Polymorphism of IL37 gene as a protective factor for autoimmune thyroid disease

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Abstract

Autoimmune thyroid disease (AITD) comprises Graves' disease (GD) and Hashimoto's thyroiditis (HT). IL37 has been recently proved to be a natural suppressor for innate immunity and acquired immunity. Therefore, this study was conducted to identify the association of IL37 genetic polymorphisms with AITD in Chinese Han population. Polymorphisms of rs3811046/rs3811047/rs2723176/rs272186 in the IL37 gene were assessed in a case-control study comprising 701 GD patients, 301 HT patients and 939 controls. Genetic variants were genotyped by multiplex polymerase chain reaction and ligase detection reaction. The frequencies of the minor allele A of rs2723176 and A of rs2723186 were significantly lower in the GD patients than in the controls ($P=0.014$, $OR=0.774$; $P=0.014$, $OR=0.777$). After gender stratification, the rs3811046 G allele and the rs3811047/rs2723186 A allele were both significantly associated with a decreased risk of GD in female patients ($P=0.030$, $OR=0.777$; $P=0.023$, $OR=0.774$; $P=0.029$, $OR=0.761$). However, none of the four single nucleotide polymorphisms of IL37 gene showed any significant association with HT. Moreover, haplotype analysis revealed the GCG haplotype conferred increased risk for GD as a whole and in female GD patients ($OR=1.213$; $OR=1.320$). The ACG haplotype was associated with an increased risk of HT as a whole ($OR=1.567$) and in male GD patients ($OR=1.820$). In contrast, the AAA haplotype showed a protective role for GD as a whole ($OR=0.760$) and in female GD patients ($OR=0.765$). Our study strongly supports that the IL37 gene variants are associated with the susceptibility to AITD.

Key Words

- ▶ IL37
- ▶ IL1F7
- ▶ single nucleotide polymorphisms (SNPs)
- ▶ autoimmune thyroid diseases (AITDs)
- ▶ Graves' disease (GD)
- ▶ Hashimoto's thyroiditis (HT)

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Introduction

Autoimmune thyroid diseases (AITDs), typically including Graves' disease (GD) and Hashimoto's thyroiditis (HT), are the most common organ-specific autoimmune disorders, affecting up to 5% of the overall population (Tomer & Huber 2009). GD and HT represent the major portion of clinical expressions of thyroid dysfunction but differ in their clinical presentations as well as pathophysiology. HT is a T cell-mediated organ-specific autoimmune disease

characterized by lymphocytic infiltration that leads to thyroid cells loss, clinically manifested as hypothyroidism (Antonelli *et al.* 2015). In contrast, GD is characterized by hyperthyroidism due to overproduction of thyroid hormone induced by specific auto-antibodies against thyrotropin receptor (TSHR) (Morshed *et al.* 2012).

The etiology of GD and HT involves common and unique pathways. Both diseases carry thyroid T cells that

escape the immune tolerance process and infiltrate the thyroid. However, distinct pathways lead thyroid-reactive T cells to cause either apoptosis of thyroid cells in HT or their overgrowth in GD (Tomer 2010). The current paradigm is that AITDs are multifactorial diseases. Several factors, including genetic, hormonal, environmental and nutritional elements, are involved in the initiation and/or development of AITDs. However, the pathophysiologic changes seen in AITDs are mediated by inflammatory cytokines (Ganesh *et al.* 2011). Cytokines are crucial regulators to promote or suppress inflammation. Single nucleotide polymorphisms (SNPs) of inflammatory cytokines may change the transcription rate and consequently the production of cytokines (Dominici *et al.* 2002, Wen *et al.* 2006, Prasad *et al.* 2010), and thus may modify individual susceptibility to AITDs. Various cytokines and respective genetic polymorphisms have been reported to be associated with AITD, but the results are inconsistent (Chen *et al.* 2005, Shiao *et al.* 2006, Anvari *et al.* 2010, Liu *et al.* 2010, Yan *et al.* 2012).

