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

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Abstract

Introduction: Our previous study revealed that anti-inflammatory cytokine gene polymorphisms increase the risk of spontaneous preterm delivery (PD) in a population of Polish women. Different genetic background of PD due to preterm premature rupture of membranes (pPROM) than PD without pPROM has been suggested. The aim of this study was to examine the relationship between the maternal carriage of polymorphic alleles of the following genes: interleukin 1β (IL-1β [+3953C>T]), interleukin 6 promoter (IL-6 [–174G>C]), tumour necrosis factor promoter (TNF-α [–308G>A]) and interleukin 1 receptor antagonist (IL-1RN) and the risk of PD caused exclusively by pPROM in a population of Polish women.

Material and methods: A case-control study. 95 Caucasian women were examined including 32 cases and 63 controls. Case subjects experienced a delivery at less than 36 weeks and 6 days of gestation due exclusively to pPROM while control subjects gave birth at term. Polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP).

Results: No statistically significant relationship between polymorphisms of examined genes and risk of PD due to pPROM in a population of Polish women was found: OR = 0.84 (95% CI: 0.34-2.01) for IL-1β, OR = 0.77 (95% CI: 0.27-2.13) for IL-6, OR = 0.72 (95% CI: 0.26-1.90) for TNF-α and OR = 1.74 (95% CI: 0.66-4.64) for IL-1RN.

Conclusions: Maternal carriage of polymorphic alleles of IL-1β, IL-6 promoter, TNF-α promoter and IL-1RA seems to have no impact on the risk of PD due to pPROM in the population of Polish women.The genetic contribution and pathomechanism of PD related to pPROM seems to differ from those of spontaneous PD without pPROM.

Key words: cytokines, prematurity, genetic polymorphism, pathomechanism.

Introduction

Preterm premature rupture of membranes (pPROM) is a rupture of fetal membranes which appears before 37 weeks of gestation and before onset of uterus contractions. Up to 50% of preterm deliveries are preceded by pPROM [1]. Ascending infections of the lower genital tract, such as bacterial vaginosis (BV), which may lead to chorioamnionitis and intrauterine

infections are thought to be the most important cause of pPROM [2]. In these states macrophages present in maternal, fetal and placental tissues undergo activation and produce a number of cytokines, among them interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) [3, 4]. These cytokines are able to stimulate production of prostaglandins by amnion, chorion and deciduas [5, 6]. Prostaglandins provoke uterine contractility which can lead to premature membrane rupture. One of the major anti-inflammatory agents encoded by the IL-1RN gene is IL-1 receptor antagonist (IL-1RA), which is a natural competitive inhibitor of IL-1β activity [7]. IL-1RA terminates IL-1β induced inflammation [8]. In mice models pre-treatment with this cytokine prevented IL-1-induced preterm parturition [9]. In *in vitro* studies it also reduces IL-1-induced prostaglandin production by amnion and chorion [10].

There are some differences among individuals in the intensity of the inflammatory response to infectious agents [3, 4, 6, 8, 10]. These differences can be caused by polymorphism of genes encoding for pro and anti-inflammatory cytokines. The gene polymorphism manifests itself in simultaneous presence in the population of different allelic forms of selected genes. Polymorphisms can concern a single nucleotide (SNP – single nucleotide polymorphism) related to deletion, insertion or substitution of a single nucleotide, or a longer, repeatedly appearing satellite sequence.

