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## Association study of CTLA-4 +49A/G gene polymorphism with recurrent pregnancy loss in the Iranian Azeri Turkish ethnic group

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**Background/aim:** Recurrent pregnancy loss (RPL) is defined as two or more pregnancy losses. T-regulatory cells play an important role in the feto-maternal interface. Cytotoxic-T-lymphocyte antigen-4 (CTLA-4) is a molecule that downregulates the activation and proliferation of T cells. The objective of the current study was to investigate the possible association of CTLA-4+49A/G gene polymorphism with RPL among patients from the Iranian Azeri Turkish ethnic group.

**Materials and methods:** The study group/patients consisted of 101 women with the experience of two or more pregnancy losses and the control group consisted of 101 women with at least two live births, without any previous history of pregnancy loss and autoimmune diseases from the same ethnic group. The CTLA-4+49A/G was detected by polymerase chain reaction-restriction fragment length polymorphisms assay.

**Results:** The distribution of CTLA-4+49A/G genotype was AA, 38.61%; AG, 51.48%; GG, 9.9% in patients and AA, 37.62%; AG, 47.52%; GG, 14.85% in controls (P-value: 0.2). Furthermore, no association in G-allele was observed in the patient and control groups (P-value: 0.5).

**Conclusion:** The results of the present study suggest that CTLA-4 does not have any association with RPL in the Iranian Azeri Turkish ethnic group.

**Key words:** CTLA-4, immune system, recurrent pregnancy loss

### 1. Introduction

Recurrent pregnancy loss (RPL) is defined by the American Society for Reproductive Medicine as two or more failed clinical pregnancies (1,2). A number of studies have shown that about one in 100 women experience RPL (3). There are several etiological factors including genetic, anatomical, endocrine, infectious, immunological, and lifestyle that are considered to be involved in RPL (4,5). A successful pregnancy is related to the interaction between mother and fetus (6). This interaction is vital since the fetus is not genetically the same as the mother. Several mechanisms have been found to establish this feto-maternal interaction. Among them, immunological mechanisms are one of the most important factors involved in tolerance toward the semiallogeneic fetus by the maternal immune system (7,8).

Regulatory T cells (Treg) of the mother's immune system play a principal protective role in maternal tolerance during the early stages of pregnancy (9). There are two different ways of T cell regulation: the first is

via interactions between T cell receptors and specific peptides expressed on the surface of antigen presenting cells (APCs), and the second is through the interactions between co-stimulatory receptors and their ligands (10). One of the co-stimulatory molecules is cytotoxic T lymphocyte antigen-4 (CTLA-4) expressed on CD+4, CD+25 regulatory T cells (11).

CTLA-4 is a 33–37 kD, transmembrane glycoprotein receptor of the immunoglobulin superfamily that is present on the surface of T-cells as a downregulator of their activation and proliferation (12,13). In addition, CTLA-4 and its homologue (CD28) in a competitive interaction bind to B7, which is a cell surface molecule on APCs. The affinity of CTLA-4 is greater than that of CD28 for B7, which results in inactivation of T-cells with CTLA-4 (13,14). The interaction of CTLA-4 and B7 reduces the proliferation of several lymphokines, such as IL-2, and leads to an extreme decrease in T cell proliferation (15). IL-2, which is known as a T-cell growth factor, plays

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a key role in response to antigens. On the other hand, when CTLA-4 binds to B7, it commences the JNK (Jun N-terminal kinase) pathway to increase the localization of transcription factor Foxo3, which leads to the prevention of IL-6 production (16).

The CTLA-4 gene, which maps to human chromosome 2q33, has 4 exons and the +49 A/G polymorphism is located at the +49 position of exon 1 (13).

Several studies have been carried out to investigate the association of CTLA-4 polymorphism with several autoimmune diseases such as diabetes and Graves disease (17). It has been suggested that this polymorphism, which leads to a change in codon 17 of leader peptide, reduces membrane CTLA-4 protein expression (18,19).

Given the importance of CTLA-4 in maternal tolerance, we performed this study to investigate the possible association of +49 A/G gene polymorphism with idiopathic RPL among women from the Iranian Azeri Turkish ethnic group.

## 2. Materials and methods

A hundred and one women with idiopathic RPL (defined by ASRM) and another 101 women with at least two live births and also without any previous history of pregnancy loss and/or autoimmune diseases were involved in this case-control study. The Ethics Committee at Tabriz University of Medical Science approved the study. All participants signed an informed consent form to provide their blood for genotyping.

