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ORIGINAL ARTICLE

The *MAGI2* gene polymorphism rs2160322 is associated with Graves' disease but not with Hashimoto's thyroiditis

X. Jia[1] · T. Zhai[2] · B. Wang[1] · J. Zhang[1] · F. Zhang[3]

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Abstract

Purpose Autoimmune thyroid diseases (AITDs) are chronic organ-specific autoimmune disorders, predominantly including Graves' disease (GD), and Hashimoto's thyroiditis (HT). This study aimed to investigate whether single-nucleotide polymorphisms (SNPs) in *MAGI2* and *MAGI3* gene contributed to the etiology of AITDs.

Methods We conducted a case–control study including 1001 patients with AITDs (625 GD, 376 HT) and 846 healthy controls. Subgroup analyses in GD and HT were also performed.

Results The genotypes of rs2160322 in *MAGI2* showed a borderline association with AITDs ($P=0.048$), and they had a strong correlation with GD ($P=0.012$). The frequency of the minor allele G of rs2160322 was significantly higher in the GD patients than in the controls ($P=0.027$; OR 1.91; 95% CI 1.020–1.391), especially for GD females ($P=0.008$; OR 1.304; 95% CI 1.072–1.587), and those who had positive family history ($P=0.011$; OR 1.412; 95% CI 1.083–1.843). For genetic model analysis, the recessive model and homozygous model of rs2160322 showed significant associations with AITDs ($P=0.009$; $P=0.019$) and GD ($P=0.004$; $P=0.005$). Nevertheless, our study could not identify any relationship between these SNPs and HT. Due to the low mutation rate of rs1343126 in *MAGI3*, we were unable to obtain a credible conclusion on its association with AITDs.

Conclusions Our study identified that *MAGI2* rs2160322 was strongly associated with GD susceptibility. The potential dysfunction of tight junction proteins and aberrant epithelial barrier caused by abnormal *MAGI2* expression may be a novel mechanism of GD.

Keywords MAGI2 · Single-nucleotide polymorphisms · Autoimmune thyroid diseases · Graves' disease · Hashimoto's thyroiditis

Introduction

Autoimmune thyroid diseases (AITDs) are a class of chronic organ-specific autoimmune diseases, predominantly including Graves' disease (GD) and Hashimoto's thyroiditis (HT) [1, 2]. The incidence of AITDs in the overall population is 2–5%, of which women have an absolute preponderance, with the ratio of female to male ranges from 5:1 to 10:1 [3–5]. The exact etiology of AITDs has not yet fully elucidated, and it is usually thought to be multifactorial, involving genetic susceptibility, impaired immune system homeostasis, and environmental factors [6–8].

Membrane-associated guanylate kinase inverted *(MAGI)2* and *MAGI3* are the main members of the tight junction (TJ) family and are closely related in function. TJ proteins, encoded by TJ gene families (*F11R*, *MAGI1*, *MAGI2*, *MAGI3*, *PARD3*, *PTEN*, and *TJP1*) [9], have been

✉ J. Zhang
zhangjinan@hotmail.com

✉ F. Zhang
jgyyzf@126.com

[1] Department of Endocrinology, Jinshan Hospital of Fudan University, No. 1508 Longhang Road, Jinshan District, Shanghai 201508, China

[2] Department of Endocrinology and Metabolism, Zhongshan Hospital of Fudan University, No. 180 Fenglin Road, Xuhui District, Shanghai 200032, China

[3] Department of Emergency, Jinshan Hospital of Fudan University, No. 1508 Longhang Road, Jinshan District, Shanghai 201508, China

demonstrated to exert a vital role in maintaining the appropriate intercellular space [10]. TJ proteins between thyroid epithelial cells can make belt-like structures jointing the luminal pole and limit the paracellular permeability [11]. In physiological situations, tight epithelial lining of thyroid gland as a paracellular barrier is crucial to euthyroidism. In contrast, under the pathological conditions, the dysfunction of TJ proteins leads to abnormal cell interval, which promotes the infiltration of T lymphocytes and might facilitate the exposure of normal secluded body antigens to immune system [11]. The above processes offer the possibility of TJ proteins dysfunction-induced chronic autoimmune inflammatory reactions and spur the occurrence of autoimmune diseases.