The interleukin 1 gene family (IL1F) cluster is located on chromosome 2q13, within a 36kb region, including IL1A, IL1B, IL1F7, IL1F9, IL1F6, IL1F8, IL1F5, IL1F10 and IL1RN (Nicklin *et al.* 2002). The IL1 molecule has extensive biological effects, among which is its ability to partly or entirely activate and enhance the function of mononuclear cells, neutrophils and phagocytes and promote them to release inflammatory mediators; it also mediates immune response and inflammation reactions. A genome-wide scan has identified that a region including IL1 family gene cluster is linked to ankylosing spondylitis (AS) susceptibility (Miceli-Richard *et al.* 2004, Timms *et al.* 2004). This linkage finding was later confirmed by one family-based association study in western Canadian family population (Zhang *et al.* 2004). IL37 was formerly termed as an IL1 family member 7 (IL1F7). Studies have shown that the biological properties of IL37 are mainly those of downregulating the production of pro-inflammatory cytokines and protecting mice from inflammatory and autoimmune diseases, as assessed in models where human IL37 is expressed in mice (McNamee *et al.* 2011, Sakai *et al.* 2012).

Recently, clinical studies have demonstrated that the IL37 gene SNP rs3811047 G>A is related to the susceptibility to autoimmune diseases, such as ankylosing spondylitis (AS) (Pan *et al.* 2010), disease activity of rheumatoid arthritis (RA) (Pei *et al.* 2012), and gastric cardiac adenocarcinoma (Wang *et al.* 2014).

In our unpublished study, we found that the mRNA level of IL37 was significantly high in peripheral blood

mononuclear cells of GD patients. To our knowledge, there has been no study to report the relationship between SNPs of IL37 and AITD patients so far. Therefore, the aim of the present study was to analyze the genetic association of the four SNPs of IL37 (rs3811046/rs3811047/rs2723176/rs2723186/) with AITD in a large cohort of Chinese Han population. Furthermore, we analyzed the association between genotypes and AITD clinical phenotypes.

Materials and methods

Ethics statement

The research protocol was approved by the Ethics Committee of Jinshan Hospital of Fudan University (2014-05-01; 2015-01-01). Written informed consents were obtained from all participants and/or their parents.

Study population

The study population ($n=1999$) consisted of 1061 unrelated Chinese Han AITD patients, including 701 patients with GD and 360 patients with HT, and 938 unrelated, ethnically and geographically matched healthy controls. All AITD patients were enrolled from the Out-patient Department and Inpatient Department of Endocrinology of Jinshan Hospital of Fudan University. The healthy controls were recruited from the Health Check-Up Center of the same hospital. The subjects were all of self-reported Chinese Han ethnicity.

Clinical assessment

Inclusion criteria: AITDs patients were diagnosed as described in our published papers (Yan *et al.* 2014). The diagnosis of GD was based on the presence of clinical and laboratory biochemical hyperthyroidism with diffuse goiter, decreased TSH value, increased levels of free thyroid hormones and supported by the positive anti-thyroid stimulating hormone receptor antibody (TRAb) and/or anti-thyroid peroxidase antibody (TPO-Ab) and/or anti-thyroglobulin (Tg-Ab) and/or exophthalmos. HT was diagnosed based on the presence of an enlarged thyroid and either TPO-Ab or Tg-Ab, with or without documented clinical and biochemical hypothyroidism. For the suspicious cases of HT, diagnoses were confirmed by fine needle aspiration biopsies (FNAB).

Exclusion criteria: all healthy controls had to exhibit no evidence of goiter, or TPO-Ab/Tg-Ab nor any personal or family history of thyroid diseases or any other autoimmune diseases.

The clinical phenotypes of AITD patients included: i) adult- or childhood-onset AITDs, according to the age at the time of disease onset (≤ 18 years vs ≥ 19 years); ii) presence or absence of ophthalmopathy in the GD group (defined as a distinctive disorder characterized by inflammation and swelling of the extraocular muscles, eyelid retraction, periorbital edema, episcleral vascular injection, conjunctive swelling and proptosis); iii) presence or absence of thyroid dysfunction in HT group: euthyroid status or hypothyroidism; and iv) presence or absence of AITD family history.

Peripheral blood DNA extraction

Peripheral venous blood of 2 ml was collected from all the study participants. Genomic DNA was extracted from peripheral blood cells using the Relax Gene Blood DNA System (Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's guidelines. The concentration and purity of each DNA sample were measured by Nano Drop 2000 Spectro-photometer (Thermo Scientific Company, Waltham, MA, USA).

