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Association between Polymorphisms of IL-23/IL-17 Pathway and Clinical Phenotypes of Autoimmune Thyroid Diseases

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ABSTRACT

Background: Several autoimmune and inflammatory disorders, including autoimmune thyroid diseases (AITD), have been linked to Th17 cells and the IL-23/IL-17 axis. Current data suggest that genetic variation contributes greatly to disease susceptibility to AITD.

Objectives: To study the role of single nucleotide polymorphisms (SNPs) of IL-23/IL-17 pathway in AITD predisposition and test the gene-gene/gene-sex interactions in these loci.

Methods: A total of 1051 patients with AITD, including 657 patients with Graves' disease (GD) and 394 patients with Hashimoto's thyroiditis (HT), and 874 healthy controls were enrolled in this case-control association study. Six SNPs were selected and genotyped by multiplex PCR combined with high-throughput sequencing. Interactions were tested by the general multifactor dimensionality reduction (GMDR) method.

Results: Allele C and combinational genotype AC+CC of rs3212227 within IL-23 were significantly associated with GD with goiter (P=0.003 and 0.014, respectively). Allele G and combinational genotype AG+GG of rs4819554 within IL-17RA were significantly related to HT with family history and the severity of HT (P=0.011 and 0.027; P=0.041 and 0.035). Also, allele T and genotype CT+TT of rs9463772 within IL-17RA were significantly correlated with the severity of HT (P=0.001 and 0.027, respectively). Moreover, high dimensional gene-sex interaction (IL-23R-IL-23-IL-17RA-IL-17F) was identified in AITD, GD, and HT patients with GMDR analysis.

Conclusions: Our study identified the novel loci and gene-sex interaction in AITD. This evidence, from another perspective, suggests that sex, IL-23/IL-17 pathway, and Th17 cells play an important role in the pathogenesis of AITD.

Keywords: Autoimmune Thyroid Diseases (AITD), Gene-gene Interaction, Gene-sex Interaction, IL-23/IL-17 Pathway, Single Nucleotide Polymorphism (SNP)

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INTRODUCTION

Autoimmune thyroid diseases (AITD) which involve both polygenetic susceptibility and environmental factors are complex and multifactorial (1, 2). Because the pathogenesis of AITD involves multiple genes, exploring the interactions of multiple genes will help better understand the genetic mechanisms underlying this complex disease (3). The single gene polymorphisms tend to show only a limited risk contribution to AITD (OR=1.2–1.5) (4). An epidemiological study showed that about 70% of the risk of developing hereditary AITD is due to the interaction of multiple genes (5). Consequently, epidemiological studies investigating the potential gene-gene and gene-environment relationship in AITD are of great interest to researchers.

Th17 cells, one type of $CD4^+$ T cell subsets, have a vital pro-inflammatory function in AITD by secreting many cytokines such as IL-17, IL-17F, IL-21, and IL-22 (6, 7). IL-23 is a crucial contributor to the process of maintenance of Th17 cells differentiation and promotion of IL-17 secretion (8). IL-23/IL-17 axis mainly includes IL-23, IL-23 receptor, Th17 cells as well as IL-17 and is an essential pathway in Th17 cells activation and maintenance. According to other studies, the IL-23/IL-17 axis participates in the pathogenesis of rheumatoid arthritis, psoriasis, systemic lupus erythematosus, and other autoimmune diseases (9, 10). The retinoic acid-related orphan receptor C (RORC) is a necessary transcription factor for Th17 cell differentiation in the immune response. RORγ and RORγT proteins are transcription factors transcribed and translated from the same gene RORC (11-13). RORγT may be directly involved in the regulation of IL17A and IL17F, so it is a landmark transcription factor of Th17 cells. Suppressor of cytokine signaling 3 (SOCS3) is a major negative regulator of Stat3-activating cytokines that negatively controls Th17 generation (14).

Since the genetic susceptibility to AITD cannot be fully elucidated by the existing found loci, we sought to study the role of single nucleotide polymorphisms (SNPs) of IL-23/IL-17 pathway for AITD predisposition and examine gene-gene/gene-sex interactions in these loci in a Chinese population.

