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Genetic polymorphism of IL-17 influences susceptibility to recurrent pregnancy loss in a Chinese population

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Abstract

The current research aims to investigate the relationship between Interleukin-17 (IL-17) polymorphism and the risk of recurrent pregnancy loss (RPL) within a Chinese population. Totally, 120 patients with RPL were selected and enrolled as the experiment group. Additionally, 210 healthy individuals undergoing routine physical examinations during the same period served as the control group. The IL-17 gene polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism method. The IL-17 rs2275913 polymorphism exhibited 3 genotypes: GG, GA, and AA. Significant associations were observed with the AA genotype and A allele (all $P < .05$), indicating women with the AA genotype were 2.06 times more likely to experience RPL compared to those with the GG genotype. Similarly, women carrying the A allele faced a 1.63 times higher risk of RPL than those with the G allele. Regarding the IL-17 rs763780 polymorphism, which also presented 3 genotypes (TT, TC, CC), significant associations were noted for the CC genotype and C allele (all $P < .05$). Women with the CC genotype had a 1.84 times greater risk of suffering from RPL compared to those with the TT genotype, and those with the C allele were 1.51 times more likely to experience RPL than those with the T allele. The IL-17 rs2275913 and rs763780 polymorphisms contribute an increased risk to RPL in the Chinese population. Further studies, with larger sample sizes and more rigorous designs, are necessary to validate or replicate our current results.

Abbreviations: BMI = body mass index, CI = confidence interval, dCTP = cytosine deoxynucleotide triphosphate, NK = natural killer, OR = odds ratio, RPL = recurrent pregnancy loss, TG = triglycerides.

Keywords: gene polymorphism, IL-17, novel marker, recurrent pregnancy loss

1. Introduction

Recurrent pregnancy loss (RPL) is defined as experiencing 2 or more miscarriages before reaching 20 weeks of gestation.[1] Currently, the prevalence of RPL among women of childbearing age in China is approximately 5%, significantly impacting pregnancy outcomes and family harmony. The causes of RPL are multifaceted, with genetic, anatomical, endocrine, and infectious factors all playing potential roles in its occurrence.[2] Furthermore, numerous immune abnormalities have been observed in women with RPL of unknown origin, including increased levels of antiphospholipid antibodies, antinuclear antibodies, anti-thyroglobulin antibodies, as well as natural killer (NK) cell cytotoxicity.

Recent research has established a strong connection between maternal-fetal immune imbalance and RPL.[3,4] High expression of Interleukin-17 (IL-17) was found compared with those in normal pregnancies. IL-17 acts a dominate effect on promoting the accumulation of neutrophils, exacerbating endometrial inflammation and damage, thereby increasing the risk of miscarriage.[5] The above evidences indicate that IL-17 may be a crucial player in the occurrence and progression of RPL. Moreover, polymorphisms in the IL-17A and IL-17F genes have been linked to the development and occurrence of various human diseases. Notably, rs2275913 and rs763780 are crucial polymorphic loci located in the coding regions of IL-17A and IL-17F, respectively,[6,7] and are closely associated with IL-17 secretion.[6]

In the last 10 years, numerous studies have delved into the association between IL-17 gene polymorphism and the risk of RPL, suggesting a link between IL-17 gene variations and RPL development.[8–10] However, these findings have been mixed and subject to controversy. Previous research primarily conducted in Iran, Egypt, and Saudi Arabia has highlighted

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the influence of ethnic and population differences on genetic polymorphisms and backgrounds. Given the significant genetic divergence between Chinese populations and those of West Asia and North Africa, it is plausible that gene polymorphisms, including those related to IL-17, may differ markedly. Consequently, there is a pressing need to explore the relationship between IL-17 gene polymorphism and RPL risk within the Chinese population, addressing this gap in the research landscape.

