Observational Study

Medicine®

OPEN

Correlation between CTLA4 genetic polymorphisms, its serum protein level and the susceptibility to recurrent spontaneous abortion

A case–control study

Li Li, PhD[a], Jia Liu, PhD[a], Shuang Qin, PhD[b], Ruiman Li, PhD[a,*]

Abstract

Present study was aimed to detect the influence of cytotoxic T-lymphocyte associated protein 4 (*CTLA4*) gene polymorphisms for the risk of recurrent spontaneous abortion (RSA), as well as the serum level of CTLA4 protein in RSA patients.

One hundred thirty-three RSA patients and 146 healthy persons were recruited in this case–control study. PCR-RFLP was used to genotype the *CTLA4* gene polymorphisms both in case and control groups. Serum level of CTLA4 was detected by ELISA kit. Quantitative variables were compared by *t* test or Mann–Whitney *U* test between groups. Qualitative variables were evaluated by χ^2 test or Fisher exact test. Association strength was expressed by odds ratios (ORs) and 95% confidence intervals (95% CIs).

G allele of rs4553808 ($P=.027$, OR = 0.570, 95% CI = 0.345–0.942) and T allele of rs5742909 ($P=.027$, OR = 0.570, 95% CI = 0.345–0.942) were distinctly associated with reduced susceptibility of RSA. Distinctly negative association has been discovered between rs231775 AA genotype and RSA susceptibility ($P=.040$, OR = 0.427, 95% CI = 0.188–0.973). CTLA4 protein had significantly higher serum level in RSA patients than in healthy controls ($P=.028$). In RSA patients, AA genotype carriers had higher CTLA4 serum level than that GG genotype carriers (17.83 ± 6.35 ng/mL vs 10.41 ± 7.28 ng/mL, $P=.039$).

Minor alleles of *CTLA4* polymorphisms might inhibit the RSA susceptibility via upregulated the protein expression level.

Abbreviations: 95% CIs = 95% confidence intervals, CTLA4 = cytotoxic T-lymphocyte associated protein 4, ELISA = enzyme-linked immunosorbent assay, HWE = Hardy–Weinberg equilibrium, IRM = idiopathic recurrent miscarriages, ORs = odds ratios, RPL = recurrent pregnancy loss, RSA = recurrent spontaneous abortion, SNP = single nucleotide polymorphism.

Keywords: *CTLA4*, polymorphisms, recurrent spontaneous abortion

1. Introduction

Recurrent spontaneous abortion (RSA), also known as recurrent pregnancy loss (RPL) is a complication of pregnancy. It is defined as 3 or more than 3 times consecutive pregnancy loss which occurred before 20 weeks of gestation with unexplained reason.[1] Morbidity of RSA was not very high in total pregnancies.[2] With the development of RSA, it will lead to complications which could threaten the life.[2] RSA might reduce the life quality of patients and bring higher mental and economic burden for the family.[3] Most part of RSA had no definite reason. But various factors have been found to be associated with RSA development, such as immunologic, endocrine factors, infections, and genetic factors.[4–6] Normal embryo is not rejected by mater due to the immune tolerance, disorders on the immune tolerance at maternal–fetal interface will lead to the occurrence of RSA.[7] During recent years, many studies found that immune factors play a crucial role in RSA development.[8]

Cytotoxic T-lymphocyte associated protein 4 (CTLA4), also known as cluster of differentiation 152 (CD152), belongs to the immunoglobulin superfamily. CTLA4 located at the surface of T cells and transmits the inhibitory signal to T cells.[9,10] CTLA4 plays an important role in many immune diseases.[11,12] CTLA4 could inhibit the autoreactive T cells proliferation and regulate the antigen specific apoptosis. Imbalance of Th1/Th2 is significantly associated with the outcomes of pregnancy.[13] Th1/Th2 cytokines ratio was significantly elevated in RSA patients than that in normal pregnant women.[14] Polymorphisms in *CTLA4* gene might alter the expression level of protein, then effects the cellular stimulation for T cells, especially the polymorphisms in the promoter and exon regions.[15]*CTLA4* gene has 4 exons, and various single nucleotide polymorphisms (SNPs) have been identified for the gene. Rs733618 (c.-1722T/C), rs4553808 (c.-1661A/G), rs5742909 (c.-318C/T) are located at the promote region, while rs231775 (c.49A/G) is located in exon 1.[16] Minor allele frequencies of them were more than 0.1 in CHB (Chinese Han in Beijing) population. All of these SNPs were widely explored in different diseases.[17–19] However, the genetic association of these SNPs with RSA occurrence were rarely reported in Chinese Han population.