MATERIALS AND METHODS

Subjects Enrollment

The study subjects comprised 1051 AITD patients (657 GD patients, 394 HT patients) and 874 healthy controls. The AITD group was recruited from the Endocrinology Department of Zhoupu Hospital of Shanghai University of Medicine & Health Sciences. The healthy control group was drawn from the same hospital's physical examination facility. Diagnostic criteria for AITD and inclusion criteria for the healthy controls have been described in detail in our previous study (15). According to the clinical data of AITD patients, we divided them into the following phenotypes: (1) the presence or absence of AITD family history; (2) the presence or absence of smoking history; (3) with or without ophthalmopathy; (4) the presence or absence of anti-thyroid peroxidase antibody (TPOAb) or anti-thyroglobulin antibodies (TGAb); (5) with or without goiter. In addition, GD patients were segmented into intractable GD and GD in remission according to the prognosis (15). HT patients were divided into euthyroid and hypothyroid subjects according to their thyroid function. The study was approved by the Ethics Committee of Zhoupu Hospital of Shanghai University of Medicine&Health Sciences. All participants provided both oral and written informed consent.

SNP Selection

SNPs were chosen for the current investigation because of their links with autoimmune diseases. Rs3212227 (IL-23), rs17375018 (IL-23R), rs9463772 (IL-17F) and rs4969170 (SOCS3) were selected for the present study because of their reported associations

with GD, HT or Graves' ophthalmopathy (GO) (16-19). Since the available data showed that rs4819554 (IL-17RA) and rs4845604 (RORC) had associations with several autoimmune disease susceptibility (20-22), they were chosen for genotyping as well.

Genomic DNA Extraction and Genotyping

Genomic DNA was extracted from 2ml peripheral venous blood using RelaxGene Blood DNA System (Tiangen Biotech, Beijing, China). Shanghai Biowing Applied Biotechnology Company (http://www.biowing.com.cn/) provided technical assistance for "Hi-SNP genotyping service". In brief, this method combines multiplex PCR and high-throughput sequencing technology. Firstly, DNA samples were amplified in a 10 μl PCR reaction system. Secondly, the purified and diluted amplified products of PCR were allotted to a random number and genotyped blindly utilizing next-generation sequencing technology with Illumina X-10 Platform (Illumina, USA). Thirdly, bioinformatics methods were used to analyze the sequencing results, differentiate the different samples, and finally obtain the mutation information of each locus. Specific primer sequences of amplifying the target DNA sequences are listed here: rs17375018 F: 5'-ATCTCCCCTTCACTGTCTAGTTAAG -3'; R: 5'- TCTAACCTTTTATATCTTTTATGTC CTG -3'; rs3212227 F: 5'- TTTAGGATCACA ATGATATCTTTGC -3';R: 5'- AACATT CCATACATCCTGGCAG -3'; rs4819554 F:5'- ACTCATGAAATGTGTAATTCGCTG -3';R: 5'- GACAGCTCCGGGCTCCAG -3';rs4845604 F: 5'- TCTGTCCAGCA TTTCTCCTCG -3';R: 5'- GTTGGACAGAG GTGGAGGAGTC -3'; rs4969170 F: 5'-TTAAGACTGGAACCTGGTACGTAG -3';R: 5'- CAGGCCTCTAATCTCCAGCG -3'; rs9463772 F: 5'- TATCTGGTACATTC ACCACAGGC -3'; R: 5'- TCATGAAATACG TTCCGCTAGC -3'.

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) of the control group was estimated by the SNPstats (http://bioinfo.iconcologia.net/SNPstats.). Logistic regression analysis was used to analyze disease associations by the software SPSS version 18.0. High dimensional gene-gene/gene-sex interactions on AITD were

Table 1. The demographic and clinical characteristics of AITD patients and controls

