A, present on promoter region have been studied. Table I lists the studies which analyzed the association of *TNF-α* and PTB. The *TNF-α*-238 G allele was reported to be associated with high transcriptional activity[23,58]. Significant association of *TNF-α* (-308G/A) polymorphism has been reported with PTB[24-27,59]. Interaction between infection, stress, obesity and *TNF-α* (-308G/A) polymorphism has also been reported, and all of these increase the risk of PTB[59]. However, in contradiction to these studies, negative or no associations were also reported[28-38]. A meta-analysis which included all studies from 1990 to 2005 found no association between *TNF-α* (-308G/A) and PTB (odds ratio=1.41; 95% confidence interval=0.90-2.19)[39]. Hence, association of polymorphisms of *TNF-α* with PTB is equivocal till date.

Interleukin-1 (IL-1)

Location, function and regulation

The *IL-1* gene is located on long arm of chromosome 2 (2q14). IL-1 is a pro-inflammatory cytokine. Its secretion is controlled by *IL-1* gene which has two subunits, *IL-1α* and *IL-1 β*. On the same chromosome, IL-1 receptor antagonist (*IL-1RA*) gene is also located which is a competitive inhibitor of *IL-1β*. *IL-1β* is the most investigated candidate gene of the pro-inflammatory cytokine family. The activity of pro-inflammatory IL-1β is counterbalanced by the action of IL-1RA which inhibits the binding of circulating IL-1β to cell surface receptors[60,61]. Therefore, IL-1RA helps in terminating the acute inflammation response but gets activated late during the course of an inflammatory event[60].

Polymorphisms in IL-1 gene complex

There are many reported polymorphisms and microsatellites in the *IL-1* gene complex, and the most studied polymorphisms are summarized in Table II. The promoter site of IL-1α consists of two polymorphisms; +4845G/T and -899C/T. *IL-1β* consists of three polymorphisms, namely, -31T/C, -511C/T and +3954C/T. Studies have reported a microsatellite in intron 2 of the *IL-1RA*[60-61]. This polymorphism results in five alleles. The most common allele is allele 2 (*IL1RN**2) with the recurrence of 4-26 per cent, whereas alleles 3, 4 and 5 are in <5 per cent of population. Allele 2 has been associated with various chronic inflammatory conditions. *IL1RA* polymorphism appears to affect both *IL-1* and *IL-1RA* gene expression. The T allele of a polymorphism at position 31 *(IL1β-31T)* is in a transcriptional start site and is likewise connected with a decrease in IL-1β production. This may be a consequence of the underlying

link between *IL1RN*2* and *IL1β-31T*. Carriers of rare alleles of *IL-1β* polymorphisms *(IL-1β -511T* and *-31C)* have shown higher levels of IL-1RA than individuals with wild-type *IL-1β* genotypes[62-73].

IL-1β has consistently been associated with increased risk of spontaneous preterm delivery. A study conducted on European population by Puchner *et al*[74] reported that with a unit increase in IL-1β level in women, there was 7.2 times increased risk of PTB. Thus, it may serve as predictive marker of PTL.

In a case-control study conducted on European[27] and Japanese[40] population, significant association was found between *IL-1* (+4845G/T) and PTB. Others reported the significant association of *IL-1β* (+3953/3954) with enhanced production of IL-1β[41,66]. On the contrary, inconsistent results were reported in case of *IL-1β (-511C/T)* and *IL-1β (-31C/T)* polymorphisms[27,32,40,41,72-74]. Various studies have reported inconsistent association of different polymorphisms of *IL-1α* and *β* with PTB. However, increased IL-1β levels are found consistently associated with PTB.

Interleukin-6 (IL-6)

Location, function and regulation

Gene for *IL-6* is located on 7q21 and commonly known as *IL-6*, IFN β-2 or rarely as hybridoma growth factor or hepatocytes-stimulating factor or B-cell stimulatory factor-2. IL-6 is a pro-inflammatory cytokine causing induction of T-lymphocytes, C-reactive protein synthesis and B-cell differentiation. It is widely expressed in the decidual tissue, placenta, foetal membrane and amniotic fluid. It mainly functions in embryo implantation and placental development, as well as in the immune adaptations, which are required for continuing pregnancy[75]. IL-6 production is stimulated by various factors, namely, IL-1, TNF-α and LPS. Increased levels of IL-6 are found in unexplained infertility, recurrent miscarriage, pre-eclampsia and preterm delivery. Altered systemic IL-6 trans-signalling in women can lead to recurrent miscarriage. IL-6 inhibits the generation of CD4+ T regulatory cells required for pregnancy tolerance[37,43,76-78].