In recent years, numerous in vivo studies have observed the involvement of TJ molecules in autoimmune diseases, such as rheumatoid arthritis [12], systemic lupus erythematosus [13], and inflammatory bowel disease (IBD) [14–16]. In TJ gene families, *MAGI2* and *MAGI3*, two closely linked genes mediated by phosphatase and tensin homologue (PTEN), have been identified as candidate susceptibility genes of IBD [14–16]. *MAGI2* is reported to be significantly associated with Crohn's disease (CD) and ulcerative colitis (UC) [16], while *MAGI3* is also proved to have close associations with CD and UC [15]. Given the fact that AITDs share many genetic variations with other autoimmune diseases, we aimed to extend the observations about *MAGI2* and *MAGI3* to encompass AITDs. In this study, one SNP in *MAGI2* gene (rs2160322) and one in *MAGI3* (rs1343126) were detected in a set of 1001 patients with AITDs and 846 healthy controls in Chinese Han population.

Materials and methods

Informed consent and approval

The experiment was approved by the ethics committee of Jinshan Hospital of Fudan University. Informed consent was signed in both the patient group and the control group. All subjects in this experiment were voluntary and did not receive any financial compensation.

Study design, setting, and size

A case–control study was conducted to explore the relationship between *MAGI2*, *MAGI3*, and AITDs. This study included 1052 Chinese Han patients with an established diagnosis of AITDs and 874 healthy Chinese Han controls at Jinshan Hospital between the years of 2016 and 2017. Among them, 51 AITDs patients and 28 normal controls (NC) were excluded, because the DNA extraction concentration was too low (< 100 μg/ml) or the unqualified DNA purity ($A_{260/280}$ was not between 1.8 and 2.0). The valid data ultimately included in this study came form 1001 AITD patients and 846 healthy controls.

Participants, diagnosis criteria, and subgroup

The AITDs' group included a random sample of 625 GD patients (186 males and 439 females) and 376 HT patients (54 males and 322 females), with no subjective selectivity. All patients and normal controls came from Jinshan Hospital. Patients were arbitrarily recruited from Endocrinology Clinics, while healthy controls were consecutively enrolled from the Healthy Check-Up Center with ethnically and geographically matching.

The diagnosis criteria for GD used in this study met international requirements [7], including clinical manifestations of thyrotoxicosis, biochemical markers of hyperthyroidism, positive circulating thyroid-stimulating hormone receptor antibody (TRAb) and diffuse goiter of the thyroid gland observed by ultrasonography or palpation. The definition of HT cases was based on thyroid enlargement and high levels of autoantibodies [thyroid peroxidase antibody (TPOAb) or thyroglobulin antibody (TgAb)].

To more accurately investigate the relationship between SNPs and different clinical phenotypes of AITDs, we set clinical classifications of GD and HT in the current study, including: (1) the onset age of GD or HT (≤ 18 years or ≥ 19 years); (2) presence or absence thyrotoxic exophthalmos in GD group; (3) goiter or normal volume of thyroid gland; (4) euthyroidism or hypothyroidism in HT group; and (5) presence or absence of AITDs family history (disease in immediate relatives within three generations).

Graves' ophthalmopathy (GO), also called thyroid-associated ophthalmopathy, is characterized by inflammation and fibrosis of the extra ocular muscles, chemosis, proptosis, excess tearing, and episcleral vascular injection [7]. The thyroid goiter was determined by palpation and was clinically divided into three degrees clinically. The definition of I degree is that the goiter cannot be seen, but it can be palpated. Degree II is defined as the visible and palpable goiter, but is still confined to the sternocleidomastoid region. The degree III is characterized by enlarged thyroid tissue beyond the sternocleidomastoid muscle [7]. Demographic statistics and clinical phenotypes of subjects in patient groups are shown in Table 1.

