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GENETICS

Association of the IL4R single-nucleotide polymorphism I50V with recurrent spontaneous abortion (RSA)

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Abstract
Background Recurrent spontaneous abortion (RSA) is defined as three or more consecutive abortions before the 20th week of gestation. There is increasing evidence to support an immunological mechanism for the occurrence of RSA. The purpose of our study was to examine whether single-nucleotide polymorphisms (SNPs) of the interleukin-4 receptor gene IL4R influence susceptibility to, recurrent spontaneous abortion.
Materials and methods This is a case-control study. We recruited 200 patients with RSA (case group) using established diagnostic criteria and 200, normal individuals (control group) at the fertility and infertility center in Yazd city and Isfahan city during 2012 to 2013. We screened the I50V variant in IL-4R in patients and controls by PCR-RFLF method, and we performed an association analysis between I50V variant and RSA.the data was analyzed by spss 16 software using Chi-square test.
Results No differences in the genotype and allele frequencies of the I50V SNPs were identified between patients with RSA and healthy controls.
Conclusions The frequency of SNP in IL-4 receptor (I50V) in patients with recurrent spontaneous abortion did not differ significantly compared with the control group. Analysis of IL4R SNP haplotypes or complex alleles suggested no dominant protection in patients with RSA.

Capsule Immunological disorder is a possible reason for Recurrent spontaneous abortion (RSA). I50V as a immunological genetic variant has no association with RSA. This is first report of frequency of I50V in RSA patients and related controls.

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Keywords Recurrent spontaneous abortion · IL4R single-nucleotide polymorphism · I50V

Introduction

The human conceptus is a semi-allograft and hence antigenically foreign to the mother [1]. Therefore, the process of implantation may include mechanisms to prevent allograft rejection, but once immunological tolerance becomes imbalanced, pathological pregnancy, such as spontaneous abortion could occur [2].

Unexplained recurrent spontaneous abortion (RSA), a condition defined as three or more consecutive pregnancy losses before the 20th week of gestation after routine screening tests (normal uterine cavity, parental karyotypes, endocrine and infection parameters), is thought to be caused by immunological rejection of the fetus by the mother [3]. CD4+ T cells have been implicated in unexplained RSA in both experimental and clinical studies that link CD4+/CD8+ augmentation, Th1/Th2 cytokine imbalance, and Treg depletion to this condition [4, 5]. However, the precise mechanisms by which the immune system is modulated in patients with unexplained RSA remain elusive. Recently, the new Th17 subpopulation of CD4+ effector T cells, distinct from the well-described Th1, Th2 and Treg cells, has been described and its discovery has substantially advanced our understanding of T cell-mediated immunity [6]. Human Th17 cells producing IL-17 may play a major role in rejecting conceptus antigens and therefore may be harmful to the maintenance of pregnancy [7, 8]. The cytokines IL-6 and tumor growth factor TGFβ are crucial for the generation of Th17 cells in the mouse, while IL-1β, IL-6 and IL-23 induce and maintain the differentiation of human Th17 cells [9]. Th17 cell development and cytokine secretion are down regulated in vitro by IFN and IL-4 produced by Th1 and Th2 cells, respectively [10]. Expression of

