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Single nucleotide polymorphisms in the promoter region of the interleukin -6 gene and the risk of recurrent pregnancy loss in japanese women

Saijo, Yasuaki ; Sata, Fumihiro ; Yamada, Hideto ; Kondo, Tomoko ; Hirayama Kato, Emi ; Kishi, Reiko

Running title

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Title page

Single nucleotide polymorphisms in the promoter region of the interleukin-6 gene and the risk of recurrent pregnancy loss in the Japanese population

Yasuaki Saijo, M.D. [a]

Fumihiro Sata, M.D. [a]

Hideto Yamada, M.D. [b]

Tomoko Kondo, Ph.D., [a]

Emi Hirayama Kato, M.D. [b]

Reiko Kishi , M.D. [a]

Where the work was done:

Department of Public Heath and Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

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Department affilation:

[a] Department of Public Heath , Hokkaido University Graduate School of Medicine, u, Sapporo, Japan

[b] Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, , Sapporo8, Japan

Reprint request:

Yasuaki Saijo, M.D., Department of Public Heath, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan

(Fax: +81 11 706 5068; e-mail:y-saijo@med.hokudai.ac.jp)

Capsule

There was a statistically significant difference in the IL-6-634C→G genotype frequency between Japanese patients with recurrent pregnancy loss (RPL) and controls. Patients who carry the -634 G allele may have a decreased risk of RPL.

Structured Abstract

Objective: To investigate the relationships between recurrent pregnancy loss (RPL) and single nucleotide polymorphisms (-634 C→G and -174 G→C genotypes) in the promoter region of the IL-6 gene in the Japanese population.

Design: A case-control study.

Setting: Department of Obstetrics and Gynecology in a university hospital.

Patient(s): 76 cases with RPL. Controls were 93 fertile women.

Intervention(s): Frequency and distribution of the promoter region of the IL-6 gene allele.

Main Outcome Measure(s): Determination of IL-6 promoter gene polymorphisms was performed by polymerase chain reaction and gel electrophoresis

Results: There was a significant difference in the -634C→G genotype frequency (CC vs. CG/GG) between the women with RPL and the controls. The risk of RPL was lower in the carrier of the G allele than in women with the wild type（CC）, with OR = 0.46 (95% CI = 0.24-0.91). On the other hand, we did not detect any carrier of -174C in the 169 subjects.

Conclusion(s): The results suggest that women carrying the -634 G allele of the IL-6 gene may have a decreased risk of RPL in the Japanese population.

Key words: polymorphism; interleukin-6; recurrent pregnancy loss

Introduction

About 10-14% of clinically recognized pregnancies end in pregnancy loss among the Japanese population as well as in Caucasians. The etiology of recurrent pregnancy loss (RPL) remains largely unclear (1-3). The possible immunological etiologies of pregnancy failure have been intensively investigated (4).

A shift to type-2 T-helper (Th2) cytokine production with abundant interleukine (IL)-6 and IL-10 is considered essential for the maintenance of normal pregnancy. There is evidence of a diminished Th2 immune response to placental antigens in women with RLP (5). Plasma levels of IL-6, IL-8 and IL-11 have been found to be decreased in such women compared with normal pregnancies (6). Additionally, IL-6 levels in maternal serum (7), amniotic fluid (8), vaginal fluid (9), and placenta (10) have been found to increase during the process of normal labor compared to the nonlabor state.

One study demonstrated an increase in the frequencies of type-1 T-helper (Th1) cytokine IL-1β gene promoter region variants IL-1B-511C and IL-1B-31T in RPL women with RLP (11). One of the Th2 cytokines, IL-10 promoter region variant (-1082G→A) was not associated with RPL (12, 13). However, relationships between many diseases and IL-6 promoter gene polymorphisms, such as IL-6 -174G→C (14, 15) and IL-6 -634C→G (16), were recently demonstrated. It is also known that the former polymorphism is frequently found in Caucasians (14, 15) and the latter in Japanese (16). There have been no reports of other frequent polymorphisms of the IL-6 gene, and the relationship between any IL-6 gene polymorphism and RPL has not yet been investigated. In the present study, therefore, we assessed these two promoter polymorphisms and investigated whether either of these IL-6 gene polymorphisms

predisposes women to RPL.