Polymorphism in IL-6 gene

At position -174 in the *IL-6* gene, C>G substitution (*i.e.* Cytosine to Guanine) causes higher transcriptional activity in response to IL-1 and LPS stimuli. A polymorphism at the -174 position (G/C) in the promoter region of the *IL-6* gene results in decreased cytokine production and therefore, decreased risk of PTB[37].

Table II shows the polymorphisms of *IL-6* and their association with PTB. Sugita *et al*[43] reported a significant association of *IL-6* (-6572 G/C) in PTB in the Japanese population. Moura *et al*[37] found strong evidence for the association of *IL-6* (-174G/C) with the PTB in the European population. Menon *et al*[79] compared amniotic fluid concentrations of IL-6 in cases of PTB and term births and found significant association (P=0.003). On the contrary, Kalinka and Bitner[32] reported no association between *IL-6* (-174G/C) and PTB but found an increased incidence of PTB with combined GG+GC genotype. Harper *et al*[30] carried out a study on 834 women with high risk of PTB and assessed the *IL-6* (-174 G/C) polymorphisms but was unable to detect any association with PTB. A study by Karakaş *et al*[80] found this polymorphism protective against PTB, while others reported that maternal *IL-6* (-174G/C) polymorphism was associated with chorioamnionitis[81-83].

Inconsistent results were found for the association of *IL-6* polymorphism with PTB. However, increased IL-6 levels have been reported in chorioamnionitis[45,84,85] which in turn leads to PTB. Further translation research in this area may be able to identify therapeutic agents to prevent PTB.

Interleukin-10 (IL-10)

Location, function and regulation

The *IL-10* gene is located on chromosome 1q31-1q32. It is also known as cytokine synthesis inhibitory factor or T-cell growth factor inhibitor. IL-10 is an anti-inflammatory cytokine produced mainly by monocytes and to a lesser extent by lymphocytes. Being pleiotropic in nature, it modulates both immune regulation and inflammation. It reduces Th1 cytokines by reducing the MHC class II antigens on macrophages and thus enhances B-cell survival, proliferation and antibody production. IL-10 can hinder NF-kappa B activity, which is a key mediator of the JAK-STAT signalling pathway[86].

Polymorphism in IL-10 gene

Table II summarizes the studied polymorphisms and their outcome in PTB. Polymorphisms located at the promoter region of *IL-10* gene are -1082G/A, -819C/T and -592C/A. Studies conducted on Caucasian population found polymorphism (rs1800896) associated with PTB[46,87]. Moura *et al*[37] conducted

two independent studies on Brazilian population and found no association between polymorphisms (*IL-10*-1082G/A, *1L-10*-819C/T and *IL-10*-592C/A) and PTB. Similar findings were reported by other studies also[36,37,44,47].

Thus, IL-10 was not consistently found to be associated with PTB. However, low levels of IL-10 were reported to be associated with PTB[47,84,85,88,89].

Conclusion

Since PTB rate has remained almost static over the past few years in the developed countries[90], researchers are now looking into possible genetic aetiology. The concept of involvement of cytokines-stimulating prostaglandin production resulting in PTB has been widely accepted. Many studies have been conducted in different populations to find out the association of *TLR-4, IL-1α, IL-1β, IL-6* and *IL-10* gene polymorphisms with PTB, yet the results are inconclusive. This can be due to differences in the ethnic groups studied or the influence of environmental factors. Further genome-wide and gene expression studies are needed that are also capable of assessing interactions with infections and environment. Accurate prediction of risk of PTB by molecular methods may help in planning appropriate antenatal care in women at risk.

