Original Article

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Association of IL-33 gene rs16924159 polymorphism and recurrent pregnancy loss in Iranian Azeri women

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Abstract

Background: Recurrent pregnancy loss (RPL) referred to two or more consecutive abortions before 20th week of pregnancy. The imbalance of inflammatory factors such as interleukins (IL) can be a significant factor in the RPL. The aim of this study was to investigate association of interleukin-33 (IL-33) gene rs16924159 polymorphism and RPL in Iranian Azeri women.

Materials and methods: This case-control study consisted of 100 women with RPL as case group and 100 healthy controls with successful delivery. Genomic DNA was extracted from whole blood samples using salting out method. The fragments of the rs16924159 polymorphism were amplified by PCR and the genotyping was performed using DNA sequencing.

Results: The obtained results showed that frequency of GA genotype and G allele of rs16924159 polymorphism in the case group was significantly more than healthy controls ($p = 0.033$).

Conclusions: Generally, we showed that the IL-33 gene rs16924159 polymorphism may play an important role in risk of RPL in the Iranian Azeri women. However, further studies on different races and geographic areas can be useful in identification of effects of rs16924159 polymorphism on RPL.

Keywords: IL-33; polymorphism; recurrent pregnancy loss.

Introduction

Recurrent pregnancy loss (RPL) is a multifactorial event consisting of two or more consecutive abortions before 20 weeks of gestation [1]. RPL is a serious reproductive problem affecting 1–5% of reproductive age woman [2]. The RPL is a heterogeneous condition, which hematological, anatomical, chromosomal, genetic, and endocrinological factors are involved in its pathogenicity [3, 4]. Also, exposure to lead and ethylene oxide are known as the environmental factors of RPL [5, 6]. Furthermore, infectious and immunological factors are involved in RPL [7, 8].

The balance between Th1 and Th2 cytokines is associated with a successful pregnancy [9]. Moreover, reduction of Th1 cytokines during pregnancy is involved in physiological evolution of human fetus [10]. The genetic background is responsible for regulation of cytokines production in human. Therefore, polymorphisms can involve in production rate of various cytokines [11].

The Interleukin 33 (IL-33) gene locus is on chromosome 9 (9p24.1) in human. IL-33 gene encodes a protein consisting 270 amino acids with a helix-turn-helix domain and an IL-1 receptor-like 1 (IL-1RL1) domain to connect and activate the ST2 receptors [12]. This cytokine plays an important role in the regulation of immune system and inflammation processes. IL-33 is further produced by endothelial cells and plays an important role in the activation of Th2 and mast cells [13]. Also, it is a member of the IL-1 family and plays a fundamental role in immune responses and various pathological and physiological processes, such as tissue homeostasis, autoimmune diseases and cancers [14]. The serum levels and production rate of IL-33 significantly increase in women with RPL in compare women with successful pregnancy [15]. This evidence suggested that dysregulation of IL-33 production and its serum levels are an important risk factor in RPL.

So far, association of IL-33 gene rs16924159 polymorphism with risk of RPL is still unclear. In the present

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study, we investigated the association of IL-33 gene rs16924159 polymorphism with RPL in the Iranian Azeri women.

Materials and methods

Patients and sample collection

This case-control study consisted of 100 women with RPL as case group, and 100 healthy controls with successful delivery. The case group was women with 20–45 years old with at least two continuous abortions before 20 weeks of pregnancy, which were classified as idiopathic RPL. The studied patients were with normal karyotypes and uterus structure and without any pregnancy loss related infections. Moreover, age, race, and ethnicity matched women with at least two successful pregnancies, without any endocrinopathies, autoimmune disorder, steroid treatment or inflammation were selected as healthy controls. The information's such as clinical characteristics, lifestyle and demographic were collected using interview and questionnaire from case and control groups. The collected information's included age (year), body mass index-BMI (kg/m^2), age at menarche (year), menopausal status, tobacco smoking, alcohol drinking, age at first delivery (year) and family history (Table 1). In order to prevent the epidemiological bias, all selected women in this study were from East Azerbaijan province of Iran and matched for age and ethnic and were genetically unrelated. The all studied subjects were informed about the study, and signed consent form, as the Declaration of Helsinki ethical standards.