SNP selection and genotyping

Four SNPs (rs3811046/rs3811047/rs2723176/rs2723186) of IL37 gene were selected from the Hapmap CHB data (<http://hapmap.ncbi.nlm.nih.gov/>) using Haploview Software 4.2 (Barrett *et al.* 2005). According to previously published literature, polymorphisms of these loci may also be associated with the susceptibility to AS or RA (Pan *et al.* 2010, Pei *et al.* 2012). All the tag SNPs met the following criteria: minor allele frequency (MAF) > 0.05 , Hardy–Weinberg equilibrium (HWE) with $P > 0.001$ and logarithm of odds (LOD) > 3.0 . The minor allele frequency of the four SNPs among Chinese Han in Beijing, China (CHB) was as follows: rs3811046 $M_G = 0.146$, rs3811047 $M_A = 0.146$, rs2723186 $M_A = 0.102$, rs2723176 $M_A = 0.103$.

The genotyping of four SNPs of IL37 was conducted by Shanghai Biowing Applied Biotechnology Company (<http://www.biowing.com.cn/>) using ligase detection reactions (LDR) platform (Yan *et al.* 2014). The target DNA sequences were amplified using multiplex polymerase chain reaction (PCR) method with specific primer sequences as follows: rs3811046 forward: 5'-GAGGCCT-TACTTGTGTGAAC-3'; reverse: 5'-AACCTCACTGCGTCT-GACTG-3'; rs3811047 forward: 5'-GAGGCCTTACTTGT-GTGAAC-3'; reverse: 5'-AACCTCACTGCGTCTGACTG-3'; rs2723176 forward: 5'-ACAGACCTCCTGCCTCAGAA-3'; reverse: 5'-AAGGTGCTGGCAGTTGTTTT-3'; rs2723186

forward: 5'-AAGGAGGCAGACACTTAGAC-3'; reverse: 5'-TTGACTCTGTGGTCCACTTC-3'.

Extracted DNA was amplified by PCR using the Gene Amp PCR System 9600 (Perkin Elmer, Waltham, MA, USA). The amplification procedure consisted of initial denaturation at 95 °C for 2 min, denaturation at 94 °C for 90 s, annealing at 65 °C for 30 s, extension at 65 °C for 30 s with 40 cycles and final extension at 65 °C for 10 min. After the reaction, 2 μ l of each product was run in a 3.0% agarose gel to determine whether the reaction had been successful. After completion of the amplification, the ligation detection reaction for each subject was carried out in a final volume of 10 μ l containing 1 μ l of buffer (1 $\times$), 1 μ l of probe mix (2 pmol/ μ l), 4 μ l of Multi-PCR product (100 ng/ μ l), 4 μ l of H₂O, 0.05 μ l of 2U/ μ l Taq DNA ligase (New England Biolabs, Ipswich, MA, USA). The LDR was performed using 40 cycles of denaturation at 95 °C for 2 min, annealing at 94 °C for 15 s and extension at 50 °C for 25 s. The LDR fluorescent product was analyzed using an ABI sequencer 377 (Applied Biosystems), and the results were analyzed with Genemapper Software (Applied Biosystems).

In addition, to test the validity of the assay, we made double positive controls (duplication of the same DNA samples) and negative controls (samples without DNA) in genotyping procedure. Quality-control analysis was also performed so that only SNPs and samples that had passed the 95% quality-control threshold were subjected to further statistical analysis.

Statistical analysis

All statistical data analyses were performed with SPSS version 17.0 Software (SPSS, Inc.). Association tests for genotype/allele frequency of each SNP were done using the two-tailed Pearson χ^2 -test. Compliance with Hardy–Weinberg equilibrium (HWE) was assessed using the HWE program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>) in the control population for each SNP. A linkage disequilibrium (LD) map used to define the haplotype blocks was constructed using Haploview Software (version 4.2; <http://www.broadinstitute.org/haploview/haploview>). The haplotype analysis was performed to compare distributions of haplotype frequencies of SNPs between cases and controls. Differences in the distributions of demographic characteristics variables, allele and genotype frequencies of four SNPs between the patients and controls were estimated by the χ^2 -test. The clinical and experimental indexes of different AITD phenotypes were compared by *t*-test. To examine whether each SNP independently contributes to susceptibility to AITD,