The standard salting out technique was applied to extract the genomic DNA of blood leukocytes. +49 A/G CTLA-4 gene polymorphism was detected using PCR-RFLP. The 25- $\mu$ L PCR reaction mixture contained 100 ng of genomic DNA, 10 pmol of each primer, and 1 U of Taq DNA polymerase (Sinagene). The cycling parameters were followed by 3 min initial denaturation at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 58 °C, 15 s at 72 °C; this was followed

by a final extension of 3 min at 72 °C. Next, the amplicons were digested by BbvI(NEB) at 37 °C overnight by RFLP. The digestion of wild-type allele produced a single fragment with 162 bp length, whereas the polymorphic allele produced two fragments of 72 bp and 90 bp. All data were analyzed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). In addition, the Hardy-Weinberg equilibrium (HWE) for the genotype frequencies was verified by the chi-square test. Probability values of 0.05 or less were regarded as statistically significant.

## 3. Results

The HWE results for genotype frequencies for both patients and control groups (patients as object and controls as expect groups) showed no significant association. Therefore, it was concluded that the studied population enjoys the HWE. Of all the 202 subjects, 101 patients with RPL as the study group and 101 healthy controls were genotyped for CTLA-4 +49A/G polymorphism. The average age of patients was 28 years. The demographic data and genotype frequencies of both patients and controls are given in the Table. The results were obtained using the chi-square test. The frequencies of AA, AG, and GG genotypes were 37.62%, 47.52%, and 14.85% in the control group and 38.61%, 51.48%, and 9.9% in the patients, respectively. The results showed no significant differences among patients and controls.

## 4. Discussion

Pregnancy is a unique condition in a woman's reproductive life (4,7). Many factors are suggested to be involved in RPL, including the immune system and its components, which could play an important role in maternal tolerance during pregnancy (6). It has been reported that autoimmune abnormalities such as antiphospholipid, antithyroid, antinuclear, and antisperm antibodies are associated with RPL (20). In such abnormalities cell-mediated immunity

**Table.** Genotypic and allelic distribution of +49 A/G CTLA-4 among patients and controls from the Iranian Azeri Turkish ethnic group.

Genotype	Patients n (F)	Controls n (F)	Chi-square	P value
AA	39 (38.61%)	38 (37.62%)	0.020	0.885
AG	52 (51.48%)	48 (47.52%)	0.316	0.573
GG	10 (9.9%)	15 (14.85%)	1.141	0.285
Allele	Patients n (F)	Control n (F)	Chi-square	P value
A	130 (64.35%)	124 (61.38%)	0.686	0.407
G	72 (35.64%)	78 (38.61%)	0.303	0.581

is increased and the level of regulatory T cells is altered (21). Regulatory T cells (Tregs) are crucial components of humeral immune response, which is important in the initiation and maintaining of pregnancy (1,22). The regulation of Tregs is mediated by antigen independent co-stimulatory signals, which act on the surface of Treg cells (1). The expression of *CTLA-4* could be downregulated by activated T cells (15).

This study was planned to investigate the possible association of *CTLA-4 A49G* gene polymorphism with RPL among women from northwest Iran and mainly originated from the Azeri Turkish ethnic group. The frequency of genotypes and alleles did not show significant differences between the controls and patients.

There are only a few studies that have investigated the polymorphisms of the *CTLA-4* gene in maternal tolerance during pregnancy. For instance, a recent study performed in the north Indian population showed no significant association between the *CTLA-4 49 A/G* (G allele) and RPL in that population (3). Moreover, Wang et al. investigated the *CTLA-4 +49 A/G* gene polymorphisms in Chinese women with unexplained spontaneous abortion (SA) and unexplained recurrent spontaneous abortion (RSA). The study showed no significant association between *CTLA-4* and RSA (9). However, another study carried out on

the Tunisian population showed a significant association between *CTLA-4 +49 A/G* gene polymorphism and RPL (22). Two other studies by Wang, with larger study groups, showed the association of the studied polymorphism with the immunopathogenesis of RSA in the Chinese population (23,24).

The results of the current study confirm the results of the Chinese and Indian reports and suggest that the +49 *A/G CTLA-4* polymorphic region might not affect RPL in the studied population. Although in the Chinese and Indian populations no association was observed between +49 *A/G CTLA-4* gene polymorphism and RPL, it is possible that there might be some other SNPs on *CTLA-4* that would show an association with RPL. Therefore, studying these SNPs may lead to stronger findings.

Our findings also indicate that, in fact, the frequency of the G allele of the *CTLA-4* gene in the study population from northwest Iran is higher than that of the other previously reported populations.