***Conflicts of Interest*:** None.

References

1. Lawn JE, Cousens S, Zupan J; Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? *Lancet* 2005; *365* : 891-900.
2. WHO. *Preterm Birth Factsheet No. 363*. Available from: *http://www.who.int/mediacentre/factsheets/fs363/en/*, accessed on October 18, 2015.
3. Awasthi S, Chauhan M, Pandey M, Singh S, Singh U. Energy and protein intake during pregnancy in relation to preterm birth: A case control study. *Indian Pediatr* 2015; *52* : 489-92.
4. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, *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.
5. Goldenberg RL. The management of preterm labor. *Obstet Gynecol* 2002; *100* : 1020-37.
6. Wen SW, Smith G, Yang Q, Walker M. Epidemiology of preterm birth and neonatal outcome. *Semin Fetal Neonatal Med* 2004; *9* : 429-35.
7. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008; *371* : 75-84.
8. Sykes L, MacIntyre DA, Yap XJ, Teoh TG, Bennett PR. The Th1:Th2 dichotomy of pregnancy and preterm labour. *Mediators Inflamm* 2012; *2012* : 967629.
9. The American College of Obstetricians and Gynecologists. Preterm (Premature) Labor and Birth: Resource Overview. Available from: *https://www.acog.org/Womens-Health/Preterm-Premature-Labor-and-Birth*, accessed on August 4, 2017.
10. Vrachnis N, Karavolos S, Iliodromiti Z, Sifakis S, Siristatidis C, Mastorakos G, *et al.* Review: Impact of mediators present in amniotic fluid on preterm labour. *In Vivo* 2012; *26* : 799-812.
11. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. *N Engl J Med* 2000; *342* : 1500-7.
12. Romero R, Espinoza J, Gonçalves LF, Kusanovic JP, Friel L, Hassan S, *et al.* The role of inflammation and infection in preterm birth. *Semin Reprod Med* 2007; *25* : 21-39.
13. Keelan JA. Pharmacological inhibition of inflammatory pathways for the prevention of preterm birth. *J Reprod Immunol* 2011; *88* : 176-84.
14. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J Immunol* 1993; *151* : 4562-73.
15. Bennett WA, Lagoo-Deenadayalan S, Whitworth NS, Stopple JA, Barber WH, Hale E, *et al.* First-trimester human chorionic villi express both immunoregulatory and inflammatory cytokines: A role for interleukin-10 in regulating the cytokine network of pregnancy. *Am J Reprod Immunol* 1999; *41* : 70-8.
16. Porter TF, Fraser AM, Hunter CY, Ward RH, Varner MW. The risk of preterm birth across generations. *Obstet Gynecol* 1997; *90* : 63-7.
17. Clausson B, Lichtenstein P, Cnattingius S. Genetic influence on birthweight and gestational length determined by studies in offspring of twins. *BJOG* 2000; *107* : 375-81.
18. Treloar SA, Macones GA, Mitchell LE, Martin NG. Genetic influences on premature parturition in an Australian twin sample. *Twin Res* 2000; *3* : 80-2.
19. Ward K, Argyle V, Meade M, Nelson L. The heritability of preterm delivery. *Obstet Gynecol* 2005; *106* : 1235-9.
20. Lorenz E, Hallman M, Marttila R, Haataja R, Schwartz DA. Association between the Asp299Gly polymorphisms in the toll-like receptor 4 and premature births in the Finnish population. *Pediatr Res* 2002; *52* : 373-6.
21. Härtel CH, Finas D, Ahrens P, Kattner E, Schaible T, Müller D, *et al.* Polymorphisms of genes involved in innate immunity: Association with preterm delivery. *Mol Hum Reprod* 2004; *10* : 911-5.
22. Bitner A, Sobala W, Kalinka J. Association between maternal and fetal *TLR4* (896A>G, 1196C>T) gene polymorphisms and the risk of pre-term birth in the polish population. *Am J Reprod Immunol* 2013; *69* : 272-80.
23. Drews-Piasecka E, Seremak-Mrozikiewicz A, Barlik M, Kurzawińska G, Wolski H, Woyciechowska A, *et al.* The

significance of TNF-alpha gene polymorphisms in preterm delivery. *Ginekol Pol* 2014; *85* : 428-34.