Materials and Methods

This case-control study was performed in Sapporo, Japan, during the years 1999-2002. We studied 76 cases aged 20-42 years with a history of RPL and 93 controls aged 21-54 years who visited Hokkaido University Hospital. The characteristics of the study groups are shown in Table 1. RPL was defined as a history of two or more spontaneous consecutive abortions and stillbirths. The primary RPL group compromised 68 women with a history of two or more pregnancy losses but no live birth. The 8 secondary RPL women experienced three or more pregnancy losses after one live birth.

All women with RLP were subjected to blood analyses for syphilis, anti-nuclear antibody (ANA), anti-DNA antibody, lupus anticoagulant, anti-cardiolipin antibody, anti-cardiolipin beta 2-glycoprotein I complex antibody and activated partial thromboplastin time, d-dimer, protein S, protein C activity, and factor XII to look for patients with anti-phospholipid antibody syndrome and thrombophilia. If ANA or anti-DNA antibody was present, further serological tests, for lupus erythematosus (LE), rheumatoid factor, anti-SSA(B) antibody, anti-RNP antibody, and anti-Sm antibody were performed, and complements were measured for the diagnosis of definite autoimmune diseases. Genetic analysis for factor V Leiden mutation was not done in this study because we previously found no Japanese having factor V Leiden mutation (17, 18).

Subjects were examined by ultrasound and hysterosalpingography for detection of anatomical abnormalities of the genital tract. All couples were also

subjected to chromosome karyotypic analyses of peripheral blood. Couples with balanced type chromosomal translocation and women with RPL having uterine conformational abnormalities such as a septate uterus were excluded from this study, because these etiologies of RPL were known to have the close cause-effect relationship. Women tested positive for autoantibodies and women with antiphospholipid antibody syndrome were included in this study, because immunological abnormalities including IL-6 possibly underlain the pathophysiology of RPL in these women. Of the 76 women, 10 (13.2%) had autoimmune disease and/or antiphospholipid antibody syndrome and 8 (10.5%) had hematologic abnormalities, but none had a history of thromboembolism.

Control women consisted of 93 volunteers experiencing at least one live birth and no abortion who had no history of endometriosis or infertility. There were no significant differences in age between cases and controls. This study was conducted with all the subjects' informed consent and approved by the institutional ethical boards for human gene and genome studies at Hokkaido University Graduate School of Medicine.

Genomic DNA was extracted from lymphocytes of peripheral blood samples by the use of standard techniques. Sequence amplification was performed by using polymerase chain reaction (PCR). To analyze the -634 C→G genotype, PCR amplifications were carried out as described by Ota et al (16), using the primers 5'-GAG AGG CCT TGA AGT AAC TG-3' and 5'-AAC CAA AGA TGT TCT GAA CTG A-3'. After the PCR product was digested with BsrBI endnuclease, the restriction digest was separated in 3% agarose gel, generating a 120-bp fragment and a 60-bp fragment. The 120-bp and 60-bp fragments represented the "G" allele. The 180–bp fragment represented the "C" allele.

To analyze the -174G→C genotype, PCR amplifications were carried out as described by Fernandez-Real et al. (14), using the primers 5'-TGA CTT CAG CTT TAC TCT TTG T -3' and 5'-CTG ATT GGA AAC CTT ATT AAG-3'. After the PCR product was digested with Hsp92II endnuclease, the restriction digest was separated in 3% agarose gel, generating a 140-bp fragment and a 58-bp fragment. The 140-bp and 58-bp fragments represented the "C" allele. The 198-bp fragment represented the "G" allele.

Statistical analysis

We calculated age-adjusted odds ratios (OR) and 95% confidence intervals (CI) associated with the IL-6 genotypes by unconditional logistic regression analysis. All analyses were conducted using SPSS software for Windows (SPSS Inc., Chicago, U.S.A.).

Results

The characteristics of the study groups are shown in Table1. The frequencies of the -634 C→G and -174 G→C genotypes in 76 cases with RPL were compared with those in 93 controls among a Japanese population (Table 2). There was a significant difference in the -634C→G genotype frequency (CC vs. CG/GG) between the women with RPL and the controls. The risk of RPL was lower in the carriers of the G allele than in women with wild type (CC), with an OR =0.46 (95% CI = 0.24-0.91). On the other hand, we detected no -174C/C or -174G/C allele in women with RPL or in the control women and all 169 subjects were found to have the -174G/G homozygote.

