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Association between IL-37 gene variants (rs4241122 and rs2723186) and Graves' disease risk

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Abstract

Introduction: Graves' Disease (GD) is the most common cause of primary hyperthyroidism. It is an autoimmune disorder characterized by the presence of thyrotropin-related antibodies that stimulate thyroidal cells, resulting in an overproduction of thyroid hormones. It has been reported that cytokines might be involved in the GD pathogenesis. So, aim of the present study was to investigate the possible associations between IL-37 Single Nucleotide Polymorphisms (SNPs) and susceptibility to GD.

Method: This case–control study included a total of 600 individuals, 305 GD patients and 295 healthy controls. All participants were genotyped for IL-37 SNP (rs4241122 and rs2723186) using amplification refractory mutation system polymerase chain reaction (ARMS-PCR).

Results: The allelic and genotype frequencies of rs4241122 showed statistically significant association with GD. We found a significantly higher frequency of AG genotype in GD patients than healthy controls [odds ratio (OR) =1.8; 95% Confidence Interval (CI) =1.0-1.3, p=0.04]. Besides, A allele frequency was more frequently detected in healthy controls than patients with a family history of thyroid disease (OR= 1.6, CI= 1.1-2.4, P=0.01). In addition, we found no correlation between rs2723186 and GD.

Conclusion: Our findings indicated that IL-37 gene polymorphisms might represent a genetic risk factor for GD in Iranian population. However, further studies with larger sample size are needed to verify these results.

Keywords: Autoimmune diseases; Graves disease; il-37; Single nucleotide polymorphisms; rs4241122; rs2723186.

Introduction

Graves' Disease (GD) is the most common cause of primary hyperthyroidism [1]. It is an autoimmune disorder characterized by the presence of thyrotropin-related antibodies that stimulate thyroidal cells, resulting in an overproduction of thyroid hormones [2]. The exact etiology of GD is unclear; however, it is believed that a combination of genetic and environmental factors might be involved [3]. Moreover, pre-clinical and clinical studies suggested a critical role of the immune system in the development of GD [4-9]. In addition, it is well recognized that cytokines are involved in the GD pathogenesis [4,9-11]. Cytokines are small molecules synthesized by different cell types and play important roles in physiological and pathological conditions [12-14]. Sufficient evidence demonstrates the putative importance of Interleukin (IL)-1, IL-2, IL-4, IL-6, Interferon Gamma (IFN-γ), Tumor Necrosis Factor Alpha (TNF- α), and IL-17/

IL-23 in the development of GD [15,16]. IL-37, a new IL-1 family member, has received increasing attention in recent years. It has been reported that IL 37 up-regulated in a variety of disorders such as cancer, chronic inflammatory and autoimmune-related disorders [17-19]. Moreover, a growing body of literature demonstrates that IL 37 plays a pivotal role in inhibiting both innate and adaptive immune responses as well as inflammatory microenvironments [20-22]. IL-37 has also been shown to suppress Th1/Th2/Th17 inflammatory mediators [23]. In particular, involvement of IL-37 in the pathogenesis of several autoimmune diseases such as GD has been reported [24]. Of note, several studies indicated the role of IL-37 gene polymorphism in the development of autoimmune diseases [25-28]. So, the aim of the present study was to determine the significance of IL-37 pathway on the pathogenesis of Graves' disease by detecting the possible association between Single Nucleotide Polymorphism (SNP) in IL-37 gene (rs4241122 and rs2723186) and susceptibility to GD in Iranian population.

Materials and methods

Participants

This case-control study was conducted in Iranian population to investigate the association between IL-37 gene polymorphism and Graves' disease. The study population consisted of 305 patients with GD (127 males and 185 females, mean age, 40.43 ± 12.99 years) from Motahhari Clinic of Shiraz and 295 sex- and age-matched healthy subjects (116 males and 179 females, mean age 38.53 ± 12.43 years) without a history of any autoimmune or inflammatory diseases. This study was conducted according to the standards of the declaration of Helsinki [29]. The Ethics Committee of the Shiraz University of Medical Sciences has also approved the research. All participants signed informed consent before their involvement in the study. Table 1 indicates the demographic and clinical characteristics of GD patients.

Table 1: Clinical characteristics of patients with Graves' Disease (GD).

