MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 1439-1451
DOI: 10.12659/MSM.911489

Received: 2018.06.02
Accepted: 2018.10.14
Published: 2019.02.23

Linkage Analysis of the Chromosome 5q31-33 Region Identifies JAKMIP2 as a Risk Factor for Graves' Disease in the Chinese Han Population

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1,2 Jia Li
ADEG 1 Weiping Teng
ABCDE 1,3 Yang Yu
B 1,4 Xin Hou
AG 1 Zhongyan Shan

1 Department of Endocrinology and Metabolism, Instituite of Endocrinology, Liaoning Provincial Key Laboratory of Endocrine Diseases, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning, P.R. China
2 Department of Endocrinology and Metabolism, The Fourth Affiliated Hospital of China Medical University, Shenyang, Liaoning, P.R. China
3 Department of Endocrinology and Metabolism, Affiliated Zhongshan Hospital of Dalian University, Dalian, Liaoning, P.R. China
4 Department of Geriatric Endocrinology and Metabolism, The First Hospital of China Medical University, Shenyang, Liaoning, P.R. China

Corresponding Author: Weiping Teng, e-mail: twp@vip.163.com
Source of support: The study was supported by a grant from the National Natural Science Foundation of China (Grant: 30670996) and the National High Technology Research and Development Program of China (863 Program, No. 2007AA02Z4Z1)

Background: This study aimed to investigate susceptibility to Graves's disease and the association with the 5q32–33.1 region on chromosome 5 in a Chinese Han population.

Material/Methods: Eighty Chinese Han multiplex families included first-degree and second-degree relatives with Graves' disease. Eight microsatellite markers on chromosome 5 at the 5q32–33.1 region underwent linkage analysis and the association between the regions D5S1480–D5S2014 were studied.

Results: The maximal heterogeneity logarithm of the odds (HLOD) score of D5S2090 was 4.29 (α=0.42) and of D5S2014 was 4.01 (α=0.34). A nonparametric linkage (NPL) score of 3.14 (P<0.001) was found for D5S2014. The D5S1480–D5S2014 region on chromosome 5 was associated with Graves' disease, with eight haplotype domains. There were significant differences in the sixth and eighth haplotype domains between patients with Graves' disease compared with normal individuals. Tagging single nucleotide polymorphisms (SNPs) of the sixth and eighth haplotype domains showed that individuals with SNP62 (rs12653715 G/C) who were GG homozygous had a significantly increased risk of Graves' disease compared GC heterozygous or CC homozygous individuals. The transmission disequilibrium test (TDT) indicated that SNP62 (rs12653715) and SNP63 (rs12653081) *loci* in the Janus kinase and microtubule interacting protein 2 (JAKMIP2) gene showed dominant transmission from heterozygous parents to the affected offspring, and SNPs in the secretoglobin family 3A member 2 (SCGB3A2) gene showed no transmission disequilibrium. The haplotype JAKMIP2-1 was identified as being particularly significant.

Conclusions: JAKMIP2 gene polymorphism require further study as potential risk factors for Graves' disease in the Chinese Han population.

MeSH Keywords: Genetic Linkage • Graves Disease • Haplotypes

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/911489

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Background

Graves' disease is an autoimmune thyroid disease (AITD), with a worldwide prevalence of approximately 1% [1]. There is epidemiological evidence that genetic factors play an important role in the pathogenesis of Graves' disease [2]. This disease shows a familial association among first-degree and second-degree relatives, known as multiplex families [3,4]. The risk of Graves' disease for family members of Graves' disease patients is 15 times higher than that of the general population [5]. The concordance rate for the prevalence of Graves' in monozygotic twins is significantly greater than that of dizygotic twins [6]. The presence of circulating anti-thyroid antibodies occurs in between 50–70% of family members patients with Graves' disease [7]. However, the concordance rate of Graves' disease prevalence for monozygotic twins (36%) is less than 100%, suggesting that other parameters, such as environmental factors, can impact the occurrence of Graves' disease, which creates a further challenge to studies on the genetic associations. Also, Graves' disease does not follow classical Mendelian inheritance, which indicates that there might be multiple genes involved in the pathogenesis [8].

Previously published studies have investigated the genetic causes of Graves' disease, but the genetic mechanisms underlying the pathogenesis of this condition remain poorly understood [9–11]. Early family studies that investigated multiplex families with Graves' disease and association analysis studies have shown that Graves' disease is associated with the expression of several genes with varied penetrance rates, as well as gene interactions and gene and environment interactions.

Family multiplex pedigree linkage analysis is based on identifying characteristics that are segregated with both genes and traits. Genetic marker analysis can be performed to indirectly locate disease-associated genes by observing the co-segregation between gene markers and sequence data. Recent studies have shown that on chromosome 5, the 5q32–33.1 region is a major susceptibility locus for Graves' disease in East Asian populations [12,13]. Also, Graves' disease susceptibility regions were identified by genetic markers and genome-wide scanning, including 6p, 7q, 8q, 10q, and 12q [12,13]. However, linkage analysis does not identify the complex gene polymorphisms and the precise positioning of Graves' disease genes, which means that it is not possible to comprehensively analyze all the genetic associations in Graves' disease. However, when related genes are identified and their functions are known, candidate gene association based on linkage analysis can identify the role of specific genes in Graves' disease. When combined with haplotype evaluation, linkage analysis provides a promising approach to begin to identify disease-related susceptibility genes [14].

A haplotype refers to a group of related single nucleotide polymorphism (SNP) alleles located in a region of the same chromosome. After many generations, with repeated chromosome recombination events, the original arrangement of ancestral chromosome fragments will change, but the unchanged gene fragments following recombination, and separated by recombination regions, constitute a haplotype [14]. Identifying the haplotype domain structure in the chromosome provides a convenient way to assess disease susceptibility genes. A tag SNP is a representative SNP that is found in a genome region with high linkage disequilibrium, and a group of SNPs forms the haplotype. A high degree of linkage disequilibrium in the haplotype domain only includes a few common haplotypes, but by detecting a small number of tag SNPs, the composition of the common haplotype in the region can be identified. This analytical approach is more economical and effective when compared with mass screening for every polymorphic locus.