We next evaluated the -634C→G genotype in both subgroups of women with

three or more pregnancy losses (PLs) and women with two PLs (Table 3). There was a significant difference in the -634C→G genotype frequency (CC vs. CG/GG) between the women with three or more PLs and the controls. The risk of RPL was very much lower in the carriers of the G allele than in women with the wild type, with OR = 0.22 (95% CI = 0.09-0.57). However, no difference in the -634C→G genotype frequency (CC vs. CG/GG) was found between the women with two PLs and the controls.

When 10 women with autoimmune disease and/or antiphospholipid antibody syndrome were excluded in the risk evaluation, we also found a significant lower risk of RPL in the carriers of the G allele than in women with wild type, with an OR =0.45 (95% CI = 0.22-0.93). The RPL risk in women with three or more PLs was also much lower in the carriers of the G allele than in women with the wild type, with OR = 0.25 (95% CI = 0.10-0.64).

Discussion

Many investigators have assessed possible associations between etiologies of RPL and gene polymorphisms including a family of enzymes responsible for metabolism of environmental toxins, glutathione S-transferase (GST) (19-22), and associations between etiologies of RPL and the GSTP1 (20) and GSTM1 polymorphisms (22) have been demonstrated. Others sought etiologies in gene polymorphisms susceptible to infection in women with RPL; these cytokine genes included tumor necrosis factor (TNF)-α (13, 22, 24), interferon (IFN)-γ (13), and IL-1B (23, 24), and anti-inflammatory cytokine IL-10 (12, 13). However, among these studies only one demonstrated an increase in the frequencies of IL-1B promoter region variants IL1B-511C and IL-1B-31T in women with RPL; a IL1B-511C variant was

found to be associated with Th1 overimmunity to trophoblast antigens (11).

In the present study, we for the first time demonstrated an association between RPL and the -634C→G genotype of the IL-6 gene, and found that RPL risk in the carriers of the G allele was lower than that in women with the wild type, with OR = 0.46. Ota et al. (16) reported frequencies of theIL-6-634C→G allele in 470 Japanese women as follows; gene frequencies of the C allele and G allele were 0.814 and 0.186, respectively. We obtained similar allelic frequencies from control women in our study, and these were as follows**;** gene frequencies of the C allele and G allele were 0.801 and 0.199, respectively. Thus, allelic frequencies of IL-6-634C→G among control women in the present study did not differ from the abovementioned allelic frequencies in a Japanese population. Therefore, population bias is not likely to affect the results in the present study.

Additionally, we found an extremely low RPL risk in the carriers of the G allele among women with three or more PLs with OR = 0.22 and no significant relation of the IL-6-634C→G genotype among women with two PLs. Women with three or more PLs have a more severe condition of RPL and poorer reproductive outcome in subsequent pregnancy than do women with two PLs. Thus, the causal association of the deviation of the IL-6-634C→G genotype with RPL was further suggested.

IL-6 is a Th2 cytokine, and abnormally decreased expression of IL-6 mRNA in the uterine endometrium in women with RPL was recently found (25). Additionally, low serum concentrations of IL-6 during early pregnancy in women with RPL were found to be associated with unsuccessful pregnancy outcome (26). The women with RPL had significantly decreased plasma levels of IL-6, IL-8 and IL-11 compared to those with normal pregnancies (6).

There have as yet been no reports concerning the relationship between the IL-6 -634 allele and IL-6 production in circulation. Ota et al. (16) speculated that a decrease in bone mineral density found in carriers of the G allele, but not in subjects with the wild type, was due to effects of transcriptional activation caused by the presence of the G allele, since IL-6 is known to stimulate the osteoclast development. Whether the IL-6-634G variant results in a lower risk of abortion through a shift toward Th2-dominance during early pregnancy and increased IL-6 production in maternal circulation or in the materno-fetal interface should be further studied.

In future studies, detailed mapping and molecular characterization of the IL- 6 promoter are needed to demonstrate that the -634 polymorphism has direct biochemical significance. The -634 polymorphism might simply be a molecular marker for a larger chromosomal region that includes both IL-6 and a nearby gene that affects pregnancy survival; this is another hypothetical explanation for the low RPL risk in the carriers of the IL-6-634G allele found in the present study.