Characteristics	Controls	AITD	GD	HT	P value
Number	874	1051	657	394	
Gender					2.54×10^{-17}
Male (%)	363 (41.5%)	247 (23.5%)	191 (29.1%)	56 (14.2%)	
Female (%)	511 (58.5%)	804 (76.5%)	466 (70.9%)	338 (85.8%)	
Age (years)	39.31±9.65	41.47±14.36	40.89±14.63	42.42±13.88	0.009
Age of onset (years)		38.38±14.13	37.52±14.15	39.82±14.01	
Family history (+, %)		201 (19.1%)	137 (20.9%)	64 (16.2%)	
Smoking history (+, %)		82 (7.8%)	65 (9.9%)	17 (4.3%)	
Ophthalmopathy (+, %)		107 (10.2%)	104 (15.8%)	3 (0.8%)	
TPOAb (+, %)		635 (60.4%)	347 (52.8%)	288 (73.1%)	
TGAb (+, %)		529 (50.3%)	243 (37.0%)	286 (72.6%)	
TRAb (+, %)			657 (100%)		
Goiter (+, %)		908 (86.4%)	522 (79.4%)	386 (98.0%)	
Prognosis of GD					
GD in remission (%)			200 (30.4%)		
Intractable GD (%)			318 (48.4%)		
Severity of HT					
HT with euthyroidism (%)				230 (58.4%)	
HT with hypothyroidism (%)				164 (41.6%)	

AITD=autoimmune thyroid disease; GD=Graves' disease; HT=Hashimoto's thyroiditis

detected by the software General multifactor dimensionality reduction (GMDR: Version 0.9) (23). The significance of parameters including cross-validation consistency, the testing balanced accuracy, the training balanced accuracy, and the sign test were depicted in detail in our previous study (15). P values<0.05 were defined statistically significant in our study.

RESULTS

Characteristics of AITD and Control Subjects

As shown in Table 1, AITD patients and controls were not matched for sex and age. There were 201 patients with AITD family history with a percentage of 19.1% and 908 patients with goiter with a percentage of 86.4%. In addition, in the GD subgroup, 200 patients were GD in remission and 318 were intractable GD. In the HT subgroup, 230 patients were euthyroid and 164 hypothyroid.

Association between SNPs and the Risk of AITD

The genotyping success rate of each SNP was greater than 96% and all loci exhibited three genotypes. Each SNP in the control group was under the HWE. There were no significant associations between the six

Table 2. Genotype and allele distribution in AITDs, GD, HT patients and controls

Gene/ SNP	Allele/ Genotype	Controls (%)	AITD (%)	P value	GD (%)	P value	HT (%)	P value
IL-23R	GG	418 (48.6%)	528 (51.4%)	0.477	336 (52.1%)	0.376	192 (50.3%)	0.937
rs17375018	AG	376 (43.7%)	418 (40.7%)		258 (40%)		160 (41.9%)	
	AA	66 (7.7%)	81 (7.9%)		51 (7.9%)		30 (7.8%)	
	G	1212 (70.5%)	1474 (71.8%)	0.381	930 (72.1%)	0.329	544 (71.2%)	0.709
	A	508 (29.5%)	580 (28.2%)		360 (27.9%)		220 (28.8%)	
IL-23	AA	281 (33.5%)	328 (32.5%)	0.824	205 (32.4%)	0.779	123 (32.5%)	0.477
rs3212227	AC	420 (49.9%)	508 (50.3%)		326 (51.6%)		182 (48.2%)	
	CC	139 (16.6%)	174 (17.2%)		101 (16%)		73 (19.3%)	
	A	982 (58.5%)	1164 (57.6%)	0.611	736 (58.2%)	0.903	428 (56.6%)	0.395
	C	698 (41.5%)	856 (42.4%)		528 (41.8%)		328 (43.4%)	
IL-17RA	AA	260 (30.5%)	335 (32.9%)	0.661	212 (33.2%)	0.460	123 (32.4%)	0.835
rs4819554	AG	434 (50.8%)	493 (48.4%)		301 (47.1%)		192 (50.5%)	
	GG	160 (18.7%)	191 (18.7%)		126 (19.7%)		65 (17.1%)	
	A	954 (55.9%)	1163 (57.1%)	0.457	725 (56.7%)	0.634	438 (57.6%)	0.411
	G	754 (44.1%)	875 (42.9%)		553 (43.3%)		322 (42.4%)	
RORC	GG	799 (93.8%)	969 (94.8%)	0.534	610 (95.2%)	0.537	359 (94.2%)	0.655
rs4845604	AG	52 (6.1%)	52 (5.1%)		31 (4.8%)		21 (5.5%)	
	AA	1 (0.1%)	1 (0.1%)		0 (0%)		1 (0.3%)	
	G	1650 (96.8%)	1990 (97.4%)	0.337	1251 (97.6%)	0.222	739 (97.0%)	0.842
	A	54 (3.2%)	54 (2.6%)		31 (2.4%)		23 (3.0%)	
SOCS3	AA	717 (84.3%)	852 (83.8%)	0.751	536 (84.3%)	0.780	316 (82.9%)	0.703
rs4969170	AG	128 (15.1%)	158 (15.5%)		95 (14.9%)		63 (16.5%)	
	GG	5 (0.6%)	7 (0.7%)		5 (0.8%)		2 (0.5%)	
	A	1562 (91.9%)	1862 (91.5%)	0.709	1167 (91.7%)	0.893	695 (91.2%)	0.575
	G	138 (8.1%)	172 (8.5%)		105 (8.3%)		67 (8.8%)	
IL-17F	CC	596 (70.1%)	723 (71.2%)	0.666	449 (70.6%)	0.768	274 (72.1%)	0.682
rs9463772	CT	143 (16.8%)	156 (15.3%)		99 (15.6%)		57 (15%)	
	TT	111 (13.1%)	137 (13.5%)		88 (13.8%)		49 (12.9%)	
	C	1335 (78.5%)	1602 (78.8%)	0.818	997 (78.4%)	0.922	605 (79.6%)	0.546
	T	365 (21.5%)	430 (21.2%)		275 (21.6%)		155 (20.4%)	

