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Association of single-nucleotide polymorphisms in the *IL27* gene with autoimmune thyroid diseases

Weiwei He[1], Bin Wang[2], Kaida Mu[3], Jing Zhang[3], Yanping Yang[3], Wei Yao[3], Sheli Li[1] and Jin-an Zhang[3]

[1]Department of Endocrinology, Affiliated Hospital of Yanan Medical University, Shaanxi, China
[2]Department of Endocrinology, Jinshan Hospital of Fudan University, Shanghai, China
[3]Department of Endocrinology, Shanghai University of Medicine & Health Sciences Affiliated Zhoupu Hospital, Shanghai, China

Correspondence should be addressed to S Li or J Zhang: ya116116@126.com or zhangjinan@hotmail.com

Abstract

Background: Accumulating data have shown that interleukin-27 (*IL27*) polymorphisms are linked to the susceptibility of some autoimmune diseases. We assessed whether there was an association between three single-nucleotide polymorphisms (SNPs) of *IL27* gene and autoimmune thyroid diseases (AITDs).

Methods: Three SNPs (rs153109, rs17855750 and rs181206) of *IL27* gene were genotyped by Hi-SNP high-throughput geneotyping in 843 patients with AITDs (516 Graves' disease (GD) and 327 Hashimoto's thyroiditis (HT)) and 677 healthy controls in Chinese Han population.

Results: Compared with controls, rs153109 displayed significant associations with GD in allele and genotype frequencies ($P = 0.002$ and $P = 0.008$, respectively) and rs17855750 displayed significant associations with HT in allele frequencies ($P = 0.02$), whereas no differences in genotype or allele frequencies were found between AITD patients and controls at rs181206.

Conclusion: Our study, for the first time, showed the significant association of the *IL27* gene SNPs with AITD.

Introduction

In autoimmune thyroid diseases (AITDs), one of the autoimmune diseases, cytokine-mediated immunity play a key role during the pathogenesis and development (1). AITDs are common endocrine autoimmune diseases with prevalence of about 5% in the general population, and iodine intake plays a different role in different regions (2, 3, 4, 5). A recent study in China suggested that the prevalence of AITD was 10.5% in men and 21.4% in women (6). It is universally acknowledged that the combined effects of genetic, environmental and immune factors are involved in pathogenesis of AITDs. More and more studies have shown that genetic susceptibility is closely related to AITDs, and may play a key role in the pathogenesis of AITDs and disease progression

(7, 8). AITDs mainly have two types of symptoms, Graves' disease (GD) and Hashimoto's thyroiditis (HT). HT is the most common thyroid inflammation characterized by abnormally elevated thyroglobulin antibodies (TGAb) and thyroid peroxidase antibodies (TPOAb). It causes hypothyroidism in the clinics and shows clinical manifestations opposite to GD (9); HT is more common in middle-aged women (10). The cause of HT is still unclear, although family aggregation that often occurs in generations of the same family has been identified in HT. GD is one of the most common causes of hyperthyroidism characterized by positive thyroid-stimulating hormone (TSH) receptor antibodies (TRAb) and is an organ-specific autoimmune disease with increased secretion of

thyroid hormone (TH). TRAb can bind to TSH receptor on the thyroid follicular cells and stimulate the production of thyroid hormones (11). GD is more common in women with the ratio of male to female of 1:4–6, particularly in patients in the 20–40 years age group. We have previously found that polymorphisms in some genes such as CTLA4 and PTPN22 (12, 13) were associated with GD.

Interleukin (IL)-27 (IL27) has an important role in shaping Th cell responses and is involved in the development of some autoimmune diseases (14, 15). IL27 is a member of IL-12/IL-6 family that includes Epstein–Barr virus-induced gene 3 and *IL27* p28 subunit, located on chromosome 16p11 (16), which is one of the crucial candidate genes for the development and differentiation of T cells. Nowadays, increasing evidences have confirmed that polymorphisms of *IL27* gene play significant roles in some inflammatory diseases and autoimmune diseases, such as inflammatory bowel disease (IBD) (14), rheumatoid arthritis (RA) (17), Behcet's disease (BD) (18) and systemic lupus erythematosus (SLE) (19). A study has shown that IL27 is upregulated in patients with active IBD (20), but not in patients with type 1 diabetes (21). Although *IL27* is closely related to many autoimmune inflammatory diseases, the relationship between *IL27* and AITD has not been well investigated. Hence, in this study, we performed a controlled case study with a large sample size to investigate the relationship between three *IL27* SNPs (rs153109, rs181206 and rs17855750) and AITDs in a Chinese Han population.

Methods

Patients and controls

A total of 843 patients with AITDs (516 GD and 327 HT) and 677 healthy controls were enrolled in the study. AITD patients were recruited from Department of Endocrinology, Jinshan Hospital of Fudan University. The inclusion criteria of all patients have been described previously (12, 22). Both GD and HT were diagnosed according to the international diagnostic criteria (14). The inclusion criteria for GD were hyperthyroidism, decreased TSH level, toxic diffuse and positive for TRAb. The inclusion criteria for HT were positive for TPOAb or TGAb as well as diffuse hypoechogenicity on thyroid, and with or without hypothyroidism. Common autoimmune diseases were excluded in the controls according to disease history and family history, such as SLE, RA, ankylosing spondylitis, ulcerative colitis (UC), IBD, multiple sclerosis,

Sjogren's syndrome and type 1 diabetes mellitus. The control group had no history of thyroid diseases or other autoimmune diseases. Controls with serious illness or chronic inflammatory diseases were also excluded. The healthy controls matched to the patients by geographic area. The study was approved by the Ethics Committee of Zhoupu Hospital and written informed consent was obtained from all participants.

