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Association of Deoxyribonuclease I Gene Polymorphisms with Graves' Disease in the Chinese Han Population

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Abstract

Background: This study aimed to investigate the association between the single nucleotide polymorphism (SNP) rs1053874 in the deoxyribonuclease I (DNASE1) gene and Graves' disease (GD) in the Han Chinese population.

Methods: Polymerase chain reaction-restriction fragment length polymorphism analysis and direct sequencing were used to identify the distribution of the SNP rs1053874 in the DNASE1 genes from 284 GD patients and 203 healthy controls, and associations between clinical manifestations of GD and the observed genotype and allele frequencies at the DNASE1 gene were analyzed.

Results: In the Han Chinese population, there were significant differences between the GD groups and the controls with respect to genotype and allele frequencies associated with theSNPrs1053874. The risk of GD was greater among carriers of the G allele than non-carriers (OR=0.65, 95% CI: 0.49- 0.86). There were significant differences in genotype and allele frequencies between the GD patients with a history of relapse and the GD patients without history of relapse; furthermore, the G allele of the SNP rs1053874 was associated with relapse in GD patients.

Conclusion: This study confirmed that the DNASE1 gene may be a GD susceptibility gene in the in the Southern Chinese Han population. The G allele at the rs1053874 SNP would be a direct genetic risk factor for GD in this population. Furthermore, this allele may be associated with disease relapse.

Keywords: Deoxyribonuclease I (DNASE1); Single nucleotide polymorphisms (SNPs); Graves' disease

Abbreviations: MAF: Minor Allele Frequency; RFLP: Restriction Fragment length polymorphism; SRED: Single Radial Enzyme Diffusion; cAMP: Cyclic Adenosine Monophosphate; CV: Coefficient of Variation; ELISA: Enzyme-Linked Immunosorbent Assay; GD: Graves' disease; Ig: immunoglobulin mRNA: Messenger Ribonucleic acid; OD: Optical Density; PCR: Polymerase Chain Reaction; qRT-PCR: Quality Real Time-Polymerase Chain Reaction; RA: Rheumatoid arthritis: SD: Standard Deviation; SLE: Systemic Lupus Erythematousdeviation; TSH: Thyrotropin; TGAb: Thyroglobulin Antibody; TPOAb: Thyroperoxidase Antibody; TRAb: TSH Receptor Antibody; TSH: thyroid-stimulating hormone; TSHR: Thyrotropin Teceptor; TSAb: Thyroid Stimulatory Antibodies; SNP: Single Nucleotide Polymorphism

Introduction

Graves' disease (GD), which is an autoimmune disease associated with the increased secretion of thyroid hormone, is the major cause of hyperthyroidism. Although the pathogenesis of GD has not been fully elucidated, it is known that genetic factors play an important role in the development of this disease [1]. The incidence of GD exhibits a clear pattern of familial aggregation; however, as a polygenic disease, GD does not follow a Mendelian pattern of inheritance. Genetic susceptibility to GD may be determined by the penetrance of a number of different genes; but the pathogenesis of GD may be affected by environmental factors [1].

Deoxyribonuclease I (DNase I; Enzyme Commission (EC) number 3.1.21.1) is the earliest discovered DNA hydrolase. Recent studies have found that DNase I is a multifunctional enzyme involved in cellular apoptosis, necrosis, and other relevant processes; this enzyme can also participate in the degradation of chromatin from ne- crotic cells [2].

DNase I is encoded by the DNASE1 gene, which is located on chromosome 16p13. 3. Many studies have demonstrated that polymorphisms in DNase I, which exist at both the gene and protein levels, are regulated by autosomal genes and are unrelated to sex chromosomes. To date, six alleles of DNASE1 (DNASE1*1–6) associated with enzyme polymorphisms have been identified; these alleles differ only by point mutations in single nucleotides in the coding region of the gene [3-5].

Among these polymorphisms, DNASE1*1 and DNASE1*2 are the two alleles that are most widely distributed in populations. The only difference between DNASE1*1 and DNASE1*2 is the single nucleotide polymorphism (SNP) rs10538746 in exon 8 of the DNASE1 gene [4]. Prior research [6] has not only revealed that DNase I exhibits reduced enzymatic activity and thermal stability in auto- immune thyroid disease (AITD) patients and their families but also that several SNPs may be associated with AITD pathogenesis. However, so far there is no other related research on the associations between DNASE1 polymorphisms and GD in Han Chinese populations. In this study, a case-control association study of the patients with GD and healthy controls was performed to analyze the distribution of a polymorphism in the DNASE1 gene in the Han Chinese population and the associations between this polymorphism and GD.