Table 1 Clinical and demographic characteristic of AITD patients and controls

Characteristics	GD patients (n=701)	HT patients (n=360)	Controls (n=938)
Gender			
Male (%)	214 (30.5)	44 (12.2)	309 (32.9)
Female (%)	487 (69.5)	316 (87.8)	629 (67.1)
Age (mean $\pm$ s.d.)	36.86 $\pm$ 14.549	35.09 $\pm$ 13.786	38.61 $\pm$ 9.202
Onset of age	33.98 $\pm$ 14.344	32.79 $\pm$ 13.465	–
≤ 18 years	113 (16.12)	51 (14.2)	–
≥ 19 years	588 (83.88)	309 (85.8)	–
Thyroid size			–
Normal size (%)	22 (3.1)	12 (3.3)	–
Degree I (%)	127 (18.1)	59 (16.4)	–
Degree II (%)	425 (60.7)	253 (70.3)	–
Degree III (%)	127 (18.1)	36 (10.0)	–
Family history (%)	174 (24.8)	79 (21.9)	–
Ophthalmopathy (%)	135 (19.3)	9 (2.5)	–
Thyroid antibodies			–
TPOAb	398 (56.7)	252 (70.0)	–
TgAb	303 (43.2)	226 (62.8)	–

conditional logistic regression analysis was conducted. *P* value <0.05 was taken as statically significant. Odds ratio (OR) and 95% CI were calculated for the association between each genotype and AITD.

Power calculation

The power of the data was evaluated using the Power and Sample Size Calculation Software (Ps; version 3.0; <http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>) (Dupont & Plummer 1998)). As the MAF of rs2723186 was the lowest among the four SNPs according to the Hapmap CHB data, we calculated the power of rs2323186. Considering the expected frequency of minor allele of rs2723186 in controls, the combined set of 760 GD patients and 938 controls gave a power of 94.1% with an OR=0.77 at the 5% significance level.

Results

Demographic and clinical characteristics of each group

The demographic and clinical characteristics of all subjects are summarized in Table 1. Among GD patients, 69.5% were females and the mean age was 36.86 years. Of the HT patients, 87.8% were females and the mean age was 35.09 years. In the control group, 67.1% individuals were females and the overall mean age was 38.61 years. There was no difference concerning the age and gender distribution between GD patients and controls. There was no difference in age distribution between HT patients

and controls, either. However, female HT patients outnumbered male HT ones by seven times.

Association study of IL37 polymorphism with AITD

The distribution of genotype frequencies for each SNP (rs3811046/rs3811047/rs2723176/rs2723186) was in Hardy–Weinberg equilibrium in both the patients and the controls (Table 2).

The genotypic and allelic distribution of four SNPs were analyzed in AITD, GD and HT patients and compared with those of controls. The results are summarized in Table 3. rs2723176 and rs2723186 polymorphisms appeared to be associated with AITD susceptibility. For rs2723176, the minor allele A frequency was significantly lower in both AITD and GD groups than in the control group and seemed to decrease the risks for AITD and GD (in AITD 13.3% vs 15.6%, *P*=0.044, OR=0.832, 95% CI=0.696–0.995; in GD, 12.5% vs 15.6%, *P*=0.014, OR=0.774, 95% CI=0.631–0.949). Similar results were also found for rs2723186. A significant decrease in the frequency of the minor A allele was observed in AITD and GD patient groups compared with that in the control

Table 2 Hardy–Weinberg *P* value of the four SNPs

SNP ID	Control	GD	HT
rs3811046 G/T	0.441	0.426	0.331
rs3811047 A/G	0.759	0.735	0.467
rs2723176 C/A	0.585	0.244	0.585
rs2723186 A/G	0.228	0.236	0.911

Table 3 Genotype and allele distribution of four SNPs in IL37 gene in AITDs patients, GD patients, HT patients and controls

	Allele			P_a		P_b		P_c
SNP	Genotype	Controls	AITD	(OR, 95% CI)	GD	(OR, 95% CI)	HT	(OR, 95% CI)
rs 3811046	GG	36 (4.1)	40 (4.0)		21 (3.2)		19 (5.5)	
	GT	266 (30.2)	288 (28.6)	0.712	177 (26.8)	0.182	111 (31.9)	0.444
	TT	578 (65.7)	680 (67.4)		462 (70.0)		218 (62.6)	
	G	338 (19.2)	368 (18.3)	0.455	219 (16.6)	0.062	149 (21.4)	0.217
	T	1422 (80.8)	1648 (81.7)		1101 (83.4)		547 (78.6)	
rs 3811047	AA	38 (4.2)	42 (4.0)		22 (3.2)		20 (5.6)	
	AG	288 (31.8)	313 (30.0)	0.677	195 (28.3)	0.153	118 (33.3)	0.417
	GG	581 (64.0)	688 (66.0)		472 (68.5)		216 (61.1)	
	A	364 (20.0)	397 (19.0)	0.416	239 (17.3)	0.052	158 (22.3)	0.210
	G	1450 (80.0)	1689 (81.0)		1139 (82.7)		550 (77.7)	
rs 2723176	AA	20 (2.2)	21 (2.0)		14 (2.0)		7 (2.0)	
	AC	245 (26.7)	235 (22.6)	0.089	143 (20.9)	0.023	92 (25.8)	0.903
	CC	651 (71.1)	786 (75.4)		528 (77.1)		258 (72.2)	
	A	285 (15.6)	277 (13.3)	0.044	171 (12.5)	0.014	106 (14.8)	0.655
	C	1547 (84.4)	1807 (86.7)	0.832 (0.696–0.995)	1199 (87.5)	0.774 (0.631–0.949)	608 (85.2)	
rs2723186	AA	19 (2.1)	23 (2.2)		15 (2.2)		8 (2.3)	
	AG	259 (28.2)	238 (23.2)	0.044	147 (21.6)	0.012	91 (26.3)	0.793
	GG	643 (69.7)	766 (74.6)		519 (76.2)		247 (71.4)	
	A	297 (16.1)	284 (13.8)		177 (13.0)	0.014	107 (15.5)	0.685
	G	1545 (83.9)	1770 (86.2)	0.835 (0.700–0.996)	1185 (87.0)	0.777 (0.636–0.950)	585 (84.5)	