Genes encoding for IL-1β, IL-6, TNF-α are polymorphic. Single nucleotide polymorphism in position +3953 of the gene encoding for IL-1β containing a C by T substitution results in appearance of the rarer allele 2 (IL-1β+3953*2). This allele is associated with the enhanced production of IL-1β *in vitro* [11]. Single nucleotide polymorphism at position –174 in a promoter region of IL-6 connected with substitution of C for G is associated with decrease in promoter activity. The C/C variant is related to reduced IL-6 production, whereas homozygous G/G or heterozygous G/C displays normal production of IL-6 [12]. The substitution of A for G at position -308 in a promoter region of TNF-α genes leads to creation of a rarer allele (TNF-α-308*2). Carriage of these allele is associated with increased TNF-α gene expression in comparison to homozygous G/G [13]. Gene encoding for IL-1RA (IL-1RN) is also polymorphic. A region within intron 2 of this gene contains a variable number of 86-base pair tandem repeats, which results in 5 alleles. The alleles 1, 2, 3, 4 and 5 represent four, two, five, three and six repeats of 86-bp tandem repeats, respectively [14]. The most prevalent in the general population is allele 1 (IL-1RN*1), while allele 2 (IL-1RN*2) occurs less often. Remaining alleles IL-1RN*3, IL-1RN*4, IL-1RN*5 are present in less than 1% of the population. Carriage of IL-1RN*2 is associated with higher IL-1RA and IL-1β concentration in plasma [15].

The results of our previous study suggest that maternal carriage of allele 2 of the IL-1RA gene increases the risk of preterm delivery due to preterm labour not preceded by pPROM in the population of Polish women [16]. This time it was decided to investigate whether this relationship is also valid for PD related exclusively to pPROM. The decision to undertake these two matters separately resulted from a suggestion that PD due to pPROM and PD related to preterm labour constitute two aetiologically distinct diseases [17]. We were interested to discover whether these differences also apply to genetic conditions of PD.

The aim of the current study was to evaluate the relationship between maternal carriage of polymorphic alleles of the following genes: IL-1β (IL-1β [+3953C>T]), IL-6 promoter (IL-6 [–174G>C]), TNF-α promoter (TNF-α [–308G>A]) and IL-1RN and the risk of PD due exclusively to pPROM in a population of Polish women.

Material and methods

The study was approved by the Committee for Bioethics of the Medical University of Łódź (number RNN/29/04/KE). Written informed consent was obtained from all patients.

Ninety-five Caucasian women who delivered in the Department of Perinatology, First Chair of Gynaecology and Obstetrics, Medical University of Lodz between 2005 and 2007 were included in the study. Women were divided into two groups. Cases were defined as those who delivered a child at less than 36^{6} weeks of gestation due to preterm premature rupture of membranes. Control subjects were defined as women who delivered after 37 weeks' gestation. In all women gestational age was estimated based on the date of the last period and confirmed by the ultrasound evaluation performed between 11 and 13^{+6} weeks of pregnancy. Mothers with incompetent cervix, congenital anomalies of uterus, fetal malformations, iatrogenic preterm delivery and preterm delivery resulting from preterm labour without pPROM were excluded from the study. Clinical and demographic information was collected by chart review and patient self-reports.

Maternal genomic DNA was isolated from white cells of peripheral blood obtained after delivery according to Higuchi. Polymerase chain reaction (PCR) was carried out using the GeneAmp PCR System 2007 and GeneAmp PCR system 2400 (Applied Biosystems, USA) following the manufacturer's recommendations.

In order to analyse IL-1β+3953 allelic variants the polymorphic region containing the Taq I restriction site was amplified with the following primers:

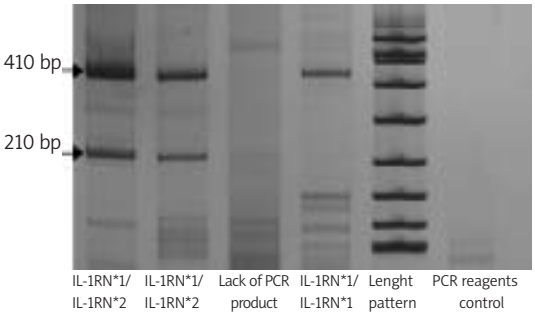

Figure 1. Methods of allelic variants identification of IL-1RA gene

5'-GTTGTCATCAGACTTTGACC-3' and 5'-TTCAGTTCA TATGGACCAGA-3'. PCR conditions consisted of a denaturing step at 95°C for 5 min followed by 30 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min. Eight millilitres of PCR product was digested with TaqI (Fermentas, Canada) at 65°C for 24 h. TaqI digestion of the 249-bp fragments results in products that either are cut into 2 fragments of 135 bp and 11 bp (IL-1β+3953*1) or remain intact (IL-1β+3953*2).