Genotyping

A standard protocol with 2 mL peripheral blood from each participant was used for DNA extraction (23, 24). Genomic DNA was extracted from peripheral blood leukocytes using standard procedures of the RelaxGene Blood DNA System (Tiangen Biotech, Beijing, China). Besides, the concentration of DNA samples and the proportion of A260/A280 were measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific Company). The *IL27* SNPs were detected using Hi-SNP high-throughput genotyping method (Shanghai Biowing Applied Biotechnology CO. LTD, Shanghai, China). The forward and reverse primers for amplification of rs153109 were 5′-GACTGGGACTGGGACTCAGCAGG-3′ and 5′-CATGGCCTCTACAGAGCAGAAACACC-3′, respectively. The forward and reverse primers for amplification of rs17855750 were 5′-CAAGCTCTCTCCCTGTCTTG-3′ and 5′-TGCATTAGGGGGACTTACAAAG-3, respectively. The forward and reverse primers for amplification of rs181206 were 5′-GGACCTTTCCTGACCATCTCCCTC-3′ and 5′-GCAGATCGCGGAGGTCCAG-3′, respectively.

Statistical analysis

The sample size calculation was performed with an expected OR of 1.40. The sample size to make a sufficient power over 80% required at least 331 cases and 265 controls for rs153109, and at least 799 cases and 640 controls for both rs181206 and rs17855750. The final sample size in our case-control study led to a statistical power of over 95% to detect a significant result. Hardy–Weinberg equilibrium (HWE) was performed using χ^2 test. STATA was used in statistical analysis with odds ratios (ORs) and 95% confidence intervals (95% CI). Chi-square test was utilized to compare the allele and genotype frequencies between cases and controls. The relationship between *IL27* and AITDs was analyzed through multiple comparison models, including allele model, dominant model, recessive model, homozygous model and additive model. Multivariate logistic regression analyses

were also performed, and age and gender were used as confounding factors. *P* value less than 0.05 was considered significant.

Results

Subject features

Table 1 shows the baseline data of all participants in this study. There were 843 AITD patients including 516 GD and 327 HT individuals, respectively, and 677 control individuals. Among the GD patients, 357 (69.19%) were female and 159 (30.81%) were male. It's worth noting that 106 (20.54%) GD patients had family history of AITDs and 82 (51.89%) had ophthalmopathy. In addition, 140 (27.13%), 239 (46.32%) and 33 (6.4%) GD patients had degree I, II and III goiter, respectively, and 104 (20.15%) GD patients had no goiter. Among the HT patients, 276 (84.40%) were female and 51 (15.60%) were male. In addition, 53 (16.21%) HT patients had family history of AITDs, 76 (23.24%), 106 (32.42%) and 8 (2.44%) HT patients had degree I, II and III goiter, respectively, and 137 (41.90%) HT patients had no goiter. Among the 677 healthy individuals, 405 (59.82%) were female and 272 (40.18%) were male. The average age was 41.82 ± 14.56 years old for GD patients, 41.84 ± 14.52 years old for HT patients and 39.48 ± 9.10 years old for healthy controls. There were no significant differences in age and gender among the GD patients, HT patients and healthy controls ($P < 0.05$). No deviation from the HWE was observed for rs153109, rs181206 and rs17855750 in the healthy controls ($P > 0.05$).

Association between rs153109 and AITDs

The genotyping results for all patients and controls are summarized in Table 2. The genotype frequencies of TT, TC and CC in rs153109 polymorphism were 41.99%, 46.38% and 11.63% in AITD patients and 38.26%, 47.56% and 14.18% in the healthy controls, respectively (Table 2). The genotype frequencies of TT, TC and CC in rs153109 polymorphism were 45.35%, 45.35% and 9.30% in GD patients and 36.70%, 48.01% and 15.29% in HT patients, respectively. These results showed that there was no statistically significant difference between AITD patients and healthy controls in both genotype distribution ($P = 0.189$) and allele frequencies of rs153109 ($P = 0.073$). It's worth noting that there were significant differences in the genotype distribution of rs153109 between GD patients and healthy controls ($P = 0.008$) and allele frequencies of rs153109 ($P = 0.002$). By contrast, there were no statistically significant differences in genotype distribution between HT patients and healthy controls ($P = 0.844$) and allele frequencies of rs153109 ($P = 0.564$).

The ORs of the associations of rs153109 of *IL27* gene with AITDs before and after adjusting for confounders (age and gender) clearly show that (1) no significant association between rs153109 of *IL27* and AITDs was observed under all comparison models (Table 3, $P > 0.05$), (2) no significant association between rs153109 of IL 27

Table 1 Baseline clinical and demographics features of patients enrolled in the study.

Items	GD	HT	Controls
Number	516	327	677
Gender			
Male	159 (30.81%)	51 (15.60%)	272 (40.18%)
Female	357 (69.19%)	276 (84.40%)	405 (59.82%)
Age (years)	41.82 ± 14.56	41.84 ± 14.52	39.48 ± 9.10
Goiter			
No goiter	104 (20.15%)	137 (41.90%)	–
Grade I goiter	140 (27.13%)	76 (23.24%)	–
Grade II goiter	239 (46.32%)	106 (32.42%)	–
Grade III goiter	33 (6.4%)	8 (2.44%)	–
Family history			
(+)	106 (20.54%)	53 (16.21%)	–
(−)	410 (79.46%)	274 (83.79%)	–
Ophthalmopathy			
(+)	82 (51.89%)	3 (0.92%)	–
(−)	434 (84.11%)	324 (99.08%)	–

GD, Graves' disease; HT, Hashimoto's thyroiditis.