Editor: Qinhong Zhang.

LL, JL, and SQ contributed equally to this work.

The authors have no conflicts of interest to disclose.

[a] Department of Obstetrics and Gynecology, First Affiliate Hospital of Jinan University, [b] Department of Obstetrics, Guangzhou Women and Children's Medical Center, Guangzhou, China.

[*] Correspondence: Ruiman Li, Department of Obstetrics and Gynecology, First Affiliate Hospital of Jinan University, Guangzhou 510000, China (e-mail: owi93ss@126.com).

Medicine (2018) 97:42(e12754)

Received: 25 April 2017 / Accepted: 17 September 2018

http://dx.doi.org/10.1097/MD.0000000000012754

In present study, we investigated the genetic effects of *CTLA4* rs733618, rs4553808, rs5742909, rs231775 on RSA susceptibility and CTLA4 serum level in Chinese Han population.

2. Materials and methods

2.1. Study subjects

Ethic committee of First affiliate hospital of Jinan university approved present study. Informed consent was signed by every subject after realized the study process. All participants were Chinese Han population. Controls were matched with cases in ages. Baseline characteristics were gathered by professional questionnaire. Women diagnosed with RSA between January 2014 and June 2016 were recruited as cases. RSA patients were diagnosed by 2 pathologists in First affiliate hospital of Jinan university. Females with the experience of normal pregnancy were enrolled as healthy controls. Individuals with the history of abortion and infectious diseases were excluded from this study.

2.2. Genotyping method

Peripheral blood was collected from the vein of every subject and put into the tubes with EDTA. Samples were centrifuged into leukocytes and serum, then stored in −80°C standby application. Genomic DNA was extracted from leukocytes using the DNA extraction kit (TIANGEN, Beijing).

CTLA4 SNPs were amplified by PCR using the primer sequences as shown in Table 1. PCR reaction included initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 40 seconds, extension at 72°C for 1 minute, and the final extension at 72°C for 5 minutes. PCR products were digested by restriction enzymes and examined by agarose gel electrophoresis (AGE).

2.3. Analysis of CTLA4 serum level

Serum level of CTLA4 in subjects was detected by CTLA4 (Human) ELISA Kit (Abnova Corporation) following the operation instruction.

2.4. Statistical analysis

Hardy–Weinberg equilibrium (HWE) test was used to detect the representativeness of the subjects. Continuous variables were presented by mean ± SD and assessed by *t* test or Mann–Whitney *U* test. Categorical variables were evaluated by χ^2 test or Fisher exact test. Association strength was expressed by odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Calculations were performed by PASW 18.0. Significant level was set to 0.05 (2-tailed) and corrected by Bonferroni method.

3. Results

3.1. Baseline characteristics

One hundred thirty-one RSA patients and 146 healthy individuals were enrolled in this study. Mean age of RSA patients was 32.38 ± 8.35 years and mean age of healthy controls was 33.60 ± 8.48 years, no significant difference existed in age difference between case and control groups.

3.2. Association between CTLA4 SNPs and RSA susceptibility

CTLA4 SNPs distributions were accorded with the HWE test both in case and controls (Table 2, $P > .05$), suggesting that these subjects could represent the general population.

