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ORIGINAL ARTICLE

Graves' disease and gene polymorphism of TNF-α, IL-2, IL-6, IL-12, and IFN-γ

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Abstract The role of genetic factors in the pathogenesis of Graves' disease (GD) is not clear. The purpose of this study was to investigate the association between single nucleotide polymorphisms in pro-inflammatory cytokine genes and GD in Iranian patients. A case–control hospital-based study was carried out on 107 GD patients and 140 healthy controls. Cytokine typing was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay. The allele and genotype frequencies of the following cytokine genes were determined: TNF-α (−308A/G, −238A/G), IL-2 (−330T/G, +166G/T), IL-6 (−174C/G, A/G nt565), IL-12 (−1188A/C), and IFN-γ (UTR 5644A/T). The following alleles and genotypes were significantly overrepresented in patients: TNF-α −308A allele ($P < 0.01$) and AA genotype ($P < 0.05$), IL-2 −330G allele ($P < 0.01$) and GG genotype ($P < 0.01$), IL-6 −174C allele ($P < 0.01$) and CC genotype ($P < 0.01$), IL-12 −1188C allele ($P < 0.01$) and CC genotype ($P < 0.01$), IFN-γ UTR5644T allele ($P < 0.01$) and TT genotype ($P < 0.01$). In conclusion, this is the first study to show a significant association between GD and IL-2 −330G, IL-12 −1188C, and IFN-γ UTR 5644T alleles. Our results support the hypothesis that polymorphism in pro-inflammatory cytokines might be involved in predisposition to GD.

Keywords Interleukin · Gene · Grave's disease · Polymorphism

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Introduction

Graves' disease (GD) is a common autoimmune cause of hyperthyroidism, which is characterized by diffuse goiter, thyrotoxicosis, and some organ-specific manifestations including Graves' ophthalmopathy and Graves' dermopathy. Although the production of thyroid-stimulating hormone (TSH) receptor antibodies is thought to be a crucial underlying etiology for this immunological process, the exact etiology of GD is still unknown [1]. Genetic predisposition is most likely due to a complex interaction between the several genetic changes and environmental factors [2]. Recent studies have proposed the role of genetic factors to account for 80% of predisposition [3].

However, among potential genetic factors, only the human leukocyte antigen class II genes and the cytotoxic T lymphocyte associated gene have been consistently reported to be involved, with a contribution of only 5% to all genetic aspects of this disease [4–7]. Further studies are necessary to elucidate the role of other contributing genes.

Pro-inflammatory cytokines are a large group of soluble protein hormones, which mediate multiple inflammatory, immunological processes. Cytokines such as IL-2, IL-6, IL-12, IFN-γ, and TNF-α are produced by the intra-thyroidal inflammatory cells and thyroidal follicular cells during the activation of local inflammation and autoimmune thyroid reactions [4, 8]. Therefore, the genetic factors affecting induction or inhibition of these cytokines are potential candidates for susceptibility to GD as an autoimmune disorder. Studies regarding the association between single nucleotide polymorphisms (SNPs) in these cytokines and autoimmune reactions, particularly in the thyroid gland, are limited. Some studies have suggested that the regulation of TNF-α production by macrophages and T lymphocytes is influenced by SNPs in autoimmune diseases, such as rheumatoid arthritis [9], systemic lupus erythematosus [10], and Crohn's disease [11], whilst there are remarkable inconsistencies in GD [3]. Additionally, association of certain polymorphisms in the IL-6 gene with elevation of its concentrations in the blood and development of autoimmune reactions of GD and juvenile rheumatic arthritis is reported in some studies [4, 8, 12]. A few studies have investigated the association of SNPs in IL-2, IL-12, and IFN-γ genes with GD [4, 8].

Genotype–phenotype association studies are of high importance in understanding the contribution of genetic mechanisms to the immunomodulatory role of pro-inflammatory cytokines. In a recent study, we showed that ophthalmologic manifestations of GD are associated with specific gene polymorphisms in pro-inflammatory cytokines [13]. In this study, we attempted to evaluate the association of SNPs in a number of important pro-inflammatory cytokines, i.e. IL-2, IL-6, IL-12, IFN-γ, and TNF-α, with GD in a case–control study of Iranian participants.