Table 2 Allele frequencies and genotype distribution of IL-27 polymorphisms in AITD patients and healthy controls.

Gene/SNP	Controls	AITD	*P* value	GD	*P* value	HT	*P* value
IL27	*n* (%)	*n* (%)	AITD vs Control	*n* (%)	GD vs Control	*n* (%)	HT vs Control
rs153109							
T	840 (62.04)	1099 (56.18)	0.073	702 (68.02)	0.002	397 (60.70)	0.564
C	514 (37.96)	587 (34.82)		330 (31.98)		257 (39.30)	
TT	259 (38.26)	354 (41.99)	0.189	234 (45.35)	0.008	120 (36.70)	0.844
TC	322 (47.56)	391 (46.38)		234 (45.35)		157 (48.01)	
CC	96 (14.18)	98 (11.63)		48 (9.30)		50 (15.29)	
rs181206							
T	1233 (91.06)	1545 (91.64)	0.575	946 (91.67)	0.604	599 (91.59)	0.696
C	121 (8.94)	141 (8.36)		86 (8.33)		55 (8.41)	
TT	564 (83.31)	707 (83.87)	0.464	432 (83.72)	0.327	275 (84.10)	0.906
TC	105 (15.51)	131 (15.54)		82 (15.89)		49 (14.98)	
CC	8 (1.18)	5 (0.59)		2 (0.39)		3 (0.92)	
rs17855750				327			
T	1230 (90.84)	1501 (89.03)	0.100	928 (89.92)	0.449	573 (87.61)	0.02
G	124 (9.16)	185 (10.97)		104 (10.08)		81 (12.39)	
TT	560 (82.72)	665 (78.89)	0.125	413 (80.04)	0.068	252 (77.06)	0.087
TG	110 (16.25)	171 (20.28)		102 (19.77)		69 (21.10)	
GG	7 (1.03)	7 (0.83)		1 (0.19)		6 (1.84)	

AITD, autoimmune diseases; GD, Graves' disease; HT, Hashimoto's thyroiditis.

gene and AITDs was observed after adjustment for age and gender (Table 3), (3) a significant association of *IL27* rs153109 with GD was observed under the allele model (OR=0.76, 95% CI 0.64–0.91, *P*=0.002), dominant model (OR=0.75, 95% CI 0.59–0.94; *P*=0.014), recessive model (OR=0.62, 95% CI 0.43–0.90, *P*=0.011) and homozygous model (OR=0.74, 95% CI 0.61–0.90, *P*=0.003) (Table 4) and (4) no significant association between rs153109 of

IL27 gene and HT was observed under all comparison models (Table 5, *P*>0.05). Multivariate logistic regression analyses indicate that rs153109 of *IL27* gene is significantly associated with GD (Table 4), but not with AITDs and HT (Tables 3 and 5). Subgroup analysis in GD patients with ophthalmopathy suggested that there was an obvious difference in the genotype distribution and allele frequencies of rs153109 between GD patients

Table 3 Odds ratios (ORs) of the associations of three polymorphisms in *IL27* gene with AITDs before and after adjusting for confounders (age and gender).

	Unadjusted estimates		Adjusted estimates[a]	
Comparison models	OR (95% CI)	*P* values	OR (95% CI)	*P* values
rs153109				
Allele model	0.87 (0.75–1.01)	0.071	0.86 (0.74–1.00)	0.053
Dominant model	0.86 (0.70–1.05)	0.140	0.84 (0.68–1.03)	0.097
Recessive model	0.80 (0.59–1.08)	0.138	0.79 (0.58–1.08)	0.140
Homozygous model	0.86 (0.74–1.02)	0.077	0.85 (0.72–1.01)	0.063
Additive model	0.89 (0.71–1.10)	0.287	0.87 (0.69–1.08)	0.203
rs181206				
Allele model	0.93 (0.72–1.20)	0.578	0.91 (0.71–1.18)	0.487
Dominant model	0.96 (0.73–1.26)	0.770	0.94 (0.71–1.24)	0.640
Recessive model	0.50 (0.16–1.53)	0.225	0.52 (0.16–1.63)	0.260
Homozygous model	0.71 (0.40–1.24)	0.224	0.72 (0.41–1.27)	0.255
Additive model	1.00 (0.75–1.32)	0.973	0.97 (0.73–1.28)	0.817
rs17855750				
Allele model	1.23 (0.96–1.56)	0.098	1.22 (0.96–1.56)	0.111
Dominant model	1.28 (0.99–1.66)	0.061	1.28 (0.98–1.66)	0.070
Recessive model	0.80 (0.28–2.30)	0.680	0.80 (0.27–2.35)	0.682
Homozygous model	0.92 (0.54–1.55)	0.749	0.91 (0.53–1.57	0.743
Additive model	1.31 (1.00–1.71)	0.046	1.30 (1.00–1.71)	0.054

[a]Age and gender were adjusted in the multivariate logistic regression analyses.
95% CI, 95% confidence interval; AITD, autoimmune diseases; OR, odds ratio.

Table 4 Odds ratios (ORs) of the associations of three polymorphisms in *IL27* gene with GD before and after adjusting for confounders (age and gender).