Higher frequencies of rs733618 TC and CC genotypes were discovered in RSA patients than that in controls, but the differences were not significant (Table 2, $P > .05$). C allele frequency was respectively 32.44% in RSA patients and 38.36% in controls. This allele slightly associated with reduced RSA susceptibility ($P > .05$). GG genotype of rs4553808 SNP has been discovered in 1 RSA patient (0.76%) and 4 healthy controls (2.74%). AG genotype had higher frequency in controls than that in RSA patients (28.08% vs 19.08%). No significant difference existed in the genotype distributions between case and control groups ($P > .05$). Rs4553808 G allele was more frequently discovered in controls, indicating a distinct association with decreased RSA susceptibility ($P = .027$, OR = 0.570, 95% CI = 0.345–0.942). CT and TT genotypes of rs5742909 SNP had lower frequencies in RSA patients, but these genotypes had no significant association with RSA susceptibility ($P > .05$). T allele frequency had significantly difference between case and control groups ($P = .027$), demonstrating that rs5742909 T allele significantly associated with decreased susceptibility for RSA (OR = 0.570, 95% CI = 0.345–0.942). Frequencies of rs231775 GG, GA and AA genotypes were 51.15%, 41.22%, 7.63% in RSA patients and 43.15%, 41.78%, 15.07% in controls, respectively. Significant difference existed in AA genotype frequency between cases and controls ($P = .040$). AA genotype might significantly correlated with reduced RSA susceptibility (OR = 0.427, 95% CI = 0.188–0.973).

3.3. Influence of rs231775 genotypes for CTLA4 serum level

Due to genotypes of rs733618, rs4553808, and rs5742909 SNPs had no significant association with RSA susceptibility, we did not

Table 1

Information of *CTLA4* polymorphisms.

SNP	Allele	Primer sequence	Restriction enzyme	Genotype	Fragments (bp)
rs733618	c.-1722T/C	F: 5′-CTAAGAGCATCCGCTTGCACCT-3′	*Bbv*I	TT	486
		R: 5′-TTGGTGTGATGCACAGAAGCCTTTT-3′		CC	257,229
rs4553808	c.-1661A/G	F: 5′-CTAAGAGCATCCGCTTGCACCT-3′	*Mse*I	AA	333,153
		R: 5′-TTGGTGTGATGCACAGAAGCCTTTT-3′		GG	486
rs5742909	c.-318C/T	F: 5′-AAATGAATTGGACTGGATGGT-3′	*Mse*I	CC	226,21
		R: 5′-TTACGAGAAAGGAAGCCGTG-3′		TT	130,96,21
rs231775	c.+49A/G	F: 5′-CCACGGCTTCCTTTCTCGTA-3′	*Bbv*I	AA	329
		R: 5′-AGTCTCACTCACCTTTGCAG-3′		GG	245,84

Note: *CTLA4* = cytotoxic T-lymphocyte associated protein 4, F = forward, R = reverse, SNP = single nucleotide polymorphism.

Table 2

Association between *CTLA4* polymorphisms and RSA susceptibility.

Genotype/allele	Case, n = 131 (%)	Control, n = 146 (%)	*P*	OR (95% CI)
rs733618				
TT	61 (46.56)	58 (39.73)	—	—
TC	55 (41.98)	64 (43.84)	.437	0.817 (0.491–1.359)
CC	15 (11.45)	24 (16.44)	.165	0.594 (0.284–1.244)
T	177 (67.56)	180 (61.64)	—	—
C	85 (32.44)	112 (38.36)	.147	0.772 (0.544–1.095)
P_{HWE}	.629	.378		
rs4553808				
AA	105 (80.15)	101 (69.18)	—	—
AG	25 (19.08)	41 (28.08)	.064	0.587 (0.333–1.034)
GG	1 (0.76)	4 (2.74)	.212	0.240 (0.026–2.188)
A	235 (89.69)	243 (83.22)	—	—
G	27 (10.31)	49 (16.78)	**.027**	**0.570 (0.345–0.942)**
P_{HWE}	.712	.947		
rs5742909				
CC	106 (80.92)	102 (69.86)	—	—
CT	23 (17.56)	39 (26.71)	.055	0.567 (0.317–1.016)
TT	2 (1.53)	5 (3.42)	.280	0.385 (0.073–2.029)
C	235 (89.69)	243 (83.22)	—	—
T	27 (10.31)	49 (16.78)	**.027**	**0.570 (0.345–0.942)**
P_{HWE}	.565	.598		
rs231775				
GG	67 (51.15)	63 (43.15)	—	—
GA	54 (41.22)	61 (41.78)	.474	0.832 (0.504–1.376)
AA	10 (7.63)	22 (15.07)	**.040**	**0.427 (0.188–0.973)**
G	188 (71.76)	187 (64.04)	—	—
A	74 (28.24)	105 (35.96)	.053	0.701 (0.489–1.005)
P_{HWE}	.846	.262		