AITD=autoimmune thyroid disease; GD=Graves' disease; HT=Hashimoto's thyroiditis; OR=odds ratio; 95% CI=95% confidence intervals; P value was adjusted for age and sex.

SNPSs and AITD, GD, and HT risk, shown in Table 2.

Association between SNPs and Clinical Phenotypes

We further investigated the association between polymorphisms and clinical phenotypes of AITD by logistic regression for adjusting sex and age. When comparing the allele and genotype distribution of rs3212227 in GD patients with or without goiter, we found that the frequency of allele C in GD with goiter was significantly reduced than that in GD without goiter (OR=0.657, P=0.003). Besides, in the dominant model, frequencies of combinational genotype AC+CC also significantly reduced in GD with goiter (OR=0.570, P=0.014).

Furthermore, we discovered links between rs4819554 and HT with or without a family history. For rs4819554, the frequency of allele G in HT with a family history significantly reduced than that in HT without a family history (OR=0.589, P=0.011). In the dominant model, the frequencies of genotype AG+GG significantly reduced in HT with family history (OR=0.530, P=0.027).

As for the severity of HT, we found that the frequency of allele G in HT with hypothyroidism significantly decreased than that in HT with euthyroidism (OR=0.736, P=0.041). In the dominant model, frequencies of combinational genotype AG+GG also significantly reduced in HT with hypothyroidism (OR=0.625, P=0.035). Conversely, for rs9463772, the frequency of allele T in HT with hypothyroidism significantly elevated than that in HT with

Table 3. Genotype and allele distribution in phenotypes of AITD patients

		GD without Goiter (%)	GD with Goiter (%)	OR (95%CI)	P value
IL-23	AA	31 (23.8%)	174 (34.7%)	1.000 (ref.)	
rs3212227	AC+CC	99 (76.2%)	328 (65.3%)	0.570 (0.364-0.892)	0.014
	A	130 (50.0%)	606 (60.4%)	0.657 (0.499-0.864)	0.003
	C	130 (50.0%)	398 (39.6%)		
		HT without FH (%)	**HT with FH (%)**	**OR (95%CI)**	**P value**
IL-17RA	AA	96 (30.1%)	27 (44.3%)	1.000 (ref.)	
rs4819554	AG+GG	223 (69.9%)	34 (55.7%)	0.530 (0.302-0.930)	0.027
	A	355 (55.6%)	83 (68.0%)	0.589 (0.391-0.889)	0.011
	G	283 (44.4%)	39 (32.0%)		
		HT with euthyroidism (%)	**HT with hypothyroidism (%)**	**OR (95%CI)**	**P value**
IL-17RA	AA	62 (28.1%)	61 (38.4%)	1.000 (ref.)	
rs4819554	AG+GG	159 (71.9%)	98 (61.6%)	0.625 (0.404-0.967)	0.035
	A	241 (54.5%)	197 (61.9%)	0.736 (0.549-0.988)	0.041
	G	201 (45.5%)	121 (38.1%)		
IL-17F	CC	169 (76.5%)	105 (66.1%)	1.000 (ref.)	
rs9463772	CT+TT	52 (23.5%)	54 (33.9%)	1.671 (1.061-2.632)	0.027
	C	370 (83.7%)	235 (73.9%)	1.815 (1.272-2.590)	0.001
	T	72 (16.3%)	83 (26.1%)		