Characteristic	Gravespatientnumber(%)
Number of subjects	305
Age (means ± SD)	40.43 ± 12.99
Sex (male/female, no%)	120(39.34%)/185(60.65%)
Family history of Graves diseases	21(6.88%)
Family history of Thyroid diseases	85(27.76%)
Ophthalmopathy	205(67.21%)

Preparation of blood sample and DNA extraction

Five milliliters of whole blood were obtained from all participants in EDTA-containing tubes. Genomic DNA was extracted through "salting out" method. Before storing in -20˚C, the purity of DNA samples was evaluated by measuring the ratio of maximum absorbance at 260 nm to 280 nm using the spectrophotometer instrument.

Genotype analysis

Two SNPs of the IL-37 gene including rs4241122 and rs2723186 were determined using Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) method. Allele specific ARMS primers and the common primer for each SNP were designed using online Primer3 software version 0.4.0 (http://fokker.wi.mit.edu/primer3/input.htm). Forward and reverse primers were as follows. Forward sense primer A (5'-CAGGCTCTAGACTGACTCCA-3') that amplify allele A and primer G (5'- CAGGCTCTAGACTGACTCCG-3') that amplify allele G. The common antisense primer (5'- TCAAACTATCAACATCAAGGCACA-3') was added in both reactions, which amplify allele-specific sequences of 355 bp for rs4241122 and Forward sense primer A (5'- GAAGAGGAGGCTTAAACCA-3') that amplify allele A and primer G (5'-AGAAGAGGAGGCTTAAACCG3') that amplify allele G. The common antisense primer (5'-GCTGAAGGGATGGATGACTTT-3') was added in both reactions, which amplify allele-specific sequences of 289 bp for rs2723186. PCR was done using 250 ng/µl DNA (5 µl), 3.0 µM MgCl2, 50 µmol dNTPs, 0.7 pg/µl of each primer, 0.1unit of Taq DNA polymerase (Fermentas, Lithuania) under the following conditions: initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation (94°C, 30 sec), annealing (61°C, 30 sec), extension (72°C, 30 sec), and a final extension at 72°C for 5 min. The amplified products were separated using electrophoresis on a 2% agarose gel stained with a safe stain. The gel was visualized under a UV transilluminator with a 50-1500 bp ladder (Figure 1).

Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 26, Graph Pad Prism (La Jolla, CA, USA) version 8.0.2, and Epi Info version 7.2.2.6 software packages were used for data analysis. The normal distribution of variables was evaluated using the Kolmogorov-Smirnov/Shapiro-Wilk's test. The two-tailed Pearson's chi-square (χ2) test also was used to compare the genotype and allele frequencies between studied groups. P values equal to or less than 0.05 were considered significant.

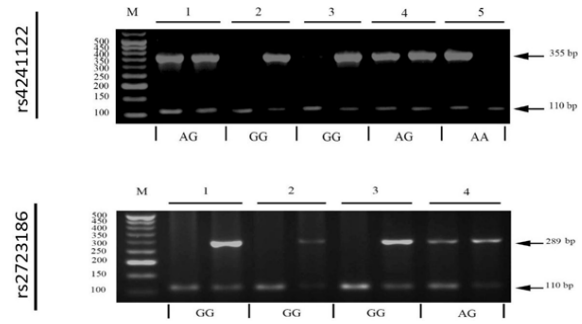

Figure 1: Representative picture showing PCR amplification fragments of SNP in IL-37 gene variants (rs4241122 and rs2723186) run on an agarose gel. bp; base pair, M; 50 bp DNA molecular weight marker, SNP; single-nucleotide polymorphism, PCR; polymerase chain reaction.

Results

Genotype and allele frequencies of il-37 rs4241122 polymorphism

The genotype distribution of rs4241122 in GD patients and controls were in Hardy-Weinberg equilibrium. A logistic regression model was used to investigate the association between genotype and allele frequencies and the risk of GD. Evaluation of the genotype distribution of rs4241122 among patients

showed that 42.6% of cases had AA, 50.6% had AG and 6.9% had GG genotypes. The frequencies of genotypes AA, AG and GG in the control group were 47.5%, 42.0% and 10.5%, respectively. As shown in Table 2, genotype analysis of rs4241122 showed that the AG genotype had a higher frequency in patients than healthy controls (OR = 1.83, 95% Confidence Interval (CI) = 1.0–1.3, p= 0.04). Of note, we found no association between rs4241122 and GD after gender stratification (data are not shown).