DNA extraction and genotyping

Extraction of genomic DNA was performed from 3 mL blood samples containing EDTA (as coagulation factor) using salting out method. The quantity and quality of extracted DNA was investigated according to OD 260/280 ratio using a Nanodrop instrument, which this ratio between 1.7 and 1.9 was desirable. Also, electrophoresis on 1% agarose gel was carried out in order to confirm the results. The fragments of the rs16924159 polymorphism were amplified by polymerase chain reaction (PCR). The used primer sequences were forward:

5'-AATAAGGGGCTAGTGCAG-3' and reverse: 5'-CCCAGAAATTATCCACAG-3' with 690 bp PCR products. The amplification was performed in 50 μL volumes using 2 μL each primers, 2 μL template DNA, 5 μL dNTP, 5 μL PCR buffer, 1.5 μL MgCl_2 , 0.5 μL Taq DNA polymerase, and 32 μL distilled water. The used PCR condition was as follows: one cycle as initial denaturation (94 °C for 5 min), 35 cycles as denaturation (94 °C for 45 s), 35 cycles as annealing (51 °C for 45 s), 35 cycles as extension (72 °C for 45 s), and one cycle as final extension (72 °C for 5 min). The amplified fragments were separated using electrophoresis on 1.5% agarose gel stained by ethidium bromide. The PCR products were then sequenced using Sanger method. Finally, the obtained sequences were analyzed and compared with the sequence of reference gene.

Statistical analysis

The statistical analysis of obtained data was carried out using statistical package for the social sciences (SPSS) software (version 21.0). The logistic regression was used to analyze association between studied polymorphism and RPL risk. The Hardy-Weinberg equilibrium (HWE) in genotypes distribution of BC patients and healthy controls were analyzed using chi-square (χ^2) test and Fisher's exact test. Also odds ratio (OR) and 95% confidence intervals (CI) were evaluated. The difference of demographic and clinical features between RPL patients and healthy controls were analyzed using independent sample *t*-test. The statistically significant was considered as $p < 0.05$.

Results

The demographic characteristics and clinical features of studied patients and controls are presented in Table 1. The statistical analysis showed that there are no significant differences in mean age, smoking, and blood groups between case and control groups ($p > 0.05$), whereas there are significant differences in BMI between case and control groups ($p = 0.004$).

According to χ^2 tests, the genotype distribution of studied polymorphism was in agreement with the Hardy-Weinberg equilibrium ($p > 0.05$). The distributions of genotype and allele frequencies of studied polymorphism in case and control groups are presented in Table 2. Our study showed an association for rs16924159 polymorphism with RPL.

The genotype frequencies of the IL-33 gene rs16924159 polymorphism were 38.0% (GG), 62.0% (GA), and 0.0% (AA) in case group; whereas were 53.0% (GG), 47.0% (GA), and 0.0% (AA) in the control group (Table 2). The frequencies of GA genotype in the case group was significantly more in compared to the healthy controls ($p = 0.033$). The allele frequencies of the IL-33 gene rs16924159 polymorphism were 69.0% (G) and 31.0% (A) in the case group, whereas were 76.5% (G) and 23.5% (A) in the healthy controls (Table 2). The frequencies of G allele in the case group was significantly more in compared to the healthy controls ($P = 0.0092$).

Table 1. Demographic variables and characteristics of women in the case and control groups.

Characteristic	Case (n = 100)	Control (n = 300)	p-value
Mean age (years)	31.10 $\pm$ 4.67	29.18 $\pm$ 2.21	0.299
Smoking (%)	12 (12%)	19 (19%)	0.494
Mean BMI (kg/m^2)	26.98 $\pm$ 2.38	21.29 $\pm$ 5.18	0.004
Blood groups			
AB	4 (4%)	7 (7%)	0.378
A	64 (64%)	55 (55%)	0.657
B	8 (8%)	9 (9%)	0.146
O	24 (24%)	29 (29%)	0.456

Significant Statistically $p < 0.05$.