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#### References

- Najafi S, Hadinedoushan H, Eslami G, Aflatoonian A. Association of IL-17A and IL-17 F gene polymorphisms with recurrent pregnancy loss in Iranian women. *J Assist Reprod Genet* 2014; 31: 1491-1496.
- The Particle Committee of American Society for Reproductive Medicine: Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *ASRM PAGES* 2012; 98: 1103-1111.
- Mojarrad M, Hassanzadeh-Nazarabadi M, Tafazoli N. Polymorphism of genes and implantation failure. *Int J Mol Cell Med* 2013; 2: 1-8.
- Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. *Rev Obstet Gynecol* 2009; 2: 76-83.
- Rull K, Nagirnaja L, Laan M. Genetics of recurrent miscarriage: challenges, current knowledge, future directions. *Front Genet* 2012; 3: 1-13.
- Christiansen OB. Reproductive immunology. *Mol Immunol* 2013; 55: 8-15.
- Molazadeh M, Karimzadeh H, Azizi M. Prevalence and clinical significance of antinuclear antibodies in Iranian women with unexplained recurrent miscarriage. *Iran J Reprod Med* 2014; 12: 221-226.
- Inada K, Shima T, Nakashima A, Aoki K, Ito M, Saito S. Characterization of regulatory T cells in decidua of miscarriage cases with abnormal or normal fetal chromosomal content. *J Reprod Immunol* 2013; 97: 104-111.
- Wang X, Ma Z, Hong Y, Lu P, Lin Q. Expression of CD28 and cytotoxic T lymphocyte antigen 4 at the maternal-fetal interface in women with unexplained pregnancy loss. *Int J Gynecol Obstet* 2006; 93: 123-129.
- Saito S, Sasaki Y, Sakai M. CD4+CD25 high regulatory T cells in human pregnancy. *J Reprod Immunol* 2005; 65: 111-120.
- Quandt D, Hoff H, Rudolph M, Fillatreau S, Brunner-Weinzierl CM. A new role of CTLA-4 on B cells in thymus-dependent immune responses in vivo. *J Immunol* 2014; 179: 7316-7324.
- Geng R, Song F, Yang X, Sun P, Hu J, Zhu C, Zhu B, Fan W. Association between cytotoxic T lymphocyte antigen-4 +49A/G, -1722T/C, and -1661A/G polymorphisms and cancer risk: a meta-analysis. *Tumour Biol* 2014; 35: 3627-3639.
- Ahmadi S, Rostamzadeh J, Khosravi D, Shariati P, Shakiba N. Association of CTLA-4 gene 49 A/G polymorphism with the incidence of type 1 diabetes mellitus in the Iranian Kurdish population. *Pak J Biol Sci* 2013; 16: 1929-1935.
- Krummel MF, Allison JP. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. *J Exp Med* 1996; 183: 2533-2540.
- Tait ED, Hunter CA. The Foxo and the hound: chasing the in vivo regulation of T cell populations during infection. *Nat Immunol* 2009; 10: 457-458.

16. Almasi S, Erfani N, Mojtahedi Z, Rajaee A, Ghaderi A. Association of CTLA-4 gene promoter polymorphisms with systemic sclerosis in Iranian population. *Genes Immun* 2006; 6: 401-406.
17. Abdel Galil SM, Hagrass HA. The role of CTLA-4 exon-1 49 A/G polymorphism and soluble CTLA-4 protein level in Egyptian patients with Behçet's disease. *Biomed Res Int* 2014; 2014: 1-6.
18. Du L, Yang J, Huang J, Ma Y, Wang H, Xiong T, Xiang Z, Zhang Y, Huang J. The associations between the polymorphisms in the CTLA-4 gene and the risk of Graves' disease in the Chinese population. *BMC Med Genet* 2013; 14: 46-54.
19. Najafi S, Hadinedoushan H, Eslami G, Aflatoonian A. Association of IL-17A and IL-17 F gene polymorphisms with recurrent pregnancy loss in Iranian women. *J Assist Reprod Genet* 2014; 31: 1491-1496.
20. Ghazeeri GS, Kutteh WH. Immunological testing and treatment in reproduction: frequency assessment of practice patterns at assisted reproduction clinics in the USA and Australia. *Hum Reprod* 2001; 16: 2130-2135.
21. Sharif FA, Ashour MJ, Badawi NT, Al-Ashi SF. Polymorphism in regulatory T-cell (Treg)-related genes is associated with unexplained recurrent pregnancy loss. *AJCEM* 2016; 4: 63-67.
22. Messaoudi S, Houas I, Yaseen Kh, Dandana M, Mahjoub T. CTLA-4 gene polymorphisms and risk of idiopathic recurrent pregnancy loss in a Tunisian population. *BMC Genomics* 2014; 15: 11-12.
23. Wang XP, Lin QD, Ma ZW, Hong Y, Zhao AM, Di W, Lu PH. A/G polymorphism at position 49 in exon 1 of CTLA-4 gene in Chinese women with unexplained recurrent spontaneous abortion. *Zhonghua Fu Chan Ke Za Zhi* 2006; 41: 155-158.
24. Wang X, Lin Q, Ma Z, Hong Y, Zhao A, Di W, Lu P. Association of the A/G polymorphism at position 49 in exon 1 of CTLA-4 with the susceptibility to unexplained recurrent spontaneous abortion in the Chinese population. *Am J Reprod Immunol* 2005; 53: 100-105.