Currently, several genes have been identified in patients with Graves' disease and have been studied in different ethnicities, including HLA-associated genes, CD40, CTLA-4, PTPN22, FCRL3, Thyroglobulin, TSHR and FOXP3 [9,15–17]. Relatively few studies have been undertaken on the 5q31-33 region, a major susceptibility locus of Graves' disease in East Asian populations. The present study investigated 80 Graves' disease multiplex families in the Chinese Han population and used tag SNPs to assess Graves' disease susceptibility genes in these individuals. This study aimed to investigate the susceptibility to Graves's disease and the association with the 5q32–33.1 region on chromosome 5 in a Chinese Han population.

Material and Methods

Selection criteria for the Graves' disease family pedigrees

The study was conducted at the First Affiliated Hospital of China Medical University. This study was approved by the Ethics Committee of the Fourth Affiliated Hospital of China. Medical University and was conducted according to the Declaration of Helsinki. Signed informed consent was obtained from each study participant.

The selection of study participants included multiplex families, consisting of individuals with Graves's disease who had first-degree and second-degree relatives with Graves' disease. All study participants were of Chinese Han ethnicity. The lowest standard for selecting pedigrees was at least four first-degree relatives, including the proband. Because age impacts the disease penetrance affected first-degree relatives <18 years of age were not included in the study. Graves' disease was diagnosed according to criteria that included a clinical diagnosis of hyperthyroidism, a diffuse goiter, eye signs that included

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exophthalmos, a positive thyroid stimulating hormone (TSH) receptor antibody (TRab) test, and a diffusely increased thyroid uptake of ^{123}I by the radioactive iodine uptake (RAIU) test. At least three of these diagnostic criteria were required for inclusion in the study as a case of Graves' disease.

Eighty Graves' disease multiplex families of Han ethnicity included 478 study participants, 201 patients with Graves' disease and an average of six individuals per pedigree or family. The study participants were recruited from ten cities in Liaoning Province. Pedigrees with one, two, three, four, and five first-degree relatives suffering from the disease were 20 (25%), 37 (46%), 16 (20%), five (6%) and two (3%), respectively. The 201 cases of Graves' disease included 45 men and 156 women, with a mean age of 43.72±14.31 years, and a male to female ratio of 1: 3. First-degree and second-degree relatives of the patients with Graves' disease included 277 cases, 136 men and 141 women, with a mean age of 48.95±16.98 years.

Microsatellite detection

From the database of published genome-wide screening microsatellite markers (STRs), within the genetic distance of about 8 cM (centimorgan) of chromosome 5 at the 5q32–33.1 region (between D5S436–D5S434), eight microsatellite markers with >70% heterozygosity, including D5S2017, D5S1480, D5S436, D5S2847, D5S2090, D5S434, D5S413, and D5S2014, were selected.

Microsatellite locus detection

Analysis of published polymerase chain reaction (PCR) primers (Supplementary Table 1), identified PCR products that were used for electrophoresis with the default program on the automated GeneScan ABI PRISM™ 3730xl DNA Analyzer (ThermoFisher Scientific, Waltham, MA, USA). The sizes of the detected fragments were analyzed using GeneMapper software version 4.0 (ThermoFisher Scientific, Waltham, MA, USA).

Construction of the domain structure of the haplotype of chromosome 5 at the 5q32–33.1 region

In the Graves' disease linkage region (D5S1480–D5S2014), 74 single nucleotide polymorphisms (SNPs) with more than 20% low-frequency alleles located around the gene coding region were selected using the dbSNP database published by the National Center for Biotechnology Information (NCBI). Except for the fourteenth and fiftieth SNPs, the remaining detected SNPs were in Hardy-Weinberg equilibrium, indicating that samples had good comparability. No information was recorded for the fourteenth and fiftieth SNPs in subsequent analysis.

Forty-eight probands were selected from the Graves' disease multiplex pedigrees that were used for gene positioning for analysis of the selected SNPs using the pyrosequencing method [18,19]. SNP genotype information of 45 normal Chinese Han individuals was obtained from the international HapMap database (www.hapmap.org) [20,21].

Detection of tag SNPs of chromosome 5 at the 5q32–33.1 region in the Chinese Han population and their association with Graves' disease

In the constructed 5q32–33.1 haplotype, the sixth and eighth haplotypes (block 6 and block 8) spanned the JAKMIP2 and SCGB3A2 genes with three and five tag SNPs, respectively. Detection was performed using the TaqMan genotyping method on the eight SNP *loci* (Supplementary Table 2) for all members of the 80 Graves' disease multiplex pedigrees [22,23].

Statistical analysis

GeneHunter software version 2.0 was used in linkage analysis to assess two-point HLOD scores, multipoint HLOD scores, and multipoint nonparametric linkage (NPL) scores for each microsatellite. Correlation analysis between single SNPs was performed using the chi-squared (χ^2) test or Fisher's exact test with SPSS version 11.5. Construction of the haplotype domain structure was performed using Haploview version 4.2 software (*www.broadinstitute.org/haploview*) [24]. The minimum value of the minor allele frequency (MAF) was set to 0.05, and tag SNPs were selected with r^2 >0.8 between the SNPs. A transmission disequilibrium test (TDT) was conducted based on pedigree samples [25,26]. A P-value <0.05 was considered to be statistically significant.