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Materials and Methods

Study subjects

284 unrelated Han Chinese patients with GD were enrolled in this study. The study was conducted at the Department of Endocrinology in the Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University. All GD patients were diagnosed on the basis of the clinical criteria and confirmed by thyroid function testing and thyroid antibody measurements. The clinical evaluation included the patient's history as well as the presence of typical symptoms and signs of hyperthyroidism. The laboratory diagnosis included elevated serum free triiodothyronine (FT3) and free thyroxine (FT4) concentrations, low or undetectable serum sensitive TSH (sTSH), and positive thyrotropin (TSH) receptor antibodies (TRAb). Meanwhile, goiter was assessed by experienced endocrinologists and confirmed by thyroid ultrasonography; Graves' ophthalmopathy (GO) was diagnosed by complete eye examination performed by experienced oph- thalmologists. The exclusion criteria were as follows: patients with other coexisting autoimmune diseases or other thyroid disease. There were 209 females (73.59%) and 75 males (26.41%), who ranged from 13–80 years of age, with a mean age of 39 years (29–50 years). The median disease duration was 1.5 years (1 month–40 years). Simultaneously, 203 healthy volunteers were recruited as the control subjects, which included 133 females (65.52%) and 70 males (34.48%). The exclusion criteria for the healthy controls were as follows: normal subjects with a history of thyroid or other autoimmune diseases and with abnormal thyroid function and autoantibodies [FT3, FT4, sTSH, thyroperoxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb), or TRAb]. All study participants were unrelated Han Chinese individuals who resided in Guangdong Province in the southern region of China. Informed consent was obtained from all participants before the study samples were collected; the study protocol was approved by the Ethics Committee of Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University and registered in the Chinese Clinical Trial Registry.

Clinical data collection and laboratory examinations

Baseline clinical and lab data for all subjects were collected. The medical histories of the GD patients were obtained, including gender, age, disease duration, family history, and history of GD relapse. Medical examinations were conducted to record patients' physical conditions conditions, including extent of goiter and ophthalmological parameters. In addition, peripheral blood samples from all subjects were collected by venipuncture in the fasting state in the morning for DNA extraction and biochemical markers. The serum FT3, FT4, sTSH, TGAb, and TPOAb were assayed by automated chemiluminescent immunoassays (Siemens ADVIA Centaur CP, MA, USA), and the serum TRAb was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (RSR Ltd., Cardiff, UK).

Detection of the rs1053874 SNP

A whole blood genomic DNA extraction kit (Omega Bio-Tek, GA, US) was used to extract genomic DNA from 0.25 ml samples of peripheral venous blood containing an anticoagulant. Polymerase chain reaction (PCR) was used to amplify target fragments. PCR primers for rs1053874 were designed by PRIMER 5.0 software (Microsoft Corp, PREMIER Biosoft International) and were synthesized by Sangon Biotech (Sangon Biotech CO. LTD, Shanghai, China) [8]. The upstream primer was 5'-ATCGTGGTTGCAGGGATGCTGCCTC-3', and the downstream primer was 5'-AGTTCAACAGGTGTGGGGAG-3'.

For each PCR amplification, the reaction system, which consisted of a total volume of 25 μl, contained 150 ng of DNA template, 2.5 pmol of each upstream and downstream primer, 500 μM of deoxyribonucleotide triphosphate (dNTP) mix, 12.5 μl of 2 ×GC Buffer I (including $MgCl_2$), and 1 U of Taq Hot Start enzyme. The following PCR conditions were utilized: an initial denaturation at 94°C for 5 minutes; 32 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 45 seconds, and 9 extension at 72°C for 1 minute; and a final extension at 72°C for 5 minutes. Restriction fragment length polymorphism (RFLP) was used to identify the A2317G genotype. More specifically, 10 μl of PCR product was uniformly mixed with 1 μl of endonuclease and 2 μl of 10 × Buffer R, and the resulting solution was incubated in a 37°C water bath for 4 hours. Then, to identify the genotypes, the products of this endonuclease digestion were analyzed by electrophoresis on a 3% agarose gel run at 70 V for 45 minutes. Samples of each genotype were sent for sequencing.