Potential bias

To rule out potential genetic background interference, we ensured that each patient was independent and had no genetic relationship. To minimize interference from environmental factors, all subjects included in this study lived in the same area (Shanghai, China). In addition, all members

Table 1 Demographic statistics and clinical phenotypes of subjects in patient groups

	AITDs (%)	GD (%)	HT (%)
Number	1001	625	376
Female	761 (76.02)	439 (70.24)	322 (85.64)
Male	240 (23.98)	186 (29.76)	54 (14.36)
Age	41.44 ± 14.41	40.82 ± 14.66	42.46 ± 13.93
Onset of age	37.92 ± 14.29	36.88 ± 14.34	39.60 ± 14.11
≤ 18	67 (6.69)	55 (8.80)	12 (3.19)
≥ 19	934 (93.31)	570 (91.20)	364 (96.81)
Ophthalmopathy (+)	100 (9.99)	97 (15.52)	3 (0.80)
Family history (+)	194 (19.38)	134 (21.44)	60 (15.96)
Thyroid size			
Normal	276 (27.57)	125 (192.31)	151 (40.16)
I degree	261 (26.07)	169 (27.04)	92 (24.47)
II degree	416 (41.56)	291 (46.56)	125 (33.24)
III degree	48 (4.80)	40 (6.40)	8 (2.13)

AITDs autoimmune thyroid diseases, *GD* Graves' disease, *HT* Hashimoto's thyroiditis

did not have any other immune disease, inflammatory infection, and chronic disease to eliminate interference from other diseases.

Sources of data and measurements

DNA sample collection and extraction

Genomic DNA was isolated by The Relax Gene Blood DNA System (Tiangen Biotech Co., Ltd., Beijing, China), from 1 ml peripheral venous blood of each subjects. To ensure that the extracted DNA had high quality, we used Nano Drop 2000 Spectro-photometer (Thermo Scientific Company, Waltham, MA, USA) to determine the concentration and purity of the extracted DNA. A DNA sample that satisfies both conditions of a concentration ≥ 100 ug/ml and $A_{260/280}$ between 1.8 and 2.0 was considered a qualified sample. Unqualified DNA samples were discarded.

SNP selection and genotyping

Two TJ-related genes and two SNPs were investigated in the present study, *MAGI2* (rs2160322) and *MAGI3* (rs1343126). The products of these two genes interact with each other and belonged to the TJ protein network [9]. The multiplex polymerase chain reaction (PCR) method was used to amplify the target DNA sequence. Specific primer sequences were designed as follows: *MAGI2* (rs2160322) upper primer—CTAAAGAAGGTGCCTCTGATTTCACTGG; lower primer—CTAGGAAGCTTTTGATTCTGCCTATTTGGG; *MAGI3* (rs1343126) upper primer—GCAGAACACATTTCCTTATCATTTTCCC; lower primer—CATTGGGGTAATCCATTTAACATTAAACG.

Statistical analysis

Statistical methods, quantitative variables, and missing data

This study used SPSS statistical version 23 (IBM, Chicago, IL, USA) to calculate all odds ratios (OR), 95% confidence intervals (95% CI), and *P* values, based on the two-tailed Pearson Chi-square test (χ^2 test) for genotype/allele frequency of each SNP. The *P* value < 0.05 was considered to be statistically significant. For each SNP, deviation from Hardy–Weinberg equilibrium (HWE) was estimated using the HWE program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl), and the *P* value (HWpval) of the two tag SNPs meets the criteria of HWpval > 0.05. Linkage analysis and haplotype analysis were also performed in this study. A linkage disequilibrium (LD) test was conducted using Haploview Software [version 4.2 (Broad Institute, Cambridge, MA, USA)]. In addition, because the mutation rate of polymorphism rs1343126 in *MAGI3* (susceptible allele T) in our DNA samples was too low (< 5%), it was not enough to obtain reliable statistical results, so we have discarded this part of data. The data results associated with rs1343126 in *MAGI3* would not appear in the next results section.

Adjustment and genotyping–clinical phenotype analysis

To consolidate the evidence, we performed an adjustment analysis and gave the *P* value before and after adjustment. Specifically, significant findings were further examined by multiple logistic regression, adjusting for potential interfering factors (gender and age) simultaneously. To obtain more detailed conclusions, we also performed an χ^2 test analysis between each clinical subgroup.

Outcomes

The main outcome of this study was to confirm whether SNPs in *MAGI2* and *MAGI3* gene contributed to the etiology of AITDs. In addition, we further derived the relationship between different AITDs subtypes and *MAGI2* gene (rs2160322).

