ORIGINAL ARTICLE: GENETICS

Interleukin-1beta -511T > C genetic variant contributes to recurrent pregnancy loss risk and peripheral natural killer cell proportion

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Objective: To identify whether interleukin gene polymorphisms are risk factors for idiopathic recurrent pregnancy loss (RPL) in Korean women.
Design: Case–control study.
Setting: Hospital-based study.
Patient(s): A cohort of 385 idiopathic RPL patients and 232 controls with Korean ethnicity.
Intervention(s): None.
Main Outcome Measure(s): Genotyping was assessed with a polymerase chain reaction–restriction fragment length polymorphism assay. We examined polymorphisms in three interleukin (IL) genes: *IL-1β*, *IL-4*, and *IL-10*.
Result(s): The *IL-1β* -511T>C polymorphism was associated with RPL (-511TT vs. -511CC: adjusted odds ratio 1.826; 95% confidence interval 1.130–2.953). Allelic gene–gene interaction analysis revealed that the T/B2/G (*IL-1β*/*IL-4*/*IL-10*) allele combination was only detected in the RPL group (adjusted odds ratio 20.046; 95% confidence interval 1.188–338.204). The proportion of peripheral natural killer cells was higher in patients with the *IL-1β* -511C allele compared with the -511T allele.
Conclusion(s): According to these results, *IL-1β* -511T>C may be a predisposing factor to RPL susceptibility. However, the mechanism underlying the function of *IL-1β* -511T>C in RPL remains to be determined, and further studies are needed to improve understanding of the roles of *IL-1β* -511T>C, using a larger and more heterogeneous cohort. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: *IL-1β* -511T>C, polymorphism, NK cell, RPL, recurrent pregnancy loss

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Interactions between the fetus and the mother's immune system play an important role in a successful pregnancy (1). The immune system of the mother is tightly controlled to defend against microbial infections, but to accept the embryo, which expresses semiallogenic paternal antigens throughout its development. In addition, immune-mediated processes like tissue growth and differentiation are decisive to pregnancy maintenance. Dysfunction of this elaborate regulation may lead to reproductive failures, such as implantation failure, pregnancy loss, preterm birth, intrauterine fetal growth restriction, and preeclampsia (1, 2).

Recurrent pregnancy loss (RPL) is defined as two or more consecutive pregnancy losses before the 20th week

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of gestation (3–5). Approximately 1%–3% of healthy (3) and 10%–20% of all pregnant women (4) experience RPL. The clinical diagnosis of RPL is based on several tests that detect parental chromosomal anomalies and maternal thrombophilic, endocrine, and immunologic disorders (6). The etiology of RPL includes several factors, such as genetics, anatomic deformities, endocrine dysfunction, placental anomaly, infection, smoking, alcohol, environmental factors, psychological trauma, and stress (7).

Despite several well-known etiologic factors, the cause of RPL cannot be determined in almost 50% of cases. Unexplained RPL has been hypothesized to have an immunologic component (8). In particular, cytokines are known to play critical roles in many reproductive events. A homeostatic balance between cytokines is essential to pregnancy maintenance, as well as in autoimmune abnormalities, coagulation, angiogenesis, vascular tone, and apoptosis (9–11). For example, high maternal helper T1 (Th1) cytokines induce RPL, whereas elevated maternal helper T2 (Th2) cytokine expression allows for normal pregnancy (12).

During early pregnancy, maternal cells encounter histoincompatible fetal cells at the maternal–fetal interface. An initial sterile inflammatory immune response occurs, causing embryonic implantation. This event is essential for embryonic development (13). Proinflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor-α, and interferon-γ, cause activities that are dangerous to the fetus, whereas anti-inflammatory cytokines, such as IL-4, IL-6, and IL-10, cause reactions that are advantageous (14). Previous studies have suggested roles for IL-1β, -4, and -10 in successful pregnancies and RPL (7, 8, 11, 14–18). Genetic variants of these cytokines can cause variation in their expression, resulting in abnormalities during embryonic development and miscarriage or infertility (13). Previous studies have indicated that IL-1, IL-4, and IL-10 are involved in vascular disease (17, 19–21). Furthermore, associations of cytokine gene polymorphisms with susceptibility to diseases, different clinical features, and outcomes of disease have been demonstrated previously (8).