Note: 95% CI = 95% confidence interval, *CTLA4* = cytotoxic T-lymphocyte associated protein 4, HWE = Hardy–Weinberg equilibrium, OR = odds ratios, RSA = recurrent spontaneous abortion.

explore the influences of these SNPs for CTLA4 serum level. Then we only detected the influence of rs231775 genotypes for CTLA4 serum levels. Serum levels of CTLA4 was significantly higher in RSA patients (12.86 ± 5.17 ng/mL) than that in healthy controls (6.54 ± 2.21 ng/mL) ($P = .028$). Serum CTLA4 levels were higher in RSA patients than in controls respectively in GG, GA, and AA genotype carriers. No significant difference exited in these groups except AA genotype (Fig. 1, 17.83 ± 5.86 ng/mL vs 8.57 ± 1.89 ng/mL, $P = .016$). Thus we suggested that rs231775 AA genotype significantly associated with RSA susceptibility might via upregulated CTLA4 level. While, serum CTLA4 level had no significant difference between genotypes of rs733618, rs4553808, rs5742909, respectively (Fig. 1, $P > .05$).

4. Discussion

Previous study proved that *CTLA4* gene played a crucial role in the immune tolerance of maternal–fetal interface which was the key factor for RSA development.[20] Very few studies focused on the association of *CTLA4* polymorphisms and RSA susceptibility. Therefore, we carried out this study.

Various studies found that rs733618 SNP significantly correlated with many immune diseases. Niknam et al[21] demonstrated that -1722C allele negatively associated with acute rejection in kidney transplant patients with active *Cytomegalovirus* infection. A potential of rs733618 SNP also has been discovered in autoimmune myasthenia gravis.[22] A meta-analysis study found a positive association between TT genotype of rs733618 and the susceptibility for systemic lupus erythematosus.[23] While, rs733618 SNP had no significant association with nonanterior uveitis.[17] However, in this study we found that heterozygote and homozygote of rs733618 T allele were mildly associated with decreased susceptibility for RSA. At the same time, no significant association has been discovered between rs733618 alleles and RSA susceptibility.

Slightly reduced RSA susceptibility also has been discovered respectively in the heterozygotes and homozygotes of rs4553808 G allele and rs5742909 T allele. Besides, rs4553808 G allele and rs5742909 T allele were significantly associated with 0.570-fold reduced RSA susceptibility respectively. But Misra et al[18] suggested that rs4553808 and rs5742909 SNP had no significant association with idiopathic recurrent miscarriages (IRM). This difference might be caused by different ethnicity, region, or sample size. While other studies indicated that these 2 SNPs may contribute to different immune diseases. Chen et al[24] found that rs4553808 G allele significantly associated with the increased risk of ulcerative colitis. −1661GG genotype might act as a risk factor for viral infection in patients with kidney transplantation.[25] A study performed by Fattah et al[26] indicated that T allele of rs5742909 distinctly associated with the activity for rheumatoid arthritis in Egyptian population.

AA genotype of rs231775 SNP more frequently discovered in healthy controls suggested that this genotype distinctly correlated with 0.427-fold decreased RSA susceptibility. However, rs231775 alleles had no significant association with RSA susceptibility. Present result accorded with previous study. Wang et al[19] indicated that GG genotype of rs231775 SNP distinctly related to enhanced RSA susceptibility. +49AA genotype negatively associated with the susceptibility of allergic rhinitis and positively associated with asthma.[27] However, Rasti et al