Results

In the current study, we examined the frequency distribution for each allele and analyzed the association for each SNP in a case–control manner. Associations of rs2160322 in *MAGI2* gene with AITDs, GD, and HT are shown in Table 2. In the

Table 2 Associations of rs2160322 in MAGI-2 gene with AITDs, GD, and HT

SNP rs2160322	Alleles	NC (%)	AITDs (%)	*P* value (AITDs vs. NC)	GD (%)	*P* value (GD vs. NC)	HT (%)	*P* value (HT vs. NC)
Total	CC	389 (45.98)	453 (45.25)	0.048	269 (43.04)	0.012	184 (48.94)	0.380
	CG	384 (45.39)	427 (42.66)		272 (43.52)		155 (41.22)	
	GG	73 (8.63)	121 (12.09)		84 (13.44)		37 (9.84)	
	C	1162 (68.68)	1333 (66.58)	0.176	810 (64.80)	0.027	523 (69.55)	0.667
	G	530 (31.32)	669 (33.42)		440 (35.20)		229 (30.45)	
Female	CC	245 (50)	352 (46.25)	0.099	189 (43.05)	0.022	163 (50.62)	0.507
	CG	208 (42.45)	325 (42.71)		197 (44.87)		128 (39.75)	
	GG	37 (7.55)	84 (11.04)		53 (12.07)		31 (9.63)	
	C	698 (71.22)	1029 (67.61)	0.056	575 (65.49)	0.008	454 (70.50)	0.752
	G	282 (28.78)	493 (32.39)		303 (34.51)		190 (29.50)	
Male	CC	144 (40.45)	101 (42.08)	0.088	80 (43.01)	0.036	21 (38.89)	0.962
	CG	176 (49.44)	102 (42.50)		75 (40.32)		27 (50.00)	
	GG	36 (10.11)	37 (15.42)		31 (16.67)		6 (11.11)	
	C	464 (65.17)	304 (63.33)	0.516	235 (63.17)	0.514	69 (63.89)	0.795
	G	248 (34.83)	176 (36.64)		137 (36.83)		39(36.11)	

AITDs autoimmune thyroid diseases, *GD* Graves' disease, *HT* Hashimoto's thyroiditis, *NC* normal controls

genotype analysis, the SNP marker in *MAGI2* (rs2160322) was weakly related to AITDs ($P=0.048$) in the subjects, but was strongly correlated with GD ($P=0.012$). The susceptibility allele G also showed a strong relationship with GD ($P=0.027$; OR 1.91; 95% CI 1.020 and 1.391), and the trend was more significantly in females ($P=0.008$; OR 1.304; 95% CI 1.072–1.587). In males, the genotype distribution displayed positive relationship ($P=0.036$), while the allele distribution did not show any difference between patient group and normal controls. We further analyzed the genotype and allele frequencies of rs2160322 in *MAGI2* in different GD clinical phenotypes, as shown in Table 3. We found that in the respect of family history, rs2160322 in *MAGI2* was strongly correlated with those who had a positive family history ($P_{genotype}=0.012$; $P_{allele}=0.011$, OR 1.412, 95% CI 1.083–1.843). However, there was neither correlation between GO and rs2160322 in *MAGI2*, nor correlation between early onset of disease and this gene locus. This suggests that female patients and those with family history are more closely related to rs2160322 compared to the whole patients group.

To analyze the relationship between rs2160322 and AITDs in more depth, we conducted genetical model analysis, which can be seen in Tables 4 and 5. From Table 4, we

Table 3 Genotype/allele frequencies of rs2160322 in MAGI-2 in different clinical phenotypes of GD

SNP	Genotype/allele frequencies, *n* (%)							
rs2160322	Controls	Total GD	GO (+)	GO (−)	Children GD	Adult GD	Family history(+)	Family history (−)
CC	389 (45.98)	269 (43.04)	38 (39.18)	231 (43.75)	28 (50.91)	241 (42.36)	51 (38.06)	218 (44.40)
CG	384 (45.39)	272 (43.52)	49 (50.52)	223 (42.23)	20 (36.36)	252 (44.29)	61 (45.52)	211 (42.97)
GG	73 (8.63)	84 (13.44)	10 (10.31)	74 (14.02)	7 (12.73)	76 (13.36)	22 (16.42)	62 (12.63)
C	1162 (68.68)	810 (64.80)	125 (64.43)	685 (64.87)	68 (66.67)	734 (64.50)	163 (60.82)	647 (65.89)
G	530 (31.32)	440 (35.20)	69 (35.57)	371 (35.13)	34 (33.33)	404 (35.50)	105 (39.18)	335 (34.11)
	P values							
	*P*1	OR (95% CI)		*P*2	OR (95% CI)		*P*3	OR (95% CI)
CC\CG\GG	0.434			0.335			0.012	
C\G	0.229	1.210(0.886–1.652)		0.671	1.096(0.717–1.676)		0.011	1.412(1.083–1.843)