Results

Linkage analysis between chromosome 5 at the 5q32–33.1 region and Graves' disease in the Chinese Han population

Graves' disease is a complex disease with significant genetic heterogeneity; however, its inheritance pattern and penetrance are unclear [27]. The linkage analysis results by the two-point parameter method are shown in Supplementary Table 3. In recessive inheritance with the 30–100% penetrance model, the selected screening microsatellite markers (STRs) reached peak two-point heterogeneity logarithm of the odds (HLOD) scores. Except for the two-point HLOD score of D5S2017, which was 1.74 (α=0.21), the HLOD scores of other *loci* were >2, and D5S2090 showed the highest HLOD score of 4.29 (α=0.42). An HLOD score of >1.9 supported linkage, while a HLOD score >3.3 indicated significant linkage [28,29]. These results indicated that the chromosome 5 region D5S1480–D5S2014 was significantly linked with Graves' disease.

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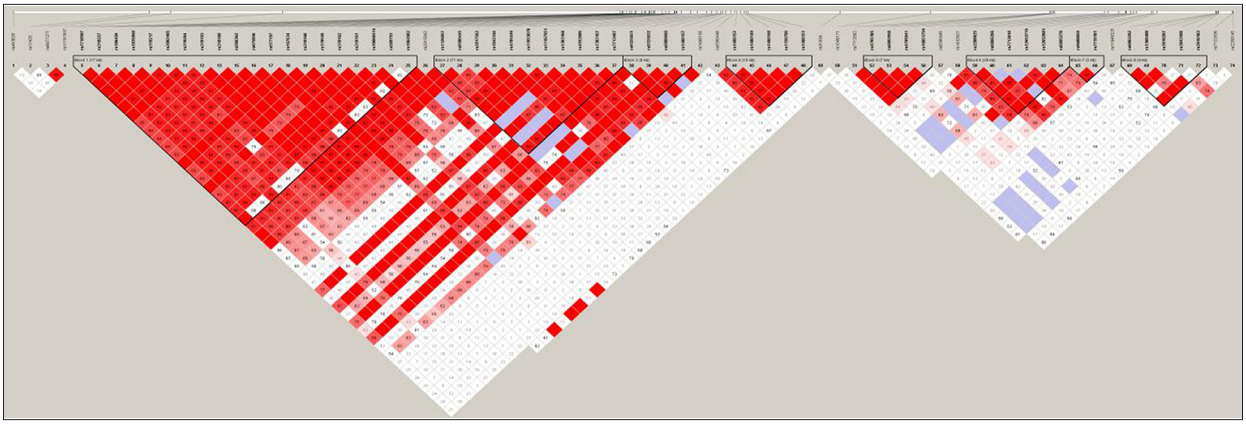

Figure 1. Haplotype structure in Chinese Han individuals and the main haplotype structure of the chromosome 5q31 region.

In linkage analysis by the multipoint parameter method (Supplementary Table 4), the results showed that in the selected STRs, except for D5S2017 whose highest multipoint HLOD score was 1.53 (α=0.19), the highest multipoint HLOD scores of the remaining 7 STRs were above 2. The highest multipoint HLOD score for D5S2014 was 4.01 (α=0.34). In linkage analysis of a single positive linkage pedigree, multipoint HLOD scores were between 0.1–0.2. This finding supported that the D5S1480–D5S2014 region on chromosome 5 was significantly linked with Graves' disease.

Linkage analysis results by the multipoint nonparametric method of selected STRs are shown in Supplementary Table 5. Except for the nonparametric linkage (NPL) score of 1.93 for D5S2017 (P=0.014), NPL scores for the remaining 7 STRs were >2 and the maximum NPL score was 3.12 (P<0.001), which occurred at D5S2014. This result also suggested that this region was significantly associated with Graves' disease.

Single nucleotide polymorphism (SNP) analysis of chromosome 5 at the 5q32–33.1 region and construction of the haplotype domain structure

Seventy-two SNPs located around the gene coding region were selected from the Graves' disease linkage region (D5S1480–D5S2014). There were statistical differences in allele distribution of the SNP71 rs3843496 locus and the remaining five *loci*, between Graves' disease patients and normal individuals (SNP71 rs3843496, P=0.0002) (SNP2 rs11435, SNP36 rs1383167, SNP60 rs6866266, SNP62 rs12653715, and SNP67 rs11948325, P<0.05). These data are summarized in Supplementary Table 6.

The Haploview software was used to construct the haplotype domain structure based on PyroSequencing and HapMap data (Figure 1). The color shades of squares in the figure indicate the linkage disequilibrium (LD) index among SNPs, and the greater the score, the darker the square and the higher the LD index. The specific chromosome region (D5S1480–D5S2014) in the Chinese Han population consisted of eight haplotype domains. The haplotype domain structures were determined by a few representative tag SNPs. The hotspot regions for recombination were within various haplotypes.

Differences in haplotype distribution between patients with Graves' disease and normal individuals

A further comparison was undertaken of the distribution of major haplotypes of the eight haplotype domain structures found in chromosome 5 at the 5q32–33.1 region, between patients with Graves' disease and normal individuals. As shown in Table 1, the proportions of AGCGTC and AACCTC haplotypes in block 6 between patients with Graves' disease and normal individuals were 19.3: 76.7; 34.5: 55.5; and 34.6: 61.4;19.5: 70.5 respectively, with a statistically significant difference (P=0.0064 and P=0.0309, respectively). The proportions of GGCCT haplotype in block 8 between patients with Graves' disease and normal individuals were 79.0: 17.0 and 61.5: 28.5, respectively, with statistical significance (P=0.0274). Single *loci* with significant differences, including SNP60, SNP60rs6866266, and SNP62 rs12653715, were located in block 6. SNP71 rs3843496 was located in block 8. Therefore, the study focused on the relationship between this region of chromosome 5 and Graves' disease.