The substitution of G for A at the rs1053874 site can produce a restriction site recognized by the restriction endonuclease XhoI. Thus, the amplification products were digested by XhoI. The three genotypes of the rs1053874 SNP in the DNASE1 gene could then be identified based on the lengths of the digested fragments. In particular, agarose gel electrophoresis of the digested fragments should result in a single band at 261bp for the AA genotype; three bands at 261bp, 239bp, and 22bp for the heterozygous AG genotype; and two bands at 239bp and 22bp for the GG genotype.

Statistical Analysis

All data were analyzed using the SPSS17.0 software package. Hardy-Weinberg equilibrium testing was used to examine whether each groups of the studied population could be representative of the overall population with respect to genotype frequencies. All data were tested for normality. Normally distributed data were expressed as the means ± standard deviation ($x \pm s$), whereas non-normally distributed data were expressed as median (interquartile range). Two independent sample t-tests were used for between-group comparisons of normally distributed data. Non-normally distributed data were analyzed using rank-based nonparametric tests. χ^2 tests were used for between-groups comparisons of genotype frequencies, allele frequencies, and complication rates. Two-sided probabilities were computed for all statistical analyses, and $P < 0.05$ was regarded as significant.

Results

Comparisons of general characteristics of the study subjects

The clinical characteristics of the patients with GD and healthy volunteers are shown in Table 1. According to the Pearson Chi-Square test, no significant difference in gender or age was exhibited between the control and GD groups ($P > 0.05$); furthermore, there was no significantly differ with respect to either age or gender in the GD patients with or without a family history (all $P > 0.05$). The patients with GO or without GO did not significantly differ with respect to either age ($P = 0.09$) or gender ($P = 0.36$). The patients with or without a history of relapse also did not significantly differ with respect to age ($P = 0.18$) or gender ($P = 0.18$).

Association of rs10583874 polymorphism between GD and control subjects

To examine the reliability of the observed data, the genotype frequencies in the control subjects for the rs1053874 SNP in DNASE1 were tested for Hardy-Weinberg equilibrium. The results demonstrated that the observed genotype frequencies did not significantly differ from the expected genotype frequencies associated with Hardy-Weinberg

	(X ± SD)								
GD-total		**GD-family history**		**GO**		**GD-recurrence**		**Controls Normal range**	
		(+)	**(-)**	**(+)**	**(-)**	**(+)**	**(-)**		
n	284	81	203	22	262	35	249	203	
Gender (M/F)	75/209	24/57	51/152	4/18	71/191	6/29	69/180	70/133	
Age (year)	38.0 ± 14.3	36.0 ± 14.5	38.8 ± 14.1	33.2 ± 12.3	38.4 ± 14.4	40.7 ± 13.6	38.0 ± 14.3	42.8 ± 13.2	
FT_3 (pmol/L)	33.0 ± 23.5	40.4 ± 24.5	36.7 ± 21.1	19.6 ± 12.7	17.8 ± 8.9	15.3 ± 7.5	19.7 ± 5.4	5.2 ± 1.3	3.5~6.5
FT_4 (pmol/L)	29.5 ± 10.9	27.1 ± 13.4	26.5 ± 14.1	23.7 ± 13.6	26.4 ± 18.2	25.6 ± 15.3	26.6 ± 18.3	14.5 ± 6.6	11.5~22.7
TSH(mU/L)	0.4 ± 1.1	0.2 ± 0.7	0.3 ± 0.6	0.3 ± 0.5	0.4 ± 0.6	0.6 ± 1.4	0.5 ± 1.0	3.50 ± 1.3	0.5~4.9

Note: GD: Graves' disease; GO: Graves' Ophthalmopathy; M: Male; F: Female; FT3: Free Triiodothyronine; FT4: Free Thyroxine; TSH: Thyrotropin

Table 1: Baseline clinical characteristics of GD patients and healthy controls.