Interleukin-6 promoter region was amplified with oligonucleotides 5'-TCCCCCTAGTTGTGTCTGGCG-3', 5'-CTGCACTTTATCCCCTAGTTGTGTCATGCC-3' and 5'-TGAGGGTGGGGCCAGAGA-3'. The PCR included 40-s denaturation at 95°C followed by 35 amplification cycles (40 s at 95°C, 45 s at 63°C and 40 s at 72°C). The size of digestion products was 94 bp for allele G and 103 bp for allele C.

Tumour necrosis factor-α promoter was amplified by polymerase chain reaction with primers 5'-GGCAA TAGGTTTTGAGGGCCAT-3' and 5'-TCCTCCCTGCTCCGA TTCCG-3'. Cycling was performed at 94°C for 4 min; then for 30 cycles of 94°C for 40 s, 60°C for 40 s and 72°C for 40 s. Polymerase chain reaction products were digested overnight at 37°C with NcoI (Fermentas). The size of the digestion products was 87 bp and 20 bp for TNF-α*1 and 107 bp for TNF-α*2.

Oligonucleotides 5'-CTCAGCAACACTCCTAT-3' and 5'-TCCTGGTCGCAGGTAA-3' were used as PCR primers in order to analyse allelic variants in intron 2 of IL-1RN. The PCR conditions were as follows: 1 cycle at 95°C for 5 min, followed by 40 cycles of 94°C for 1 min, 63°C for 1 min and 72°C for 1 min. Polymerase chain reaction products were 410 bp (IL-1RN*1), 240 bp (IL-1RN*2) and 500 bp (IL-1RN*3) in size. All DNA fragments were analysed on 10% polyacrylamide gels with Mini Protein II and Mini Protein III equipment (Bio-Rad, USA) and stained with ethidium bromide (Figure 1).

Genotype frequencies in study and control groups were compared using an exact logistic regression model. Analysis was used to estimate the odds ratio (OR) and its 95% confidence intervals (95% CI). Analysis was performed with the statistical analysis package R version 2.7.2 (http://www.r-project.org). Assuming the power of the test as 0.80 and level of significance $\alpha = 0.05$ with a population size of 95 women, OR of 3.7 or above will be detected as significant.

Results

We studied 32 women with PD due to pPROM occurring before 36^6 weeks' gestation and 63 women who delivered at or after 37 weeks' gestation (reference group). There was no difference in the percentage of caesarean sections performed, maternal age and weight at delivery between term and preterm subjects. In the study group significantly more women smoked cigarettes and had a history of at least one preterm delivery than in the control group. As defined by our parameters, the mean gestational age at delivery and the mean newborn birth weight were significantly lower among women who delivered prematurely. These data are illustrated in Table I.

The frequency of polymorphic allele 2 of the IL-1β gene was 43.8% in women who gave birth to a premature baby and 51.8% in women who delivered at term. We found no statistically significant influence of polymorphism of the IL-1β (+3953C>T) gene on the risk of PD as a result of pPROM, OR = 0.84 (95% CI: 0.34-2.01).

The analysis of frequencies of individual genotypes of IL-6 gene promoter shows CC, GC and GG genotype in 40.6%, 31.3%, and 28.1% of case subjects and 39.6%, 39.6%, and 20.8% of control subjects respectively. No statistically significant

Table I. Demographic characteristics of examined population

Parameter	Preterm delivery (n = 32)	Term delivery (n = 63)	Value of p
Maternal age at delivery [years]	31.2	29.0	NS
Mean maternal weight at delivery [kg]	75.0	74	NS
Smoking during pregnancy [%]	31.0	17.5	< 0.05
History of preterm delivery [%]	20.7	4.6	< 0.05
Percentage of caesarean section [%]	31.0	31.7	< 0.05
Gestational age at delivery [week]	32.2	39.2	< 0.05
Newborns' birth weight [g]	1980	3470	< 0.05

influence of IL-6 gene promoter polymorphism on the risk of PD due to pPROM was found. Odds ratio and its 95% CI for GC genotype were OR = 0.77 (95% CI: 0.27-2.13) and for GG genotype OR = 1.32 (95% CI: 0.43-4.08).

