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Original Article

Replication of association of nine susceptibility loci with Graves' disease in the Chinese Han population

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Abstract: This study is to evaluate the association of 9 single nucleotide polymorphisms (SNPs) with Graves' disease (GD) in different homogenous samples of the Chinese Han population. A total of 2,865 unrelated individuals were enrolled from Linyi City, Shandong Province, China, including 1,139 patients of GD and 1,726 controls. All 9 SNPs showed significant associations with GD ($P < 1.3\times10^{-4}$, Bonferroni corrected $P_c < 0.001$). The most significant association was detected at rs2281388 at the *HLA-DPB1* locus ($P=1.3\times10^{-21}$; OR=1.62, 95% CI: 1.47-1.79). After adjusting for gender and age, 7 SNPs remained significantly associated with GD ($P < 3.4\times10^{-4}$, $P_c < 0.003$). The risk of GD caused by any of these SNPs was not significantly different between female and male participants ($P_{het} > 0.15$). Four SNPs located in *MHC* regions were significantly associated with GD in different ages ($P < 8.4\times10^{-4}$, $P_c < 0.04$). The risks of any SNP leading to the development of GD did not differ significantly in different ages (*P_trend* > 0.02, $P_c > 0.18$). The rs6457617 at the *HLA-DR-DQ* locus was significantly correlated with gender in GD patients ($P=0.004$, $P_c=0.04$). No significant correlation was found between any SNP and age of diagnosis in GD patients ($P > 0.02$, $P_c > 0.17$). The 9 previously identified SNPs are associated with GD in the Chinese Han population. And, gender and age may not influence the associations between the 9 SNPs and GD.

Keywords: Graves' disease, polymorphism, association, gender, age of diagnosis

Introduction

Graves' disease (GD), characterized by production of autoantibodies against thyroid-stimulating hormone (TSH) receptor and hyperthyroidism, is one of the most common forms of autoimmune thyroid disease (AITD) [1]. A study in twins suggests that 79% of the risk of developing GD is attributable to genetic factors [2].

Single nucleotide polymorphisms (SNPs), which are the most common form of DNA variation, are closely related with GD. Candidate gene association studies and genome-wide association studies (GWAS) have identified several susceptibility loci for GD in different ethnic populations. These susceptibility loci include immune-related genes, such as *HLA* (6p21), *CTLA4* (2q33), *SCGB3A2* (5q32), *CD40* (20q12), *PTPN22* (1p13), *FCRL3* (1q21) and the thyroid-specific gene *TSHR* (14q31) [3-8]. Two new susceptibility loci for GD, *RNASET2* (6q27) and *RHOH-CHRNA9* (4p14), have been identified from a previous GWAS based on 1,536 cases and 1,516 controls in the Chinese Han population [7]. The presence of these two loci has been confirmed in Polish and U.K. Caucasians [9, 10]. A subsequent three-stage study, which was performed by including additional 5,781 cases and 6,332 controls, identified five new risk loci for GD, including *GPR174-ITM2A* (Xq21.1), *C1QTNF6-RAC2* (22q12.3-13.1), *SLAMF6* (1q23.2), *ABO* (9q34.2), and an intergenic region harboring two non-coding RNAs (14q32.2) [11]. Another study performed in U.K. Caucasians of European ancestry using a custom-made ImmunoChip identified seven new risk loci for AITD, including *MMEL1* (1p36.32), *TRIB2* (2p25.1), *LPP* (3q37.3/3q28), *BACH2*

Table 1. The detailed information of 9 SNPs analyzed in this study

Chromosome	SNP	Chromosome position	Annotated gene
1q21	rs3761959	157,669,278	*FCRL3*
2q33	rs1024161	204,721,752	*CTLA4*
4p14	rs6832151	40,303,633	*RHOH, CHRNA9*
6p21	rs4947296	31,058,178	*MUC21, C6orf15*
6p21	rs6903608	32,428,285	*HLA-DR-DQ*
6p21	rs6457617	32,663,851	*HLA-DR-DQ*
6p21	rs2281388	33,060,118	*HLA-DPB1*
6q27	rs9355610	167,383,075	*RNASET2*
14q31	rs12101261	81,451,229	*TSHR*

Table 2. Demographic characteristics (gender and age) in GD group and control group

		Gender		Age (years)*	
Group	N	Male	Female	Range	Mean ± SD
GD	1139	231	908	11-83	35.2±11.9†
Control	1726	318	1408	30-87	48.0±10.7

*Age at diagnosis for cases and age at enrollment for controls. †$P < 0.0001$ when compared to the control group (t-test).