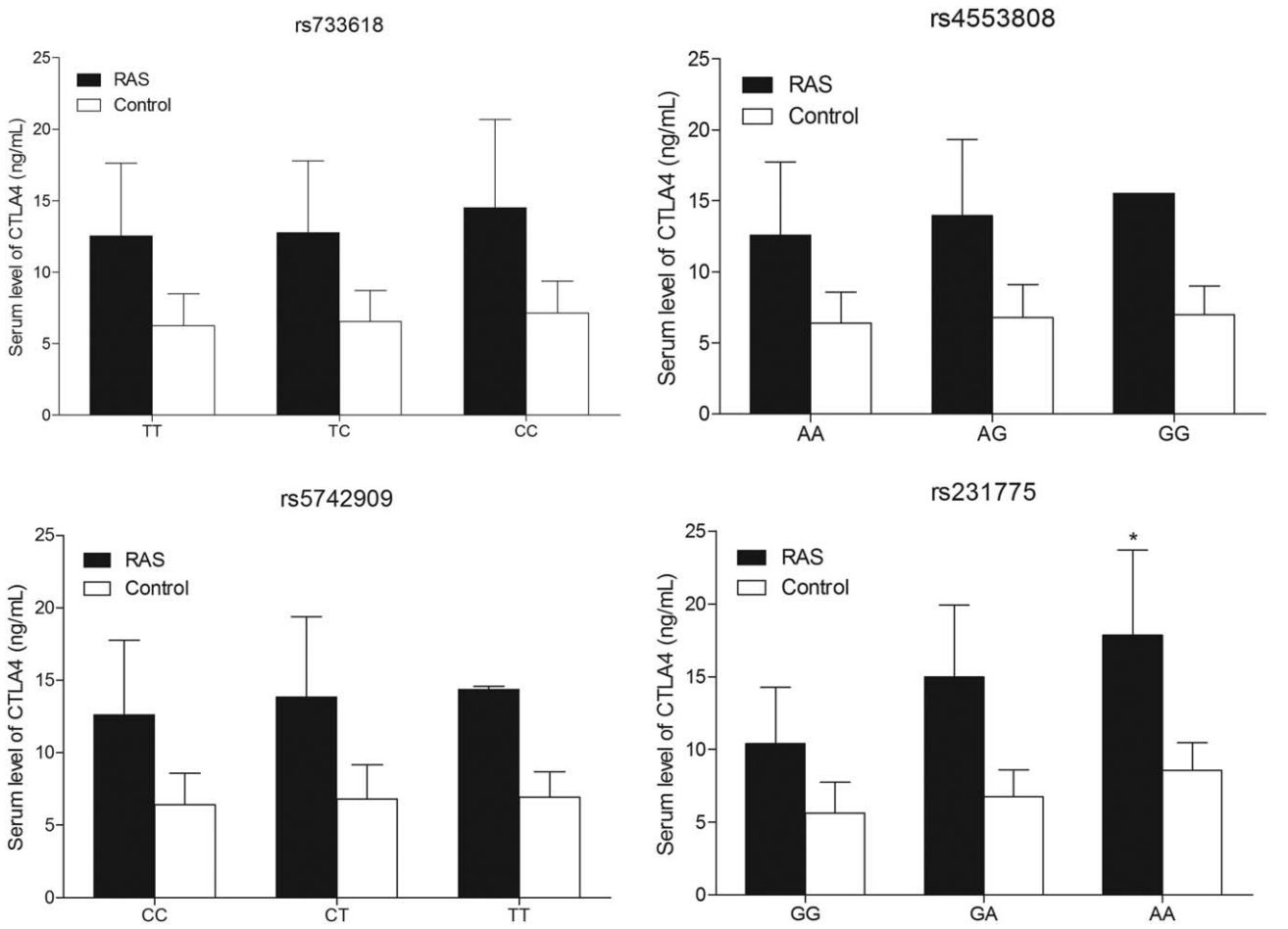

Figure 1. Influence of rs231775 genotypes for CTLA4 serum level. In RSA patients, AA genotype carriers had higher CTLA4 serum level than that in GG genotype carriers (17.83 ± 5.86 ng/mL vs 10.41 ± 3.86 ng/mL, $P = .039$). Serum CTLA4 level was significantly higher in RSA patients than in controls respectively carrying AA genotype (17.83 ± 5.86 ng/mL vs 8.57 ± 1.89 ng/mL, $P = .016$). While, serum CTLA4 level had no significant difference between genotypes of rs733618, rs4553808, rs5742909, respectively ($P > .05$).

found that rs231775 G allele carriers had higher susceptibility to RSA in an Iran population.[28] This difference might be caused by the divergences in study population.