Comparison models	Unadjusted estimates		Adjusted estimates[a]	
	OR (95% CI)	*P* values	OR (95% CI)	*P* values
rs153109				
Allele model	0.76 (0.64–0.91)	0.002	0.76 (0.64–0.90)	0.002
Dominant model	0.75 (0.59–0.94)	0.014	0.74 (0.58–0.93)	0.011
Recessive model	0.62 (0.43–0.90)	0.011	0.62 (0.43–0.90)	0.012
Homozygous model	0.74 (0.61–0.90)	0.003	0.74 (0.61–0.90)	0.003
Additive model	0.80 (0.63–1.03)	0.080	0.79 (0.62–1.01)	0.061
rs181206				
Allele model	0.93 (0.70–1.24)	0.606	0.91 (0.68–1.22)	0.537
Dominant model	0.97 (0.71–1.32)	0.849	0.95 (0.70–1.30)	0.751
Recessive model	0.33 (0.69–1.54)	0.157	0.34 (0.07–1.61)	0.174
Homozygous model	0.57 (0.26–1.24)	0.158	0.58 (0.27–1.27)	0.171
Additive model	1.02 (0.74–1.40)	0.904	1.00 (0.73–1.37)	0.981
rs17855750				
Allele model	1.12 (0.84–1.47)	0.443	1.12 (0.85–1.48)	0.429
Dominant model	1.19 (0.89–1.60)	0.237	1.19 (0.89–1.61)	0.241
Recessive model	0.19 (0.02–1.52)	0.116	0.21 (0.02–1.69)	0.141
Homozygous model	0.44 (0.15–1.26)	0.125	0.46 (0.16–1.33)	0.155
Additive model	1.26 (0.93–1.69)	0.132	1.25 (0.93–1.70)	0.141

[a]Age and gender were adjusted in the multivariate logistic regression analyses.
95% CI, 95% confidence interval; GD, Graves' disease; OR, odds ratio.

without ophthalmopathy and controls ($P=0.007$ and $P=0.004$ respectively; Supplementary Table 1, see section on supplementary data given at the end of this article). Subgroup analysis by gender also found that rs153109 was significantly associated with GD in females for both the genotype distribution ($P=0.024$) and the allele frequencies ($P=0.007$) (Supplementary Tables 2 and 3).

Association between rs181206 and AITD

It is clear from Table 2 that there was (1) no significant difference between AITD patients and healthy controls in both genotype distribution ($P=0.464$) and allele frequency of *IL27* rs181206 ($P=0.575$), (2) no significant difference between GD patients and healthy controls

Table 5 Odds ratios (ORs) of the associations of three polymorphisms in *IL27* gene with HT before and after adjusting for confounders (age and gender).

Comparison models	Unadjusted estimates		Adjusted estimates[a]	
	OR (95% CI)	*P* values	OR (95% CI)	*P* values
rs153109				
Allele model	1.06 (0.87–1.28)	0.563	1.04 (0.85–1.28)	0.681
Dominant model	1.07 (0.81–1.40)	0.633	1.04 (0.78–1.39)	0.777
Recessive model	1.09 (0.75–1.58)	0.640	1.18 (0.74–1.60)	0.684
Homozygous model	1.06 (0.87–1.30)	0.571	1.05 (0.85–1.29)	0.664
Additive model	1.05 (0.79–1.40)	0.729	1.03 (0.76–139)	0.842
rs181206				
Allele model	0.94 (0.68–1.30)	0.702	0.95 (0.68–1.34)	0.780
Dominant model	0.94 (0.66–1.35)	0.752	0.95 (0.66–1.38)	0.807
Recessive model	0.77 (0.20–2.94)	0.707	0.85 (0.21–3.40)	0.818
Homozygous model	0.88 (0.45–1.71)	0.700	0.92 (0.64–1.84)	0.814
Additive model	0.96 (0.66–1.38)	0.816	0.96 (0.66–1.41)	0.84
rs17855750				
Allele model	1.39 (1.04–1.87)	0.028	1.37 (1.01–1.87)	0.046
Dominant model	1.42 (1.03–1.97)	0.033	1.39 (0.99–1.95)	0.059
Recessive model	1.79 (0.60–5.37)	0.299	1.95 (0.60–6.34)	0.269
Homozygous model	1.38 (0.80–2.40)	0.251	1.43 (0.79–2.60)	0.236
Additive model	1.40 (1.00–1.95)	0.052	1.35 (0.95–1.91)	0.093

[a]Age and gender were adjusted in the multivariate logistic regression analyses.
95% CI, 95% confidence interval; HT, Hashimoto's thyroiditis; OR, odds ratio.

in both the genotype distribution (*P*=0.327) and allele frequencies of rs181206 (*P*=0.604) and (3) no significant difference between HT patients and healthy controls in the genotype distribution (*P*=0.906) and the allele frequency of rs181206 (*P*=0.696).

Table 3 shows no significant association of *IL27* rs181206 with AITDs under all comparison models before and after adjustment for age and gender (*P*>0.05). Tables 4 and 5 show no association between *IL27* rs181206 and GD or HT under all comparison models (*P*>0.05).

Association between rs17855750 and AITD

Table 2 shows that the allele frequencies of T and G in rs17855750 polymorphism were 89.03% and 10.97% in AITD patients, 90.84% and 9.16% in healthy controls, 89.92% and 10.08% in GD patients, and 87.61% and 12.39% in HT patients, respectively (Table 2). Our results show that the allele frequencies of rs17855750 were statistically and significantly different between HT patients and healthy controls (*P*=0.02).