The relationship between target SNPs and Graves' disease

Region 6 and region 8 associated with Graves' disease were selected from the constructed 5q32–33.1 haplotype domain structures and the tag SNPs were selected as target SNPs. Block 6 and block 8 (the sixth and eighth haplotypes) spanned the JAKMIP2 and SCGB3A2 genes with three and five tag SNPs, respectively (Figure 2). Genotype distribution for each SNP locus followed the Hardy-Weinberg equilibrium. The distribution of each SNP allele and genotype in the case-control group is shown in Table 2. The G allele of the rs12653715 locus was significantly associated with Graves' disease (P=0.106). The genotype distribution of the locus in the case-control group

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Table 1. Distribution of main haplotypes in each haplotype domain structure between GD cases and controls.

Haplotype	Frequency	Case: Control ratio	Chi square	*P* value
Block 1				
TGCCGAGGAAAGTCATGACC	0.345	35.2: 60.8, 29.0: 61.0	0.415	0.5194
CGCTGAGGAAAGCCAGGGCA	0.247	22.0: 74.0, 23.9: 66.1	0.340	0.5599
CTTCAGAATGGATTGTAGTC	0.223	20.6: 75.4, 21.0: 69.0	0.096	0.7561
Block 2				
TTCCTTCAGGG	0.328	26.0: 70.0, 35.0: 55.0	2.939	0.0865
TTCCTCTGAAG	0.22	26.0: 70.0, 15.0: 75.0	2.934	0.0867
GCATCCTAAGA	0.181	17.9: 78.1, 15.8: 74.2	0.041	0.8388
Block 3				
CTCT	0.374	30.7: 65.3, 38.9: 51.1	2.467	0.1163
CTCG	0.218	22.3: 73.7, 18.1: 71.9	0.263	0.6082
ACAG	0.215	25.0: 71.0, 15.0: 75.0	2.406	0.1209
CCCG	0.18	17.7: 78.3, 15.9: 74.1	0.019	0.8892
Block 4				
AACGG	0.489	41.0: 53.0, 49.0: 41.0	2.157	0.1419
GGTAT	0.462	48.0: 46.0, 37.0: 53.0	1.832	0.1759
Block 5				
TCAT	0.575	55.0: 41.0, 52.0: 38.0	0.004	0.9466
TTAA	0.301	30.0: 66.0, 26.0: 64.0	0.123	0.7257
CTGA	0.108	8.0: 88.0, 12.0: 78.0	1.210	0.2713
Block 6				
AACCTC	0.291	34.6: 61.4, 19.5: 70.5	4.656	0.0309*
AGCGTC	0.289	19.3: 76.7, 34.5: 55.5	7.444	0.0064**
GGCGCT	0.237	22.0: 74.0, 22.0: 68.0	0.060	0.8064
GGTGCC	0.134	14.0: 82.0, 11.0: 79.0	0.222	0.6373
Block 7				
GG	0.474	38.9: 57.1, 49.3: 40.7	3.753	0.0527
CA	0.424	45.9: 50.1, 33.0: 57.0	2.378	0.1231
Block 8				
GGCCT	0.756	79.0: 17.0, 61.5: 28.5	4.864	0.0274*

* P<0.05, ** P<0.01.

was different (P=0.117), and further analysis showed that individuals who were GG homozygous at the rs12653715 locus were more likely to suffer from Graves' disease compared with individuals who were with GC heterozygous and CC homozygous (GG *vs.* GC + CC, OR=1.46; 95% CI, 1.011–2.108; P=0.043).

Transmission disequilibrium testing of the JAKMIP2 and SCGB3A1 genes

As shown in Table 3, *loci* with transmission disequilibrium were SNP62 (rs12653715) and SNP63 (rs12652081) in the

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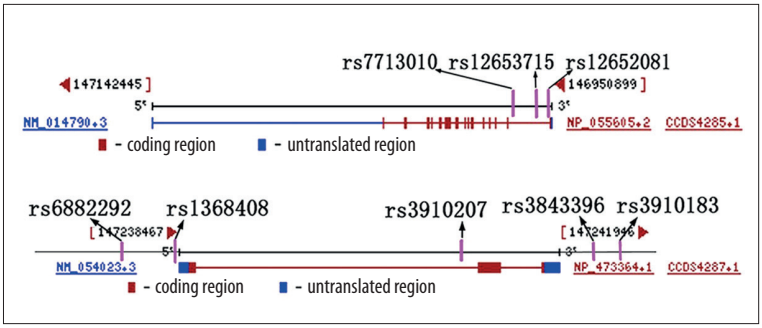

Figure 2. Locations of target single nucleotide polymorphisms (SNPs) in the corresponding genes.

Table 2. Alleles of target SNPs and allele frequencies between GD cases and controls.

SNPs	Genotype frequency			*P* value	Allele frequency		*P* value
rs7713010	CC	CT	TT		C	T	
Case	141	37	5		319	47	
Control	207	63	3	0.351	477	69	0.928
rs12653715	GG	GC	CC		G	C	
Case	97	78	25		272	128	
Control	109	132	37	0.117	350	206	0.106
rs12652081	TT	CT	CC		T	C	
Case	61	100	39		222	178	
Control	97	144	42	0.360	338	228	0.191
rs6882292	GG	GA	AA		G	A	
Case	168	14	0		350	14	
Control	251	21	1	0.596	523	23	0.754
rs1368408	GG	GA	AA		G	A	
Case	144	53	3		341	59	
Control	194	78	10	0.360	466	98	0.277
rs3910207	CC	CT	TT		C	T	
Case	152	30	1		334	32	
Control	236	41	2	0.866	513	45	0.928
rs3843496	CC	CT	TT		C	T	
Case	116	96	7		328	110	
Control	151	113	16	0.356	415	145	0.780
rs3910183	TT	TG	GG		T	G	
Case	167	31	0		365	31	
Control	235	43	0	0.955	513	43	0.957
rs2853697	AA	AC	CC		A	C	
Case	108	70	5		286	80	
Control	177	85	11	0.268	439	107	0.407

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Table 3. Transmission disequilibrium test for each SNPs locus in the *JAKMIP2* and *SCGB3A2* genes.