In order to certify the exact mechanism of *CTLA4* gene for RSA, we researched the influence of rs231775 genotype for CTLA4 serum level. Then we found that serum level of CTLA4 was significantly increased in RSA patients than in healthy individuals. In different rs231775 genotype carriers, the serum level of CTLA4 also had difference. Individuals carrying heterozygote and homozygote of +49A allele had orderly increased CTLA4 serum level both in case and control groups. But only in RSA patients with +49AA genotype had significantly higher CTLA4 serum level than in +49GG genotype carriers. Similar result has been discovered in previous study. Misra et al found that mutant genotypes of rs231775 SNP significantly associated with reduced CTLA4 serum level in IRM patients.[25] Rs231775 polymorphism might have the capacity to influence the transcription activity of the gene. Polymorphisms in promoter or exon regions might alter the expression or structure of the gene, these alterations usually induced the different physiological or pathological changes. However, effects of polymorphisms in *CTLA4* gene for RSA development were not clear until now.

In summary, we suggested that SNPs in promoter region and exon 1 of *CTLA4* gene might significantly associated with reduced RSA susceptibility via the upregulation of CTLA4. In spite of this, it should be accepted that several limitations existed in present study. Firstly, sample size was not large enough to obtain a higher test power. Secondly, application range for this study was limited by the ethnicity number. Thirdly, a variety of factors contribute to the development of RSA, but interactions between *CTLA4* gene and other genes, as well as environmental factors were not detect in present study. Haplotype between SNPs might alter the effects of each SNP for RSA susceptibility independently. However, the haplotype analysis was not performed in this study because the correlation coefficient of linkage disequilibrium (D′ and r2) was not high enough. Finally, current results were not adjusted by confounding factors that might affect the stability thereof. As a consequence, well designed studies with enlarged sample size are needed in the future so as to certify the pathogenesis of *CTLA4* in RSA development.

Author contributions

Conceptualization: Li Li, Jia Liu.
Data curation: Li Li, Jia Liu.
Formal analysis: Li Li, Jia Liu.
Funding acquisition: Li Li, Jia Liu.
Investigation: Li Li, Jia Liu.

Methodology: Shuang Qin.
Project administration: Shuang Qin.
Resources: Shuang Qin.
Software: Shuang Qin.
Supervision: Shuang Qin.
Validation: Ruiman Li.
Visualization: Ruiman Li.
Writing – original draft: Ruiman Li.
Writing – review & editing: Ruiman Li.

References

[1] Obstetrics Subgroup, Chinese Society of Obstetrics and Gynecology, Chinese Medical Association[Chinese expert consensus on the diagnosis and treatment of recurrent spontaneous abortion]. Zhonghua Fu Chan Ke Za Zhi 2016;51:3–9.

[2] Kessous R, Shoham-Vardi I, Parientel G, et al. [Association between pregnancy complications and long term maternal cardiovascular morbidity]. Harefuah 2016;155:286–90. 322.

[3] Mevorach-Zussman N, Bolotin A, Shalev H, et al. Anxiety and deterioration of quality of life factors associated with recurrent miscarriage in an observational study. J Perin Med 2012;40:495–501.

[4] Ruixue W, Hongli Z, Zhihong Z, et al. The impact of semen quality, occupational exposure to environmental factors and lifestyle on recurrent pregnancy loss. J Assist Reprod Genet 2013;30:1513–8.

[5] Abdi-Shayan S, Monfaredan A, Moradi Z, et al. Association of CD46 IVS1-1724 C>G single nucleotide polymorphism in Iranian women with unexplained recurrent spontaneous abortion (URSA). Iran J Allergy Asthma Immunol 2016;15:303–8.

[6] Yamashita M, Hayashi S, Endo M, et al. Incidence and risk factors for recurrent spontaneous preterm birth: a retrospective cohort study in Japan. J Obstet Gynaecol Res 2015;41:1708–14.

[7] Hu XH, Tang MX, Mor G, et al. Tim-3: expression on immune cells and roles at the maternal-fetal interface. J Reprod Immunol 2016;118:92–9.

[8] Mumusoglu S, Beksac MS, Ekiz A, et al. Does the presence of autoantibodies without autoimmune diseases and hereditary thrombophilia have an effect on recurrent pregnancy loss? J Matern Fetal Neonatal Med 2016;29:2352–7.