Results

Out of 107 patients, 36 patients (33.6%) had a family history of GD, and three (2.8%) had a family history of other autoimmune diseases (Hashimoto thyroiditis and type 1 diabetes). Patients did not have any autoimmune diseases other than GD. There was no significant difference between the groups in age or sex. The mean (SEM) age of onset and duration of GD were 29.07 $\pm$ 0.87 and 4.12 $\pm$ 0.52 years, respectively.

Full details of the association between GD and different alleles and genotypes are presented in Tables 1 and 2. For TNF-α, the frequency of the -308A allele ($P < 0.01$) and the AA genotype ($P < 0.05$) was significantly higher in patients than controls. For IL-2, the -330G allele ($P < 0.01$) and the GG genotype ($P < 0.01$) were significantly more common in patients than controls. For IL-6, the -174C allele ($P < 0.01$) and the CC genotype

Table 1 Association of eight polymorphisms with Graves' disease

Cytokine	Position	Alleles	Ctrl ($n = 140$) *N* (%)	GD ($n = 107$) *N* (%)	OR (95% CI)
TNF-α	-308	G	235 (85.8)	156 (74.3)	1
		A	39 (14.2)	54 (25.7)	2.09 (1.32–3.30)**
TNF-α	-238	G	215 (78.5)	181 (84.6)	1
		A	59 (21.5)	33 (15.4)	0.66 (0.42–1.06)
IL-2	-330	T	168 (60.4)	96 (44.9)	1
		G	110 (39.6)	118 (55.1)	1.88 (1.31–2.69)**
IL-2	+166	G	219 (78.8)	155 (72.4)	1
		T	59 (21.2)	59 (27.6)	1.41(0.93–2.14)
IL-6	-174	G	177 (63.7)	97 (45.3)	1
		C	101 (36.3)	117 (54.7)	2.11 (1.47–3.04)**
IL-6	nt 565	G	228 (82)	162 (75.7)	1
		A	50 (18)	52 (25.2)	1.46 (0.95–2.27)
IL-12	-1188	A	204 (72.9)	121 (56.5)	1
		C	76 (27.1)	93 (43.5)	2.06 (1.41–3.01)**
IFN-γ	UTR 5644	A	140 (50.7)	71 (33.2)	1
		T	136 (49.3)	143 (66.8)	2.07 (1.43–3.00)**

** $P < 0.01$

Table 2 Association of six polymorphisms and Graves' disease

Cytokine	Position	Genotypes	Ctrl ($n = 140$) N (%)	GD ($n = 107$) N (%)	OR (95% CI)
TNF-α	−308	GG	98 (71.5)	56 (53.3)	1
		AG	39 (28.5)	44 (41.9)	1.97 (1.15–3.40)
		AA	0 (0)	5 (4.8)	19.18 (1.04–353.5)*
TNF-α	−238	GG	79 (57.7)	74 (69.2)	1
		AG	57 (41.6)	33 (30.8)	0.62 (0.36–1.05)
		AA	1 (0.7)	0 (0.0)	0.36 (0.01–8.88)
IL-2	−330	TT	37 (26.6)	17 (15.9)	1
		TG	94 (67.6)	62 (57.9)	1.44 (0.74–2.77)
		GG	8 (5.8)	28 (26.2)	7.62 (2.88–20.17)**
IL-2	+166	GG	82 (59)	53 (49.5)	1
		TG	55 (39.6)	49 (45.8)	1.38 (0.82–2.31)
		TT	2 (1.4)	5 (4.7)	3.87 (0.72–20.68)
IL-6	−174	GG	42 (30.2)	17 (15.9)	1
		CG	93 (66.9)	63 (58.9)	1.67 (0.88–3.20)
		CC	4 (2.9)	27 (25.2)	16.68 (5.06–54.93)**
IL-6	nt 565	GG	93 (66.9)	61 (57.0)	1
		AG	42 (30.2)	40 (37.4)	1.45 (0.85–2.49)
		AA	4 (2.9)	6 (5.6)	2.29 (0.62–8.44)
IL-12	−1188	AA	72 (51.4)	34 (31.8)	1
		AC	60 (42.9)	53 (49.5)	1.87 (1.08–3.2)
		CC	8 (5.7)	20 (18.7)	5.29 (2.12–13.23)**
IFN-γ	UTR 5644	AA	43 (31.2)	15 (14.0)	1
		AT	54 (39.1)	41 (38.3)	2.18 (1.07–4.45)
		TT	41 (29.7)	51 (47.7)	3.57 (1.74–7.31)**