2. Materials and methods

2.1. Study subjects

The research was organized and divided into RPL group and control group. We recruited 120 patients diagnosed with RPL who visited our hospital. The inclusion criteria for the RPL group were as follows: the RPL patient had experienced ≥2 times spontaneous abortions without any history of live births; absence of uterine and cervical abnormalities; the couple was not closely related by blood, with no embryonic chromosome abnormalities or family history of genetic disorders; absence of reproductive tract infections; no endocrine, metabolic, or autoimmune-related diseases; and normal male semen analysis results, with the female's menstrual cycle being relatively stable and ultrasound monitoring indicating normal ovulation. For the control group, we selected 210 healthy early pregnant women who attended the hospital for routine checkups during the same period. They were healthy without a history of abnormal pregnancies that were willing to participate in the study. The current investigation was authorized and approved by the Ethics Committee of Affiliated Hospital of Xuzhou Medical University and RPL group and control group signed the written consent.

2.2. Main reagents and instruments

For DNA extraction, we used the DNA Blood Microkits from Thermo Scientific (Waltham, MA). The amplification of the IL-17 gene rs2275913 and rs763780 primers was carried out using primers obtained from Shanghai Shengong. Ampli-TaqGold polymerase, cytosine deoxynucleotide triphosphate, and the polymerase chain reaction (PCR) amplification kit were all sourced from Thermo Scientific, USA, and Takara, Japan, respectively. For visualizing the PCR products, Kodak X-OMAT Film from Reek Medical Devices Co., LTD. (Shanghai, China) was utilized. DNA concentration and purity were assessed using an ND-1000 spectrophotometer, procured from Nano-drop Technologies (Waltham, MA). Lastly, sequencing was performed with a gene sequencer from American Life Tecch Company.

2.3. Sample collection and DNA extraction

The venous blood was collected using ethylene diamine tetraacetic acid as an anticoagulant. Genomic DNA was extracted following the instructions provided with the kit. The purity and concentration of DNA were assessed by applying a nucleic acid analyzer, with samples stored at −80°C. DNA samples exhibiting an absorbance ratio of 260 to 280 nm between 1.7 and 2.0 were deemed suitable for further PCR genotyping. The genotyping of IL-17A and IL-17F was conducted using the polymerase chain reaction-restriction fragment length polymorphism technique. The Primer Premier 5.0 software was responsible for designing the primers, according to their respective sequences. The forward and reverse primers for IL-17A (rs2275913) are 5'-TCTCCATCTCCATCACCTTTG-3' and 5'-GTCCAAATCAGCAAGAGCATC-3', respectively. For IL-17F (rs763780), the forward and reverse primers are 5'-CACTGGTGCTCTGATGAGGA-3' and 5'-CATTGTGCTTTGGCTTGCT-3', respectively.

2.4. PCR reaction and gene polymorphism detection

A DNA sample of 3 μL was combined with a 10 μL PCR mixture, which included the primer, deoxynucleotide triphosphate, magnesium chloride, Ampli-TaqGold polymerase, cytosine deoxynucleotide triphosphate, and 1 μL of 10× buffer, and then processed according to standard PCR procedures. Initially, the mixture underwent the PCR procedure follows the classic steps. For PCR product purification, 10 μL of the PCR product was treated with 2U Exonuclease I enzyme and 5U SAP enzyme, incubated in a water bath at 37°C for 60 minutes, and then inactivated at 75°C for 12 minutes. The extension product was purified by adding 1 U of SAP enzyme to 10 μL of the product, followed by incubation in a water bath at 37°C for 60 minutes and then 75°C for 12 minutes. All PCR products underwent incubation with endonuclease PstI at 37°C and were subjected to electrophoresis on a 3.0% agarose gel at 80 V for 100 minutes. The enzyme-digested products were visualized using ethidium bromide staining to determine the genotypes. In this study, genotypes were interpreted independently by 2 individuals using a blinded method, with inconsistent interpretations subjected to retesting.

2.5. Statistical analysis

SPSS 19.0 software was responsible for the whole analysis, such as Chi-square (χ^2) test, *t* test and 1-way analysis of variance. The Hardy-Weinberg equilibrium was applied to verify genotype frequencies. The association between IL-17 genotypes and RPL risk was examined through logistic regression, with the relative risk represented by the odds ratio (OR) and 95% confidence interval (CI). All the statistical methods applied in current study considered $P < .05$ to have a statistical difference.