Gene	SNPs	Over-transmitted Allele	80 pedigrees		Positive linkage pedigrees	
			T: U	*P* value	T: U	*P* value
JAKMIP2	SNP61-rs7713010	T	26: 18	0.2278	7: 4	0.3657
	SNP62-rs12653715	G	53: 34	0.0416*	21: 9	0.0285*
	SNP63-rs12652081	C	63: 40	0.0234*	33: 10	0.0005**
SCGB3A2	SNP68-rs6882292	G	10: 9	0.8185	3: 3	1.0000
	SNP69-rs1368408	G	30: 26	0.593	13: 8	0.2752
	SNP70-rs3910207	T	17: 16	0.8618	6: 3	0.3173
	SNP71-rs3843496	C	45: 36	0.3173	18: 12	0.2733
	SNP72-rs3910183	T	18: 15	0.6015	7: 3	0.2059

T – transmission; U – non-transmission. * $P<0.05$; ** $P<0.01$.

Table 4. Transmission disequilibrium test for haplotypes in the *JAKMIP2* and *SCGB3A2* genes.

Gene	Haplotype	Frequency	80 pedigrees		Positive linkage pedigrees	
			T: U	*P* value	T: U	*P* value
JAKMIP2	CGC +TGC	0.410	63.0: 38.0	0.0128*	33.0: 10.0	0.0005**
	CCT	0.372	35.4: 51.5	0.0838	10.5: 21.6	0.0489*
	CGT	0.214	30.6: 36.5	0.4732	8.6: 20.5	0.0282*
SCGB3A2	GGCCT	0.740	41: 35	0.4912	14.0: 11.0	0.5476
	GGCTT	0.093	18: 18	1.0000	10.0: 7.0	0.4643
	GACTT	0.077	8: 13	0.2754	4.0: 8.0	0.2434
	GATTG	0.038	8: 7	0.7963	0.0: 3.0	0.0845
	AATTG	0.036	8: 7.9	0.9754	2.0: 1.0	0.3128

T – transmission; U – non-transmission. * $P<0.05$; ** $P<0.01$.

JAKMIP2 gene. The SNP62 and SNP63 *loci* in the JAKMIP2 gene were associated with susceptibility to Graves' disease (all pedigrees, P=0.0416 and P=0.0234; positive linkage pedigrees, P=0.0285 and P=0.0005). Also, transmission of alleles G and C from heterozygous parents to their children with Graves' disease was found. SNPs in the SCGB3A2 gene showed no dominant transmission from heterozygous parents to the affected offspring (all P>0.05).

As shown in Table 4, in all pedigrees, including positive linkage pedigrees, there was transmission disequilibrium in the GC haplotype. The G allele at the SNP62 (rs12653715) locus and C allele at the SNP63 (rs12652081) locus (P=0.0128 and P=0.0005) were found in the JAKMIP2 gene. This haplotype was termed 'JAKMIP2-1,' which might represent a Graves' disease high-risk haplotype. Also, in positive linkage pedigrees, the other two haplotypes with the T allele, including the SNP63 locus, also showed transmission disequilibrium (P=0.0489 and P=0282). However, these alleles were rarely passed onto the offspring of heterozygous parents with Graves' disease due to the protective effects of these haplotypes. Several haplotypes in the SCGB3A2 gene did not reveal dominant transmission from heterozygous parents to affected offspring.

Discussion

Graves' disease is a common organ-specific autoimmune disease that involves the thyroid gland. Recent studies have shown that genetic factors are an important cause of the familial association with Graves' disease. This study used pedigree case-control testing with the two-point parametric testing, multi-point parametric testing, and multi-point nonparametric linkage (NPL) methods to perform linkage analysis with Graves'

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disease on chromosome 5 at the 5q32–33.1 region (D5S2017–D5S2014). Also, the D5S1480–D5S2014 region of chromosome 5 was associated with Graves' disease. A recently published study using linkage analysis with parametric linkage and non-parametric linkage (NPL) methods demonstrated that D5S436 and D5S2090 of the 5q31 region of chromosome 5 were significantly associated with Graves' disease, indicating that one of the major susceptibility *loci* of Graves' disease is located in the 5q31 region, with genetic heterogeneity [12]. Further studies have also shown that the 5q31 region, as well as 5q32–33.1 region on chromosome 5, were Graves' disease susceptibility *loci* in an Asian population [13,30]. However, inconsistent findings have been reported for Caucasian populations [31–33], possibly due differences in the phenotype and genotype of Graves' disease in different ethnic groups. In line with previous studies in East Asian patients with Graves' disease, the present study confirmed that on chromosome 5, the 5q32–33.1 region was a Graves' disease linkage region, suggesting that East Asians may have the same Graves' disease susceptibility *loci*.

The complex single nucleotide polymorphisms (SNPs) in this study was a third-generation marker and involved in all known SNPs of the genes of the study subject genes in combination with common haplotypes. The efficiency of this study was largely improved by the use of tag SNPs, avoiding sequencing problems for multiple SNPs. A parametric method-based linkage analysis was used for the selected eight microsatellite markers in chromosome 5 at the 5q32–33.1 region (D5S2017–D5S2014) and derived two-point HLOD scores with different inheritance modes (dominant, recessive) and penetrance rates (30%, 60%, 90%, and 100%). The maximum two-point HLOD score was obtained for each screening microsatellite marker (STR) when θ was equal to 0; α was the proportion of positive linkage pedigree (HLOD score >+0.1). The two-stage method established by Thomas et al. was applied to construct the haplotype domain structure of chromosome 5 at the 5q32–33.1 region in the Chinese Han population, and determine representative tag single nucleotide polymorphisms (SNPs) for linkage analysis of Graves' disease susceptibility genes.