24. 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.
25. Jones NM, Holzman C, Friderici KH, Jernigan K, Chung H, Wirth J, *et al.* Interplay of cytokine polymorphisms and bacterial vaginosis in the etiology of preterm delivery. *J Reprod Immunol* 2010; *87* : 82-9.
26. Liang M, Wang X, Li J, Yang F, Fang Z, Wang L, *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.
27. Yılmaz Y, Verdi H, Taneri A, Yazıcı AC, Ecevit AN, Karakaş NM, *et al.* Maternal-fetal proinflammatory cytokine gene polymorphism and preterm birth. *DNA Cell Biol* 2012; *31* : 92-7.
28. 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.
29. Heinzmann A, Mailaparambil B, Mingirulli N, Krueger M. Association of interleukin-13/-4 and toll-like receptor 10 with preterm births. *Neonatology* 2009; *96* : 175-81.
30. Harper M, Zheng SL, Thom E, Klebanoff MA, Thorp J Jr., Sorokin Y, *et al.* Cytokine gene polymorphisms and length of gestation. *Obstet Gynecol* 2011; *117* : 125-30.
31. Andalas M, Hakimi M, Nurdiati DS, Astuti I, Imran I, Harapan H. Association of 308G/A TNF-α gene polymorphism and spontaneous preterm birth in Acehnese Ethnic Group, Indonesia: This polymorphism is not associated with preterm birth. *Egypt J Med Hum Genet* 2016; *17* : 33-40.
32. 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.
33. Amory JH, Adams KM, Lin MT, Hansen JA, Eschenbach DA, Hitti J, *et al.* Adverse outcomes after preterm labor are associated with tumor necrosis factor-alpha polymorphism -863, but not -308, in mother-infant pairs. *Am J Obstet Gynecol* 2004; *191* : 1362-7.
34. Jafarzadeh L, Danesh A, Sadeghi M, Heybati F, Hashemzadeh M. Analysis of relationship between tumor necrosis factor alpha gene (G308A polymorphism) with preterm labor. *Int J Prev Med* 2013; *4* : 896-901.
35. Speer EM, Gentile DA, Zeevi A, Pillage G, Huo D, Skoner DP, *et al.* Role of single nucleotide polymorphisms of cytokine genes in spontaneous preterm delivery. *Hum Immunol* 2006; *67* : 915-23.
36. Nuk M, Orendi K, Rosenberger S, Griesbacher A, Holzapfel-Bauer M, Resch B, *et al.* Genetic variations in fetal and maternal tumor necrosis factor-α and interleukin 10: Is there an association with preterm birth or periventricular leucomalacia? *J Perinatol* 2012; *32* : 27-32.
37. Moura E, Mattar R, de Souza E, Torloni MR, Gonçalves-Primo A, Daher S, *et al.* Inflammatory cytokine gene polymorphisms and spontaneous preterm birth. *J Reprod Immunol* 2009; *80* : 115-21.
38. Mattar R, de Souza E, Daher S. Preterm delivery and cytokine gene polymorphisms. *J Reprod Med* 2006; *51* : 317-20.
39. Menon R, Merialdi M, Betrán AP, Dolan S, Jiang L, Fortunato SJ, *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.
40. Sata F, Toya S, Yamada H, Suzuki K, Saijo Y, Yamazaki A, *et al.* Proinflammatory cytokine polymorphisms and the risk of preterm birth and low birthweight in a Japanese population. *Mol Hum Reprod* 2009; *15* : 121-30.
41. Schmid M, Haslinger P, Stary S, Leipold H, Egarter C, Grimm C, *et al.* Interleukin-1 beta gene polymorphisms and preterm birth. *Eur J Obstet Gynecol Reprod Biol* 2012; *165* : 33-6.
42. Hollegaard MV, Grove J, Thorsen P, Wang X, Mandrup S, Christiansen M, *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.
43. Sugita N, Kobayashi T, Kikuchi A, Shimada Y, Hirano E, Sasahara J, *et al.* Immunoregulatory gene polymorphisms in Japanese women with preterm births and periodontitis. *J Reprod Immunol* 2012; *93* : 94-101.
44. Pandey M, Awasthi S. Prognostic role of interluekin-1 α and β gene polymorphisms in preterm birth. *Gene Rep* 2016; *4* : 112-7.
45. Wu W, Clark EA, Stoddard GJ, Watkins WS, Esplin MS, Manuck TA, *et al.* Effect of interleukin-6 polymorphism on risk. *BMC Genet* 2013; *14* : 30.
46. Stonek F, Metzenbauer M, Hafner E, Philipp K, Tempfer C. Interleukin-10 -1082 G/A promoter polymorphism and pregnancy complications: Results of a prospective cohort study in 1,616 pregnant women. *Acta Obstet Gynecol Scand* 2008; *87* : 430-3.
47. Szarka A, Rigó J Jr., Lázár L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunol* 2010; *11* : 59.
48. Rey G, Pereyra S, Velazquez T, Grasso D, Alonso J, Bertoni B, *et al.* The effect of inflammation on preterm birth. In: Morrison J, editor. *Preterm birth: Mother and child*. Rijeka, Croatia: InTech; 2012.
49. Smirnova I, Hamblin MT, McBride C, Beutler B, Di Rienzo A. Excess of rare amino acid polymorphisms in the toll-like receptor 4 in humans. *Genetics* 2001; *158* : 1657-64.
50. Kim YM, Romero R, Chaiworapongsa T, Kim GJ, Kim MR, Kuivaniemi H, *et al.* Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis. *Am J Obstet Gynecol* 2004; *191* : 1346-55.