3. Results

3.1. General information of study subjects

We examined a range of critical and relevant characteristics, such as age, number of RPL, number of children, triglycerides (TG). Statistical analysis revealed a significant association solely with the number of RPL ($P < .05$). The comprehensive data are presented in Table 1.

3.2. Genotyping and allele distribution of IL-17 rs2275913 polymorphism

Three genotypes, GG, GA, and AA were identified. Significant associations were detected for both the AA genotype and the A allele (all $P < .05$). Women possessing the AA genotype had a

Table 1

Participates characteristics of both RPL group and control group.

Characteristics	RPL group (N = 120)	Control group (N = 210)	*P*
Age (yr)	32.8 ± 6.8	31.6 ± 7.2	.138
Number of RPL	3.0 ± 0.8	0 ± 0	<.001
Number of children	1.8 ± 0.6	1.7 ± 0.5	.106
BMI (kg/m^2)	26.8 ± 8.5	25.9 ± 6.9	.246
WC (cm)	92.9 ± 16.8	90.8 ± 15.4	.273
TC (mg/dL)	198.1 ± 56.7	194.7 ± 55.2	.511
TG (mg/dL)	160.7 ± 45.2	154.6 ± 43.1	.234
HDL (mg/dL)	35.8 ± 11.8	37.1 ± 12.6	.161
LDL (mg/dL)	102.8 ± 46.8	104.8 ± 48.7	.716

BMI = body mass index, HDI = high-density lipoprotein, LDL = low-density lipoprotein, RPL = recurrent pregnancy loss, TC = total cholesterol, TG = triglycerides, WC = waist circumference.

2.06-fold increased risk of experiencing RPL compared to those with the GG genotype. Similarly, women carrying the A allele faced a 1.63 times higher risk of RPL. Detailed data are presented in Table 2.

3.3. Genotyping and allele distribution of IL-17 rs763780 polymorphism

Three genotypes, TT, TC, and CC were identified. Significant associations were observed for the CC genotype and the C allele (all $P < .05$). Women with the CC genotype had a 1.84-fold increased risk of experiencing RPL compared to those with the TT genotype. Furthermore, women carrying the C allele were 1.51 times more likely to suffer from RPL. Detailed data are provided in Table 3.

4. Discussion

The prevalence rate of RPL is approximately 1.0%. The causes of RPL are multifaceted, encompassing genetics, anatomy, infections, endocrine factors, and immune responses. Despite extensive research in this area, the underlying causes of more than half of RPL cases remain unidentified. However, recent advancements in cytogenetics and immunogenetics have deepened our understanding of the mechanisms behind embryo implantation and the maternal-fetal interface at the molecular level. The advent and utilization of genomics technology have propelled research into the essence of life and disease mechanisms into the post-genomic era. RPL is a complex condition influenced by a myriad of factors, among which genetic elements are increasingly recognized for their crucial role in its pathogenesis. Consequently, identifying genes associated with RPL susceptibility is vital for elucidating its underlying mechanisms. This endeavor has become a focal point and challenge in reproductive research, spotlighting the importance of targeted treatment strategies.

Pregnancy represents a unique process of immune tolerance to an allograft, from embryo implantation to successful childbirth, critically dependent on establishing immune tolerance at the maternal-fetal interface. The inflammatory response, a key component in forming immune tolerance, has garnered increasing attention. NK cells, T cells, and their secreted cytokines orchestrate an immune network at the interface between mother and fetus. The shift from a *helper T 1 cells* (Th1) pro-inflammatory immune response to a Th2 anti-inflammatory immune response is crucial for pregnancy success. Failure to transition from Th1 to Th2 in a timely manner allows the Th1-type pro-inflammatory immune response to predominate, disrupting the Th1/Th2 cytokine balance and the immune tolerance balance at the maternal-fetal interface. This continuous Th1-type pro-inflammatory immune reaction can lead to miscarriage. When the Th1-type pro-inflammatory immune response is dominant, NK cells are stimulated by cytokines such as IL-2, tumor necrosis factor, and interferon to become lymphokine activated killer cells, which damage trophoblastic cells and result in abortion. Many patients with RPL exhibit this abnormal immune response at the maternal-fetal interface to varying extents during pregnancy.

