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ORIGINAL ARTICLE

An association of interleukin-10 gene polymorphisms with Graves' disease in two Chinese populations

Nan Liu · Hao Lu · Feng Tao · Ting Guo · Changqin Liu · Bin Cui · Guang Ning

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Abstract Graves' disease (GD) is a common autoimmune disorder with a genetic predisposition. The cytokine interleukin-10 (IL-10) has a central role in mediating inflammation, which may affect the outcome of the patients with GD. To elucidate the impact of *IL-10* gene polymorphisms, we performed a two-stage case–control association study of five single-nucleotide polymorphisms (SNPs) within the *IL-10* gene as well as a meta-analysis of two SNP's rs1800896 and rs1800872 covering three previous studies from Iran, Taiwan, and the United Kingdom. The five SNPs were genotyped by SNPstream Genotyping and Taqman PCR. There was a significant increase of G allele of rs1800896 in the two cohorts ($P_{\text{allele}} = 2.6 \times 10^{-4}$ and 0.0082 for cohort Shanghai and Xiamen, respectively) compared with the controls. The meta-analysis showed the risk-increasing effects for the G allele of rs1800896 in GD (OR = 1.88; $P < 0.00001$). The allele and haplotype analysis results suggested that the polymorphisms of IL-10 were associated with GD susceptibility in the Chinese population.

Keywords Interleukin-10(IL-10) · Graves' disease (GD) · Single-nucleotide polymorphism (SNP)

Introduction

Graves' disease (GD) is an autoimmune disorder occurring primarily in women, with an incidence of approximately 0.25–1.09% in the Chinese population [1]. It is characterized by the presence of anti-thyroid stimulating hormone receptor antibodies leading to hyperthyroidism, diffuse goiter, and Graves' ophthalmopathy and Graves' dermopathy [2]. Data from large familial clustering and twin studies suggested that about 80% of the susceptibility to GD may be due to genetic factors with the remainder influenced by environmental or other factors [3]. Many genetic studies on GD have been carried out, and several immune-regulatory candidate genes, such as human leukocyte antigen, cytotoxic T-lymphocyte associated antigen 4 and interleukin-8, have been linked to GD susceptibility [4–6].

The pathogenesis of GD was thought to be mediated by $CD4^+$ T lymphocytes, which orchestrate inflammation and tissue remodeling through the expression and release of cytokines. The cytokine interleukin-10 (IL-10) has anti-inflammatory and immunosuppressive effects by decreasing the production of pro-inflammatory mediators, T cell stimulation and inducing T cell energy. As a T-cell helper type 2 cell-derived cytokine, IL-10 has also been shown to

Nan Liu and Hao Lu contributed equally to this study.

N. Liu · T. Guo · B. Cui · G. Ning
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai JiaoTong University School of Medicine, 197 RuiJin Er Lu, Shanghai 200025, People's Republic of China

H. Lu · F. Tao
Department of Diabetes Centre, Shuguang Hospital, Affiliated with Shanghai Traditional Chinese Medicine University, 528 ZhangHeng Road, Shanghai 201203, People's Republic of China

C. Liu
Xiamen Diabetes Institute and Department of Endocrinology and Metabolism, The First Hospital of Xiamen Affiliated to the Fujian Medical University, Xiamen 361003, People's Republic of China

B. Cui (✉)
225 South ChongQing Road, Shanghai 200025, People's Republic of China
e-mail: bcui@sibs.ac.cn

inhibit the secretion of T-cell helper type 1 cell-derived cytokines. IL-10 has also been shown to regulate the differentiation and proliferation of several immune cells [7]. A role for IL-10 in GD has been indicated by recent reports of increased levels of serum IL-10 and IgG3-secreting cells in the patients with intractable GD [8]. The gene encoding IL-10 is located at Chr.1q32.2. A number of previous studies have identified a significant association between autoimmune diseases and three of the most characterized single-nucleotide polymorphisms (SNPs) in the promoter region of *IL-10*, positions −1082 A/G (rs1800896), −819 T/C (rs1800871), and absolute-link −592 A/C (rs1800872) [9].

Several GD genetic association studies provided some suggestive evidence for a link between the polymorphisms of IL-10 and GD. The rs1800896 (A-1082G) and rs1800872 (A-592C) polymorphisms have been revealed to may be genetic risk factors for GD [9].To investigate the relationship between the *IL-10* gene and susceptibility to GD, we performed two independent case–control association studies with Chinese GD patients targeting SNP genotyping and haplotype analysis. We also performed a meta-analysis combining current and previously reported data to evaluate the effect of the two polymorphisms on the patient susceptibility to GD.