GD Graves' disease, *GO* Graves' ophthalmopathy, *NC* normal controls, *P1* GO vs. NC, *P2* children GD vs. NC, *P3* family history (+) GD vs. NC

Table 4 Associations of the polymorphism rs2160322 in the MAGI-2 gene with AITDs before and after adjusting for cofounders (age and gender)

Groups	Comparison models	Unadjusted estimates		Adjusted estimates	
		OR (95% CI)	*P* values	OR (95% CI)	*P* values
Total	Allele model	1.10 (0.96–1.26)	0.176	1.13 (0.99–1.31)	0.080
	Dominant model	1.05 (0.88–1.27)	0.576	1.10 (0.91–1.33)	0.341
	Recessive model	1.46 (1.07–1.98)	0.016	1.53 (1.11–2.09)	0.009
	Homozygous model	1.19 (1.02–1.40)	0.031	1.22 (1.03–1.44)	0.019
	Additive model	0.95 (0.79–1.16)	0.640	0.99 (0.81–1.21)	0.911
Female	Allele model	1.19 (0.99–1.41)	0.057	1.18 (0.99–1.41)	0.061
	Dominant model	1.20 (0.95–1.50)	0.126	1.20 (0.95–1.50)	0.126
	Recessive model	1.52 (1.01–2.28)	0.043	1.50 (1.00–2.25)	0.050
	Homozygous model	1.26 (1.02–1.55)	0.033	1.25 (1.01–1.54)	0.041
	Additive model	1.09 (0.86–1.38)	0.490	1.09 (0.86–1.38)	0.477
Family history (+)	Allele model	1.25 (0.98–1.59)	0.065	1.31 (1.03–1.67)	0.027
	Dominant model	1.28 (0.93–1.75)	0.133	1.36 (0.98–1.88)	0.062
	Recessive model	1.57 (0.97–2.54)	0.069	1.66 (1.02–2.71)	0.043
	Homozygous model	1.30 (1.00–1.68)	0.047	1.34 (1.03–1.74)	0.031
	Additive model	1.15 (0.83–1.61)	0.400	1.24 (0.88–1.73)	0.219

AITDs autoimmune thyroid diseases, *GD* Graves' disease, *HT* Hashimoto's thyroiditis, *NC* normal controls, *allele Model* G vs. C, *dominant Model* (GG + GC) VS CC, *recessive model* GG VS (GC + CC), *homozygous model* GG VS CC, *additive model* GC VS CC

Table 5 Associations of the polymorphism rs2160322 in the MAGI-2 gene with GD before and after adjusting for cofounders (age and gender)

Groups	Comparison models	Unadjusted estimates		Adjusted estimates	
		OR (95% CI)	*P* values	OR (95% CI)	*P* values
Total	Allele model	1.21 (1.04–1.40)	0.011	1.21 (1.05–1.40)	0.010
	Dominant model	1.19 (0.99–1.46)	0.069	1.21 (0.99–1.47)	0.061
	Recessive model	1.57 (1.16–2.12)	0.003	1.56 (1.15–2.12)	0.004
	Homozygous model	1.28 (1.09–1.50)	0.003	1.26 (1.08–1.48)	0.005
	Additive model	1.07 (0.88–1.32)	0.491	1.07 (0.87–1.32)	0.496
Female	Allele model	1.29 (1.08–1.53)	0.005	1.29 (1.08–1.53)	0.005
	Dominant model	1.37 (1.08–1.73)	0.008	1.37 (1.08–1.73)	0.008
	Recessive model	1.50 (1.03–2.20)	0.036	1.50 (1.03–2.20)	0.036
	Homozygous model	1.30 (1.06–1.58)	0.010	1.29 (1.06–1.58)	0.012
	Additive model	1.27 (0.99–1.62)	0.061	1.27 (0.99–1.62)	0.061
Family history (+)	Allele model	1.44 (1.10–1.90)	0.008	1.49 (1.13–1.97)	0.004
	Dominant model	1.43 (0.98–2.07)	0.062	1.49 (1.02–2.16)	0.039
	Recessive model	2.14 (1.28–3.59)	0.004	2.22 (1.32–3.72)	0.002
	Homozygous model	1.54 (1.17–2.03)	0.002	1.56 (1.18–2.07)	0.002
	Additive model	1.21 (0.81–1.79)	0.350	1.27 (0.85–1.89)	0.240