IL-17 is also increased in autoimmune conditions, inflammatory disease and immunological rejection disease, and neutralization of IL-17 by an IL-17R-IgG-Fc fusion protein has been shown to prevent acute rejection of aortic allografts and prolong survival of cardiac allografts [11]. Whether a similar treatment to inhibit IL-17 or Th17 cells would prevent unexplained RSA. And understanding the mechanisms of Th17 regulation in human disease is essential for the development of novel, targeted therapies and to guide therapeutic decision-making [12]. Several findings suggest that the Th2 cytokine IL-4 and its receptor may be of particular interest in the control of Th17-induced inflammation. In humans, a diminished response to IL-4 is thought to contribute to autoimmune inflammation. A single nucleotide polymorphism (SNP) in the coding region of the IL-4R,I50V [rs1805010] governs the presence of isoleucine (I) versus valine (V) at position 50 in the amino acid sequence. This polymorphism in IL-4R is functionally important because it affects the strength of signaling through the receptor and may be able to regulate IL-17 production [13, 14]. With this regards, RSA is now suspected to be driven by pathogenic Th17 cells that secrete IL-17 and can be regulated by IL-4. And inherited polymorphism of the IL-4R (I50V) may be controls the ability of the human immune system to regulate the magnitude of IL-17 production. So the aim of this study was to evaluate a frequency of functional I50V variant in IL-4 receptor gene and to determine an association between functional I50V variant in IL-4 receptor gene and Recurrent Spontaneous Abortion.

Material and methods

Subjects: patients and controls

We carried out a case–control study. The recruitment of the study individuals was performed at the fertility and infertility center in Yazd city and Isfahan city during 2012 to 2013. We analyzed a total of 200 patients with three or more recurrent spontaneous abortions (as a case group) and 200 healthy women without any history of abortion (as a control group). During the entire investigation period the current laws of ethical committee were followed; the patients gave their informed consent for use of their blood collected. Studied patients were without anatomical, microbial, viral, genetical disease hormone profile tests and tests for ovulation and tubal patency of them were normal. Male partner's patients were with normal investigations included in the study. The investigations included semen analysis in other hand, according to medical evidences, etiology of patients abortions was unexplained. The following data for the patients were obtained: age, age at each abortion, numbers of abortions, time of abortion during each pregnancy, familial history of abortion, occurrence of bleeding and pain during abortion.

Genotyping of the I50V ln IL-4R variant

The blood samples of the control group and of the patients were collected in tubes containing EDTA. The molecular analyses were performed using DNA extracted from peripheral blood leukocytes with a routine salting out procedure. The I50V polymorphism was genotyped using the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism assay (PCR-RFLP). We performed PCR using Amplicon master mix and a primer annealing temperature of 55 °C.analysis using the primer pair 5-GGCAGGTGTGAGGAGCATCC, 252–233 bp upstream of the Ile50Val polymorphic site and 5-GCCT CCGTTGTTCTCAGGTA (nn 399–418). The cycling conditions used were: 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 30 s; and a final cycle extension at 72 °C for 5 min. The 275-bp PCR product was run on a 1 % agarosa gel. Green viewer (containing ethidium bromide) was used for staining and visualization on a UV transiluminator. For RFLP digestion, 5 ul of amplified PCR product was digested with 1U of Rsa1 enzyme incubated overnight at 37 °C and subsequently analyzed on 2 % agarose. The I allele is seen as 273 bps band and V allele produces two fragments of 254 bp and 11 bp as shown in Fig. 1.

Statistical analysis

The data were analyzed by using the Chi-square test in the presence and absence of I50V polymorphismin IL-4R expressing individuals. Odds ratio was calculated with a confidence interval of 95 %. The data were processed by SPSS 16 software. The significance level of the tests for considering P-values as significant was set to <0.05.

Result

The statistical analysis showed that there is no significant difference between mean age in the case group and control

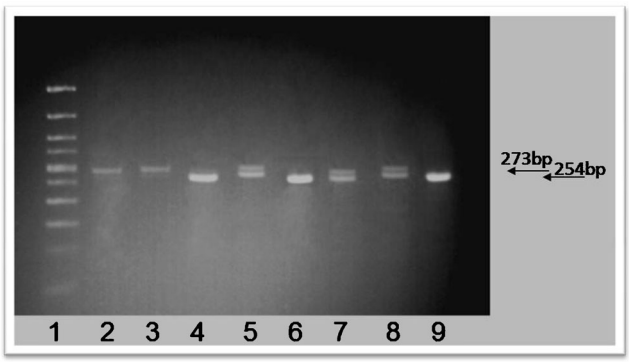


Fig. 1 Genotyping of the Ile50Val polymorphism of IL4R by PCR-RFLP method in eight unrelated subjects. Line 1 is a 50bp ladder; 2,3 homozygote for the Ile50 allele; 4,6,9 homozygote for the Val50 allele and 5,7,8 heterozygote for the Ile50 and Val50 allele