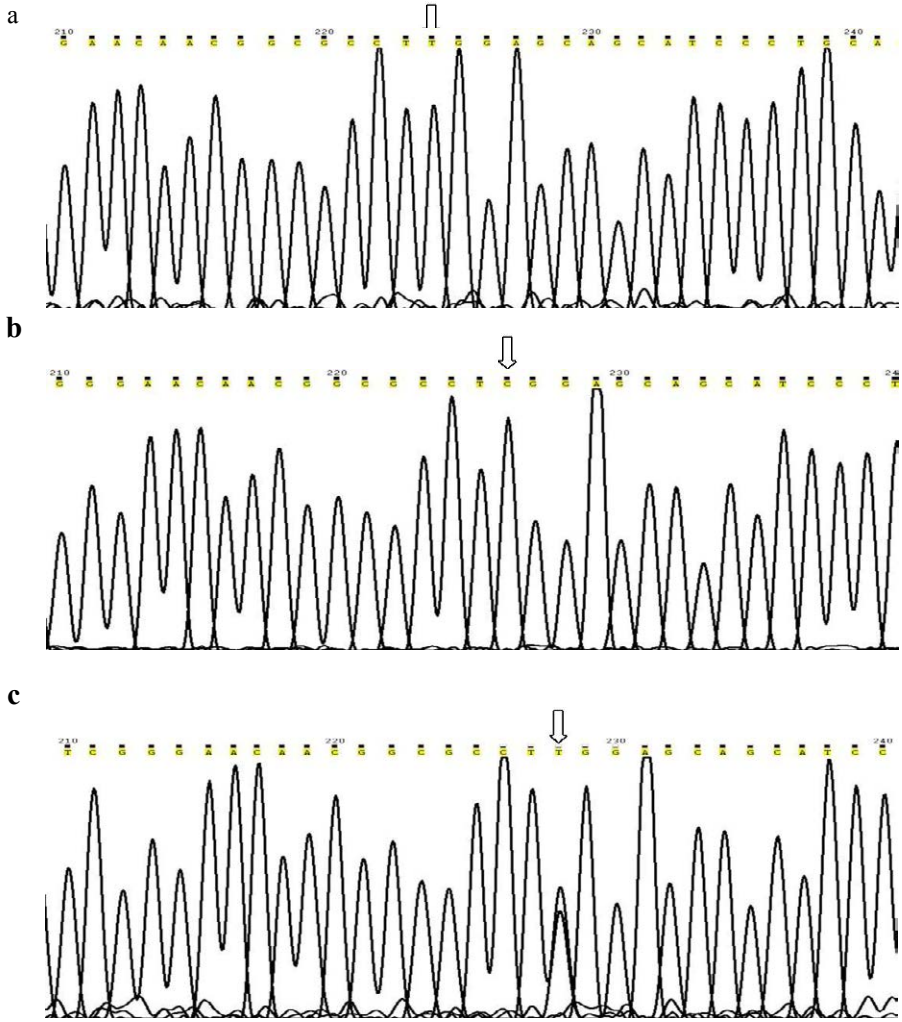

Figure 1: Sequencing results of rs1053874 in the in the Southern Chinese Han population. The three genotypes of the rs1053874 Single-nucleotide polymorphism (SNP) in the DNASE1 gene were performed by PCR-RFLP method in the in the Southern Chinese Han population.
a) The sequence from the patient with AA genotype of rs1053874;
b) The sequence from the patient with GG genotype of rs1053874;
c) The sequence from the patient with AG genotype of rs1053874.

equilibrium (χ^2 = 1.35, P = 0.25), suggesting that the subjects were representative of the overall population of interest. The frequencies of the A/A, A/G, and G/G genotypes in the control groups were 29.56%, 45.81%, and 24.63%, respectively; while 23.94%, 38.03%, and 38.03% in the GD group, respectively (Figure 1). The differences in the genotype frequencies of the controls and GD groups were significant (χ^2 = 9.71, P = 0.00). In the controls groups, the major allele was A (52.46%), whereas in the GD groups, the major allele was G (57.04%). The differences in the allele frequencies of the controls and GD groups were significant (χ^2= 8.59, P = 0.00). The number of instances and proportion of each genotype and allele in the two groups are presented in Table 2A.

Among the GD patients, the median ages of GD onset for the three

Genotype frequencies	Control(%)	GD(%) OR (95%CI)	χ2	P
A/A	60(29.56)	68(23.94)		
A/G	93(45.81)	108(38.03)	9.71	0.01
G/G	50(24.63)	108(38.03)		
Allele frequencies				
A	213(52.46)	244(42.96) 0.68[a](0.53-0.88)	8.59	0
G	193(47.54)	324(57.04)		

GD: Graves' Disease; M: Male; F: Female

Table 2a: Genotype distribution and allele frequencies of rs1053874 In GD patients and healthy controls.

genotypes of A/A, A/G, and G/G were 30 years (24–44 years), 34 years (25–42 years), and 32 years (25–43 years), respectively. There were no significant differences in the ages of onset associated with these three genotypes (χ^2 = 0.34, P = 0.84).