GD Graves' disease, *HT* Hashimoto's thyroiditis, *NC* normal controls, *allele model* G vs. C, *dominant model* (GG + GC) VS CC, *recessive model* GG VS (GC + CC), *homozygous MODEL* GG VS CC, *additive model* GC VS CC

can acquire the information that for the total AITDs patients, both the recessive model and homozygous model embodied a strong correlation with AITDs ($P = 0.016$; $P = 0.031$, respectively), especially after the adjusting of the possible cofounders (age and gender) ($P = 0.009$; $P = 0.019$, respectively). As shown in Table 5, the GD group displayed significant association with either allele model, recessive model, or homozygous model of rs2160322 ($P = 0.011$, $P = 0.003$, and $P = 0.003$, respectively), despite after sex and age adjustment ($P = 0.010$, $P = 0.004$ and $P = 0.005$). It should be noted that although there was no obvious difference between the general GD populations and healthy controls in dominant model ($P = 0.061$), a statistically significant P value was observed between females' GD patients and normal controls after

adjusting for age ($P=0.008$). In patients with positive GD family history, the allele model, dominant model, recessive model and homozygous model all displayed a strong positive P value after adjustment ($P=0.004$, $P=0.039$, $P=0.002$, and $P=0.002$, respectively). The results of this study suggested that there was a significant gender difference in the relationship between rs2160322 and AITDs, and their relationship was more pronounced in patients with a family history. No correlation between rs2160322 in *MAGI2* and the HT group was observed in our experiment.

Discussion

In the present study, we found a strong correlation between the genotypes of rs2160322 in *MAGI2* and individual's susceptibility to GD. The frequency of the minor allele G was significantly higher in the GD patients than in the healthy controls, especially for GD females and those with family history.

GD, as an autoimmune disease, has a complex genetic background [7]. Our research team has previously found that GD is associated with SNPs of various immune genes, including *CD40*, *CTLA4*, *STAT4*, *IL37,* and so on [17–19]. Because autoimmune diseases have a degree of similarity in immune imbalance and genetic background, it is often found that certain genes are associated with multiple autoimmune diseases. The previous studies have found that *MAGI2* and *MAGI3* are associated with IBD [15, 16]. In this study, we, for the first time, found that *MAGI2* rs2160322 was significantly related to GD. Our results suggest that the role of rs2160322 in promoting GD is more pronounced in female patients, which is consistent with the prevalence of gender differences in the incidence of GD. The dominant mechanism of women in GD has not been fully understood, and may be related to genetic differences, sex hormones, and psychological factors [20, 21].

Although GD is not a hereditary disease in the traditional sense, it has a certain degree of family aggregation and genetic susceptibility [22]. The probability of hyperthyroidism occurring in identical twins is as high as 30–60%, while fraternal twins are only 3–9% [1]. Furthermore, GD has the most obvious genetic predisposition in all the types of hyperthyroidism, while other types of hyperthyroidism appear to be not directly related to heredity [1]. Our study found that the relationship between rs2160322 and GD was pronounced in patients with family history in the subgroup analysis. Thus, mutations in the rs2160322 loci of *MAGI2* gene may play a greater role in GD patients with family history.

MAGI2, located on chromosome 7, is a gene that contains roughly 1.4 megabases with 21 exons [16]. It encodes a scaffolding TJ protein of 2410 amino acids, which maintains the architecture of cell junctions and regulates the cell spacing [23]. MAGI2 protein includes six PDZ domains, two WW domains, and a guanylate kinase (GK) domain [24, 25]. The function of the above domains is quite sophisticated that endows *MAGI2* with the capacity to interact with many extracellular compartments (cell adhesion molecules, receptors, and lumens) and intracellular signaling structures [26].