P_a value, AITD vs controls; P_b value, GD vs controls; P_c value, HT vs controls; OR, odd ratio.

group (in AITD, 13.8% vs 16.1%, $P=0.044$, OR=0.835, 95% CI=0.700–0.996; in GD, 13.0% vs 16.1%, $P=0.014$, OR=0.777, 95% CI=0.636–0.950). Comparisons of the genotype frequencies also showed obvious differences in genotype distributions between GD patients and healthy controls (rs2723176, $P=0.023$; rs2723186, $P=0.012$). However, we could not find significant differences in the genotype or allele frequencies of two SNPs (rs3811046 and rs3811047) between AITD patients, GD patients, HT patients and healthy controls.

In further analysis for the association of four SNPs with susceptibility to AITD in female and male groups (Table 4), the minor G allele of rs3811046 and the minor A allele of rs3811047 were significantly associated with a decrease in GD risk in female group ($P=0.030$, OR=0.777, 95% CI=0.618–0.976; $P=0.023$, OR=0.774, 95% CI=0.620–0.966 respectively). The frequencies of rs2723186 genotypes in female GD patients differed significantly from those in the controls ($P=0.023$); the A allele frequency was significantly lower than that of healthy controls ($P=0.029$, OR=0.761, 95% CI=0.596–0.973). It was found that the frequency of A allele of rs2723176 was lower in GD female group but without statistical significance ($P=0.053$). However, all four SNPs of IL37 gene did not reveal any significant associations with HT in female or male patients.

To evaluate the independent genetic effect of each SNP on the susceptibility to AITD, conditional logistic regression analysis was performed. As shown in Table 5, the association of rs2723186 remained significant after adjustment for rs2723176 SNP genotypes. Adjusted P values (P adjusted) for rs2723186 under the dominant model were 0.041 and 0.036 in AITD and GD after adjustment for rs2723176. The results suggest that rs2723186 SNP is independently associated with GD.

Analysis of haplotypic association

The four SNPs of IL37 gene were tightly linked to each other; a strong linkage disequilibrium was observed among rs3811047, rs2723176 and rs2723186 in controls using the Haploview 4.2, especially these three SNPs that were in the same LD block (Table 6). To further identify whether haplotypes of the IL37 gene were correlated with AITD, we created an LD map (Fig. 1) and analyzed haplotype frequency differences between AITD patients and controls (Table 7). Haplotype analysis showed that three haplotypes, GCG, AAA and ACG, were detected in the IL37 gene. The frequency of haplotype GCG with GD patients was significantly higher than that in their control group ($P=0.034$), the GCG haplotype conferred a significant increased risk for GD as a whole (OR=1.213,

Table 4 Analysis of genotype and allele distribution of four SNPs in GD patients, HT patients and controls after gender stratification