Genotype and allele frequencies of IL-37 rs2723186 polymorphism

The genotype distribution of rs2723186 in GD patients and controls were in Hardy-Weinberg equilibrium. A logistic regression model was used to investigate the association between genotype and allele frequencies and the risk of GD. Evaluation of the genotype distribution of rs2723186 among patients showed that 91.8% of cases had GG, 8.2% had AG and 0.0% had AA genotypes. The frequencies of genotypes GG, AG and AA in the control group were 95.2%, 4.8% and 0.0%, respectively. On the other hand, the allele frequencies among the patients were 95.5% and 4.1% for alleles G and A, respectively. Healthy controls had 97.6% allele G and 2.4% allele A. As shown in table 2, no significant difference was observed between studied groups. Of note, due to the lack of AA genotype and low frequency of AG genotype, we were not able to determined correlation between rs2723186 and clinical manifestation.

Association between rs4241122 polymorphisms and GD clinical manifestations

Evaluation of the clinical manifestations of GD among patients with IL-37 polymorphism revealed significant statistical associations between rs4241122 and family history of thyroid (Table 3). The A allele frequency was significantly higher in healthy controls compared to patients with a family history of thyroid disease (OR= 1.6, CI= 1.1-2.4, P=0.01). Furthermore, analysis of rs4241122 polymorphism showed that opthalmopathy manifestation is more frequently seen in GD patients with AG genotype (OR= 2.2, CI= 0.8-5.4, P=0.08), however this was not statistically significant.

Table 2: Genotype and allele frequencies of IL-37 rs4241122 and rs2723186 polymorphism in GD patients and healthy controls.

IL-37 SNPs		Patients (n=305)	Controls (n=295)	OR (95%CI)	p-value
rs4241122	Genotypes				
	GG	21(6.9)	31(10.5)	1.00(reference)	
	AG	154(50.6)	124(42.0)	1.83(1.0-1.3)	0.04
	AA	130(42.6)	140(47.5)	1.37(0.7-2.5)	0.30
	Alleles				
	G	196(32.13)	186(31.5)	1.00(reference)	
	A	414(67.87)	404(68.4)	0.97(0.7-1.2)	0.82
	Genotype combinations				
	GG	21(6.9)	31(10.5)	1.00(reference)	
	AA+AG	284(93.1)	264(89.5)	1.58(0.8-2.8)	0.11
	AA	130(42.6)	140(47.5)	1.00(reference)	
	GG+AG	175(57.4)	155(62.5)	1.21(0.8-1.6)	0.23
rs2723186	Genotypes				
	GG	280(91.8)	281(95.2)	1.00(reference)	
	AG	25(8.2)	14(4.8)	0.5(0.2–1.09)	0.09
	AA	0	0	-	-
	Alleles				
	G	585(95.9)	576(97.6)	1.00(reference)	
	A	25(4.1)	14(2.4)	0.5(0.2–1.1)	0.10

OR, Odds ratio; CI, confidence interval. Data are presented as number (%).

Discussion

In this study, we examined rs4241122 and rs2723186 in IL-37 gene for its association with Graves' disease in Iranian population. We found no correlation between rs2723186 and GD susceptibility, while the allelic and genotype frequencies of rs4241122 showed a statistically significant association with GD. We found a significantly higher frequency of AG genotype in GD patients than healthy controls. Our study also highlights that the A allele has a protective role in a family history of thyroid disease.

Previous studies acknowledged the predominant anti-inflammatory properties of IL-37 [30]. In fact, It is thought that IL-37 acts as a natural suppressor of innate and adaptive immunity, so any changes either in protein or mRNA levels might be involved in the development of autoimmune related disorders such as Rheumatoid Arthritis (RA), Multiple Sclerosis (MS), Type 1 Diabetes (T1D) and GD [24,31-33]. It also has been reported that specific genotypes might be involved in the severity and clinical features of the disease. A significant association between IL-37 polymorphisms (rs3811046, rs3811047, rs2723176, rs272186) and susceptibility to GD has been reported in Chinese population [34]. Ni Yan et al. demonstrated that the A allele of rs2723176, rs2723186, rs3811047 and G allele