(6q15), *PRICKLE1* (12q12), *ITGAM* (16p11.2), and an unconfirmed MS locus at 11q21 [10]. However, most of these susceptibility loci have not been verified in different ethnic and geographic populations. Previous studies suggest that the susceptibility variants might vary in individuals from different geographic populations [4, 12].

In this study, the relationship between 9 previously identified SNPs and GD in different homogenous individuals of the Chinese Han population was investigated. These 9 SNPs included rs3761959 at the *FCRL3* locus, rs1024161 at the *CTLA4* locus, rs6832151 at the *RHOH-CHRNA9* locus, rs4947296 at the *MUC21-C6orf15* locus, rs6903608 and rs6457617 at the *HLA-DR-DQ* locus, rs2281388 at the *HLA-DPB1* locus, rs9355610 at the *RNASET2* locus, and rs12101261 at the *TSHR* locus. And, the risks of any SNP causing GD were analyzed in different gender and ages.

Materials and methods

Subjects and clinical characteristics

A total of 2,865 unrelated individuals from the Chinese Han population were enrolled from Linyi City, Shandong Province, China, including 1,139 cases with Graves's disease (GD group) and 1,726 geographically matched healthy controls (control group). There were 231 males and 908 females in GD group with the average age of 35.2±11.9 years old, and 318 males and 1408 females in the control group with the average age of 48.0 ±10.7 years old.

The diagnosis of GD was based on symptoms of hyperthyroidism and diffuse goiter, and the presence of at least one of the following symptoms: positive in the test of TSH receptor antibody, diffusely increased I-131 (iodine-131) uptake in the thyroid gland, or exophthalmos [4, 12]. All individuals of GD group were interviewed and examined by experienced clinicians. Control subjects were collected from the same geographic region as that of GD patients and had similar ethnic backgrounds. They had no known personal or family history of GD or other autoimmune diseases. They were all over 30 years old. This age criteria in the control group were designed to exclude individuals who might develop GD later on, considering that GD and other autoimmune thyroid diseases occur disproportionately in the young female population.

The study protocol was approved by the Ethics Committee for Human Research of Linyi People's Hospital. Informed consent was obtained from all participants after explanation of the nature and possible consequences of the study.

Genomic DNA extraction and genotyping

Peripheral blood was collected from the median cubital veins of GD patients and healthy control individuals. Genomic DNA was extracted from peripheral blood using a commercially available genomic DNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. As previously described [7, 13], SNP genotyping was performed using TaqMan SNP Genotyping Assays on an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The detailed information of these 9 SNPs was listed in **Table 1**.

Table 3. Characteristics and distribution of the SNPs

Chromosome	SNP	Chromosome position*	Annotated gene	Alleles (A/B)†	Genotype count (AA/AB/BB)†	
					GD group	Control group
1q21	rs3761959	157,669,278	*FCRL3*	A/G	318/810/524	261/818/647
2q33	rs1024161	204,721,752	*CTLA4*	C/T	94/645/913	156/759/811
4p14	rs6832151	40,303,633	*RHOH, CHRNA9*	G/T	257/816/579	211/780/735
6p21	rs4947296	31,058,178	*MUC21, C6orf15*	C/T	73/490/1089	49/313/1364
6p21	rs6903608	32,428,285	*HLA-DR-DQ*	C/T	339/831/482	250/825/651
6p21	rs6457617	32,663,851	*HLA-DR-DQ*	T/C	386/832/434	304/825/597
6p21	rs2281388	33,060,118	*HLA-DPB1*	T/C	187/1030/435	165/750/811
6q27	rs9355610	167,383,075	*RNASET2*	G/A	468/773/411	377/871/478
14q31	rs12101261	81,451,229	*TSHR*	C/T	144/674/834	194/808/724

No *Chromosomal position is based on NCBI Build 37.3 (National Center for Biotechnology Information, Bethesda, MD, U.S.). †A represents the minor allele and B represents the common allele.