Table 1 Clinical characteristics of patients with RSA

Patients (n=200)		n(%)
Number of abortions	2	29(14.6 %)
	3	96(48 %)
	4	55(27.6 %)
	5	20(9.8 %)
Time of abortion during gestation	1–5	83(41.5 %)
	6–10	90(45 %)
	11–15	22(11 %)
	16–19	5(2.5 %)
Bleeding and pain during abortion	Yes	160(80 %)
	No	40(20 %)

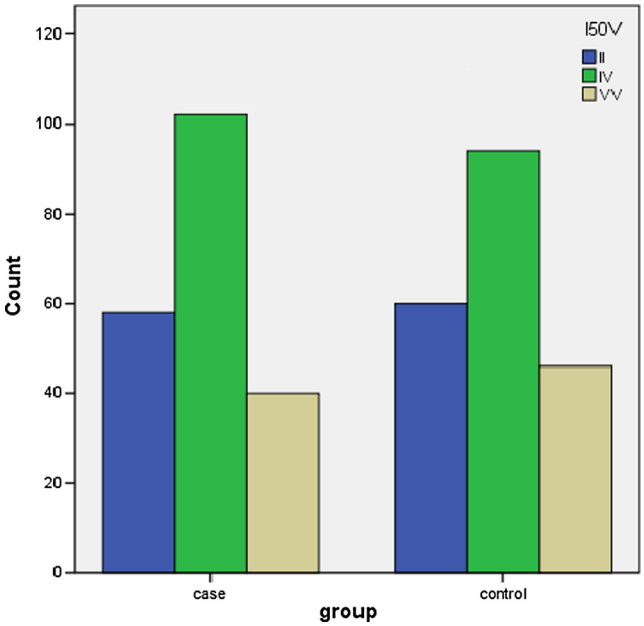
group (the mean age in the case group was:35.3 yr±5.8 (range 19–43), the mean age in the control group was: 34.9 yr±3.2, (range 20–41) P value =0.40), in addition, some features of patients shown in Table 1. To examine the possible contribution of I50V polymorphism of IL4R to RSA susceptibility, we performed a case–control association study. The distribution of the genotypes in the RSA patient and control groups is shown in Table 2. I50V polymorphism was in Hardy-Weinberg equilibrium, and the frequencies were consistent with those previously reported by other investigators. There were no significant differences in the genotype and allele frequencies between RSA patients and healthy controls (p value =0.67; CI:95 %). Moreover, no differences between the RSA patient and control cohorts were observed in the distribution of possible IL4R haplotypes calculated from the frequencies of this SNP. Thus, this change-in-function IL4R polymorphism do not appear to be associated with RSA susceptibility as shown in graphs 1, 2, 3 and 4.