It was found that the serum IL-6 concentration of the carriers of the -174C/C allele was lower than that of the carriers of the -174G/G allele in Caucasian subjects (15), and that the frequency of the -174C allele was 0.40-0.45 in Caucasians (14, 15). Although we carried out analyses for the IL-6-174G→C genotype, we detected no -174C/C or -174G/C allele in women with RPL or in the control women. Recently, no -174C allele was found among 388 Japanese men (27), and only one person with the -174C allele was found in 259 Chinese men (28). Another report found that -174G/C polymorphism was rare in Koreans (29). Thus, our results concerning the IL-6-174G→C genotype in Japanese were compatible with these previous results. Carriers of the -174C allele seem to be extremely rare among East Asians.

Numerous investigations have been performed focusing on the possible roles of immunological abnormalities in RPL. Murine studies have provided evidence that Th1 cytokines are harmful to pregnancy, causing fetal death, whereas Th2 cytokines produced at the materno-fetal interface are beneficial to the maintenance of pregnancy by suppressing cellular cytotoxicity (30, 31). Abnormal Th1/Th2 balance with Th1 dominance of peripheral mononuclear cells in response to trophoblast antigens has been found to be associated with the etiology of RPL (32).

As regards another immunological abnormality found in women with RPL, high peripheral blood natural-killer (NK) cell activities before conception (33) and early in gestation (34) have been associated with subsequent miscarriage. Additionally, decreased serum levels of macrophage migration inhibition factor during early gestation were recently found in women with RPL with subsequent miscarriage of a normal fetal karyotype (35). This evidence suggested that immunological abnormalities were causally associated with RPL.

Based on the results of the present and previous studies (11, 20, 22), we suggest that not only one but many genetic factors might be associated with RPL as predisposing factors. Whether genetic factors predisposing to RPL are reflected by the abovementioned immunological abnormalities should be further investigated. If the relation between the genetic factors and the immunological abnormalities is elucidated, genetic polymorphisms such as IL-6 -174G→C could be a marker for selecting therapeutic options and for counseling of women with RPL.

Some couples may be predisposed to produce embryos with high rates of somatic mosaicism, leading to abnormal development and RPL (38, 39). However, there are no investigations of the relationships between cytokine gene polymorphisms

and somatic mosaicism of zygotes, and this point also should be further clarified. In conclusion, it is suggested that women with the -634 G allele of the IL-6 gene have a decreased risk of RPL.

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References

1 Stirrat GM. Recurrent abortion. II. Clinical associations, causes, and management. Lancet 1999; 336: 728-33.

2 Parazzini F, Bocciolone L, Fedele L, Negri E, La Vecchia C, Acaia B. Risk factors for spontaneous abortion. Int J Epidemiol 1991; 20: 157-61.

3 Cramer DW, Wise LA. The epidemiology of recurrent pregnancy loss. Semin Reprod Med 2000; 18: 331-9.

4 Clark DA, Arck, PC, Chaouat, G.. Why did your mother reject you? Immunogenetic determinants of the response to environmental selective pressure expressed at the uterine level. Am J Reprod Immunol 1999; 41: 5-22.

5 Raghupathy R, Makhseed M, Azizieh F, Hassan N, Al-Azemi M, Al-Shamali E. Maternal Th1- and Th2-type reactivity to placental antigens in normal human

pregnancy and unexplained recurrent spontaneous abortions. Cell Immunol 1999; 196: 122-30.

6 Koumantaki Y, Matalliotakis I, Sifakis S, Kyriakou D, Neonaki M, Goymenou A, et al. Detection of interleukin-6, interleukin-8, and interleukin-11 in plasma from women with spontaneous abortion. Eur J Obstet Gynecol Reprod Biol 2001; 98: 66-71.

7 Arntzen KJ, Lien E, Austgulen R. Maternal serum levels of interleukin-6 and clinical characteristics of normal delivery at term. Acta Obstet Gynecol Scand 1997; 76: 55-60.

8 Olah KS, Vince GS, Neilson JP, Deniz G, Johnson PM. Interleukin-6, interferon-gamma, interleukin-8, and granulocyte-macrophage colony stimulating factor levels in human amniotic fluid at term. J Reprod Immunol 1996; 32: 89-98.