Table 4. Association of the SNPs with GD

SNP	Annotated gene	Minor allele	MAF of GD group	MAF of control group	*P**	OR (95% CI)
rs3761959	*FCRL3*	A	0.44	0.39	3.6×10^{-5}	1.23 (1.11-1.35)
rs1024161	*CTLA4*	C	0.25	0.31	1.1×10^{-7}	0.75 (0.67-0.83)
rs6832151	*RHOH-CHRNA9*	G	0.40	0.35	4.0×10^{-6}	1.26 (1.14-1.39)
rs4947296	*MUC21-C6orf15*	C	0.19	0.12	7.6×10^{-17}	1.76 (1.54-2.02)
rs6903608	*HLA-DR-DQ*	C	0.46	0.38	1.3×10^{-9}	1.35 (1.23-1.49)
rs6457617	*HLA-DR-DQ*	T	0.49	0.42	6.2×10^{-9}	1.33 (1.21-1.46)
rs2281388	*HLA-DPB1*	T	0.42	0.31	1.3×10^{-21}	1.62 (1.47-1.79)
rs9355610	*RNASET2*	G	0.52	0.47	1.3×10^{-4}	1.21 (1.10-1.33)
rs12101261	*TSHR*	C	0.29	0.35	1.1×10^{-6}	0.77 (0.70-0.86)

MAF, minor allele frequency. *The Bonferroni corrected significance level was 0.006 (0.05/9).

Statistical analysis

The data were expressed as mean ± SD (standard deviation). *P* value less than 0.05 was considered to be significantly different. The demographic characteristics of GD group and control group were compared using either the X^2 test for categorical data or t-test for numerical data. Data analyses for SNPs were performed using PLINK (v1.07) [14]. Hardy-Weinberg equilibrium was assessed using the X^2 test. Minor allele frequencies (MAF) of each SNP between GD patients and controls were compared using the X^2 test. The odds ratio (OR) and 95% confidence interval (CI) were calculated using logistic regression. The associations between the SNPs and GD were further evaluated using logistic regression after adjusting gender and age. An additive effects model was applied to analysis of allele dosage in which the genotypes AA, AB, and BB were coded 0, 1, and 2, respectively. "A" represented the rare allele and "B" represented the common allele.

Analyses stratified by gender and age of diagnosis were performed. The Breslow-Day test was used to test for the heterogeneity for associations between men and women. The Cochran-Armitage trend test was used to test for the trend on odds ratios across various age groups. Correlation of the SNPs with gender in patients with GD was tested using the X^2 test. Correlation of the SNPs with age of diagnosis in patients with GD was tested using linear regression. Multiple comparisons were corrected using the Bonferroni method.

Results

Comparison of clinical features and SNPs of subjects

The general clinical features were compared between GD group and control group. The data of gender and age of 1,139 GD cases and 1,726 controls enrolled in this study were summarized in **Table 2**. There was no significant dif-

Table 5. Association of the SNPs with GD after gender stratification

SNP	Annotated gene	Minor allele	Male				Female				*P_het*†
			MAF of GD group	MAF of control group	*P**	OR (95% CI)	MAF of GD group	MAF of control group	*P**	OR (95% CI)	
rs3761959	*FCRL3*	A	0.47	0.40	0.02	1.31 (1.04-1.64)	0.43	0.39	5.2×10^{-4}	1.21 (1.09-1.35)	0.54
rs1024161	*CTLA4*	C	0.25	0.28	0.15	0.83 (0.64-1.07)	0.25	0.32	2.1×10^{-7}	0.73 (0.65-0.82)	0.38
rs6832151	*RHOH-CHRNA9*	G	0.44	0.36	0.006	1.38 (1.10-1.73)	0.40	0.35	1.4×10^{-4}	1.24 (1.11-1.38)	0.40
rs4947296	*MUC21-C6orf15*	C	0.18	0.13	0.02	1.44 (1.06-1.96)	0.19	0.12	4.3×10^{-16}	1.85 (1.59-2.15)	0.15
rs6903608	*HLA-DR-DQ*	C	0.45	0.39	0.04	1.26 (1.01-1.58)	0.46	0.38	8.6×10^{-9}	1.37 (1.23-1.53)	0.53
rs6457617	*HLA-DR-DQ*	T	0.54	0.44	6.9×10^{-4}	1.47 (1.18-1.84)	0.47	0.41	1.3×10^{-6}	1.30 (1.17-1.45)	0.32
rs2281388	*HLA-DPB1*	T	0.42	0.28	8.1×10^{-7}	1.81 (1.43-2.30)	0.43	0.32	1.8×10^{-16}	1.59 (1.42-1.77)	0.32
rs9355610	*RNASET2*	G	0.51	0.48	0.26	1.14 (0.91-1.42)	0.52	0.47	2.2×10^{-4}	1.22 (1.10-1.36)	0.57
rs12101261	*TSHR*	C	0.31	0.35	0.07	0.80 (0.63-1.02)	0.29	0.34	6.0×10^{-6}	0.77 (0.69-0.86)	0.76