Discussion

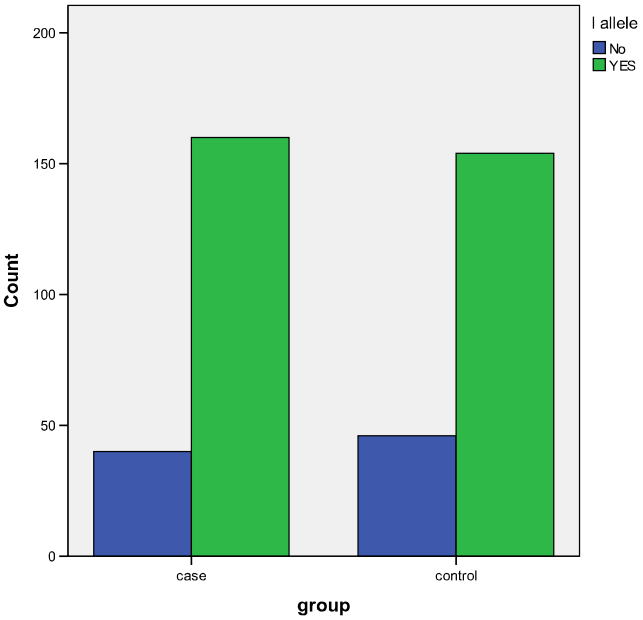
At the maternal-fetal interface, where maternal cells encounter histo-incompatible fetal cells, an initial sterile inflammatory immune response to danger signals from embryonic implantation is established, which is essential for angiogenesis and trophoblast development [15]. The dominant T helper 1 inflammatory immune response (TNFα, IFN) is switched to a T helper 2 interleukin (IL-4, IL-10) immune response and induction of maternal immune tolerance to the allogenic fetus assures successful implantation and reproductive outcome [16, 17]. Various cellular immune responses involving NK cells, cytotoxic T cells, natural killer-T (NKT) cells, regulatory T (Treg) cells and cytokine networks have been reported to be related to this process [18]. In addition, regulatory molecules, such as human leukocyte antigen HLA-G, inhibitory T cell co-stimulatory molecules, complement regulatory proteins of trophoblasts, and the immunosuppressive enzyme, indoleamine 2, 3 dioxygenase have been suggested as protection mechanisms for the allogenic fetus from maternal immune responses [19, 20]. Therefore, a complex cellular and molecular network is utilized for successful implantation and pregnancy. Th17 cells have been reported as a new unique subpopulation of CD4+ T cells, characterized by the expression of IL-17A, IL-17 F, IL-21, IL-22, IL-6 and TNFα [21]. Th17 cells have been implicated in either the initiation or progression of inflammatory diseases, autoimmune diseases and transplant rejection in humans. Previous studies demonstrated that the proportions of Th17 (CD4+IL-17A+) cells in both peripheral blood and decidua were significantly higher in unexplained RSA than in normal pregnant women [22]. Th17 cells were relatively enriched in the decidua of unexplained RSA patients, suggesting that these cells may play a key role in the maternal–fetal local immunological rejection [23]. Also IL-17, which is secreted by Th17 cells was also suggested to play a major role in angiogenesis and/or immune regulation [24]. IL-17 is localized in both cyto and syncytiotrophoblasts [25]. And expression of IL-17A on CD4+ T cells was significantly higher in unexplained RSA than in normal pregnant women [12, 26]. We propose that factors promoting immune tolerance and rejection may co-exist at the maternal-fetal interface, and the balance between pro-inflammatory factors and immune regulation has a profound impact on the outcome of a pregnancy [27]. A predominance of Th17 cells coupled with a decrease in Treg cells in peripheral blood and decidua

Table 2 The distribution of I50V polymorphism in case and control group

			I50V			Total
			II	IV	VV	
Group	Case	Count	58	102	40	200
		% within group	29.0 %	51.0 %	20.0 %	100.0 %
	control	Count	60	94	46	200
		% within group	30.0 %	47.0 %	23.0 %	100.0 %
Total		Count	118	196	86	400
		% within group	29.5 %	49.0 %	21.5 %	100.0 %