* $P < 0.05$; ** $P < 0.01$

($P < 0.01$) were significantly more common in patients than controls. For IL-12, the −1188C allele ($P < 0.01$) and the CC genotype ($P < 0.01$) were significantly more common among patients than controls. For IFN-γ, the UTR 5644T allele ($P < 0.01$) and the TT genotype ($P < 0.01$) were significantly more frequent in patient than controls. The results of haplotypic analysis are shown in Table 3.

Discussion

Polymorphisms in pro-inflammatory cytokine genes may affect the susceptibility to the autoimmune thyroid dysfunctions, particularly GD. We focused on five important pro-inflammatory cytokines in this study. Regarding TNF-α gene polymorphisms, the association between GD and −238A [14, 15], −308A [14, 16], −1031C [17], and −863A [17, 18] alleles has been shown. The increased frequency of the −308A allele and the AA genotype in Iranian patients is in agreement with previous studies. One cannot, however, rule out the possibility that the observed association has actually been due to an association between GD and the HLA locus, given that the locus for HLA (6p21.3) might be in linkage disequilibrium with the TNF-α locus. The −238A/G polymorphism did not have a significant association with GD in our series. One reason for this inconsistency with previous reports may be related to ethnic differences, as we have previously demonstrated for other cytokine gene polymorphisms [19]. Sample size difference between studies is another potential explanation.

To the best of our knowledge, the present study is the first to demonstrate a significant association between IL-2 polymorphisms and GD. A significant epistatic interaction between IL-2 (330T/G) and systemic sclerosis has recently been reported [20]. Our data revealed significantly increased frequencies of the G allele and the GG genotype. Our study was also the first to evaluate the role of +166G/T polymorphisms in GD. We did not find a significant association regarding this SNP.

Polymorphisms in the IL-6 gene promoter region have been associated with the development of carotid atherosclerosis, multiple myeloma, and juvenile arthritis [8, 21–23]. Considering its important role in growth and differentiation of lymphocytes, IL-6 might contribute to the promotion of thyroid receptor antibody synthesis during the course of GD [8, 24, 25]. The observation that the −174C allele and the CC genotype had higher frequencies in our patients is in contrast with a previous study in Polish-

Table 3 The results of haplotypic analysis

Cytokine	Haplotypes	Control ($n = 140$) N (%)	Patient ($n = 107$) N (%)	OR (95% CI)
TNF-α (−308, −238)	GG	176 (64.2)	138 (65.7)	1.07 (0.73–1.56)
	AG	39 (14.2)	42 (20.0)	1.51(0.93–2.43)
	GA	59 (21.5)	18 (8.6)	0.34 (0.19–0.60)**
	AA	0 (0)	12 (5.7)	34.57 (2.03–587.77)**
IL-2 (−330, +166)	TG	112 (40.6)	54 (25.2)	0.49 (0.33–0.73)**
	GG	107 (38.7)	101 (47.2)	1.41 (0.98–2.03)
	GT	1 (0.4)	17 (7.9)	23.73 (3.13–179.89)**
	TT	56 (20.3)	42 (19.6)	0.96 (0.61–1.50)
IL-6 (−174, nt 565)	GG	173 (62.2)	95 (44.4)	0.48 (0.34–0.70)**
	CG	55 (19.8)	67 (31.3)	1.85 (1.22–2.79)**
	GA	4 (1.4)	2 (0.9)	0.65 (0.12–3.56)
	CA	46 (16.6)	50 (23.4)	1.54 (0.98–2.41)

** $P < 0.01$

Caucasian patients [26]. There was no significant association between A/G nt565 and GD in our study. We showed a significant increase in IL-12 −1188C allele and the CC genotype in patients with GD. This association does not seem to be present in Japanese [27, 28] and European [29] populations. Our results regarding the association between the C allele and GD are not surprising given that IL-12 levels are elevated in the hyperthyroid phase of GD [27–29].