51. Park JE, Kim YI, Yi AK. Protein kinase D1 is essential for MyD88-dependent TLR signaling pathway. *J Immunol* 2009; *182* : 6316-27.
52. Awomoyi AA, Rallabhandi P, Pollin TI, Lorenz E, Sztein MB, Boukhvalova MS, *et al.* Association of TLR4 polymorphisms with symptomatic respiratory syncytial virus infection in high-risk infants and young children. *J Immunol* 2007; *179* : 3171-7.
53. Lavoie PM, Ladd M, Hirschfeld AF, Huusko J, Mahlman M, Speert DP, *et al.* Influence of common non-synonymous toll-like receptor 4 polymorphisms on bronchopulmonary dysplasia and prematurity in human infants. *PLoS One* 2012; *7* : e31351.
54. Pawelczyk E, Nowicki BJ, Izban MG, Pratap S, Sashti NA, Sanderson M, *et al.* Spontaneous preterm labor is associated with an increase in the proinflammatory signal transducer TLR4 receptor on maternal blood monocytes. *BMC Pregnancy Childbirth* 2010; *10* : 66.
55. So T, Ito A, Sato T, Mori Y, Hirakawa S. Tumor necrosis factor-alpha stimulates the biosynthesis of matrix metalloproteinases and plasminogen activator in cultured human chorionic cells. *Biol Reprod* 1992; *46* : 772-8.
56. Anum EA, Springel EH, Shriver MD, Strauss JF 3rd. Genetic contributions to disparities in preterm birth. *Pediatr Res* 2009; *65* : 1-9.
57. Haider S, Knöfler M. Human tumour necrosis factor: Physiological and pathological roles in placenta and endometrium. *Placenta* 2009; *30* : 111-23.
58. Huizinga TW, Westendorp RG, Bollen EL, Keijsers V, Brinkman BM, Langermans JA, *et al.* TNF-alpha promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients. *J Neuroimmunol* 1997; *72* : 149-53.
59. Crider KS, Whitehead N, Buus RM. Genetic variation associated with preterm birth: A HuGE review. *Genet Med* 2005; *7* : 593-604.
60. Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: Role in biology. *Annu Rev Immunol* 1998; *16* : 27-55.
61. Arend WP. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev* 2002; *13* : 323-40.
62. Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. *Clin Infect Dis* 2002; *34* : 204-9.
63. Hall SK, Perregaux DG, Gabel CA, Woodworth T, Durham LK, Huizinga TW, *et al.* Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. *Arthritis Rheum* 2004; *50* : 1976-83.
64. Addas-Carvalho M, Origa AF, Saad ST. Interleukin 1 beta and tumor necrosis factor levels in stored platelet concentrates and the association with gene polymorphisms. *Transfusion* 2004; *44* : 996-1003.
65. Barber MD, Powell JJ, Lynch SF, Fearon KC, Ross JA. A polymorphism of the interleukin-1 beta gene influences survival in pancreatic cancer. *Br J Cancer* 2000; *83* : 1443-7.
66. Pociot F, Mølvig J, Wogensen L, Worsaae H, Nerup J. 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.
67. Maury CP, Liljeström M, Laiho K, Tiitinen S, Kaarela K, Hurme M, *et al.* Anaemia of chronic disease in AA amyloidosis is associated with allele 2 of the interleukin-1beta-511 promoter gene and raised levels of interleukin-1beta and interleukin-18. *J Intern Med* 2004; *256* : 145-52.
68. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. *Eur J Immunol* 1998; *28* : 2598-602.
69. Danis VA, Millington M, Hyland VJ, Grennan D. Cytokine production by normal human monocytes: Inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. *Clin Exp Immunol* 1995; *99* : 303-10.
70. Hacker UT, Erhardt S, Tschöp K, Jelinek T, Endres S. Influence of the IL-1Ra gene polymorphism on *in vivo* synthesis of IL-1Ra and IL-1beta after live yellow fever vaccination. *Clin Exp Immunol* 2001; *125* : 465-9.
71. Schrijver HM, van As J, Crusius JB, Dijkstra CD, Uitdehaag BM. Interleukin (IL)-1 gene polymorphisms: Relevance of disease severity associated alleles with IL-1beta and IL-1ra production in multiple sclerosis. *Mediators Inflamm* 2003; *12* : 89-94.
72. Vamvakopoulos J, Green C, Metcalfe S. Genetic control of IL-1beta bioactivity through differential regulation of the IL-1 receptor antagonist. *Eur J Immunol* 2002; *32* : 2988-96.
73. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production *in vitro*. *Scand J Immunol* 1998; *47* : 195-8.
74. Puchner K, Iavazzo C, Gourgiotis D, Boutsikou M, Baka S, Hassiakos D, *et al.* Mid-trimester amniotic fluid interleukins (IL-1β, IL-10 and IL-18) as possible predictors of preterm delivery. *In Vivo* 2011; *25* : 141-8.
75. Prins JR, Gomez-Lopez N, Robertson SA. Interleukin-6 in pregnancy and gestational disorders. *J Reprod Immunol* 2012; *95* : 1-4.
76. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection. *J Clin Invest* 1990; *85* : 1392-400.
77. El-Bastawissi AY, Williams MA, Riley DE, Hitti J, Krieger JN. Amniotic fluid interleukin-6 and preterm delivery: A review. *Obstet Gynecol* 2000; *95* : 1056-64.
78. Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, *et al.* The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. *Am J Obstet Gynecol* 2000; *183* : 1124-9.
79. Menon R, Camargo MC, Thorsen P, Lombardi SJ, Fortunato SJ. Amniotic fluid interleukin-6 increase is an indicator of spontaneous preterm birth in white but not black Americans. *Am J Obstet Gynecol* 2008; *198* : 77.e1-7.