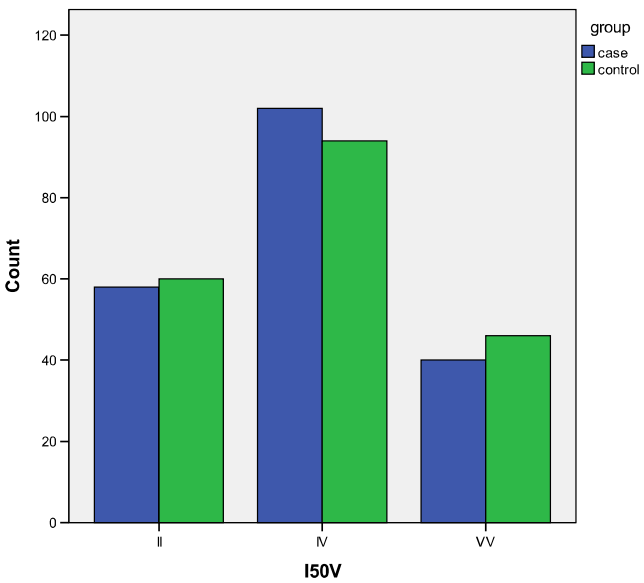


Graph 1 The distribution of I50V polymorphism in case and control group

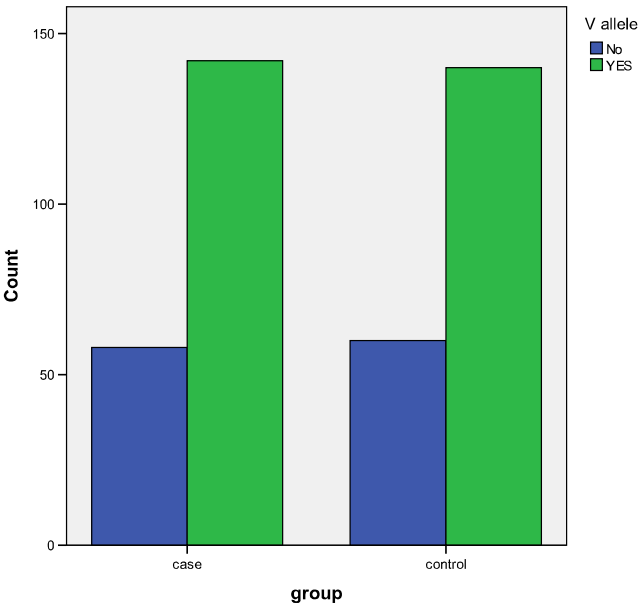


Graph 3 The distribution of I allele in case and control group

in unexplained RSA patients suggests that an immunologic imbalance and subsequent immune dysregulation by the altered Th17/Treg cell populations strongly influences pregnancy outcomes [28]. Tregs and Th17 cells are two lymphocyte subsets with opposing actions. In normal pregnancy, Tregs prevent the generation of an immune response against fetal tissue and a decrease in the number of Tregs is associated with abortion [29]. In contrast to the Tregs, Th17 cells promote inflammatory, autoimmunity and transplant rejection in humans and increased Th17 cells companied with decreased Tregs had been shown in unexplained RSA [30]. Determining the regulatory mechanisms that could suppress Th17 cells might lead to novel approaches to the treatment of RSA. At the maternal-fetal interface, a Th2 type cytokine response is orchestrated by trophoblasts and decidual leukocytes [31]. IL-4 expression of the first trimester chorionic villi has been demonstrated in human villous and extravillous cytotrophoblasts [32], and Since Th17 could mediated RSA, the investigation of the inhibitory effect of IL4R allelic variants on the transcription of relevant molecules is of high priority in understanding the nature of the newly identified association between the I50V IL4R SNP and RSA. Our present study is the first to demonstrate the role of a single nucleotide polymorphism in the IL-4R to determine an