Block 6 was located in JAKMIP2, and there was linkage disequilibrium (LD) in the SNP61 (rs7713010 C/T), SNP62 (rs12653715 G/C), and SNP63 (rs12653081 T/C) *loci* of this haplotype. The transmission disequilibrium test (TDT) is a method that uses pedigree samples to detect the associations of genetic *loci* with diseases. In a random population, since pedigree samples were directly used for genome-wide scanning, the major susceptibility of Graves' disease members in most of the pedigrees should be around chromosome 5 at the 5q32–33.1 region. It is better to exclude the influence of common genetic heterogeneity factors in multiple genetic diseases. If some genetic *loci* (microsatellite or SNP *loci*) are associated with the prevalence of a disease, the probability of passing this locus from heterozygous parents to the affected offspring in the pedigree should be significantly greater than the odds of passing them to the unaffected descendants. This study included 80 Graves' disease multiplex pedigrees (478 members) and 24 positive linkage pedigrees (LOD score >0.1 in linkage analysis), respectively, to perform transmission disequilibrium test for each SNP locus located in the JAKMIP2 and SCGB3A2 genes and haplotype. The results showed that the G allele of the SNP62 locus (P=0.0416) and C allele of the SNP63 locus (P=0.0005) had dominant transmission from heterozygous parents to the affected offspring. Also, the JAKMIP2-1 haplotype determined by SNP62 G+ SNP63 C also had significant dominant transmission (P=0.0005). Alleles and genotypes in other *loci* had similar distribution patterns in case and control groups, and the difference in distribution frequencies did not reach statistical significance. The above findings indicated that the JAKMIP2 gene may be associated with Graves' disease prevalence.

The Janus kinase and microtubule interacting protein 2 (JAKMIP2) gene is also known as neuroendocrine long coiled-coil protein-1 (NECC1) gene and is located in the 5q32 region of the human chromosome, containing 21 exons with a full length of 199kb. The JAKMIP2 protein is preferentially expressed in the neuroendocrine tissues of vertebrates, including in the central nervous system, the pituitary, the adrenal gland, and the testes, and has been identified as a component of the Golgi matrix [34–36]. Currently, limited information is available regarding the function of JAKMIP2. Cruz-Garcia and colleagues have reported an inverse relationship between JAKMIP2 expression and hormone secretion in pituitary melanotropes in frogs [37], and have further demonstrated that JAKMIP2 acts as a negative modulator of the regulated secretory pathway in PC12 cells *in vitro* [38]. The potential roles of JAKMIP2 in thyroid hormone regulation and in the etiology of Graves' disease requires further investigation.

Recent studies on gene structure and function have shown that introns can be self-excising, and regulate gene expression [39,40]. SNPs located in the exon-exon junction region can affect mRNA excision by promoting the partial deletion of the exon sequence or maintain the intron sequence uncut, leading to disease development. Mutations in the middle of an intron can cause disease by activating the recessive cleavage site that affects mRNA excision [39]. In this study, SNP62 and SNP63 were located in the intron of the JAKMIP2 gene, but how introns affect JAKMIP2 protein function requires further study.

The secretoglobin family 3A, member 2 (SCGB3A2) gene, also known as the uteroglobin-related protein 1(UGRP1) gene, is a member of the immunoglobulin superfamily [41]. The SCGB3A2 gene is found on human chromosome 5 at the 5q33.1 region,

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with a length of 2.9 kb, and has three exons and twelve introns. SCGB3A2 is mainly expressed in lung tissues but is also expressed in thyroid tissues [42]. The SCGB3A2 secreted protein binds to specific receptors on the surface of target cells. In previous studies, SCGB3A2 has been reported to be associated with Graves' disease in two Caucasian populations and one Han population [32,33,43]. The results from the present study showed that the allele, the genotype, and the haplotype of SCGB3A2 and each SNP locus had similar distributions in the cases of Graves' disease and control groups, and the difference between them was not statistically significant. Also, there was no dominant transmission from heterozygous parents to the affected offspring. Therefore, in the assessed Chinese Han pedigrees, SCGB3A2 may not be a major susceptibility gene for Graves' disease.

This study analyzed the association of the JAKMIP2 gene and Graves' disease using comparative tests of pedigree cases and found that JAKMIP2 might be a genetic risk factor for Graves' disease. However, this study had several limitations. Although 80 Graves' disease multiplex pedigrees or families were recruited, the sample size was relatively small. The results cannot explain the role of JAKMIP2 gene abnormalities in the pathogenesis of Graves' disease. Also, this study only showed that the expression of the JAKMIP2 gene might be a molecular marker for the risk of developing Graves' disease, but whether or not the JAKMIP2 gene is associated with the clinical parameters of Graves' disease requires clarification and the specific mechanism by which the JAKMIP2 gene increases the risk of Graves' disease remains to be investigated. Further genetic, epidemiologic, and functional studies are required to elucidate the relationship between JAKMIP2 gene polymorphisms, including the haplotype JAKMIP2-1, and Graves' disease.

Conclusions

This study showed that the expression of the JAKMIP2 gene polymorphisms was associated with the presence of Graves' disease in a Chinese Han population. The JAKMIP2-1 haplotype was identified as being particularly significant. The mechanisms underlying this gene association in the Chinese Han population and in other populations requires further study.

Acknowledgments

We are grateful to all the patients and their family members who participated in this study and the physicians who referred the family members and verified the diagnoses. We also acknowledge the help of Dr. Ying Jin from the Human Medical Genetics Program.

Conflict of interest

None.