MAF, minor allele frequency. *The Bonferroni corrected significance level was 0.003 (0.05/9/2). †*P* values obtained from the Breslow-Day test for testing for the heterogeneity of association between males and females.

Table 6. Association of the SNPs with GD after age stratification

SNP	Annotated gene	Minor allele	Age of diagnosis (year)										*P_trend*†
			< 20 (n=125)		20-30 (n=269)		30-40 (n=303)		40-50 (n=255)		≥ 50 (n=187)		
			*P**	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	
rs3761959	*FCRL3*	A	0.53	1.09(0.84-1.41)	0.04	1.21(1.01-1.46)	0.02	1.23 (1.04-1.47)	0.005	1.31 (1.08-1.57)	0.60	0.94 (0.76-1.18)	0.21
rs1024161	*CTLA4*	C	0.32	0.86 (0.65-1.15)	0.02	0.79 (0.64-0.97)	0.003	0.74 (0.61-0.91)	0.002	0.71 (0.58-0.89)	0.17	0.85 (0.67-1.07)	0.18
rs6832151	*RHOH-CHRNA9*	G	0.10	1.25 (0.96-1.62)	0.002	1.34 (1.11–1.61)	0.18	1.13 (0.94-1.35)	0.02	1.25 (1.03-1.51)	0.01	1.33 (1.07-1.65)	0.35
rs4947296	*MUC21-C6orf15*	C	3.0×10^{-8}	2.34 (1.72-3.18)	3.0×10^{-7}	1.84 (1.45–2.32)	3.4×10^{-7}	1.79 (1.43-2.24)	0.02	1.38 (1.06-1.79)	8.4×10^{-4}	1.62 (1.22-2.14)	0.02
rs6903608	*HLA-DR-DQ*	C	0.04	1.30 (1.01-1.69)	9.8×10^{-5}	1.44 (1.20–1.72)	1.6×10^{-5}	1.46 (1.23-1.74)	1.5×10^{-7}	1.64 (1.36-1.98)	0.06	1.23 (0.99-1.52)	0.13
rs6457617	*HLA-DR-DQ*	T	0.006	1.43(1.11-1.85)	9.5×10^{-6}	1.51 (1.26-1.81)	3.3×10^{-4}	1.37 (1.15-1.63)	0.002	1.34 (1.12-1.62)	0.02	1.29 (1.04-1.60)	0.29
rs2281388	*HLA-DPB1*	T	1.0×10^{-7}	2.00(1.54-2.58)	1.2×10^{-10}	1.82 (1.52-2.19)	9.6×10^{-7}	1.55 (1.30-1.85)	1.3×10^{-5}	1.53 (1.26-1.85)	7.7×10^{-4}	1.45 (1.17-1.81)	0.06
rs9355610	*RNASET2*	G	0.13	1.22(0.94-1.58)	0.004	1.31 (1.09-1.57)	0.02	1.23 (1.04-1.47)	0.04	1.22 (1.01-1.47)	0.69	0.96 (0.77-1.19)	0.39
rs12101261	*TSHR*	C	0.03	0.73(0.55-0.98)	0.06	0.83 (0.68-1.01)	0.009	0.78 (0.64-0.94)	0.001	0.71 (0.57-0.87)	0.03	0.78 (0.61-0.98)	0.32

MAF, minor allele frequency. *The Bonferroni corrected significance level was 0.001 (0.05/9/5). †One-tailed *P* values obtained from Cochran-Armitage trend test for testing trend on OR across various age groups. The Bonferroni corrected significance level was 0.006 (0.05/9).

ference in gender between GD group and control group ($P > 0.05$). However, the differences in average ages between GD group and control group were statistically significant ($P < 0.0001$). The 9 SNPs were identified by genotyping. Characteristics and genotype counts of the 9 SNPs were summarized in **Table 3**. All SNPs were in Hardy-Weinberg equilibrium in control group. However, the SNPs of GD group were not in Hardy-Weinberg equilibrium.