Graph 2 The distribution of each genotype in case and control



Graph 4 The distribution of V allele in case and control group

association between functional I50V variant in IL-4 receptor gene and Recurrent Spontaneous Abortion. Previous studies showed that CD4 T cells isolated from individuals homozygous for the I50 allele demonstrated significantly better responses to IL-4 than CD4 T cells isolated from V50/V50 individuals [33]. I50/I50 CD4 T cells reacted more effectively to the effect of IL-4 on GATA-3 expression, a key transcription factor in Th2 cell differentiation [34], and to the inhibitory effect of IL-4 on IL-17 secretion, The reduced up-regulation of a Th2-relevant transcription factor and the diminished down modulation of a IL-17 secretion based on surface receptor by IL-4 in CD4 T cells from V50/V50 individuals as demonstrated here could explain the association of the V50 homozygosity with RSA [35]. Alternatively, diminished responsiveness to the regulatory effects of IL-4 on other inflammatory cells involved in the pathogenesis of RSA, such as B cells, and macrophages, could also contribute to the association of this decrease-in-function IL4R polymorphism and RSA [35]. Together, these findings provide a mechanism that might underlie the association between the I50V SNP of IL4R and occurance of RSA, Delineating the mechanisms that underlie genetic associations in RSA is critical for understanding the pathogenesis of the disease. In this study, we investigated the association between IL4R and occurance of RSA. Although no association was observed with the occurrence of RSA. Previous studies indicated that a polymorphism in IL-4R in part controls production of IL-17 by Th17 cells cultured from healthy individuals. Specifically, IL-4 significantly inhibited IL-17 production by cells from subjects with the I/I genotype and the I/V genotype, but not the *V/V* genotype. But our study has failed to confirm an association of the I50V polymorphism with RSA.

References

1. Lim KJ, Odukoya OA, Ajjan RA, Li T-C, Weetman AP, Cooke ID. The role of T-helper cytokines in human reproduction. Fertil Steril. 2000;73(1):136–42.
2. Lunghi L, Ferretti ME, Medici S, Biondi C, Vesce F. Control of human trophoblast function. Reprod Biol Endocrinol. 2007;5(6).
3. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Hum Reprod. 2000;15(3):713–8.
4. Rezaei A, Dabbagh A. T-helper (1) cytokines increase during early pregnancy in women with a history of recurrent spontaneous abortion. Med Sci Monit. 2002;8(8):CR607–CR10.
5. Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+ CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod. 2004;10(5):347–53.
6. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441(7090):235–8.
7. Saito S, Nakashima A, Shima T, Ito MREVIEWARTICLE. Th1/Th2/Th17 and Regulatory T-Cell Paradigm in Pregnancy. Am J Reprod Immunol. 2010;63(6):601–10.
8. Nakashima A, Ito M, Yoneda S, Shiozaki A, Hidaka T, Saito S. SHORT COMMUNICATION: Circulating and Decidual Th17 Cell Levels in Healthy Pregnancy. Am J Reprod Immunol. 2010;63(2):104–9.
9. Korn T, Mitsdoerffer M, Croxford AL, Awasthi A, Dardalhon VA, Galileos G, et al. IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. Proc Natl Acad Sci. 2008;105(47):18460–5.
10. Steinman L. A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell–mediated tissue damage. Nat Med. 2007;13(2):139–45.
11. Afzali B, Lombardi G, Lechler R, Lord G. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol. 2007;148(1):32–46.
12. Wang W-J, Hao C-F, Yin G-J, Bao S-H, Qiu L-H, Lin Q-D. Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexplained recurrent spontaneous abortion patients. J Reprod Immunol. 2010;84(2):164–70.
13. Kruse S, Japha T, Tedner M, Sparholt S, Forster J, Kuehr J, et al. The polymorphisms S503P and Q576R in the interleukin-4 receptor gene are associated with atopy and influence the signal transduction. IMMUNOL-OXFORD-. 1999;96:365–71.
14. Risma KA, Wang N, Andrews RP, Cunningham CM, Ericksen MB, Bernstein JA, et al. V75R576 IL-4 receptor α is associated with allergic asthma and enhanced IL-4 receptor function. J Immunol. 2002;169(3):1604–10.
15. Mellor A, Munn D. Immunology at the maternal-fetal interface: lessons for T cell tolerance and suppression. Annu Rev Immunol. 2000;18(1):367–91.
16. Soares MJ. The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. Reprod Biol Endocrinol. 2004;2(10.1186):1477–7827.
17. Kwak-Kim J, Yang KM, Gilman-Sachs A. Recurrent pregnancy loss: a disease of inflammation and coagulation. J Obstet Gynaecol Res. 2009;35(4):609–22.
18. Boyson JE, Rybalov B, Koopman LA, Exley M, Balk SP, Racke FK, et al. CD1d and invariant NKT cells at the human maternal–fetal interface. Proc Natl Acad Sci. 2002;99(21):13741–6.
19. Entrican G. Immune regulation during pregnancy and host–pathogen interactions in infectious abortion. J Comp Pathol. 2002;126(2):79–94.
20. Hunt JS, Petroff MG, McIntire RH, Ober C. HLA-G and immune tolerance in pregnancy. FASEB J. 2005;19(7):681–93.
21. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol. 2009;27:485–517.
22. Mandal M, Marzouk AC, Donnelly R, Ponzio NM. Maternal immune stimulation during pregnancy affects adaptive immunity in offspring to promote development of TH17 cells. Brain Behav Immun. 2011;25(5):863–71.
23. Lee SK, Kim JY, Lee M. Gilman-Sachs A, Kwak-Kim J. Th17 and regulatory T cells in women with recurrent pregnancy loss. Am J Reprod Immunol. 2012;67(4):311–8.
24. Numasaki M. Fukushi J-i, Ono M, Narula SK, Zavodny PJ, Kudo T, et al. Interleukin-17 promotes angiogenesis and tumor growth. Blood. 2003;101(7):2620–7.
25. Pongcharoen S, Somran J, Sritippayawan S, Niumsup P, Chanchan P, Butkhamchot P, et al. Interleukin-17 expression in the human placenta. Placenta. 2007;28(1):59–63.
26. Liu YS, Wu L, Tong XH, Wu LM, He GP, Zhou GX, et al. Study on the relationship between Th17 cells and unexplained recurrent spontaneous abortion. Am J Reprod Immunol. 2011;65(5):503–11.