In both groups of women the most frequent genotype was IL-1RN*1/IL-1RN*1. Among women who delivered before term it was found in 43.8% while among women who delivered at term this genotype was more frequent (56.6% of women).

The genotype IL-1RN*1/IL-1RN*2 was present in 40.6% of the study group and 30.2% in controls. In the remaining women we detected IL-1RN*2/IL-1RN*2 genotype or other rare genotypes: IL-1RN*1/IL-1RN*3, IL-1RN*2/IL-1RN*3. We did not reveal a statistically significant influence of IL-1RA gene polymorphism on the risk of pPROM and subsequent PD. Odds ratio for pPROM occurrence in women who carried one polymorphic allele 2 (IL-1RN*2) was OR = 1.74 (95% CI: 0.66-4.64) and in the case of carriage of two polymorphic alleles 2 or allele 3 (IL-1RN*3) OR = 1.53 (95% CI: 0.39-5.67).

When we analysed the frequency of individual genotypes of the TNF-α gene we found that in both groups of women the most frequent genotype was TNF-α*1/TNF-α*1. We found this genotype in 75% of women who delivered prematurely and in 68.5% of women who delivered at term. No statistically significant relationship between carriage of polymorphic allele 2 of the TNF-α gene (TNF-α*2) and the risk of preterm delivery as a result of pPROM was found, OR = 0.72 (95% CI: 0.26-1.90). Results are illustrated in Figure 2.

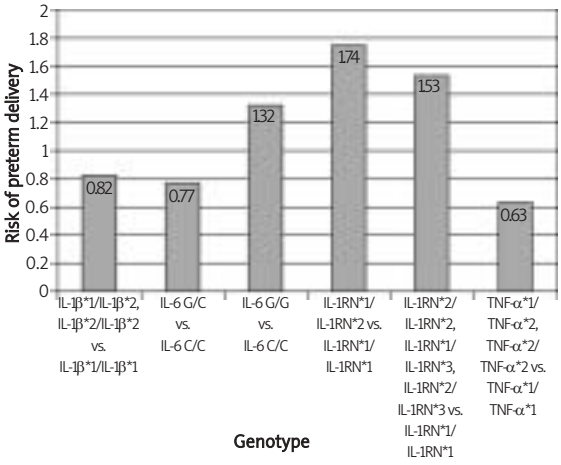

Figure 2. Relationship between selected cytokine genotype and the risk of PD due to pPROM in the population of Polish women. Data statistically not significant

Discussion

According to our knowledge this is one of the first studies where the relationship between maternal carriage of polymorphic alleles of pro and anti-inflammatory cytokines and the risk of PD resulting exclusively from pPROM was analysed. In our research we have not identified a statistically significant influence of maternal carriage of polymorphic genes encoding for IL-1β, IL-6 promoter, TNF-α promoter and IL-1RA on the risk of preterm delivery caused exclusively by preterm premature rupture of membranes.

Until now the majority of authors have studied the relationship between gene polymorphisms and the risk of PD regardless of the cause. Most researchers agree that both maternal and fetal carriage of allele 2 of the IL-1RA gene increases the risk of spontaneous preterm delivery [3, 7, 18]. In contrast, the impact of polymorphisms of remaining genes that we have examined, i.e. IL-1β, IL-6 promoter, and TNF-α promoter, on the risk of prematurity is being negated [12, 19, 20]. In our previous study we revealed that maternal carriage of IL-1RN allele 2 significantly increases the risk of preterm delivery due to preterm contractions, not preceded by pPROM in the population of Polish women, OR = 3.12 (95% CI: 1.14-9.00) [16]. It seems that lack of an influence of IL-1RN gene polymorphism on the risk of PD due to pPROM and at the same time a distinct impact of this polymorphism on the risk of spontaneous preterm delivery, due to uterus contractions not preceded by pPROM, might indicate that these two states have different pathomechanisms and different genetic contributions. These observations seem to confirm the findings reported by Roberts *et al*. [21]. These authors did not identify a statistically significant impact of carriage of a polymorphic allele of the TNF-α promoter gene on the risk of PD. However, they observed that the rarer allele TNF-α*2 was more common in women who delivered prematurely due to pPROM compared to women in whom PD was not associated with pPROM, OR = 3.18 (95% CI: 1.33-7.83). Menon *et al*. even suggest that PD due to pPROM and PD without pPROM should be considered as two separate diseases due to different aetiology [20].