A mild association of *IL27* rs17855750 with AITDs was observed in additive model before adjustment for age and gender (Table 3, *P*=0.046). But there was no significant difference in both genotype distribution and allele frequencies of rs17855750 between GD patients and healthy controls (Table 4, *P*>0.05). However, as shown in Table 5, a significant association of *IL27* rs17855750 with HT was observed under the allele model (OR=1.39, 95% CI 1.04–1.87, *P*=0.028) and dominant model (OR=1.42, 95% CI 1.03–1.97; *P*=0.033). Multivariate logistic regression analyses show that the association was still significant after adjustment for age and gender in allele model (OR=1.37, 95% CI 1.01–1.87; *P*=0.046) (Table 5). In addition, Table 5 shows a slight but not significant association between rs17855750 of *IL27* and HT in additive model before adjustment (OR=1.40; 95% CI 1.00–1.95; *P*=0.052). Subgroup analysis by gender found that rs17855750 was mildly associated with HT in females for both the genotype distribution (*P*=0.042) and allele frequencies (*P*=0.023), which suggested that rs17855750 was possibly involved in the pathogenesis of HT, but the association in males was insignificant (Supplementary Tables 2 and 3). Outcomes of subgroup analyses stratified by family history and goiter degree are shown in the Supplementary Tables 4, 5, 6 and 7.

Discussion

Our study explored the association of three SNPs of *IL27* in autoimmune thyroid disease and found that both

genotype frequency and allele frequency of rs153109 has a strong correlation with GD. In addition, rs17855750 has a correlation with HT in both allele and dominant models, while rs181206 has no significant positive relationship with GD and HT.

To the best of our knowledge, the present study is the first on the association of *IL27* polymorphism with GD predisposition. With the advancement of modern medicine, although the pathogenesis of GD has gradually surfaced, due to its complexity, it has not yet been accurately determined. It is generally believed that interactions between environment and genes play a crucial role in the pathogenesis of GD. Although many genes have been reported to be associated with the susceptibility of GD, there is no well-recognized report to date. The polymorphisms of *IL27* gene has been extensively studied in many autoimmune diseases on account of *IL27*, which is a critical cytokine that functions as a mediator between the innate and adaptive immune system (25). As we all know, GD is closely related to the abnormal activation of T lymphocytes, and IL27 is a proinflammatory cytokine that plays an essential role in transcriptional activation and regulation of T lymphocytes (26). IL27, a special IL-12/IL-6 family cytokine, is a heterodimeric cytokine mainly produced by antigen-presenting cells, including DCs and monocytes/macrophages (27). IL27 is a pleiotropic cytokine that has multiple roles in immune function. IL27 has significant antitumor activity because it enhances antitumor immunity by regulating NK cell activity, with strong direct anti-antigen and anti-metastatic effects (28), and may play a key role in the antitumor immune response by regulating the production of T helper 1 (Th1)/Th2 bias and regulatory T cells (Treg) (29). IL27 induces cytotoxic T lymphocyte production by inducing transcription factor T-bet and cytotoxic effect granzyme B and perforin expression (30). A previous study pointed out that IL-27R-deficient mice develop profound or fatal T-cell-mediated pathology without modulating IL27 signaling (31). *IL27* was viewed as a proinflammatory cytokine before, due to its support to the development of interferon (IFN)-secreting T helper cells (Th). However, IL27 has recently been shown to act as a negative regulator of ongoing immune responses during infection and autoimmune inflammation because it can limit the production of proinflammatory cytokines by CD4 T cells including IFN and resist the role of IL-6 (32). Therefore, we examined the association of *IL27* gene with several autoimmune and inflammatory diseases, analyzed the contribution of three potentially functional SNPs of *IL27* and demonstrated a

clear association between *IL27* polymorphisms and GD susceptibility.

Previous studies showed that rs153109, rs17855750 and rs181206 of *IL27* gene displayed significant associations with dilated cardiomyopathy (DCM) (33), atrial fibrillation (AF) (33), allergic rhinitis (34), pre-eclampsia (35) and renal cell carcinoma (RCC) (36). Thyroid autoimmunity is frequently related to polyglandular endocrine syndromes (37, 38). The number of AITD patients with autoimmune polyglandular syndromes in our study was not very common, and the information above was not recorded in detail, so the relationship between autoimmune polyglandular syndromes and *IL27* SNPs could not be analyzed. Currently, there are no studies on the relationship between autoimmune polyglandular syndromes and IL27 polymorphisms, and more studies are needed to explore the relationship between them in the future. Existing studies reported that rs153109 AG and AG/GG genotypes, rs17855750 GT and GT/GG genotypes of *IL27* gene significantly increased the risk of PTC, both rs153109 and rs17855750 of *IL27* gene contributed to the increased risk of PTC (22). Moreover, there is another study which showed that rs153109 of *IL27* gene may be associated with the decreased risk for lymph node metastasis of PTC (39). In our present study, rs153109 locus shows a strong correlation with GD and rs17855750 locus shows a significant correlation with HT, but there is no significant association between rs181206 and AITDs including GD and HT.