Supplementary Tables

Supplementary Table 1. Primer sequences of microsatellites.

STRs	Forward	Reverse
D5S1072	5'-GAGTCCTCTCACTGATATTTTTGTA-3'	5'-GTCTATCCTTCCAGATGGTTC-3'
D5S1082	5'-TTGGGAAGAATAGCTTTCCC-3'	5'-TTCTAGCTTCCCCCTATGCT-3'
D5S638	5'-TATGTGCCAGGCATTACGCT-3'	5'-GTCTCCACCCACAGCAGG-3'
D5S2748 (GATA139B09)	5'-GACTTCTCCACCCCCATAAC-3'	5'-TTAGTCAGGTTCTCCAGAGAGG-3'
D5S2009	5'-CATGGGCATGTTTCAAAAT-3'	5'-AGTACCTCCTTAGTAACTCTGGGC-3'
D5S343	5'-CTTGAAATGTTCCCAACACA-3'	5'-TGCACAGATGAGGAAACTGA-3'
D5S324	5'-AGTCACCTTCTCTGTCTCCA-3'	5'-AGGCCTCATTCAAAATCTGT-3'
D5S2034	5'-AGCTACTACCAGCAGCATTC-3'	5'-CTACATTATTATTATTGTGTGTCCG-3'

Primer sequences were from UniSTS database.

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Supplementary Table 2. Information on target SNPs.

SNPs No.	Name	Position in UCSC (bp)	Alleles	Genes	*Loci*
SNP61	rs7713010	147724712	C/T	JAKMIP2	Intron1
SNP62	rs12653715	147732794	C/G	JAKMIP2	Intron1
SNP63	rs12652081	147737372	T/C	JAKMIP2	Intron1
SNP68	rs6882292	147877993	G/A	SCGB3A2	Near-gene-5'
SNP69	rs1368408	147878599	G/A	SCGB3A2	Near-gene-5'
SNP70	rs3910207	1477881921	C/T	SCGB3A2	Intron1
SNP71	rs3843496	147882249	C/T	SCGB3A2	Near-gene-3'
SNP72	rs3910183	1477882352	T/G	SCGB3A2	Near-gene-3'

The positions of the SNPs were based on UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) assembly.

Supplementary Table 3. Microsatellite-marked two-point HLOD scores of different penetrance rates of various genetic modes.

Microsatellite	HLOD(α) of each penetrance of dominant inheritance				HLOD (LO of each penetrance of recessive inheritance			
	30%	60%	90%	100%	30%	60%	90%	100%
D5S2017	0.25 (0.18)	0.15 (0.12)	0.06 (0.05)	0.00 (0.01)	0.64 (0.15)	0.99 (0.18)	1.47 (0.20)	1.74 (0.21)
D5S1480	1.56 (0.43)	1.43 (0.37)	0.70 (0.20)	0.04 (0.04)	2.05 (0.30)	2.54 (0.32)	2.83 (0.30)	2.79 (0.27)
D5S436	2.00 (0.47)	2.03 (0.43)	1.01 (0.23)	0.04 (0.03)	2.25 (0.28)	2.69 (0.29)	2.94 (0.27)	2.66 (0.23)
D5S2847	0.95 (0.38)	0.88 (0.32)	0.51 (0.18)	0.00 (0.00)	2.79 (0.36)	3.27 (0.37)	3.78 (0.35)	3.79 (0.33)
D5S2090	1.79 (0.48)	1.65 (0.42)	0.64 (0.20)	0.06 (0.06)	3.34 (0.44)	2.46 (0.29)	4.29 (0.42)	4.10 (0.38)
D5S434	0.43 (0.24)	0.28 (0.17)	0.07 (0.07)	0.00 (0.00)	2.07 (0.28)	3.83 (1)	2.90 (0.27)	2.80 (0.25)
D5S413	1.17 (0.43)	0.93 (0.35)	0.23 (0.12)	0.00 (0.00)	2.41 (0.33)	3.05 (0.36)	3.79 (0.37)	3.69 (0.29)
D5S2014	2.70 (0.52)	2.13 (0.41)	0.73 (0.17)	0.02 (0.02)	3.30 (0.35)	3.64 (0.36)	3.56 (0.32)	3.09 (0.26)

α, ratio of positive linkage pedigrees. Blue, HLOD >+1.9; Red, HLOD >+3.3.

Supplementary Table 4. Microsatellite-marked maximum multi-point HLOD scores for different penetrance rates.

Microsatellite	Genetic distance (cm)	Multi-point HLOD value (oi)	Penetrance/genetic mode
D5S2017	145.21	1.53 (0.19)	100%/recessive inheritance
D5S1480	147.49	3.06 (0.26)	90%/recessive inheritance
D5S436	147.49	3.53 (0.27)	90%/recessive inheritance
D5S2847	149.48	3.60 (0.24)	100%/recessive inheritance
D5S2090	150.34	3.59 (0.24)	100%/recessive inheritance
D5S434	150.34	3.63 (0.27)	100%/recessive inheritance
D5S413	150.34	3.62 (0.27)	90%/recessive inheritance
D5S2014	153.17	4.01 (0.34)	60%/recessive inheritance

Genetic distance was selected from Marshfield database; i, average distance between genders; α, ratio of positive linkage pedigrees. Blue, HLOD >+1.9; Red, HLOD >+3.3.

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Supplementary Table 5. Multipoint NPL scores for microsatellites in chromosome 5q32-33.1.

Microsatellite	Genetic distance (cm)	NPL-score	P-value	Information
D5S2017	145.21	1.92	0.014*	0.89
D5S1480	147.49	2.13	0.008**	0.91
D5S436	147.49	2.30	0.005**	0.90
D5S2847	149.48	2.03	0.011*	0.93
D5S2090	150.34	2.03	0.011*	0.94
D5S434	150.34	2.10	0.009**	0.94
D5S413	150.34	2.78	0.001**	0.92
D5S2014	153.17	3.12	<0.001**	0.91

Genetic distance was selected from the Marshfield database, i, average distance between genders. * $P<0.05$; ** $P<0.01$.