Materials and methods

Subjects

Two independent cohorts of GD cases and controls were included in this study. Our initial cohort consisted of 727 (180 males and 547 females aged 10–81 years with a mean age of 37.9 ± 14.2 years) cases and 701 (175 males and 526 females aged 17–85 years with a mean age 40.1 ± 15.3 years) controls recruited in Ruijin Hospital from Shanghai in the eastern region of China. The follow-up replication cohort consisted of 376 cases and 318 gender-matched control individuals collected from Xiamen Island in the southern part of China. All GD patients were diagnosed on the basis of the clinical evidence of hyperthyroidism, diffuse goiter investigated by ultrasound, and thyroid eye disease, and the biochemical criteria of hyperthyroidism [TSH < 0.05 mIU/l; free T3 > 6.49 pmol/l (normal range: 2.62–6.49 pmol/l) and/or free T4 > 19.04 pmol/l (normal range: 9.01–19.04 pmol/l), Abbott Laboratories, USA and the presence of TSHR antibodies (normal range: <5.0 U/l)] [10]. Healthy volunteers from the same region without a family history of GD or other autoimmune diseases served as controls. Informed consent was obtained from all participants, and the study was approved by the Institutional Ethics Board of School of Medicine, Shanghai Jiao Tong University.

SNPs selection and genotyping

For the selection of tag-SNPs, Haploview software (http://www.broad.mit.edu/mpg/haploview) was applied to conduct linkage disequilibrium (LD) and haplotype block analyses using HapMap genotype data for the chromosomal region 1: 205, 004, 571-205, 015, 462 [CHB database, HapMap release 27 (Feb 2009)] [11]. The region of interest was an approximately 11-kb region, which included the *IL-10* gene and approximately 3 kb upstream and 3 kb downstream of the gene. Any marker that was not eventually chosen as a tagging marker was considered to be strongly correlated with at least one of the tagging markers with $r^2 > 0.8$. Two SNPs (rs1800896 and rs1800872) located at position −1082 and −592 in the promoter region were designated as tag-SNPs because of previous reports. Three other tag-SNPs were chosen from HapMap SNPs with minor allele frequencies >5% (rs3790622, rs3021094, and rs3024496). With these five tag-SNPs, we were able to capture all the SNPs of the HapMap phase CHB common variation in the *IL-10* gene.

Genomic DNA from peripheral blood of the subjects was extracted using a commercially available kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The five selected SNPs were genotyped using GenomeLab SNPstream 12-plex Genotyping System (Beckman & Coulter Inc. Fullerton, CA) and Taqman PCR (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The clinical data were expressed as means ± SD. The Hardy–Weinberg Equilibrium was performed using Excel (Microsoft Office Excel, Microsoft Corp., Redmond, WA). Genotype and allele frequencies for individual polymorphisms were compared for statistically significant differences between GD and control groups using the χ^2 test and Fisher's exact test. The associations of haplotype and frequencies with GD were estimated using SNPStats Program software (http://bioinfo.iconcologia.net/snpstats/start.htm) [12]. Meta-analysis was performed using Review Manager Software (version 4.2, the Cochrane Collaboration, Oxford, England; http://www.cc-ims.net/RevMan/).

Results

Single variant association

The observed genotype distributions in the GD patients and controls were in accordance with Hardy–Weinberg equilibrium. Table 1 shows the full genotype and allele frequency data of the two cohorts of GD patients and normal