9 Imseis HM, Greig PC, Livengood CH 3rd, Shunior E, Durda P, Erikson M. Characterization of the inflammatory cytokines in the vagina during pregnancy and labor and with bacterial vaginosis. J Soc Gynecol Investig 1997; 4: 90-4.

10 Steinborn A, Gunes H, Roddiger S, Halberstadt E. Elevated placental cytokine release, a process associated with preterm labor in the absence of intrauterine infection. Obstet Gynecol 1996; 88: 534-9.

11 Wang ZC, Yunis EJ, De los Santos MJ, Xiao L, Anderson DJ, Hill JA. T helper 1-type immunity to trophoblast antigens in women with a history of recurrent pregnancy loss is associated with polymorphism of the IL1B promoter region. Genes Immun 2002; 3: 38-42.

12 Karhukorpi J, Laitinen T, Karttunen R, Tiilikainen AS. The functionally important IL-10 promoter polymorphism (-1082G-->A) is not a major genetic regulator in

recurrent spontaneous abortions. Mol Hum Reprod 2001; 7: 201-3.

13 Babbage SJ, Arkwright PD, Vince GS, Perrey C, Pravica V, Quenby S, et al. Cytokine promoter gene polymorphisms and idiopathic recurrent pregnancy loss. J Reprod Immunol 2001; 51: 21-7.

14 Fernandez-Real JM, Broch M, Vendrell J, Gutierrez C, Casamitjana R, Pugeat M, et al. Interleukin-6 gene polymorphism and lipid abnormalities in healthy subjects. J Clin Endocrinol Metab 2000; 85: 1334-9.

15 Fishman D, Faulds G., Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, 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.

16 Ota N, Nakajima T, Nakazawa I, Suzuki T, Hosoi T, Orimo H, et al. A nucleotide variant in the promoter region of the interleukin-6 gene associated with decreased bone mineral density. J Hum Genet 2001; 46: 267-72.

17 Yamada H, Kato EH, Kobashi G, Ebina Y, Shimada S, Morikawa M, et al. Recurrent pregnancy loss: etiology of thrombophilia. Semin Thromb Hemost 2001; 27: 121-9.

18 Kobashi G, Yamada H, Asano T, Nagano S, Hata A, Kishi R, et al. The factor V Leiden mutation is not a common cause of pregnancy-induced hypertension in Japan. Semin Thromb Hemost 1999; 25: 487-9.

19 Hirvonen A, Taylor JA, Wilcox A, Berkowitz G., Schachter B, Chaparro C, et al. Xenobiotic metabolism genes and the risk of recurrent spontaneous abortion. Epidemiology 1996; 7: 206-8.

20 Zusterzeel PL, Nelen WL, Roelofs HM, Peters WH, Blom HJ, Steegers EA.

Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. Mol. Hum. Reprod 2000: 6: 474-8.

21 Mendola P, Moysich KB, Freudenheim J, Shields, PG, Schisterman EF, Graham S, et al. Risk of recurrent spontaneous abortion, cigarette smoking, and genetic polymorphisms in NAT2 and GSTM1. Epidemiology 1998; 9: 666-8.

22 Sata F, Yamada H, Kondo T, Gong Y, Tozaki S, Kobashi G, et al. Glutathione S-transferase M1 and T1 polymorphisms and the risk of recurrent pregnancy loss. Mol Hum Reprod 2003; 9: 165-9

23 Baxter N, Sumiya M, Cheng S, Erlich H, Regan L, Simons A, et al. Recurrent miscarriage and variant alleles of mannose binding lectin, tumour necrosis factor and lymphotoxin alpha genes. Clin Exp Immunol 2001; 126: 529-34.

24 Reid JG., Simpson NA., Walker RG., Economidou O, Shillito J, Gooi HC, et al. The carriage of pro-inflammatory cytokine gene polymorphisms in recurrent pregnancy loss. Am J Reprod Immunol 2001; 45: 35-40.

25 Wolff MV, Thaler CJ, Strowitzki T, Broome J, Stolz W, Tabibzadeh S. Regulated expression of cytokines in human endometrium throughout the menstrual cycle: dysregulation in habitual abortion. Mol Hum Reprod 2000; 6: 627-34.