Association of the SNPs with GD

To investigate the association between SNPs and GD, logistic regression was performed. As shown in **Table 4**, all of 9 SNPs showed significant associations with GD ($P < 1.3\times10^{-4}$). These associations survived Bonferroni correction for multiple tests of 9 SNPs ($P_c < 0.001$). The rs2281388 at the *HLA-DPB1* locus ($P=1.3\times10^{-21}$; OR=1.62, 95% CI: 1.47-1.79) showed the most significant association with GD. The rs4947296 at the *MUC21-C6orf15* locus ($P=7.6\times10^{-17}$; OR=1.76, 95% CI: 1.54-2.02) had the second most significant association with GD. The other 2 SNPs at the *HLA-DR-DQ* locus (rs6903608 and rs6457617) showed less significant association with GD ($P=1.3\times10^{-9}$ and 6.2×10^{-9}, respectively; OR=1.35 and 1.33, respectively). These 4 SNPs of rs2281388, rs4947296, rs6903608 and rs6457617 in the *MHC* region were not in linkage disequilibrium ($r^2 < 0.06$). Another 3 SNPs that demonstrated a significant association with GD were rs3761959 at the *FCRL3* locus ($P=3.6\times10^{-5}$; OR=1.23, 95% CI: 1.11-1.35), rs6832151 at the *RHOH-CHRNA9* locus ($P=4.0\times10^{-6}$; OR=1.26, 95% CI: 1.14-1.39), and rs9355610 at the *RNASET2* locus ($P=1.3\times10^{-4}$; OR=1.21, 95% CI: 1.10-1.33). The other 2 SNPs (rs1024161 at the *CTLA4* locus and rs12101261 at the *TSHR* locus), whose MAF were less common in GD group than control group, also showed a significant association with GD ($P=1.1\times10^{-7}$ and 1.1×10^{-6} respectively; OR=0.75 and 0.77, respectively).

The associations between the SNPs and GD were further evaluated using logistic regression after adjusting gender and age. A total of 7 SNPs (rs1024161, rs6832151, rs4947296, rs6903608, rs6457617, rs2281388, and rs12101261) remained significantly associated with GD ($P < 3.4\times10^{-4}$, $P_c < 0.003$). However, the associations between the other 2 SNPs (rs3761959 and rs9355610) and GD did not survive the Bonferroni correction ($P > 0.009$, $P_c > 0.08$; data not shown).

Association of the SNPs with GD after gender stratification

The association of the SNPs with GD was further analyzed after gender stratification. In females, all of 9 SNPs were significantly associated with GD ($P < 5.2\times10^{-4}$, $P_c < 0.009$; **Table 5**). While in males, only 2 SNPs (rs6457617 and rs2281388) were significantly associated with GD ($P < 6.9\times10^{-4}$, $P_c < 0.01$). After Bonferroni correction, the other 7 SNPs in males (rs3761959, rs1024161, rs6832151, rs4947296, rs6903608, rs9355610, and rs12101261) were still not significantly associated with GD ($P > 0.006$, $P_c > 0.1$). However, the associations between all SNPs and GD were not significantly heterogeneous between females and males, as analyzed by Breslow-Day test ($P_{het} > 0.15$) (**Table 5**).