Table 1 Association of the *IL-10* gene polymorphisms with GD

Snp rs number		Shanghai cohort			Xiamen cohort		
		GD ($n = 727$)	Control ($n = 701$)	P value[a] [OR (95% CI)]	GD ($n = 376$)	Control ($n = 318$)	P value[a] [OR (95% CI)]
rs1800896							
Genotype	AA	608 (83.6)	629 (89.7)	**0.0016**	318 (84.6)	290 (91.2)	**0.023**
	AG	109 (15)	69 (9.8)		54 (14.4)	27 (8.5)	
	GG	10 (1.4)	3 (0.4)		4 (1.1)	1 (0.3)	
Allele	A	1325 (91)	1327 (95)	**2.6×10^{-4}**	690 (92)	607 (95)	**0.0057**
	G	129 (9)	75 (5)	**1.72 (1.28–2.31)**	62 (8)	29 (5)	**1.88 (1.19–2.9)**
rs1800872							
Genotype	AA	321 (44.2)	299 (43)	0.58	169 (45)	133 (41.8)	0.49
	AC	326 (45)	310 (44.5)		163 (43.4)	152 (47.8)	
	CC	78 (10.8)	87 (12.5)		44 (11.7)	33 (10.4)	
Allele	A	968 (67)	908 (65)	0.389	501 (67)	418 (66)	0.724
	C	482 (33)	484 (35)	0.93 (0.80–1.09)	251 (33)	218 (34)	0.96 (0.77–1.20)
rs3790622							
Genotype	CC	633 (87.1)	588 (84.4)	0.33	331 (88)	269 (84.6)	0.34
	TC	92 (12.7)	106 (15.2)		43 (11.4)	48 (15.1)	
	TT	2 (0.3)	2 (0.4)		2 (0.5)	1 (0.3)	
Allele	C	1358 (93)	1282 (92)	0.142	705 (94)	585 (92)	0.241
	T	96 (7)	112 (8)	0.81 (0.61–1.07)	47 (6)	50 (8)	0.78 (0.52–1.18)
rs3021094							
Genotype	AA	231 (31.8)	194 (27.7)	**0.0082**	113 (30.1)	77 (24.2)	0.074
	AC	375 (51.6)	346 (49.4)		201 (53.5)	170 (53.5)	
	CC	121 (16.6)	161 (23)		62 (16.5)	71 (22.3)	
Allele	A	837 (58)	734 (52)	**0.0051**	427 (57)	324 (51)	**0.0296**
	C	617 (42)	668 (48)	**1.24 (1.07–1.43)**	325 (43)	312 (49)	**1.27 (1.02–1.56)**
rs3024496							
Genotype	TT	665 (91.5)	648 (92.4)	0.79	355 (94.4)	295 (92.8)	0.38
	TC	61 (8.4)	52 (7.4)		21 (5.6)	23 (7.2)	
	CC	1 (0.1)	1 (0.1)		0	0	
Allele	T	1319 (96)	1348 (96)	0.516	731 (97)	613 (96)	0.383
	C	63 (4)	54 (4)	1.13 (0.78–1.64)	21 (3)	23 (4)	0.76 (0.42–1.40)

CI confidence interval; *OR* odds ratio; *GD* Graves' disease

[a] Significant P values <0.05 are marked in bold

controls. In the initial cohort from Shanghai, there was significant association of rs1800896 and rs3021094 between GD patients compared with normal controls. The frequency of the G allele of rs1800896 variant was significantly higher in the GD group than in control [OR = 1.72 (95% CI: 1.28–2.31), $P_{\text{allele}} = 2.6 \times 10^{-4}$]. For the rs3021094 variant, the A allele was significantly associated with the GD group [OR = 1.24 (95% CI: 1.07–1.43), $P_{\text{allele}} = 0.0051$]. In the follow-up replication cohort from Xiamen Island, the G allele of rs1800896 was also significantly higher in GD patients [OR = 1.88 (95% CI: 1.19–2.90), $P_{\text{allele}} = 0.0057$] than in controls, and a similar increase in the frequency of the A allele of rs3021094 was found in the GD group [OR = 1.27 (95% CI: 1.02–1.56), $P_{\text{allele}} = 0.\ 0296$] compared to the controls. There were no statistical differences in the distributions of genotype and allele frequencies for rs1800872, rs3790622, and rs3024496 in the two cohorts.

Haplotype association

Three haplotypes in the *IL-10* gene (frequencies > 0.10) were identified, and each haplotype was composed of five SNPs (rs1800896, rs1800872, rs3790622, rs3021094, and rs3024496) in the two cohorts. The most common haplotype (AACCT) served as a reference haplotype in our

Table 2 IL-10 haplotype associations with GD

rs1800896	rs1800872	rs3790622	rs3021094	rs3024496	Control/case	*P*	OR	95% CI
A	A	C	C	T	C1 0.408/0.352	–	1	–
					C2 0.399/0.326	–	1	–
A	C	C	A	T	C1 0.281/0.209	0.24	0.89	0.73–1.08
					C2 0.269/0.265	0.27	1.18	0.88–1.57
A	**A**	**C**	**A**	**T**	**C1 0.154/0.249**	**5.91×10^{-9}**	**1.93**	**1.54–2.42**
					C2 0.171/0.238	**0.0017**	**1.65**	**1.51–2.25**

GD Graves' disease; *OR* odds ratio; *CI* confidence interval; *C1* cohort of Shanghai; *C2* cohort of Xiamen

The most common haplotype was defined as the reference. Data are presented as frequency. *P* values <0.05 are considered significant marked in bold

analysis (Table 2). Compared with the controls, the frequency of the AACAT haplotype was significantly increased for GD patients [24.9 vs. 15.4%, OR = 1.93 (95% CI: 1.54–2.42), $P = 5.9 \times 10^{-9}$], and we found the same result in the replication cohort [23.6 vs. 17.1%, OR = 1.65 (95% CI: 1.51-2.25), $P = 0.0017$].

Meta-analysis of SNPs and GD

There were three reported studies of a genetic association of the two shared SNPs (rs1800896 and rs1800872) with GD susceptibility from Iran, Taiwan, and the UK [9, 13, 14]. All three studies were independent, and the data were in Hardy–Weinberg equilibrium. The forest plots for the allele model are shown in Fig. 1. Combining the study of the Iranian population data sets and the two present populations, we analyzed the rs1800896 polymorphism involving 1210 GD cases and 1159 controls in the meta-analysis.