SNP	Allele	Genotype				P_a				P_b	
		Controls		GD		HT		OR (95% CI)		OR (95% CI)	
		M	F	M	F	M	F	M	F	M	F
rs3811046	GG	9 (3.1)	27 (4.6)	7 (3.5)	14 (3.0)	2 (4.7)	17 (5.6)	0.911	0.106	0.675	0.468
	GT	92 (31.3)	174 (29.7)	59 (29.8)	118 (25.5)	11 (25.6)	100 (32.8)				
	TT	193 (65.6)	385 (65.7)	132 (66.7)	330 (71.5)	30 (69.7)	188 (61.6)				
	G	110 (18.7)	228 (19.5)	73 (18.4)	146 (15.8)	15 (17.4)	134 (22.0)	0.914	0.030	0.778	0.211
	T	478 (81.3)	944 (80.5)	323 (81.6)	778 (84.2)	71 (82.6)	476 (78.0)				
rs3811047	AA	9 (3.0)	29 (4.8)	7 (3.3)	15 (3.1)	2 (4.7)	18 (5.8)	0.944	0.083	0.681	0.447
	AG	101 (33.4)	187 (30.9)	68 (32.3)	127 (26.6)	12 (27.9)	106 (34.1)				
	GG	192 (63.6)	389 (64.3)	136 (64.4)	336 (70.3)	29 (67.4)	187 (60.1)				
	A	119 (19.7)	245 (20.2)	82 (19.4)	157 (16.4)	14 (16.7)	142 (22.8)	0.914	0.023	0.509	0.200
	G	485 (80.3)	965 (79.8)	340 (80.6)	799 (83.6)	70 (83.3)	480 (77.2)				
rs2723176	AA	5 (1.6)	15 (2.5)	2 (0.9)	12 (2.5)	0 (0.0)	7 (2.2)	0.302	0.068	0.565	0.974
	AC	89 (29.2)	156 (25.5)	50 (23.8)	93 (19.6)	11 (25.0)	81 (25.9)				
	CC	211 (69.2)	440 (72.0)	158 (75.3)	370 (77.9)	33 (75.0)	225 (71.9)				
	A	99 (16.2)	186 (15.2)	54 (12.9)	117 (12.3)	11 (12.5)	95 (15.2)	0.135	0.053	0.369	0.98
	C	511 (83.8)	1036 (84.8)	366 (87.1)	833 (87.7)	77 (87.5)	531 (84.8)				
rs2723186	AA	5 (1.6)	14 (2.3)	3 (1.4)	12 (2.5)	0 (0.0)	8 (2.6)	0.458	0.023	0.454	0.932
	AG	91 (29.6)	168 (27.4)	52 (24.8)	95 (20.2)	10 (23.3)	81 (26.7)				
	GG	211 (68.8)	432 (70.3)	155 (73.8)	364 (77.3)	33 (76.7)	214 (70.7)				
	A	101 (16.4)	196 (16.0)	58 (13.8)	119 (12.6)	10 (11.6)	97 (16.0)	0.248	0.029	0.252	0.98
	G	513 (83.6)	1032 (84.0)	362 (86.2)	823 (87.4)	76 (88.4)	509 (84.0)				

M, male; F, female; P_a value, GD vs controls; P_b value, HT vs controls; OR, odd ratio.

95% CI=1.015–1.450); in females, the GCG haplotype was also associated with an increase risk of GD (OR=1.320, 95% CI=1.061–1.642, $P=0.012$). The frequency of haplotype ACG was significantly higher in HT patients than in the controls ($P=0.013$); the ACG haplotype was associated with an obviously increased risk of HT (OR=1.567, 95% CI=1.096–2.241, $P=0.013$). In males, the frequencies of the ACG haplotype in AITD patients and GD patients were significantly higher than those in control groups ($P=0.037$, 0.036), indicating that ACG

was a risk haplotype for GD (OR=1.820, 95% CI=1.033–3.205), but not for HT. In contrast, the frequencies of haplotype AAA were significantly lower in AITD and GD patient groups than those in their control groups ($P=0.037$, 0.009), suggesting that the AAA haplotype showed a protective role for AITD susceptibility as a whole (OR=0.826, 95% CI=0.691–0.989), especially for GD (OR=0.760, 95% CI=0.619–0.934). In females, the AAA haplotype was also associated with a decreased risk for GD (OR=0.765, 95% CI=0.596–0.983).

Table 5 Interaction analysis of gene-gene in AITD, GD and HT patients by logistic regression^a.

	rs3811047			rs2723176			rs2723186		
Dominant model									
rs3811046	0.999	0.999	0.999	0.095	0.198	0.119	0.242	0.283	0.437
rs3811047				0.999	0.999	0.999			
rs2723176							0.041	0.036	0.161
Recessive SNP									
rs3811046	1.0	1.0	0.587	1.0	1.0	0.945	1.0	1.0	–
rs3811047				1.0	1.0				
rs2723176							0.901	0.547	0.947
Co-dominant model SNP									
rs3811046	0.677			0.892			0.999		
rs3811047				1.0					
rs2723176							0.999		

^aThe data are presented as $P_{adjusted}$ values (AITD/ GD/ HT) from a multiplicative interaction model. The regression models were adjusted for age and sex.