80. Karakaş NM, Ecevit AN, Yalçın Y, Özdemir B, Verdi H, Tekindal MA, *et al.* Effect of maternal and neonatal interleukin-6-174G/C polymorphism on preterm birth and neonatal morbidity. *J Matern Fetal Neonatal Med* 2017; *23*: 1-7.

81. Resch B, Radinger A, Mannhalter C, Horvath B, Binder A, Zenz W, *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.

82. Djukic M, Gibson CS, Maclennan AH, Goldwater PN, Haan EA, McMichael G, *et al.* Genetic susceptibility to viral exposure may increase the risk of cerebral palsy. *Aust N Z J Obstet Gynaecol* 2009; *49* : 247-53.

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

84. Popowski T, Goffinet F, Batteux F, Maillard F, Kayem G. Prediction of maternofetal infection in preterm premature rupture of membranes: Serum maternal markers. *Gynecol Obstet Fertil* 2011; *39* : 302-8.

85. Makhseed M, Raghupathy R, El-Shazly S, Azizieh F, Al-Harmi JA, Al-Azemi MM, *et al.* Pro-inflammatory maternal cytokine profile in preterm delivery. *Am J Reprod Immunol* 2003; *49* : 308-18.

86. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Crit Rev Immunol* 2012; *32* : 23-63.

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

88. Wommack JC, Ruiz RJ, Marti CN, Stowe RP, Brown CE, Murphey C, *et al.* Interleukin-10 predicts preterm birth in acculturated hispanics. *Biol Res Nurs* 2013; *15* : 78-85.

89. Ruiz RJ, Jallo N, Murphey C, Marti CN, Godbold E, Pickler RH, *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.

90. Keelan JA, Newnham JP. Recent advances in the prevention of preterm birth. *F1000Research* 2017; 6. pii : F1000Faculty Rev-1139.

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