GD=Graves' disease; HT=Hashimoto's thyroiditis; FH=Family history; OR=odds ratio; 95% CI=95% confidence intervals. P value was adjusted for age and sex.

euthyroidism (OR=1.815, P=0.001). In the dominant model, the frequencies of genotype CT+TT also significantly increased in HT with hypothyroidism (OR=1.671, P=0.027). The above data is shown in Table 3.

Gene-gene Interactions Analysis via GMDR

In the current study, no gene-gene interactions affecting AITD risk were discovered (Table 4).

Gene-sex Interactions Analysis via GMDR

Two to seven-factor models of gene-sex interactions on the risk of AITD, GD, and HT adjusted by age are shown in Table 5. Possible interactions between gene-sex of AITD, GD, and HT risk were discovered. No matter in which group of patients, the best gene-sex interaction was the model of IL-23R-IL-23-IL-17RA-IL-17F-sex (for AITD: P=0.001, testing balanced accuracy=58.11%, cross-validation consistency=10/10; for GD: P=0.001, testing balanced accuracy=56.51%, cross-validation consistency=10/10; for HT: P=0.001, testing balanced accuracy=60.38%, cross-validation consistency=10/10). These suggested a latent gene-sex interaction between the five variants that influences the risk of AITD.

DISCUSSION

The current hypothesis is that in complex diseases, a large number of associated genetic variations influence the expression of nearby genes, by this means contributing to the onset of the disease. In the present study, we made a hypothesis that the joint effect of various SNPs in genes of a pathway might have a stronger effect on gene expression of that pathway than

Table 4. GMDR models of gene-gene interactions and AITD risk

Factor numbers	Best model	Training balance accuracy	Testing balance accuracy	Cross-validation Consistency	Sign test (P value)
		AITD			
2	IL-23R-IL-17RA	0.5282	0.4830	6/10	0 (1.0000)
3	IL-23R- IL-23-IL-17RA	0.5449	0.4945	7/10	4 (0.8281)
4	IL-23R- IL-23-IL-17RA- IL-17F	0.5695	0.5034	10/10	4 (0.8281)
5	IL-23R- IL-23-IL-17RA- SOCS3-IL-17F	0.5903	0.4978	10/10	5 (0.6230)
6	IL-23R-IL-23-IL-17RA-RORC-SOCS3-IL-17F	0.6034	0.4839	10/10	2 (0.9893)
		GD			
2	IL-17RA -SOCS3	0.5353	0.4880	5/10	3 (0.9453)
3	IL-23-IL-17RA- IL-17F	0.5548	0.4999	7/10	6 (0.3770)
4	IL-23R- IL-23-IL-17RA- IL-17F	0.5810	0.5022	9/10	6 (0.3770)
5	IL-23R- IL-23-IL-17RA- SOCS3-IL-17F	0.6056	0.5197	10/10	8 (0.0547)
6	IL-23R-IL-23-IL-17RA-RORC-SOCS3-IL-17F	0.6203	0.5071	10/10	5 (0.6230)
		HT			
2	IL-23-SOCS3	0.5422	0.5085	7/10	7 (0.1719)
3	IL-23- IL-17RA - IL-17F	0.5648	0.4774	6/10	3 (0.9453)
4	IL-23R- IL-23-IL-17RA- IL-17F	0.5978	0.5360	10/10	8 (0.0547)
5	IL-23R- IL-23-IL-17RA- SOCS3-IL-17F	0.6232	0.5040	10/10	4 (0.8281)
6	IL-23R-IL-23-IL-17RA-RORC-SOCS3-IL-17F	0.6380	0.4892	10/10	3 (0.9453)

AITD=autoimmune thyroid disease; GD=Graves' disease; HT=Hashimoto's thyroiditis; For SNP: 0=no risk alleles, 1=1 risk allele, 2=2 risk alleles; For Sex: 1=male, 2=female. P value was adjusted for sex and age.