Supplementary Table 6. Results of association analysis between SNPs and GD.

No.	SNP	Allele	Case: Control ratio	Chi Square	P value
1	rs443033	T	45: 43, 36: 54	2.225	0.1358
2	rs11435	G	72: 22, 56: 34	4.487	0.0342*
3	rs6877277	T	81: 15, 75: 15	0.037	0.8469
4	rs11167937	C	82: 10, 75: 15	1.290	0.2560
5	rs7718587	T	35: 57, 33: 57	0.037	0.8478
6	rs319227	G	71: 25, 66: 24	0.009	0.923
7	rs186459	T	26: 70, 24: 66	0.004	0.9489
8	rs1835950	T	34: 60, 31: 59	0.06	0.8066
9	rs319217	A	26: 70, 24: 66	0.004	0.9489
10	rs2082405	A	66: 26, 63: 27	0.067	0.7963
11	rs319204	A	26: 70, 23: 65	0.021	0.8846
12	rs319193	G	69: 27, 60: 30	0.593	0.4413
13	rs319189	A	69: 27, 60: 30	0.593	0.4413
15	rs586362	A	68: 26, 60: 30	0.699	0.4031
16	rs675846	A	67: 27, 60: 30	0.457	0.4990
17	rs577197	G	70: 26, 63: 27	0.194	0.6597
18	rs167634	T	68: 26, 64: 26	0.034	0.8531
19	rs319166	C	70: 26, 63: 27	0.194	0.6597
20	rs178549	A	70: 26, 63: 27	0.194	0.6597
21	rs319162	T	68: 28, 57: 33	1.186	0.2762
22	rs319161	G	71: 25, 63: 27	0.361	0.5478
23	rs10068414	A	40: 54, 30: 60	1.658	0.1978
24	rs589793	C	69: 27, 64: 26	0.013	0.9082
25	rs1864982	C	69: 25, 64: 26	0.121	0.7283
26	rs2915842	A	27: 63, 25: 65	0.108	0.7422
27	rs1126057	G	24: 72, 20: 70	0.198	0.6560
28	rs6580445	C	24: 72, 20: 70	0.198	0.6560
29	rs2017562	A	21: 75, 18: 72	0.099	0.7536

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No.	SNP	Allele	Case: Control ratio	Chi Square	P value
30	rs1842346	T	20: 72, 18: 72	0.083	0.7729
31	rs4705449	T	67: 27, 61: 29	0.266	0.6061
32	rs11953078	C	65: 31, 49: 41	3.445	0.0635
33	rs11167951	T	67: 27, 54: 36	2.597	0.1071
34	rs1383168	G	27: 67, 16: 74	3.076	0.0795
35	rs4552686	A	66: 28, 54: 36	2.114	0.1460
36	rs1383167	A	28: 68, 15: 75	4.084	0.0433*
37	rs7713487	A	37: 57, 29: 59	0.807	0.3689
38	rs9325031	A	25: 71, 16: 74	1.846	0.1742
39	rs9325032	C	41: 51, 33: 57	1.176	0.2781
40	rs6880660	A	24: 68, 15: 75	2.398	0.1215
41	rs1480157	G	65: 31, 50: 40	2.907	0.0882
42	rs1480155	C	23: 73, 15: 73	1.339	0.2472
43	rs6580448	T	43: 49, 40: 50	0.097	0.7560
44	rs1480152	G	54: 42, 40: 50	2.59	0.1075
45	rs1480149	G	48: 46, 38: 52	1.444	0.2295
46	rs1480150	T	52: 42, 41: 49	1.753	0.1854
47	rs1156700	A	50: 42, 41: 49	1.407	0.2356
48	rs1480151	T	49: 43, 41: 49	1.081	0.2986
49	rs31039	T	62: 32, 56: 34	0.279	0.5974
51	rs7713582	A	88: 8, 78: 12	1.210	0.2713
52	rs4705185	T	87: 9, 78: 12	0.727	0.3940
53	rs6892958	T	41: 55, 38: 52	0.004	0.9466
54	rs4705041	A	88: 8, 76: 12	1.333	0.2483
56	rs10051794	T	57: 39, 52: 38	0.049	0.8251
57	rs6580495	G	89: 7, 78: 10	0.908	0.3407
58	rs1432827	T	42: 50, 41: 49	0.001	0.9896
59	rs2288825	G	39: 55, 33: 57	0.449	0.5028
60	rs6866266	A	38: 58, 21: 67	5.208	0.0225*
61	rs7713010	T	14: 82, 13: 77	0.001	0.9786
62	rs12653715	C	38: 58, 21: 69	5.663	0.0173*
63	rs12652081	C	41: 55, 35: 55	0.280	0.5964
64	rs6895278	C	74: 22, 68: 22	0.060	0.8064
65	rs9688059	C	47: 49, 33: 57	2.863	0.0906
66	rs7719181	A	52: 40, 40: 46	1.784	0.1817
67	rs11948325	G	49: 47, 33: 57	3.894	0.0485*
68	rs6882292	G	92: 4, 84: 6	0.571	0.4500
69	rs1368408	G	80: 16, 70: 20	0.919	0.3379
70	rs3910207	C	88: 8, 73: 11	1.076	0.2996
71	rs3843496	C	87: 9, 62: 28	13.772	0.0002**
72	rs3910183	T	88: 8, 79: 11	0.766	0.3815
73	rs7722926	T	74: 22, 63: 27	1.201	0.2731
74	rs2250145	A	67: 29, 56: 34	1.188	0.2757

* P<0.05; **P<0.01.

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