Association of the SNPs with GD after age stratification

To investigate the association of the SNPs with GD in different ages, the GD patients were divided into 5 groups according to the age at diagnosis (patients of younger than 20 years old, 20-30 years old, 30-40 years old, 40-50 years old, and older than 50 years old) and Cochran-Armitage trend test was performed. As shown in **Table 6**, four SNPs in the *MHC* regions (rs2281388, rs4947296, rs6903608, and rs6457617) were significantly associated with GD in either all or some age groups ($P < 8.4\times10^{-4}$, $P_c < 0.04$). The rs2281388 at the *HLA-DPB1* locus showed a significant association with GD in all 5 age groups ($P < 7.7\times10^{-4}$, $P_c < 0.03$). The rs4947296 at the *MUC21-C6orf15* locus showed a significant association with GD in all age groups except the age group with age between 40 and 50 ($P < 8.4\times10^{-4}$, $P_c < 0.04$). The rs6903608 at the *HLA-DR-DQ* locus was significantly associated with GD in 3 age groups of 20-30, 30-40, and 40-50 years old ($P < 9.8\times10^{-5}$, $P_c < 0.004$). The rs6457617 at the *HLA-DR-DQ* locus was significantly associated with GD in 2 age groups of 20-30 and 30-40 years old ($P < 3.3\times10^{-4}$, $P_c < 0.01$). The other 5 SNPs (rs3761959, rs1024161, rs6832151, rs9355610, and rs12101261), located in the non-*MHC* regions, were not found to be significantly associated with GD in any age groups ($P > 0.001$, $P_c > 0.05$). However, the risks of GD caused by any SNP were not significantly different among different age groups ($P_{trend} > 0.02$, $P_c > 0.18$).

Table 7. Correlation of the SNPs with gender in GD group

SNP	Annotated gene	Minor allele	Female MAF	Male MAF	P^*	OR (95% CI)
rs3761959	*FCRL3*	A	0.43	0.47	0.11	0.87 (0.73-1.04)
rs1024161	*CTLA4*	C	0.25	0.25	0.81	1.03 (0.84-1.26)
rs6832151	*RHOH-CHRNA9*	G	0.40	0.44	0.07	0.85 (0.71-1.02)
rs4947296	*MUC21-C6orf15*	C	0.19	0.18	0.46	1.09 (0.87-1.37)
rs6903608	*HLA-DR-DQ*	C	0.46	0.45	0.58	1.05 (0.88-1.26)
rs6457617	*HLA-DR-DQ*	T	0.47	0.54	**0.004**	0.77 (0.65-0.92)
rs2281388	*HLA-DPB1*	T	0.43	0.42	0.59	1.05 (0.88-1.26)
rs9355610	*RNASET2*	G	0.52	0.51	0.63	0.96 (0.80-1.14)
rs12101261	*TSHR*	C	0.29	0.31	0.41	0.92 (0.76-1.12)

MAF, minor allele frequency. *The Bonferroni corrected significance level was 0.006 (0.05/9). Significant *P* values are highlighted in bold.

Table 8. Correlation of the SNPs with age of diagnosis in GD group

SNP	Annotated gene	Minor allele	MAF	*β*	*s.e.*	P^*
rs3761959	*FCRL3*	A	0.44	-0.04	0.03	0.20
rs1024161	*CTLA4*	C	0.25	-0.01	0.03	0.75
rs6832151	*RHOH-CHRNA9*	G	0.40	0.02	0.03	0.47
rs4947296	*MUC21-C6orf15*	C	0.19	-0.06	0.03	0.05
rs6903608	*HLA-DR-DQ*	C	0.46	-0.01	0.03	0.74
rs6457617	*HLA-DR-DQ*	T	0.49	-0.02	0.03	0.42
rs2281388	*HLA-DPB1*	T	0.42	-0.07	0.03	0.02
rs9355610	*RNASET2*	G	0.52	0.06	0.03	0.06
rs12101261	*TSHR*	C	0.29	-0.01	0.03	0.64

MAF, minor allele frequency. *The Bonferroni corrected significance level was 0.006 (0.05/9).

Genotype-phenotype correlations in GD patients

The rs6457617 at the *HLA-DR-DQ* locus was significantly correlated with gender in patients with GD (P=0.004, P_c=0.04; **Table 7**). The minor allele T was less common in females than males (0.47 vs. 0.54; OR=0.77, 95% CI: 0.65-0.92). The other SNPs were not significantly correlated with gender in GD patients ($P > 0.07$, $P_c > 0.63$). Linear regression did not indicate any significant correlation between any SNP and age of diagnosis in patients with GD ($P > 0.02$, $P_c > 0.17$; **Table 8**).