Table 5. GMDR models of gene-sex interactions and AITD risk

Factor numbers	Best model	Training balance accuracy	Testing balance accuracy	Cross-validation Consistency	Sign test (P value)
		AITD			
2	SOCS3-sex	0.5920	0.5903	7/10	10 (0.0010)
3	IL-23R-SOCS3-sex	0.5959	0.5733	2/10	9 (0.0107)
4	IL-23-IL-17RA-IL-17F-sex	0.6076	0.5682	6/10	10 (0.0010)
5	IL-23R-IL-23-IL-17RA-IL17F-sex	0.6348	0.5811	10/10	10 (0.0010)
6	IL-23R-IL-23-IL-17RA-SOCS3-IL-17F-sex	0.6574	0.5701	10/10	10 (0.0010)
7	IL-23R-IL-23-IL-17RA-RORC-SOCS3-IL-17F-sex	0.6692	0.5501	10/10	10 (0.0010)
		GD			
2	IL-23R-sex	0.5654	0.5516	6/10	8 (0.0547)
3	IL-23R-IL-17F-sex	0.5746	0.5520	8/10	9 (0.0107)
4	IL-23-IL-17RA-IL-17F-sex	0.5968	0.5384	7/10	7 (0.1719)
5	IL-23R-IL-23-IL-17RA-IL17F-sex	0.6325	0.5651	10/10	10 (0.0010)
6	IL-23R-IL-23-IL-17RA-SOCS3-IL-17F-sex	0.6616	0.5569	10/10	10 (0.0010)
7	IL-23R-IL-23-IL-17RA-RORC-SOCS3-IL-17F-sex	0.6739	0.5443	10/10	9 (0.0107)
		HT			
2	SOCS3-sex	0.6395	0.6394	9/10	10 (0.0010)
3	IL-23-IL-17F-sex	0.6440	0.6132	6/10	10 (0.0010)
4	IL-23-IL-17RA-IL-17F-sex	0.6554	0.6038	8/10	10 (0.0010)
5	IL-23R-IL-23-IL-17RA-IL-17F-sex	0.6829	0.6038	10/10	10 (0.0010)
6	Il-23R-IL-23-IL-17RA-SOCS3-IL-17F-sex	0.7059	0.5877	10/10	10 (0.0010)
7	IL-23R-IL-23-IL-17RA-RORC-SOCS3-IL-17F-sex	0.7224	0.5918	10/10	10 (0.0010)

AITD=autoimmune thyroid disease; GD=Graves' disease; HT=Hashimoto's thyroiditis; For SNP: 0=no risk alleles, 1=1 risk allele, 2=2 risk alleles; For Sex: 1=male, 2=female. P value was adjusted for age.

a single locus. Consequently, we created a genetic risk model for the IL23/IL17 pathway. It was unfortunate, however, that there were no substantial connections between SNP rs17375018, rs3212227, rs4819554, rs4845604, rs4969170, and rs9463772 with AITD risk.

Interestingly, subsequent clinical phenotypes analyses showed the association between SNPs and AITD sub-phenotypes. Goiter is typical for AITD, although it is not a consistent manifestation. Some patients may not have goiter at the time of diagnosis of Graves' hyperthyroidism (24, 25). In this study, about 79.4% of GD patients were diagnosed with goiter by ultrasound. Previous investigations have reported that the presence of an enlarged thyroid could affect the disease severity, treatment, and prognosis of GD patients (26-28). The enlarged volume of the thyroid positively connected with the high levels of thyrotropin receptor antibody (TRAb) in serum, which could influence the remission induction and sustenance in GD (26). Rs3212227 is located in the 3′-untranslated regions of IL12B which encodes the p40 subunit of IL-23 and IL-12, so it is involved in both the IL12/Th1 pathway and IL23/Th17 pathway (29). The presence of the major allele (A) confers a risk of psoriasis and psoriatic arthritis (C allele: protective effect) (30). Similarly, in this study, we found that allele C also played a protective role in

the pathogenesis of GD in goiter. Allele C and genotype AC+CC of rs3212227 were associated with GD patients with goiter, which generated a reduced risk by 34.3% and 43.0% (OR=0.657 and 0.570). Therefore, we assumed that allele C of rs3212227 might be correlated with the treatment and prognosis of GD patients in China, although more technical research is required to substantiate this stance.