IFN-γ has a critical role in enhancing the expression HLA class I, class II, and some adhesion molecules on thyrocytes, including intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3). Polymorphisms in the IFN-γ gene may contribute to the autoimmune response in pathogenesis of GD. In particular, the +874A/T polymorphism has been associated with GD in the Japanese population [30]. The UTR 5644T allele had a higher frequency in patients with GD than in controls in our study. This association has not been shown in previous work. However, we cannot eliminate the possibility that our locus is in linkage disequilibrium with +874A/T.

The results of this study support the idea that pro-inflammatory cytokines might be involved in predisposition to and development of GD. We revealed new associations, which need to be further investigated in other populations. Haplotypic analysis, as carried out in this study on a limited number of positions, can provide additional insights. A better picture of genetic contributions to susceptibility to autoimmune conditions would certainly help improve our understanding of the pathophysiology of diseases such as GD. Although our study has enough statistical power to show many significant associations, our non-significant findings need to be confirmed in future studies with larger samples.

Patients and methods

Participants

This case–control hospital-based study was conducted from February 2005 to September 2008 and comprised a total of 107 unrelated Iranian patients (33 males), diagnosed with GD and 140 healthy controls. The patients were enrolled from the outpatient endocrine clinic of a large university general hospital. Controls were subjects who had no clinical evidence or family history of any type of autoimmune disorders and were selected from the healthy staffs. Patients and controls were all residents of Tehran. The study was approved by the local ethics committee of Tehran University of Medical Sciences. Informed consent was obtained from all participants. GD was diagnosed by an endocrinologist with substantial experience in thyroid diseases. Diagnosis was based on suggestive history, compatible physical examination, and confirmatory laboratory tests including sensitive TSH, free T4, total T3, and anti-thyroglobulin antibody. 24-h radioactive iodine uptake was used to exclude thyrotoxicosis not caused by hyperthyroidism.

DNA analysis

Cytokine typing was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany). Briefly, amplification was carried out using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. After electrophoresis, the gel was placed on a UV transilluminator and a picture for interpretation and documentation was taken. Each of the

primer mixes contained a control primer pair that amplified either a part of the β-globin gene or a part of the C-reactive protein gene. The β-globin control primers produce a 89-bp fragment, while the primer pairs amplifying the CRP gene produced a 440-bp amplicon. The allele and genotype frequencies of the following cytokine genes were determined: TNF-α ($-$308A/G, $-$238A/G), IL-2 ($-$330T/G, +166G/T), IL-6 ($-$174C/G, A/G nt565), IL-12 ($-$1188A/C), and IFN-γ (UTR 5644A/T).

Statistical analysis

The statistical package SPSS 16 (Chicago, IL, USA) was used for analysis. Continuous variables are expressed as mean $\pm$ standard error of mean (SEM). The required sample size for this study, using $\alpha = 0.05$, power $= 0.80$, and OR $= 2.16$ (for the $-$308A vs. $-$308G allele [14]), was calculated to be 101 patients. Prevalence of genotypes and alleles were determined in each group and the corresponding odds ratios (OR) with 95% confidence intervals (95% CI) were calculated. For determining ORs, the following alleles (and their corresponding homozygous genotypes) were considered as the reference category: TNF-α ($-$308G, $-$238G), IL-2 ($-$330T, +166G), IL-6 ($-$174G, G nt565), IL-12 ($-$1188A), and IFN-γ (UTR 5644A). Two-sided *P*-values were determined by the chi-squared or Fisher's exact tests as appropriate. *P*-values were corrected using the Bonferroni method for multiple testing. A P_c-value smaller than 0.05 was considered statistically significant.

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