We have previously shown that thyroid-stimulating hormone receptor (TSHR) (40), CD40 (13), microRNA (23) and IL17 (41) are associated with the pathogenesis of GD and found that polymorphisms of *PTPN22* and *CTLA4* are associated with the pathogenesis of GD, suggesting that genetic polymorphism plays a key role in the pathogenesis and progression of GD (12, 13). Signal transducer and activator of transcription 3 (*STAT3*) rs3816769 and rs744166 have been shown to be associated with AITD risk and thyroid antibody levels in the Polish population (42). In this study, we further demonstrate a clear association between polymorphism of *IL27* and AITDs, and provide genetics evidence for the role of *IL27* in the pathogenesis of GD. Though our study indicated *IL27* rs153109 locus is a susceptible factor to GD and rs17855750 locus is a susceptible factor to HT, the molecular mechanisms underlying the roles of *IL27* rs153109 and *IL27* rs17855750 in autoimmune diseases are still unclear. Moreover, IL-23/IL-17 axis plays a crucial role in the pathogenesis of inflammatory and autoimmune diseases (43). There is a study which suggested that the rs2275913 of the

IL17/IL23R pathway is associated with rheumatic heart disease (RHD) in South Indian populations (44). The associations found in the rs11209026 of *IL23R* and the rs187238 of *IL18* genes suggest that a genetically determined high activity of the IL23/IL17 pathway was associated with increased risk of ankylosing spondylitis (45). Yu *et al.* confirmed the association of SNPs of *IL23R* and *IL17A* with UC risk in Chinese Han population (46).

Although our study confirms that *IL27* has a significant association with GD, there are still limitations in this study. First, our study found for the first time that the genetic susceptibility of *IL27* is clearly associated with GD in Asian populations, but whether it is also true in other ethnic groups needs to be further explored with large sample size. Second, this study only evaluated the association between *IL27* gene SNPs and GD susceptibility, and did not analyze the role of *IL27* gene SNPs in predicting treatment outcomes. Third, our study tested only three potential SNP sites of *IL27* in GD patients and healthy controls. The association of other SNP sites with GD needs to be further evaluated.

In conclusion, this is the first identification of the association of *IL27* rs153109 with GD and rs17855750 with HT. Our findings add new data on the genetic contribution to GD susceptibility and support the crucial role of *IL27* in GD pathogenesis. However, further researches are warranted to elucidate the molecular mechanisms underlying the relationship between *IL27* and GD.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/EC-18-0370.

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.

References

1 Caturegli P, Kimura H, Rocchi R & Rose NR. Autoimmune thyroid diseases. *Current Opinion in Rheumatology* 2007 **19** 44–48. (https://doi.org/10.1097/BOR.0b013e3280113d1a)

2 Valdes S, Maldonado-Araque C, Lago-Sampedro A, Lillo JA, Garcia-Fuentes E, Perez-Valero V, Gutierrez-Repiso C, Ocon-Sanchez P, Goday A, Urrutia I, *et al*. Population-based national prevalence of

thyroid dysfunction in Spain and associated factors: diabetes study. *Thyroid* 2017 **27** 156–166. (https://doi.org/10.1089/thy.2016.0353)

3 Khattak RM, Ittermann T, Nauck M, Below H & Volzke H. Monitoring the prevalence of thyroid disorders in the adult population of Northeast Germany. *Population Health Metrics* 2016 **14** 39. (https://doi.org/10.1186/s12963-016-0111-3)

4 Shan Z, Chen L, Lian X, Liu C, Shi B, Shi L, Tong N, Wang S, Weng J, Zhao J, *et al*. Iodine status and prevalence of thyroid disorders after introduction of mandatory universal salt iodization for 16 years in China: a cross-sectional study in 10 cities. *Thyroid* 2016 **26** 1125–1130. (https://doi.org/10.1089/thy.2015.0613)

5 McLeod DS, Cooper DS, Ladenson PW, Whiteman DC & Jordan SJ. Race/Ethnicity and the prevalence of thyrotoxicosis in young Americans. *Thyroid* 2015 **25** 621–628. (https://doi.org/10.1089/thy.2014.0504)

6 Chen C, Xu H, Chen Y, Chen Y, Li Q, Hu J, Liang W, Cheng J, Xia F, Wang C, *et al*. Iodized salt intake and its association with urinary iodine, thyroid peroxidase antibodies, and thyroglobulin antibodies among urban Chinese. *Thyroid* 2017 **27** 1566–1573. (https://doi.org/10.1089/thy.2017.0385)

7 Tomer Y. Mechanisms of autoimmune thyroid diseases: from genetics to epigenetics. *Annual Review of Pathology* 2014 **9** 147–156. (https://doi.org/10.1146/annurev-pathol-012513-104713)

8 Lee HJ, Li CW, Hammerstad SS, Stefan M & Tomer Y. Immunogenetics of autoimmune thyroid diseases: a comprehensive review. *Journal of Autoimmunity* 2015 **64** 82–90. (https://doi.org/10.1016/j.jaut.2015.07.009)

9 Brix TH, Kyvik KO, Christensen K & Hegedus L. Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 930–934. (https://doi.org/10.1210/jcem.86.2.7242)

10 Ajjan RA & Weetman AP. The pathogenesis of Hashimoto's thyroiditis: further developments in our understanding. *Hormone and Metabolic Research* 2015 **47** 702–710. (https://doi.org/10.1055/s-0035-1548832)

11 Burch HB & Cooper DS. Management of Graves disease: a review. *JAMA* 2015 **314** 2544–2554. (https://doi.org/10.1001/jama.2015.16535)

12 Heward JM, Brand OJ, Barrett JC, Carr-Smith JD, Franklyn JA & Gough SC. Association of PTPN22 haplotypes with Graves' disease. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 685–690. (https://doi.org/10.1210/jc.2006-2064)