Data from family and twin studies provided compelling support for the genetic basis of AITD by the molecular genetic studies. Several prevalence surveys had reported that AITD patients had a family history of thyroid dysfunction and other types of thyroid disease (31). In our research, we found out that there existed associations between rs4819554 and HT with family history. For rs4819554, allele G and genotype AG+GG were found to be associated with HT patients with family history, which generated a reduced risk by 41.1% and 47.0% (OR=0.589 and 0.530). The general pathogenesis of HT is abnormal immune responses against thyroid tissue, so it is the most common cause of autoimmune hypothyroidism (32). Therefore, the detection of thyroid function in HT patients is of particular clinical importance. In the present study, 41.6% of HT patients developed severe hypothyroidism and were treated daily, while 58.3% patients were in a euthyroid status. Rs4819554 also associated with disease severity of HT in our study. Allele G and genotype AG+GG of rs4819554 conferred a reduced risk by 26.4% and 37.5% for HT with hypothyroidism (OR=0.736 and 0.625). Rs4819554 is located in the IL17RA promoter region and the allele A would create a site for the transcription factor activating enhancer binding protein 4 (AP-4) whose core binding sequence is CAGCTG (33). Besides, rs4819554 was reported to be linked to gene expression differences between the genotypes. Expression levels of IL17RA mRNA and protein in peripheral leukocytes and CD14+ monocytes of patients with asthma were significantly elevated in patients with AA genotype than in those with GG genotype (34). Therefore, these findings suggested that the association between rs4819554 and some phenotypes of HT could be explained by its effect on the gene expression of IL17RA. On the contrary, allele T and genotype CT+TT of rs9463772 offered an increased risk for HT with hypothyroidism (OR=1.815 and OR=1.671). Rs9463772 is located in the 5 flanking sequence of IL-17F gene and allele T was reported to increase the risk of GD in a Chinese population (18). Via function prediction analysis, rs9463772 polymorphism might influence transcription factors binding, such as GATA-2, GATA-1 and NF-κB (18). Nevertheless, in the present study, allele T was only discovered to be associated with HT with hypothyroidism as a risk factor. This dichotomy could be attributed to regional differences or other confounding elements. Taken together, we concluded that rs4819554 and rs9463772 in the IL-17RA and IL-17F gene conferred predisposition to clinical genotypes of HT. The evidence supporting our above conclusion is that many cytokines related to Th17 cells are involved in the pathogenesis of HT. Ruggeri RM et al. reported HT patients had significantly higher positive detection rates and serum concentrations of IL-23 in comparison with the healthy controls (35). IL-22, another pro-inflammatory cytokine produced by Th17 or Th22 cells, increased in serum of newly diagnosed, untreated HT patients, which indicated IL-22 was involved in the pathogenesis of HT (36). IL-37 is a key regulatory factor in the contexts of pro-inflammatory and anti-inflammatory pathways and may inhibit Th1/Th17-mediated response (37, 38). It has been reported that IL-37 is up-regulated in HT and may exert a protective role by counteracting oxidative stress and inflammation (39).

AITD affects women 5 to 10 times more than men. However, the underlying causes of the difference in gender prevalence have not been understood. The preponderance of AITD in women can be explained by non-genetic or genetic factors which can influence

female dominance of AITD through X chromosome loci (40). In our study, in terms of high-dimensional gene-sex interaction, there was a five-way interaction between IL-23R, IL-23, IL-17RA, IL-17F and sex in patients with AITD, GD and HT, which had the highest testing-balanced accuracy and cross-validation consistency. This finding may suggest that the interaction between these sites and sex plays a role in AITD susceptibility, because polymorphisms of these genes were associated with clinical AITD phenotypes. It is important to note that hypothesis generation is the goal of GMDR analysis. As a result, the precise process by which these loci link with sex needs to be studied extensively. Therefore, the exact mechanism by which these loci interact with sex remains to be further investigated.

In conclusion, we observed the association between SNPs of IL-23/IL-17 pathway and AITD clinical phenotypes and identified the gene-sex interaction in AITD patients. Therefore, this evidence, from another perspective, suggests that sex, the IL-23/IL-17 pathway, and Th17 cells play an important role in the pathogenesis of AITD. However, our study also had some limitations, such as the small number of selected loci for this pathway and failure to correct for more confounding factors. Further studies are needed to confirm the conclusion subsequently.

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