A few authors have tried to explain the relationship between the carriage of polymorphic alleles of genes encoding for cytokines and the risk of PD due to pPROM. Kalish *et al.* in a study carried out in the US revealed that fetal carriage of IL-1RN*2 was associated with increased risk of pPROM and subsequent PD [7]. Our research does not show these relationships in a population of Polish women. In a study carried out in a population of Afro-American women, the authors demonstrated that carriage of a polymorphic allele of the TNF-α gene increases the risk of pPROM [21]. Tumour necrosis factor-α is able to stimulate numerous

tissues, among them fetal membranes and cervix uteri, to produce matrix metalloproteinases (MMP), which are able to degrade collagen. The degradation of collagen in fetal membranes and in cervix uteri might be responsible for preterm premature rupture of membranes and dilatation of the cervix. These data suggest that the presence of the polymorphic allele TNF-α*2, which is associated with higher expression of the TNF-α gene, might increase the risk of pPROM. Our findings do not confirm this hypothesis in a population of Polish women. The different results are probably associated with ethnic disparity. Many authors have emphasized the distinct influence of individual allelic variants on the risk of preterm delivery in different populations [22, 23]. Genç *et al.* revealed that fetal carriage of allele 1 of the IL-1α gene increases the risk of preterm delivery in Afro-American women, but carriage of allele 2 of this gene is associated with higher risk of prematurity in women of Hispanic origin [3]. Dissimilar influence of allelic variants in distinct populations might be associated with the extremely high number of SNP in the human genome and with interactions between these polymorphisms. It has been estimated that there are approximately 200 000 single nucleotide polymorphisms within the encoding regions of the 80 000 human genes [3]. Recent studies have shown that the carriers of polymorphic alleles of TLR4 (Toll-like receptor 4), which plays a fundamental role in pathogen recognition and activation of innate immunity, are at increased risk of pPROM and subsequent PD [24]. It has also been revealed that the carriage of polymorphic alleles encoding for MMP1 (matrix metalloproteinase 1), a protein involved in the breakdown of extracellular matrix, influences the risk of pPROM [25].

We realize that our research is limited by the small groups and by the power of the study. Further larger studies conducted on a prospective basis are necessary to elucidate differences in patho-mechanism and genetic contributions between PD related and not related to pPROM.

In conclusion, it should be said that individual genes interacting with numerous polymorphic alleles might have a different influence on the duration of pregnancy. We can hope that in future it will be possible to define a group of genes responsible for preterm delivery in specific populations. This would allow us to identify patients at high risk for giving birth to a premature baby so that prevention programmes could be implemented before any symptoms of preterm delivery appear.

The genetic contributions and pathomechanism of PD related exclusively to pPROM seem to differ from those of spontaneous PD without pPROM. Maternal carriage of polymorphic alleles of IL-1β, IL-6 promoter, TNF-α promoter and IL-1RA genes seems to have no impact on risk of PD due to pPROM, while carriage of IL-1RN allele 2 probably increases the risk of prematurity in the population of Polish women.