Table 3: Genotype and allele frequencies of IL-37 rs4241122 and rs2723186 polymorphism in GD patients and healthy controls.

rs4241122	Family history of Graves (Yes)	Family history of Graves (No)	OR (95%CI)	p-value
Genotypes				
GG	1(3.7)	20(7.04)	1.00(reference)	
AG	7(33.3)	147(51.7)	0.95 (0.11-8.14)	0.96
AA	13(61.9)	117(41.1)	2.22 (0.27-17.94)	0.45
Alleles				
G	9(20.9)	187(32.9)	1.00(reference)	
A	34(79.06)	381(67.07)	1.85 (0.87-3.94)	0.1
	Family history of Thyroid (Yes)	**Family history of Thyroid (No)**	**OR (95%CI)**	**p-value**
Genotypes				
GG	6(7.05)	15(6.81)	1.00(reference)	
AG	30(35.2)	124(56.3)	0.60 (0.21-1.68)	0.33
AA	49(57.6)	81(36.8)	1.5 (0.55-4.15)	0.42
Alleles				
G	42(24.7)	154(35.0)	1.00(reference)	
	128(75.2)	286(65.0)	1.6 (1.1-2.44)	0.01
	Opthalmopathy (Yes)	Opthalmopathy(No)	OR(95%CI)	p-value
Genotypes				
GG	11(5.36)	11(10.8)	1.00(reference)	
AG	106(51.7)	48(47.5)	2.2 (0.89-5.44)	0.08
AA	88(42.9)	42(41.5)	2.09 (0.84-5.22)	0.11
Alleles				
G	128(31.2)	70(34.6)	1.00(reference)	
A	282(68.7)	132(65.3)	1.16 (0.81-1.67)	0.39

OR, Odds ratio; CI, confidence interval. Data are presented as number (%).

of rs3811046 have a protective role in GD [34]. In contrast we found no correlation between rs2723186 and GD. Of note, we could not detect AA genotype in patients and controls. Overall, based on previous studies it seems that the AA genotype frequency is relatively low [28,35] that might be responsible for these contrasting results. Interestingly, in our previous study in Behcet's disease also we were not able to detect AA genotype in Iranian population [36]. These observation might simply indicate the differences in ethnicity, clinical heterogeneity or sample size and probably further studies with larger sample size could address this issue.

The present study also analyzed the association between rs4241122 and GD susceptibility. The results showed the correlation between rs4241122 AG genotype and GD. To the best of our knowledge this study reports a significant association between rs4241122 and GD for the first time. Indeed, few studies have investigated the role of IL-37 rs4241122 in autoimmune-related disorders. Zhang et al. found no correlation between IL-37 SNPs (rs2723186, rs3811046, rs4241122, rs4364030, rs4392270) and susceptibility to RA [37]. GholiJani et al. also did not find any association between IL-37 rs4241122 and Behcet's disease [36]. These discrepancies between studies mainly reflect the nature of the disease and also might point out a pathogenetic role of IL37 rs4241122 in GD. To rule out a significant pathogenic role of IL-37 gene in GD, further studies with larger sample size and other ethnic populations are recommended. Besides studying other SNPs in IL-37 gene might represent valuable results and also might be helpful in determining the exact role of IL-37 in the GD pathogenesis.

Conclusion

In summary, the present study demonstrates that IL-37 (rs4241122) polymorphism might contribute to the increased risk of graves in an Iranian population. However, to confirm the possible role of IL-37 in the GD development further studies with larger sample size and other ethnic populations are recommended.

Statements and declarations

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Conflict of interest: The authors declare no conflict of interest.

Ethical approval and informed consent to participate and publication: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation set by the committee of Ethical Standards of shiraz University, shiraz-Iran. Informed consent for it was obtained from all patients for being included in the study and for the data to be published. All patients and healthy participants provided written, informed consent to participate and for the publication of data in this study. This experiment was carried out in compliance with the Declaration of Helsinki.

Author contributions: N. G: Conceptualized the study, experimental design, interpretation of the data, final approval of the manuscript; G. D: Conceptualized the study, experimental design, interpretation of the data, final approval of the manuscript; M. M: data collection, performed the analysis and drafted the manuscript, final approval of the manuscript; FR. K: Conceptualized the study, experimental design, interpretation of the data, final approval of the manuscript, revising the manuscript and overall supervision.

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