13 Yang J, Qin Q, Yan N, Zhu YF, Li C, Yang XJ, Wang X, Pandey M, Hou P & Zhang JA. CD40 C/T(-1) and CTLA-4 A/G(49) SNPs are associated with autoimmune thyroid diseases in the Chinese population. *Endocrine* 2012 **41** 111–115. (https://doi.org/10.1007/s12020-011-9510-1)

14 Li CS, Zhang Q, Lee KJ, Cho SW, Lee KM, Hahm KB, Choi SC, Yun KJ, Chung HT & Chae SC. Interleukin-27 polymorphisms are associated with inflammatory bowel diseases in a Korean population. *Journal of Gastroenterology and Hepatology* 2009 **24** 1692–1696. (https://doi.org/10.1111/j.1440-1746.2009.05901.x)

15 Shen H, Xia L, Xiao W & Lu J. Increased levels of interleukin-27 in patients with rheumatoid arthritis. *Arthritis and Rheumatism* 2011 **63** 860–861. (https://doi.org/10.1002/art.30180)

16 Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J, Hibbert L, Churakova T, Travis M, Vaisberg E, *et al*. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells. *Immunity* 2002 **16** 779–790. (https://doi.org/10.1016/S1074-7613(02)00324-2)

17 Paradowska-Gorycka A, Raszkiewicz B, Jurkowska M, Felis-Giemza A, Romanowska-Prochnicka K, Manczak M & Olesinska M. Association of single nucleotide polymorphisms in the IL27 gene with rheumatoid arthritis. *Scandinavian Journal of Immunology* 2014 **80** 298–305. (https://doi.org/10.1111/sji.12209)

18 Dehghanzadeh R, Babaloo Z, Sakhinia E, Khabazi A, Shanehbandi D, Sadigh-Eteghad S & Gharibi T. IL-27 gene polymorphisms in Iranian patients with Behcet's disease. *Clinical Laboratory* 2016 **62** 855–861. (https://doi.org/10.7754/Clin.Lab.2015.150843)

19 Paradowska-Gorycka A, Sowinska A, Stypinska B, Grobelna MK, Walczyk M, Olesinska M, Piotrowski P & Jagodzinski PP. Genetic variants in IL-12B and IL-27 in the polish patients with systemic lupus erythematosus. *Scandinavian Journal of Immunology* 2016 **84** 49–60. (https://doi.org/10.1111/sji.12439)

20 Furuzawa Carballeda J, Fonseca Camarillo G & Yamamoto-Furusho JK. Interleukin 27 is up-regulated in patients with active inflammatory bowel disease. *Immunologic Research* 2016 **64** 901–907. (https://doi.org/10.1007/s12026-016-8804-z)

21 Santos AS, Melo ME, Crisostomo LG, Fukui RT, Matioli SR & Silva ME. Lack of association between IL27 gene variants and type 1 diabetes susceptibility. *Cytokine* 2013 **61** 349–352. (https://doi.org/10.1016/j.cyto.2012.12.003)

22 Nie X, Yuan F, Chen P, Pu Y, Zhu J, Wang Y, Xiao X, Che G, Gao L & Zhang L. Association between IL-27 gene polymorphisms and risk of papillary thyroid carcinoma. *Biomarkers in Medicine* 2017 **11** 141–149. (https://doi.org/10.2217/bmm-2016-0283)

23 Cai T, Li J, An X, Yan N, Li D, Jiang Y, Wang W, Shi L, Qin Q, Song R, *et al*. Polymorphisms in MIR499A and MIR125A gene are associated with autoimmune thyroid diseases. *Molecular and Cellular Endocrinology* 2017 **440** 106–115. (https://doi.org/10.1016/j.mce.2016.11.017)

24 Song RH, Qin Q, Yan N, Muhali FS, Meng S, He ST & Zhang JA. Variants in IRAK1-MECP2 region confer susceptibility to autoimmune thyroid diseases. *Molecular and Cellular Endocrinology* 2015 **399** 244–249. (https://doi.org/10.1016/j.mce.2014.10.013)

25 Wynick C, Petes C & Gee K. Interleukin-27 mediates inflammation during chronic disease. *Journal of Interferon and Cytokine Research* 2014 **34** 741–749. (https://doi.org/10.1089/jir.2013.0154)

26 Ouaked N, Mantel PY, Bassin C, Burgler S, Siegmund K, Akdis CA & Schmidt-Weber CB. Regulation of the foxp3 gene by the Th1 cytokines: the role of IL-27-induced STAT1. *Journal of Immunology* 2009 **182** 1041–1049. (https://doi.org/10.4049/jimmunol.182.2.1041)

27 Jung JY, Roberts LL & Robinson CM. The presence of interleukin-27 during monocyte-derived dendritic cell differentiation promotes improved antigen processing and stimulation of T cells. *Immunology* 2015 **144** 649–660. (https://doi.org/10.1111/imm.12417)

28 Pontrelli P, Rascio F, Zaza G, Accetturo M, Simone S, Infante B, Furian L, Castellano G, Ditonno P, Battaglia M, *et al*. Interleukin-27 is a potential marker for the onset of post-transplant malignancies. *Nephrology Dialysis Transplantation* 2019 **34** 157–166. (https://doi.org/10.1093/ndt/gfy206)

29 Hasegawa H, Mizoguchi I, Chiba Y, Ohashi M, Xu M & Yoshimoto T. Expanding diversity in molecular structures and functions of the IL-6/IL-12 heterodimeric cytokine family. *Frontiers in Immunology* 2016 **7** 479. (https://doi.org/10.3389/fimmu.2016.00479)