TJ is a complex of several integral membrane-spanning components, partly including claudin, zonula occludens (ZO), occludin, and junctional adhesion molecule (JAM) [10]. *MAGI2* is associated with epithelial tight proteins assembly and appropriate localization [25–27]. Accordingly, it is plausible to consider that defects in MAGI2 expression or function may participate in the pathogenesis of AITDs by disassembly of TJ protein networks. In a previous study, high expression of three connexins was detected in GD, namely, ZO-1, JAM-A, and claudin [11]. However, HT displays a significant difference in TJ protein expression with GD. In HT, a high claudin level was demonstrated, while the expression of JAM-A and ZO-1 was lower than that of GD [11]. This discrepancy may partly explain why the SNP rs2160322 in *MAGI2* does not play a great role in HT like in GD, as our result shows.

Lymphocyte transmigration has been demonstrated in the thyroid tissues of AITDs' patient and is deemed to be a pathological feature of AITDs [3, 4], while MAGI2 has been proved to inhibit cell migration and proliferation [28]. Infiltrating T-helper 1 (Th1) cells have been found to impair the epithelial barrier seriously, through releasing IFN-γ, a cytokine that can downregulate a junction protein (claudin-1) in cultured thyroid tissue of GD patients in vitro [29, 30]. Nilsson et al. revealed that IL-1α, local-delivered by thyroid follicular epithelial cells and monocytes, can negatively regulate the TJ complex and provoke paracellular flow in cultured thyrocytes [31]. Similarly, IL-1β has been confirmed a depressor of TJ proteins' (Claudin and ZO-1) expression and may alter their sub-cellar distribution in the thyroid of AITDs patients [11]. In addition, Rebuffat et al. pointed out that the epithelium destruction or alteration of follicular tightness in AITDs may promote the contact of the autoantigen with immune system [11]. Based on the above-mentioned studies and consisted with our present experiment, we can make a reasonable assumption that the variants of *MAGI2* could influence the lymphocyte trans-endothelial infiltration and contribute to the etiology of GD.

The important role of apoptosis in the occurrence and development of AITD are widely recognized, and *MAGI2* dysfunction-induced cell apoptosis has been demonstrated in the previous literatures [32, 33]. Nevertheless, the effects of *MAGI2* dysfunction-induced thyroid apoptosis have not been clearly established yet. There is a close functional relation between *MAGI2* and PTEN [14, 27], one other TJ gene

that is relevant to thyroid cell apoptosis [34]. Under normal conditions, *MAGI2* upregulates PTEN expression by reducing protein degradation [26, 35], and improves PTEN stability [35]. PTEN, localized on chromosome 10q23.3, encodes a protective lipid phosphatase protein that deregulates the phosphatidylinositol 3-kinase (PI3K) [35]. PI3K is a ubiquitous and proapoptotic lipid kinase that plays a crucial role in cell apoptosis and inflammatory response through activating chemokine receptors and promoting leukocyte migration [36]. PTEN inactivation caused by the deletion or mutation of *MAGI2* could activate the PI3K pathway, with consequent thyroid apoptosis and immune dysregulation, manifesting as autoimmune disorders and chronic inflammation [36]. In addition, DNA released from apoptotic cells stimulates the immune response, forming a positive feedback loop that further initiates and continues the autoimmune process [37]. Therefore, besides effects on junctional assembly, alterations in the balance of epithelial cell viability and apoptosis may represent a supplemental consequence of *MAGI2* gene mutations associated with GD.

Our results first confirmed that the variants of *MAGI2* gene were novel risk factors for GD and made a reasonable assumption for the pathophysiological mechanisms behind it. However, our research is limited to the Chinese Han population, and there may be racial bias. It is necessary to expand the scope of research to populations of different regions and races to get a more authoritative conclusion. Moreover, we need to conduct further researches about other TJ genes, so as to clarify the biological mechanism between TJ gene families and AITDs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiment was approved by the ethics committee of Jinshan Hospital of Fudan University.

Informed consent Informed consent was signed in both the patient group and the control group. All subjects in this experiment were voluntary and did not receive any financial compensation.

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