Single nucleotide polymorphism represents one of the most prevalent forms of heritable human variation, accounting for approximately 90% of all genetic polymorphisms. These variations are generated through the conversion, transposition, deletion, and insertion of single nucleotides, forming genetic markers that can alter gene transcriptional activity or function. Such changes can impact the expression or activity of proteins. Specifically, single nucleotide polymorphisms in certain cytokine genes may affect the production and activation of corresponding cytokines, leading to variations in cytokine expression levels and, consequently, the emergence of various diseases.

In our study, we discovered that polymorphisms in IL-17 rs2275913 and rs763780 contribute an increased risk to RPL in the Chinese population. Our results do not fully align with those reported in previous literature, a discrepancy we consider to be reasonable.[11–17] Contradictory findings are not uncommon in genetic research due to the influence of race, region, and other factors. Genetic polymorphisms vary significantly

Table 2

Comparison of genotype and allele frequency between RPL group and control group.

	Control group (N = 210)		RPL group (N = 120)			
IL-17 A (rs2275913)	n	%	n	%	OR (95% CI) [a]	*P**
GG	66	31.4	24	20.0	1.00[REF]	
GA	64	30.5	36	30.0	1.55 (0.83–2.88)	.220
AA	80	38.1	60	50.0	2.06 (1.16–3.66)	.018
G	196	46.7	84	35.0	1.00[REF]	
A	224	53.3	156	65.0	1.63 (1.17–2.25)	.005

CI = confidential index, IL = Interleukin, OR = odds ratio, RPL = recurrent pregnancy loss.

*Adjusted for sex and age by logistic regression model.

Table 3

Comparison of genotype and allele frequency between RPL group and control group.

	Control group (N = 210)		RPL group (N = 120)			
IL-17 F (rs763780)	n	%	n	%	OR (95% CI) [a]	*P**
TT	64	30.5	25	20.8	1.00[REF]	
TC	64	30.5	36	30.0	1.44 (0.78–2.67)	.246
CC	82	39.0	59	49.2	1.84 (1.04–3.26)	.035
T	192	45.7	86	35.8	1.00[REF]	
C	228	54.3	154	64.2	1.51 (1.09–2.09)	.013

CI = confidential index, IL = Interleukin, OR = odds ratio, RPL = recurrent pregnancy loss.

*Adjusted for sex and age by logistic regression model.

across different countries and regions, impacted by the distinct racial backgrounds present.[18–24] Our research focuses on the Chinese population, whose genetic polymorphisms differ from those observed in Egypt and Saudi Arabia. Given that Egypt is in North Africa and Saudi Arabia is in West Asia, their genetic profiles are markedly different from those of the Chinese population.

5. Conclusion

In conclusion, our findings suggest that polymorphisms in IL-17 rs2275913 and rs763780 contribute an increased risk to RPL in the Chinese population. To confirm our results, further research involving larger sample sizes and more rigorously designed studies will be necessary in the future.