Table 6 Linkage disequilibrium in controls

L1	L2	D'	r ²
rs3811047	rs2723176	1.0	0.721
rs3811047	rs2723186	0.95	0.684
rs2723176	rs2723186	1.0	0.952

Genotype and clinical phenotype correlations

The genotypes and alleles of the selected four SNPs were tested for interactions with such risk factors of AITD as cigarette smoking and family history, but without any notable effect (data not shown). Clinical parameters of AITD, including age of onset (≤ 18 years or ≥ 19 years), GD with or without ophthalmopathy, intractable GD or GD in remission and HT patients with thyroid dysfunction (hypothyroidism or normal), were not associated with genotype or allele variants of the four tested SNPs either (data not shown).

Discussion

To the best of our knowledge, the present study was the first demonstration that the IL37 polymorphism is associated with susceptibility to AITD. The results of our study showed that carriers of the minor A allele of rs2723176 and rs2723186 were associated with a significantly decreased risk of GD; Moreover, the minor G allele of rs3811046 and the minor A allele of rs3811047 also significantly reduced the risk of GD in female patients. This suggests that the minor A allele of rs2723176/rs2723186/ rs3811047 and the minor G allele of rs3811046 were the protective factors for GD. Our study contributes to the understanding of individual susceptibility to AITD when it is a carrier of protective alleles of IL37, highlighting the relevance of polymorphisms in inflammation-related gene as molecular marker for AITD.

IL37 (IL1F7) has five splice variants (IL37a–e) and is the only IL1 family member that is not found in mice (Boraschi *et al.* 2011). Recent studies revealed that IL37 has a strong anti-inflammatory effect during an innate immune response as well as in the development of inflammation (Nold *et al.* 2010, Banchereau *et al.* 2012). Published literature suggests that IL37 is expressed in various normal tissues, tumors (Pan *et al.* 2001, Kumar *et al.* 2002) and by human peripheral blood mononuclear cells under inflammatory conditions, such as systemic lupus erythematosus (SLE) (Song *et al.* 2013), Behcet's disease (BD) (Ye *et al.* 2014) and acute coronary syndrome (Ji *et al.* 2014). Genetic variants in inflammation-related

cytokine genes may have the potential to alter the regulation of transcript production or function. Considering the role of the inflammation-related cytokines in the pathogenesis of the autoimmune response involved in AITD, a change in the function or the quantity of a particular cytokine may lead to the initiation or inhibition of the inflammatory process. The importance of IL37 in the immunological response is well established in existing literature. Thus, SNPs in the cytokine may have a role in the susceptibility to HT and GD in which the immune response is a major feature.

SNP rs3811046 (G/T transition) and rs3811047 (A/G transition) were located in exon 2 (amino acid position 31, 42 respectively). Both of the SNPs were missense mutations resulting in different amino acid codons (rs3811046: Gly/Val; rs3811047: Thr/Ala). Polymorphism of rs3811047 has been implicated in susceptibility to RA (Pei *et al.* 2012, Shi *et al.* 2013 33), AS (Pan *et al.* 2010), and gastric cardiac adenocarcinoma (Wang *et al.* 2014). A study which recruited subjects (276 controls and 276 RA patients) from central plains of Chinese Han population indicated no significant correlation between RA

Figure 1

Four tag SNPs on the LD map of IL37 gene. Patterns of linkage disequilibrium (LD) between the IL37 single nucleotide polymorphisms (SNPs). The strength of LD measured by D' multiplying 100 is displayed in the red diamonds. A red cell without number indicates a complete LD. A larger number in the cell indicates a higher degree of LD. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JME-15-0144>.