27. NG SC, GILMAN-SACHS A, THAKER P, BEAMAN KD, BEER AE, KWAK-KIM J. Expression of intracellular Th1 and Th2 cytokines in women with recurrent spontaneous abortion, implantation failures after IVF/ET or normal pregnancy. Am J Reprod Immunol. 2002;48(2):77–86.
28. Lee YK, Mukasa R, Hatton RD, Weaver CT. Developmental plasticity of Th17 and Treg cells. Curr Opin Immunol. 2009;21(3):274–80.
29. Saito S, Sasaki Y, Sakai M. CD4⁺CD25^{high}regulatory T cells in human pregnancy. J Reprod Immunol. 2005;65(2):111–20.
30. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, de St Groth BF, et al. Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol. 2009;183(11):7023–30.
31. Dimitriadis E, White C, Jones R, Salamonsen L. Cytokines, Chemokines and growth factors in endometrium related to implantation. Hum Reprod Update. 2005;11(6):613–30.
32. Jerzak M, Bischof P. Apoptosis in the first trimester human placenta: the role in maintaining immune privilege at the maternal–fetal interface and in the trophoblast remodeling. Eur J Obstet Gynecol Reprod Biol. 2002;100(2):138–42.
33. W-p Z, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997;89(4):587–96.
34. Skapenko A, Leipe J, Niesner U, Devriendt K, Beetz R, Radbruch A, et al. GATA-3 in human T cell helper type 2 development. J Exp Med. 2004;199(3):423–8.
35. Wallis SK, Cooney LA, Endres JL, Lee MJ, Ryu J, Somers EC, et al. A polymorphism in the interleukin-4 receptor affects the ability of interleukin-4 to regulate Th17 cells: a possible immunoregulatory mechanism for genetic control of the severity of rheumatoid arthritis. Arthritis Res Ther. 2011;13(1):R15.