26 Makhseed M, Raghupathy R, Azizieh F, Farhat R, Hassan N, Bandar A. Circulating cytokines and CD30 in normal human pregnancy and recurrent spontaneous abortions. Hum Reprod 2000; 15: 2011-7.

27 Hayakawa T, Takamura T, Hisada A, Abe T, Nomura G, Kobayashi K. IL-6 gene polymorphism -174G/C does not contribute substantially to hyperlipidaemia and Type II diabetes mellitus in Japanese men. Diabetologia 2002; 245: 453-4.

28 Zhai R, Liu G., Yang C, Huang C, Wu C, Christiani DC. The G to C polymorphism

at -174 of the interleukin-6 gene is rare in a Southern Chinese population. Pharmacogenetics 2001; 11: 699-701.

29 Lim CS, Zheng S, Kim YS, Ahn C, Han JS, Kim S, et al. The --174 G to C polymorphism of interleukin-6 gene is very rare in Koreans. Cytokine 2002; 19: 52-4

30 Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993; 14: 353-6.

31 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.

32 Suthanthiran M, Strom T. Immunobiology and immunopharmacology of organ allograft rejection. J Clin Immunol 1995; 15: 161-71.

33 Hill JA, Polgar K, Anderson DJ. T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. JAMA 1995; 273: 1933-6.

34 Aoki K, Kajiura S, Matsumoto Y, Ogasawara M, Okada S, Yagami Y, et al. Preconceptional natural-killer-cell activity as a predictor of miscarriage. Lancet 1995; 345: 1340-2.

35 Yamada H, Kato EH, Kobashi G, Ebina Y, Shimada S, Sakuragi S, et al. High NK cell activity in early pregnancy correlates with subsequent abortion with normal chromosomes in women with recurrent miscarriage. Am J Reprod. Immunol 2001; 46: 132-6

36 Yamada H, Kato EH, Morikawa M, Shimada S, Saito H, Watari M, et al: Decreased serum levels of macrophage migration inhibitory factor in spontaneous abortions

with normal chromosome karyotype. Hum Reprod 2003; 18: 616-20

37 Kalousek DK. Pathogenesis of chromosomal mosaicism and its effect on early human development. Am J Med Genet 2000; 91: 39-45.

38 Schubert R, Eggermann T, Hofstaetter C, von Netzer B, Knopfle G, Schwanitz G. Clinical, cytogenetic, and molecular findings in 45,X/47,XX,+18 mosaicism: clinical report and review of the literature. Am J Med Genet 2002; 110: 278-82.

39 Kalousek DK. Pathogenesis of chromosomal mosaicism and its effect on early human development. Am J Med Genet 2000; 91: 39-45.

TABLE 1. Characteristics of 76 cases with recurrent pregnancy loss (RPL) and 93 controls in a Japanese population

	Cases		Controls	
	Number	%	Number	%
Age				
20-29	28	36.8	36	38.7
30-39	41	53.9	48	51.6
≥ 40	7	9.2	9	9.7
Number of previous pregnancy losses				
2	29	38.2	—	—
3	32	42.1	—	—
≥ 4	15	19.7	—	—

TABLE 2. Distribution of Il-6 genotypes among 76 cases with RPL and 93 controls

	Cases		Controls			
	Number	%	Number	%	OR* (95% CI)	P value
IL-6						
-634 C→G						
CC	58	76.3	56	60.2	1.00	
CG/GG	18	23.7	37	39.8	0.46 (0.24-0.91)	0.026
-174 G→C						
GG	76	100	93	100	-	
CG/CC	0	0	0	0	-	

*Age-adjusted logistic regression analysis.

TABLE 3. Distribution of Il-6 -634 genotypes among the cases with three or more pregnancy losses (PLs) and with two PLs and controls

	Cases		Controls			
	Number	%	Number	%	OR* (95% CI)	P value
Three or more PLs						
IL-6-634 C→G						
CC	41	87.2	56	60.2	1.00	
CG/GG	6	12.8	37	39.8	0.22 (0.09-0.57)	0.002
Two PLs						
IL-6-634 C→G						
CC	17	58.6	56	60.2	1.00	
CG/GG	12	41.4	37	39.8	1.05(0.45-2.46)	NS

*Age-adjusted logistic regression analysis.