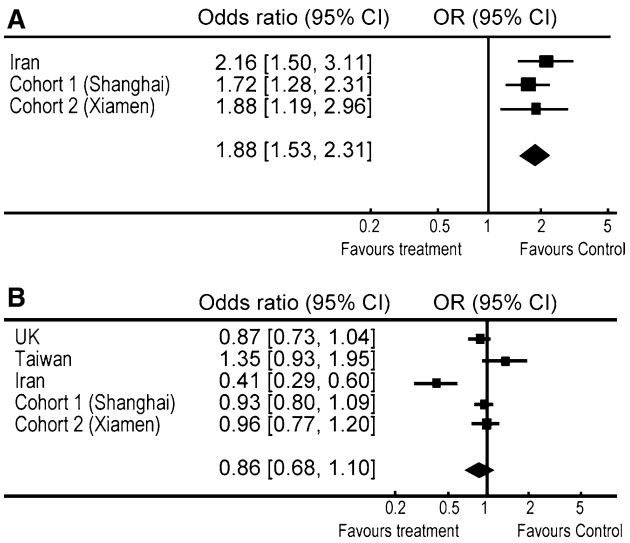

Fig. 1 The ORs for the association between the rs1800896 polymorphism (**a**) and the rs1800872 polymorphism (**b**) and risk of GD. The *size* of the *gray box* is proportional to the weight of the corresponding study. The pooled ORs and 95% CIs are represented by the *shaded diamonds*

No significant inter-study heterogeneity was observed ($I^2 = 0\%$, *P* value for heterogeneity = 0.63). The result demonstrated that the G allele for rs1800896 was significantly associated with GD by a fixed effect [pooled OR = 1.88 (95% CI: 1.53–2.31), $P < 0.00001$]. With the five studies on the rs1800872 polymorphism, the pooled populations originating from UK, Iran, Taiwan, Shanghai, and Xiamen Island included 1971 GD cases and 2132 controls. The I^2 statistic suggested that there was significant heterogeneity among the GD group ($I^2 = 81.5\%$), and the pooled OR was calculated by random effects approaches of DerSimonian and Laird methods. We did not find any statistically significant difference for rs1800872 compared to the controls [pooled OR = 0.86 (95% CI: 0.68–1.10), $P = 0.23$].

Discussion

Cytokines are involved in the antibody-mediated immune response and, therefore, have the potential to be determinants for autoimmune diseases. IL-10, one of the major anti-inflammatory cytokines, is secreted by activated T cells, monocytes, B cells, and thymocytes. IL-10 has been found associated with several autoimmune diseases, such as encephalomyelitis and hemolytic anemia, which suggest that IL-10 might be a regulatory role involved in inflammation and reversing immune-pathology [15, 16]. Interestingly, it was reported that the serum concentration of cytokine IL-10 was much higher in GD patients compared with controls [17].

Polymorphisms in *IL-10* gene were also investigated and showed an association with GD. In this study, we investigated the role of five tag-SNPs of the *IL-10* gene for the risk of GD susceptibility in an initial Shanghai cohort. We further investigated this relationship in an independent replication cohort from Xiamen Island to confirm the preliminary results. The G allele of rs1800896, located at position −1082 of the promoter region, was significantly higher in the GD group than in the controls in both cohorts ($P_{\text{allele}} = 2.6 \times 10^{-4}$ and 0.0082 for cohort Shanghai and

Xiamen, respectively). In addition, a meta-analysis combining our present data with that of another Asian population (Iran) indicated that carriers of the G allele of rs1800896 showed a significantly increased risk for GD (OR = 1.88). Similarly, the rs1800896 polymorphism in the *IL-10* gene has also been reported to affect the susceptibility and severity of infectious, autoimmune, and neoplastic diseases [18, 19].

The G allele and the homozygous GG of rs1800896 led to a higher IL-10 serum level, and subjects negative for the A allele exhibited a higher production of IL-10 than those who were positive for the A allele [20]. In vitro, the transcription factor Sp1 was identified to bind to the G allele of the rs1800896 polymorphism, and the expression of IL-10 was greatly increased for GG genotypes compared with AA genotypes in lipopolysaccharide (LPS)-stimulated B-cells [21]. These results are in concordance with reported data that the G allele was associated with higher IL-10 production in mononuclear cells after LPS stimulation [22]. This result of G-allele aggregation of rs1800896 in GD patients was concomitant with higher serum levels of IL-10 in primary GD.

In conclusion, this study revealed that the G allele of the rs1800896 polymorphism and the AACAT haplotype (rs1800896, rs1800872, rs3790622, rs3021094, and rs3024496) of the *IL-10* gene were significantly associated with susceptibility to GD and should be considered as risk factors for GD in the Chinese population.

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