BMI: Body Mass Index.

Table 2. Genotype and allele frequencies of rs16924159 polymorphism in the case and control groups.

Polymorphism	Inheritance model	Genotype and Allele	Case (n = 100)	Control (n = 100)	p-value	OR (95% CI)
IL-33 rs16924159	Codominant	GG	38 (38.0%)	53 (53.0%)	Ref	Ref = 1
		GA	62 (62.0%)	47 (47.0%)	0.033	4.537 (0.43–4.77)
		AA	0 (0.0%)	0 (0.0%)	1	1
	Dominant	GG	38 (38.0%)	53 (53.0%)	Ref	Ref = 1
		GA + AA	62 (62.0%)	47 (47.0%)	0.003	3.981 (1.22–2.13)
	Recessive	AA	0 (0.0%)	0 (0.0%)	Ref	Ref = 1
		GA + GG	100 (50%)	100 (50%)	1	1
	Overdominant	GA	62 (62.0%)	47 (47.0%)	Ref	Ref = 1
		GG + AA	38 (38.0%)	53 (53.0%)	0.412	2.823 (0.12–2.11)
	Alleles	G wild	138 (69.0%)	152 (76.5%)	Ref	Ref = 1
		A mutant	62 (31.0%)	46 (23.5%)	0.0092	2.837 (0.36–1.18)

Significant Statistically $p < 0.05$.

OR: Odds Ratio. CI: Confidence Interval.

Discussion

The role of IL-33 polymorphisms in RPL pathogenesis remains unknown. Due to the multifactorial nature of the RPL, various factors investigated to identify cause of this condition, such as coagulation factors, infection factors, immunological factors, anatomical problems, and chromosomal abnormality [7, 16, 17]. This study focused on the rs16924159 polymorphism of IL-33 gene to investigate its association with RPL in Iranian Azeri women.

Previous studies have evaluated the effects of BMI on RPL, with inconsistent results. Some studies reported a significant effect of BMI on women with RPL. In present study, we demonstrated a significant difference between patients with RPL and healthy controls in term of BMI. This result were in agreement with the study of Yue et al. [18], but were in disagreement with the study of Liu et al. [19].

So far, very limited studies have reported to correlation between IL-33 gene polymorphisms and RPL. In a study by Soheilyfar et al., reported that IL-33 polymorphism (rs1929992) was associated with RPL in Iranian women (16). Our study showed an association between IL-33 gene polymorphism (rs16924159) and RPL in Iranian Azeri women. Our study demonstrated for the first time that heterozygous genotype (GA) of rs16924159 polymorphism is a risk factor in RPL in Iranian Azeri women. In a study by Yue et al. (2016) on Chinese women with RPL showed a relationship between rs16924159 polymorphism and RPL and homozygous mutant genotype of rs16924159 was a risk factor in RPL [20].

Differences in the results of mentioned studies can be due to other involved genes, and differences in geographic area, sample size and selection bias, ethnicity and race heterogeneity, and environmental factors [21].

Conclusions

Generally, our study indicated that IL-33 gene rs16924159 polymorphism may be associated with RPL risk in the Iranian Azeri women. However, the exact role and effects of rs16924159 polymorphism in RPL is not fully identified. Therefore, for better understanding association of this polymorphism with RPL, However, further studies on different races and geographic areas with larger sample sizes are recommended to identification of effects of rs16924159 polymorphism on RPL.

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Informed consent: Informed consent has been obtained from all the individuals included in the study.

Ethical approval: The research related to human use complied with all the relevant national regulations and institutional policies, was performed in accordance with the tenets of the Helsinki Declaration and has been approved by the authors institutional review board or equivalent committee.

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