Table 7 Haplotype analysis of AITD patients and controls

Haplotype	Controls (frequency)	AITD	P	OR (95%CI)	GD	P	OR (95%CI)	HT	P	OR (95%CI)
Total										
GCG	1456 (79.3)	1685 (80.7)	0.273	–	1131 (82.3)	0.034	1.213 1.015–1.450	553 (77.5)	0.316	–
AAA	283 (15.4)	273 (13.1)	0.037	0.826 0.691–0.989	167 (12.2)	0.009	0.760 0.619–0.934	106 (14.8)	0.728	–
ACG	86 (4.7)	120 (5.7)	0.134	–	70 (5.4)	0.588	–	51 (7.1)	0.013	1.567 1.096–2.241
Female										
GCG	965 (78.9)	1276 (80.7)	0.2577	–	795 (83.1)	0.012	1.320 1.061–1.642	481 (76.9)	0.3135	–
AAA	184 (15.0)	209 (23.2)	0.1684	–	114 (11.9)	0.036	0.765 0.596–0.983	95 (15.2)	0.9352	–
ACG	65 (5.3)	88 (5.6)	0.7014	–	42 (4.4)	0.3539	–	46 (7.3)	0.0761	–
Male										
GCG	491 (79.9)	410 (80.0)	0.9548	–	338 (79.7)	0.9347	–	72 (81.7)	0.692	–
AAA	99 (16.1)	65 (12.7)	0.1044	–	54 (12.7)	0.1302	–	11 (12.5)	0.3818	–
ACG	592 (3.7)	33 (6.5)	0.037	1.773 1.027–3.061	28 (6.6)	0.036	1.82 1.033–3.205	77 (5.8)	0.332	–

OR, odd ratio.

susceptibility and rs3811047 SNP in IL37 gene; however, it also showed that RA patients with A allele were significantly better than those without A allele in joint swelling index, rest pain, HAQ scoring and blood sedimentation (Shi *et al.* 2013). Another Chinese group had synchronously reported no statistically significant differences in the genetic polymorphism (genotypes and allele frequencies) of rs3811047 in the RA patients compared with controls (Pei *et al.* 2012). They also found that those carrying A allele had less severe disease activity, suggesting that the A allele of rs3811047 may have a protective effect on RA. Wang *et al.* detected the allelic distribution of rs3811047 G>A polymorphism in 243 gastric cardiac adenocarcinoma cases and 476 controls, and observed that the rs3811047 G>A polymorphism was significantly associated with a decreased risk of gastric cardiac adenocarcinoma either in the single locus analysis or the recessive genetic model (Wang *et al.* 2014). In agreement with existing studies, our study reports associations of the minor A allele of rs3811047 and the minor G allele of rs3811046 with protective effects from developing GD in female patients but not HT patients in a large case-control Chinese Han population.

SNP rs2723176 and rs2723186 were both in the intron region. As far as we know, there is hardly any study on the polymorphism of the above two SNPs. We analyzed for the first time the associations of rs2723176/rs2723186 polymorphisms with susceptibility to AITD. Our results demonstrated that the minor A allele of rs2723176/rs2723186 may be a protective factor for GD, can significantly decrease the risk of GD (OR=0.774, 0.777 respectively), but does not associate with HT. After gender stratification, only the A allele of rs2723186 is significantly associated with a decreased risk in female GD patients (OR=0.761).

Haplotype analysis is likely to continue to play an important role in genetic epidemiology studies (Schaid 2004) because it effectively captures both the joint marker correlations and the evolutionary history. Therefore, we carried out the haplotype analysis of the tested four SNPs between AITD patients and controls. The rate of haplotype GCG was significantly higher in GD, conferring a significant degree of risk for GD as a whole and in female GD patients. The rate of haplotype ACG was significantly higher in HT, suggesting an obviously increased risk of HT. In contrast, the rate of haplotype AAA was significantly lower in GD, showing a protective role for GD susceptibility. These results are consistent with those of the allele analysis of the four SNPs.

It is worthwhile to point out that several limitations exist in our study. Firstly, because of limited SNPs included in our study, future studies using the high-throughput analytic methods available (i.e., gene sequencing) may help clarify our findings and provide further insights into the role of IL37 in AITD. In addition, the mechanisms by which four SNPs exert their roles are not comprehensively understood. Therefore, further studies are required to determine the functional variants of IL37 SNPs in AITD. Finally, findings from this study cannot be extrapolated to other ethnic groups. The relevance of IL37 gene risk variants to additional autoimmune diseases, autoimmune disease susceptibility in other populations and specific disease sub-phenotypes and outcomes needs to be addressed in large, well-characterized cohorts.

Conclusion

We were the first to evaluate the correlation of polymorphisms in IL37 gene with the susceptibility to AITD. Our study suggests that the minor A allele of rs3811047/rs2723176/rs2723186 and the minor G allele of rs3811046 have a protective influence on GD susceptibility.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

N Y, S M, Q Q, X W, and R S carried out the work. J X, W J, L S, Q Y and Y J helped with the collection of specimens. N Y conducted the data analysis and wrote the manuscript and J Z coordinated the research team. All authors have read and approved the final manuscript.

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