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References

1. Parry S, Strauss JF 3rd. Premature rupture of the fetal membranes. N Engl J Med 1998; 338: 663-70.
2. 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.
3. Genç MR, Gerber S, Nesin M, Witkin SS. Polymorphism in the interleukin-1 gene complex and spontaneous preterm delivery. Am J Obstet Gynecol 2002; 187: 157-63.
4. Simhan HN, Krohn MA, Roberts JM, Zeevi A, Caritis SN. Interleukin-6 promoter –174 polymorphism and spontaneous preterm birth. Am J Obstet Gynecol 2003; 189: 915-8.
5. Kent AS, Sullivan MH, Sun MY, Zosmer A, Elder MG. Effects of interleukin-6 and tumor necrosis factor-alpha on prostaglandin production by cultured human fetal membranes. Prostaglandins 1993; 46: 351-9.
6. Jana B, Kozłowska A, Andronowska A, Jedlińska-Krakowska M. The effect of tumor necrosis factor-alpha (TNF-alpha), interleukin (IL)-1 beta and IL-6 on chorioamnion secretion of prostaglandins (PG)F 2 alpha and E2 in pigs. Reprod Biol 2008; 8: 57-68.
7. Kalish RB, Vardhana S, Gupta M, Chasen ST, Perni SC, Witkin SS. Interleukin-1 receptor antagonist gene polymorphism and multifetal pregnancy outcome. Am J Obstet Gynecol. 2003; 189: 911-4.
8. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991; 77: 1627-52.
9. Romero R, Tartakovsky B. The natural interleukin-1 receptor antagonist prevents interleukin-1-induced preterm delivery in mice. Am J Obstet Gynecol 1992; 167: 1041-5.
10. Romero R, Sepulveda W, Mazor M, et al. The natural interleukin-1 receptor antagonist in term and preterm parturition. Am J Obstet Gynecol 1992; 167: 863-72.
11. Pociot F, Mølvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992; 22: 396-402.
12. Stonek F, Metzenbauer M, Hafner E, Philipp K, Tempfer C. Interleukin 6-174 G/C promoter polymorphism and pregnancy complications: results of a prospective cohort study in 1626 pregnant women. Am J Reprod Immunol 2008; 59: 347-51.
13. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci; 94: 3195-9.
14. Bessler H, Osovsky M, Sirota L. Association between IL-1RA gene polymorphism and premature delivery. Biol Neonate 2004; 85: 179-83.

15. 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.
16. Kalinka J, Bitner A. Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and the risk of spontaneous preterm delivery in the population of Polish women. Arch Perinatal Med 2008; 14: 33-6.
17. Fortunato SJ, Menon R. Distinct molecular events suggest different pathways for preterm labor and premature rupture of membranes. Am J Obstet Gynecol 2001; 184: 1399-405.
18. Murtha AP, Nieves A, Hauser ER, et al. Association of maternal IL-1 receptor antagonist intron 2 gene polymorphism and preterm birth. Am J Obstet Gynecol 2006; 195: 1249-53.
19. Edwards RK, Ferguson RJ, Duff P. The interleukin-1 beta +3953 single nucleotide polymorphism: cervical protein concentration and preterm delivery risk. Am J Reprod Immunol 2006; 55: 259-64.
20. 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.
21. Roberts AK, Monzon-Bordonaba F, Van Deerlin PG, et al. Association of polymorphism within the promoter of the tumor necrosis factor alpha gene with increased risk of preterm premature rupture of the fetal membranes. Am J Obstet Gynecol 1999; 180: 1297-302.
22. Menon R, Velez DR, Morgan N, Lombardi SJ, Fortunato SJ, Williams SM. Genetic regulation of amniotic fluid TNF-alpha and soluble TNF receptor concentrations affected by race and preterm birth. Hum Genet 2008; 124: 243-53.
23. Menon R. Spontaneous preterm birth, a clinical dilemma: etiologic, pathophysiologic and genetic heterogeneities and racial disparity. Acta Obstet Gynecol Scand 2008; 87: 590-600.
24. Rey G, Skowronek F, Alciaturi J, Alonso J, Bertoni B, Sapiro R. Toll receptor 4 Asp299Gly polymorphism and its association with preterm birth and premature rupture of membranes in a South American population. Mol Hum Reprod 2008; 14: 555-9.
25. Wang H, Ogawa M, Wood JR, et al. Genetic and epigenetic mechanisms combine to control MMP1 expression and its association with preterm premature rupture of membranes. Hum Mol Genet 2008; 17: 1087-96.