[9] Walunas TL, Lenschow DJ, Bakker CY, et al. CTLA-4 can function as a negative regulator of T cell activation. Immunity 1994;1:405–13.

[10] Paterson AM, Lovitch SB, Sage PT, et al. Deletion of CTLA-4 on regulatory T cells during adulthood leads to resistance to autoimmunity. J Exp Med 2015;212:1603–21.

[11] Humblet-Baron S, Schonefeldt S, Garcia-Perez JE, et al. Cytotoxic T-lymphocyte-associated protein 4-Ig effectively controls immune activation and inflammatory disease in a novel murine model of leaky severe combined immunodeficiency. J Allergy Clin Immunol 2017;140:1394. e8–403.e8.

[12] Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer 2016;54:139–48.

[13] Wu M, Zhu Y, Zhao J, et al. Soluble costimulatory molecule sTim3 regulates the differentiation of Th1 and Th2 in patients with unexplained recurrent spontaneous abortion. Int J Clin Exp Med 2015;8:8812–9.

[14] Yuan J, Li J, Huang SY, et al. Characterization of the subsets of human NKT-like cells and the expression of Th1/Th2 cytokines in patients with unexplained recurrent spontaneous abortion. J Reprod Immunol 2015;110:81–8.

[15] Ligers A, Teleshova N, Masterman T, et al. CTLA-4 gene expression is influenced by promoter and exon 1 polymorphisms. Genes Immun 2001; 2:145–52.

[16] 28097051 CTLA-4 polymorphisms associate with breast cancer susceptibility in Asians: a meta-analysis

[17] Leon Rodriguez DA, Serrano Lopera A, Cordero-Coma M, et al. Study of association of CTLA4 gene variants to non-anterior uveitis. Tissue Antigens 2015;86:373–6.

[18] Misra MK, Mishra A, Phadke SR, et al. Association of functional genetic variants of CTLA4 with reduced serum CTLA4 protein levels and increased risk of idiopathic recurrent miscarriages. Fertil Steril 2016;106:1115.e6–23.e6.

[19] Wang X, Lin Q, Ma Z, et al. 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;53:100–5.

[20] Tsai AF, Kaufman KA, Walker MA, et al. Transmission disequilibrium of maternally-inherited CTLA-4 microsatellite alleles in idiopathic recurrent miscarriage. J Reprod Immunol 1998;40:147–57.

[21] Niknam A, Karimi MH, Yaghobi R, et al. The association between viral infections and co-stimulatory gene polymorphisms in kidney transplant outcomes. Jundishapur J Microbiol 2016;9:e31338.

[22] Li HF, Hong Y, Zhang X, et al. Gene polymorphisms for both autoantigen and immune-modulating proteins are associated with the susceptibility of autoimmune myasthenia gravis. Mol Neurobiol 2016;54:4771–80.

[23] Zhu JM, Li BK, Chen GM, et al. CTLA-4-1722T/C polymorphism and systemic lupus erythematosus susceptibility: a meta-analysis involving ten separate studies. Immunol Invest 2013;42:91–105.

[24] Chen Z, Brant SR, Li C, et al. CTLA4-1661A/G and 3'UTR long repeat polymorphisms are associated with ulcerative colitis and influence CTLA4 mRNA and protein expression. Genes Immun 2010;11:573–83.

[25] Guo Y, Guo F, Wei C, et al. CTLA4 gene polymorphisms influence the incidence of infection after renal transplantation in Chinese recipients. PLoS ONE 2013;8:e70824.

[26] Fattah SA, Ghattas MH, Saleh SM, et al. Cytotoxic T-lymphocyte-associated protein 4 gene polymorphism is related to rheumatoid arthritis in Egyptian population. Arch Physiol Biochem 2017;123:50–3.

[27] Song SH, Wang XQ, Shen Y, et al. Association between PTPN22/CTLA-4 gene polymorphism and allergic rhinitis with asthma in children. Iran J Allergy Asthma Immunol 2016;15:413–9.

[28] Zarnegar R, Mahboobeh N. Association of the +49A/G polymorphism of CTLA4 gene with idiopathic recurrent spontaneous abortion in women in southwest of Iran. J Reprod Infertil 2016;17:151–6.