Discussion

In the present study, the associations of 9 SNPs with GD were evaluated in the Chinese Han population. These 9 SNPs were identified previously and were susceptibility loci for GD. We found that 4 SNPs (rs2281388, rs4947296, rs6903608, and rs6457617), located in the *MHC* region, were significantly associated with GD. And, they were not in linkage disequilibrium, suggesting that these SNPs are independently associated with GD. Notably, the rs2281388 at the *HLA-DPB1* locus was most significantly associated with GD. The rs2281388 was a perfect predictor of *DPB1*05:01* allele, which has been found to be associated with GD in various Asian populations [15-19]. Previously, it has been reported that the gene regions of the *RNASET2* and *RHOH-CHRNA9* were associated with GD in Polish and U.K. Caucasians [9, 10]. And, the *FCRL3* [20, 21], *CTLA4* [22], and *TSHR* [23, 24] regions were also related with GD. Consistently, our results showed that the other 5 SNPs of rs3761959 at the *FCRL3* locus, rs6832151 at the *RHOH-CHRNA9* locus, rs9355610 at the *RNASET2* locus, rs1024161 at the *CTLA4* locus, and rs12101261 at the *TSHR* locus were also significantly associated with GD. These results indicate that the 9 SNPs are also susceptibility loci for GD in the Chinese Han population.

The prevalence of GD can be different in different gender and different ages, and it is most preponderant in young female individuals [25]. In the present study, the influences of gender and age on associations of the 9 SNPs with GD were investigated. Stratified analysis showed that all SNPs were significantly associated with GD in females, while only 2 SNPs (rs6457617 and rs2281388) were significantly associated with GD in males. However, none of the associations between all SNPs and GD were significantly heterogeneous between females and males ($P_{het} > 0.15$), suggesting that gender might not influence the associations of these 9

SNPs with GD. Because that there were fewer males than females in both the GD group and control group, the negative associations between the 7 SNPs (rs3761959, rs1024161, rs6832151, rs4947296, rs6903608, rs9355610, and rs12101261) and GD in males might be a false negative due to the limited sample size. Power calculations suggest that these samples have 97.9% power to detect variants with MAF of 0.15 and odds ratio of 1.5 at significance level of 0.05 in women but only 47.0% power in males.

To analyze the effect of age on the association of SNPs with GD, GD patients were divided into 5 age groups (patients of younger than 20 years old, 20-30 years old, 30-40 years old, 40-50 years old, and older than 50 years old). The results showed that 4 SNPs in the *MHC* regions (rs2281388, rs4947296, rs6903608, and rs6457617) were found to be significantly associated with GD in some age groups. However, the other 5 SNPs, located in non-*MHC* regions (rs3761959, rs1024161, rs6832151, rs9355610, and rs12101261), were not significantly associated with GD in any age group. These results suggest that the variants in the *MHC* regions were more strongly associated with GD than the variants in the non-*MHC* regions. However, the risks of GD caused by any SNP were not significantly different in different ages (P_{trend} > 0.02), suggesting that these genetic variants may not contribute to the difference in prevalence of the disease in different ages.

Population stratification is a major concern in genetic association studies. Results of the previous studies suggest that the susceptibility variants of GD might vary in populations from different geographic regions [4, 12]. One of the strengths of the present study was the ethnically and geographically matched GD cases and controls. The association observed in the present study was unlikely to be affected by population stratification. Another major strength of the present study was the relatively large sample size in both the GD and control groups. Power calculations suggest that our study may have sufficient power to detect common variants with moderate genetic effects. Specifically, the present study showed 99.3% power to detect variants with MAF of 0.15 and odds ratio of 1.5 at significance level of 0.05. However, rare variants at the susceptibility loci of GD were not investigated in this study. Thus, the existence of rare variants in these genomic regions that might be associated with GD cannot be ruled out. Further comprehensive studies are needed to evaluate all possible variants at the susceptibility loci of GD in the Chinese Han population.

In summary, our results suggest that there are significant associations between 9 previously identified SNPs and GD in the Chinese Han population. And, gender and age may not influence the associations between the 9 SNPs and GD.

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Disclosure of conflict of interest

None.

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