Because cytokines are the major determinants of Th1 and Th2 cell reactions, and considering that polymorphic variants may result in variable production expression, we investigated three polymorphisms in cytokine genes (*IL-1β* -511T>C, *IL-4* intron 3 VNTR [variable number tandem repeat], and *IL-10* -1082A>G) in RPL patients and in control groups. These three polymorphisms are located in the promoter regions of the *IL-1β* and *IL-10* genes and located in the intron region of the *IL-4* gene. These regions are well known to be regulatory for mRNA transcription. Many previous studies have shown that minor alleles containing individual single nucleotide polymorphisms (SNPs) were differentiated for transcriptional effects (22–26). Three examples of minor alleles that effect transcriptional activity are *IL-1β* -511T (22), *IL-4* intron 3B2 (23, 24), and *IL-10* -1082A (25, 26). We hypothesized that cytokine gene polymorphisms have a role in idiopathic RPL. The aim of this study was to investigate whether three polymorphisms affecting the IL genes—*IL-1β* -511T>C, VNTR of *IL-4* intron 3, and *IL-10* -1082A>G—could present positive associations with RPL in Korean women.

MATERIALS AND METHODS

Participants

Blood samples were collected from 385 patients with idiopathic RPL (age [mean ± SD] 33.20 ± 4.55 years; body mass index [BMI] 21.49 ± 3.86 kg/m^2) and 232 control participants (age 33.34 ± 5.74 years; BMI 21.64 ± 3.42 kg/m^2). All RPL patients had experienced at least two consecutive spontaneous abortions. The RPL blood samples were collected between March 1999 and February 2012 in the Department of Obstetrics and Gynecology and the Fertility Center of CHA Bundang Medical Center in Seongnam, South Korea. The RPL patients were diagnosed by analysis of hCG levels, ultrasound, and/or physical examination. None had a history of smoking or alcohol use. Patients with RPL due to anatomic, hormonal, chromosomal, infectious, autoimmune, or thrombotic causes were excluded from the study group. Fertile female controls were recruited from CHA Bundang Medical Center. All control patients had a history of at least one successful, naturally conceived pregnancy, no history of pregnancy loss, and karyotype 46,XX. The institutional review board of CHA Bundang Medical Center approved the study, and all patients gave written, informed consent.

Genotyping

Genomic DNA was extracted from anticoagulated peripheral blood using the G-DEX blood extraction kit (Intron). Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis was used to detect genetic variants because it is more economical and cost-effective than sequencing entire genes. Nucleotide changes were determined by PCR-RFLP analysis using the isolated genomic DNA as a template. *IL-1β* -511T>C (rs16944), *IL-4* intron 3 VNTR (rs2234665), and *IL-10* -1082A>G (rs1800896) were selected using the human genome SNP database (dbSNP: www.ncbi.nlm.nih.gov/snp).

The *IL-1β* -511T>C (rs16944) polymorphism was detected by PCR-RFLP analysis using the sense primer 5′-CGT TGT GCA GTT GAT GTC CA-3′ and the antisense primer 5′-TGA ACC CTG CAT ACC GTA TGT T-3′. The 343-bp PCR product was then digested with *Ava*I (New England Biolabs) for 16 hours at 37°C. A digestion product of 343 bp represented the CC genotype; fragments of 343 bp, 254 bp, and 89 bp represented the CT genotype; and fragments of 254 bp and 89 bp represented the TT genotype.

The *IL-4* intron 3 VNTR (rs2234665) was detected by PCR amplification. The primers used for amplification of *IL-4* intron3 VNTR regions were as follows: sense 5′-AGG CTG AAA GGG GGA AAG C-3′ and antisense 5′-CTG TTC ACC TCA ACT GCT CC-3′. The B1 allele produced a PCR product of 181 bp, and the B2 allele produced a PCR product of 251 bp (15). To validate the accuracy and reproducibility of this method, each set of PCR reactions included internal controls for each genotype.

The *IL-10* -1082A>G genotype was detected using allelic discrimination real-time PCR (RG-3000; Corbett Research). Primers and TaqMan probes were designed using Primer Express Software (version 2.0) and synthesized and supplied by