30 Zhou H, Qiu J-h, Wang T, Yu Y-y, Liu X-n, Li X, Wang Y-w, Hou Y, Li L-z, Liu X-g, *et al*. Interleukin 27 inhibits cytotoxic T-lymphocyte-mediated platelet destruction in primary immune thrombocytopenia. *Blood* 2014 **124** 3316–3319. (https://doi.org/10.1182/blood-2014-06-580084)

31 Liu G, Xu J, Wu H, Sun D, Zhang X, Zhu X, Magez S & Shi M. IL-27 signaling is crucial for survival of mice infected with African trypanosomes via preventing lethal effects of CD4+ T cells and IFN-gamma. *PLOS Pathogens* 2015 **11** e1005065. (https://doi.org/10.1371/journal.ppat.1005065)

32 Wang H, Li Z, Yang B, Yu S & Wu C. IL-27 suppresses the production of IL-22 in human CD4(+) T cells by inducing the expression of SOCS1. *Immunology Letters* 2013 **152** 96–103. (https://doi.org/10.1016/j.imlet.2013.05.001)

33 Chen Y, Zeng J, Zhang R, Zeng L, Li Y, Wei H & Yang Q. Effect of interleukin-27 genetic variants on atrial fibrillation susceptibility. *Genetic Testing and Molecular Biomarkers* 2017 **21** 97–101. (https://doi.org/10.1089/gtmb.2016.0219)

34 Ke X, Shen Y, Hu X, Yuan XD, Kang HY, Wang XQ & Hong SL. [Association between IL-27 gene polymorphisms and susceptibility to allergic rhinitis]. *Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke za Zhi* 2016 **30** 684–688. (https://doi.org/10.13201/j.issn.1001-1781.2016.09.004)

35 Chen P, Gong Y, Pu Y, Wang Y, Zhou B, Song Y, Wang T & Zhang L. Association between polymorphisms in IL-27 gene and pre-eclampsia. *Placenta* 2016 **37** 61–64. (https://doi.org/10.1016/j.placenta.2015.11.003)

36 Pu Y, Chen P, Zhou B, Zhang P, Wang Y, Song Y & Zhang L. Association between polymorphisms in IL27 gene and renal cell carcinoma. *Biomarkers* 2015 **20** 202–205. (https://doi.org/10.3109/1354750X.2015.1062555)

37 Horie I, Kawasaki E, Ando T, Kuwahara H, Abiru N, Usa T, Yamasaki H, Ejima E & Kawakami A. Clinical and genetic characteristics of autoimmune polyglandular syndrome type 3 variant in the Japanese population. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** E1043–E1050. (https://doi.org/10.1210/jc.2011-3109)

38 Wemeau JL, Proust-Lemoine E, Ryndak A & Vanhove L. Thyroid autoimmunity and polyglandular endocrine syndromes. *Hormones* 2013 **12** 39–45. (https://doi.org/10.1007/BF03401285)

39 Zhang S, Gao X, Wang Y, Jia J, Zhang Q & Ji Z. Interleukin 27 -964A > G genetic polymorphism and serum IL-27p28 levels in Chinese patients with papillary thyroid cancer. *Tumor Biology* 2015 **36** 8207–8211. (https://doi.org/10.1007/s13277-015-3570-4)

40 Liu L, Wu HQ, Wang Q, Zhu YF, Zhang W, Guan LJ & Zhang JA. Association between thyroid stimulating hormone receptor gene intron polymorphisms and autoimmune thyroid disease in a Chinese Han population. *Endocrine Journal* 2012 **59** 717–723. (https://doi.org/10.1507/endocrj.EJ12-0024)

41 Yan N, Yu YL, Yang J, Qin Q, Zhu YF, Wang X, Song RH & Zhang JA. Association of interleukin-17A and -17F gene single-nucleotide polymorphisms with autoimmune thyroid diseases. *Autoimmunity* 2012 **45** 533–539. (https://doi.org/10.3109/08916934.2012.702814)

42 Kotkowska A, Sewerynek E, Domanska D, Pastuszak-Lewandoska D & Brzezianska E. Single nucleotide polymorphisms in the STAT3 gene influence AITD susceptibility, thyroid autoantibody levels, and IL6 and IL17 secretion. *Cellular and Molecular Biology Letters* 2015 **20** 88–101. (https://doi.org/10.1515/cmble-2015-0004)

43 Gaffen SL, Jain R, Garg AV & Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nature Reviews Immunology* 2014 **14** 585–600. (https://doi.org/10.1038/nri3707)

44 Poomarimuthu M, Elango S, Solomon PR, Soundrapandian S & Mariakuttikan J. Association of IL17 and IL23R gene polymorphisms with rheumatic heart disease in South Indian population. *Immunological Investigations* 2018 **47** 754–764. (https://doi.org/10.1080/08820139.2018.1493053)

45 Sode J, Bank S, Vogel U, Andersen PS, Sorensen SB, Bojesen AB, Andersen MR, Brandslund I, Dessau RB, Hoffmann HJ, *et al.* Genetically determined high activities of the TNF-alpha, IL23/IL17, and NFkB pathways were associated with increased risk of ankylosing spondylitis. *BMC Medical Genetics* 2018 **19** 165. (https://doi.org/10.1186/s12881-018-0680-z)

46 Yu P, Shen F, Zhang X, Cao R, Zhao X, Liu P, Tu H, Yang X, Shi R & Zhang H. Association of single nucleotide polymorphisms of IL23R and IL17 with ulcerative colitis risk in a Chinese Han population. *PLoS ONE* 2012 **7** e44380. (https://doi.org/10.1371/journal.pone.0044380)