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References

[1] La X, Wang W, Zhang M, Liang L. Definition and multiple factors of recurrent spontaneous abortion. Adv Exp Med Biol. 2021;1300:231–57.
[2] Fan T, Zhong XM, Wei XC, et al. The alteration and potential relationship of vaginal microbiota and chemokines for unexplained recurrent spontaneous abortion. Medicine (Baltim). 2020;99:e23558.
[3] Zhu J, Jin J, Qi Q, et al. The association of gut microbiome with recurrent pregnancy loss: a comprehensive review. Drug Discov Ther. 2023;17:157–69.
[4] Krechetova LV, Vanko LV, Vtorushina VV, Nikolaeva MA, Inviyaeva EV, Tetruashvili NK. Lymphocyte activation in the development of immune tolerance in women with recurrent pregnancy loss. Biochemistry (Mosc). 2020;85:583–93.
[5] Sahota DS, Leung TY, Leung TN, Chan OK, Lau TK. Fetal crown-rump length and estimation of gestational age in an ethnic Chinese population. Ultrasound Obstet Gynecol. 2009;33:157–60.
[6] Gao S, Mao C, Cheng J, Deng Q, Sheng W. Association of IL-17A-197G/A and IL-17F-7488T/C polymorphisms and osteoarthritis susceptibility: a meta-analysis. Int J Rheum Dis. 2020;23:37–46.
[7] Mazurek-Mochol M, Kozak M, Malinowski D, Safranow K, Pawlik A. IL-17F gene rs763780 and IL-17A rs2275913 polymorphisms in patients with periodontitis. Int J Environ Res Public Health. 2021;18:1081.
[8] 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;31:1491–6.
[9] Zidan HE, Rezk NA, Alnemr AA, Moniem MI. Interleukin-17 and leptin genes polymorphisms and their levels in relation to recurrent pregnancy loss in Egyptian females. Immunogenetics. 2015;67:665–73.
[10] Bahadori M, Zarei S, Zarnani AH, et al. IL-6, IL-10 and IL-17 gene polymorphisms in Iranian women with recurrent miscarriage. Iran J Immunol. 2014;11:97–104.
[11] Chen X, Wang Z, Yan Y, et al. XRCC3 C18067T polymorphism contributes a decreased risk to both basal cell carcinoma and squamous cell carcinoma: evidence from a meta-analysis. PLoS One. 2014;9:e84195.
[12] Qin LY, Chen X, Li P, Yang Z, Mo WN. Association between the XRCC3 Thr241Met polymorphism and cervical cancer risk: a meta-analysis. Asian Pac J Cancer Prev. 2014;14:6703–7.
[13] Wang Z, Chen X, Liu B, Li S, Liu M, Xue H. Quantitative assessment of the associations between DNA repair gene XRCC3 Thr241Met polymorphism and gastric cancer. Tumour Biol. 2014;35:1589–98.
[14] Yan Y, Chen X, Li T, Li M, Liang H. Association of OGG1 Ser326Cys polymorphism and pancreatic cancer susceptibility: evidence from a meta-analysis. Tumour Biol. 2014;35:2397–402.
[15] Chen X, Yan Y, Li P, Yang Z, Qin L, Mo W. Association of GSTP1 -313A/G polymorphisms and endometriosis risk: a meta-analysis of case-control studies. Eur J Obstet Gynecol Reprod Biol. 2013;171:362–7.
[16] Chen X, Mo W, Peng Q, Su X. Lack of association between Fas rs180082 polymorphism and risk of cervical cancer: an update by meta-analysis. BMC Med Genet. 2013;14:71.
[17] Si D, Yao Y, Chen X, Qiu J. Ethnicity-stratified analysis of the association between P53 rs1042522 polymorphism and women HPV infection: a meta-analysis. Microb Pathog. 2021;161:105099.
[18] Niu K, Chen X, Lu Y. COL3A1 rs1800255 polymorphism is associated with pelvic organ prolapse susceptibility in Caucasian individuals: evidence from a meta-analysis. PLoS One. 2021;16:e0250943.
[19] Jin X, Wu Y, Yin S, Chen X, Zhang Y. Association between the IL-10 and IL-6 polymorphisms and brucellosis susceptibility: a meta-analysis. BMC Med Genet. 2020;21:63.
[20] Jin X, Yin S, Zhang Y, Chen X. Association between TLR2 Arg677Trp polymorphism and tuberculosis susceptibility: a meta-analysis. Microb Pathog. 2020;144:104173.
[21] Yuanyuan G, Xue Y, Yachao L, Xiao F, Xu C. Association between IL-18 -607 C/A polymorphism and the risk of prostate cancer: a meta-analysis of case-control studies. Asian Pac J Cancer Prev. 2019;20:1595–602.
[22] Jin X, Yin S, Zhang Y, Chen X. Quantitative assessment of the association between IL-10 -592 A/C polymorphism and Kawasaki disease risk in Chinese population: evidence from a meta-analysis. Cardiol Young. 2018;28:811–5.
[23] Jin X, Yin S, Zhang Y, Chen X. Association between TLR2 + 2477G/A polymorphism and bacterial meningitis: a meta-analysis. Epidemiol Infect. 2018;146:642–7.
[24] Chen X, Jiang M, Zhao RK, Gu GH. Quantitative assessment of the association between ABC polymorphisms and osteosarcoma response: a meta-analysis. Asian Pac J Cancer Prev. 2015;16:4659–64.