Comparisons of genotype and allele frequencies for rs1053874 after stratification

Stratification by gender: The data revealed that A/G was the most common genotype among males of the control groups (5.71%) but that G/G was the most common genotype among males of the GD groups (38.66%). Male subjects from the controls and GD groups exhibited no significant differences in genotype distribution (χ^2 = 4.09, P = 0.13). While for female subjects, the major genotype was A/G in both the control and GD groups (45.86% and 40.67%, respectively), and the two groups exhibited a significant difference in genotype distribution (χ^2 = 7.63, P = 0.02) (Table 2B).

In addition, among males from the control and GD groups, the frequencies of the A allele were 51.43% and 46.00%, respectively, whereas the frequencies of the G allele were 48.57% and 54.00%, respectively. There was no significant difference in allele frequency between male subjects from the control and GD groups (χ^2 = 0.85, P = 0.36). Furthermore, among females from the control and GD groups, the frequencies of the A allele were 53.01% and 41.87%, respectively, whereas the frequencies of the G allele were 46.99% and 58.13%, respectively. Female subjects from the control and GD groups significantly differed with respect to allele frequency (χ^2 = 8.12, P = 0.00) (Table 2C). Moreover, between male and female subjects from the GD groups, there was no significant difference in genotype distribution (χ^2 =3.39, P = 0.18), also in allele frequencies (χ^2 =0.77, P = 0.38) (Table 2D).

Stratification by ophthalmopathy: The frequencies of the A/A, A/G, and G/G genotypes were 36.36%, 13.64%, and 50.00%, respectively, in the GO patients and 29.56%, 51.72%, and 47.78%, respectively, in the No-GO patients. There were significant differences in the overall distribution of genotypes among the controls, the GO patients, and the No-GO patients (χ^2 = 15.60, P = 0.00). Compared with the controls, the GO patients and the No-GO patients exhibited significantly different genotype distributions (χ^2 = 9.83 and 8.40, respectively; P = 0.00 and 0.02, respectively). In addition, there was a significant difference between the genotype distributions of the GO patients and the No-GO patients (χ^2 = 6.17, P = 0.046). The three aforementioned groups exhibited significantly different frequencies of the A and G alleles (χ^2 = 8.59, P = 0.01). In the groups of GO patients and the groups of No-GO patients, the major allele was the G allele, which was found at frequencies of 56.82% and 57.06%, respectively; these frequencies were higher than the frequency of the G allele in the control groups (47.54%). Comparisons revealed a significant difference in allele frequency between the No-GO patients and the controls (χ^2 = 8.33, P = 0.00) but no significant differences in allele frequency between the GO patients and either the con- trols (χ^2 = 1.37, P = 0.24) or the No-GO patients (χ^2 = 0.00, P = 0.98) (for detailed information, Table 2E).

Stratification by family history: The frequencies of the A/A, A/G, and G/G genotypes were 29.56%, 45.81%, and 24.63%, respectively, in the control groups; 20.99%, 45.68%, and 33.33%, respectively, in the groups of GD patients with a family history; and 25.12%, 34.98%, and 39.90%, respectively, in the GD patients without family history. There were significant differences among these three groups with respect to the overall distribution of genotypes (χ^2 = 12.46, P = 0.01). The genotype distributions of the control and the GD patients with no family history significantly differed (χ^2 = 11.02, P = 0.00). When the groups of GD patients with a family history was compared first with the control groups and subsequently with the groups of GD patients with no family history of the disease, no significant differences in genotype

	Controls between male and female				
Gender	Genotype frequencies	Control(%)	GD(%)	χ^2	P
	A/A	20(28.57)	23(30.67)		
Male	A/G	32(45.71)	23(30.67)	4.09	0.13
	G/G	18(25.72)	29(38.66)		
	A/A	40(30.08)	45(21.53)		
Female	A/G	61(45.86)	85(40.67)	7.63	0.02
	G/G	32(24.06)	79(37.80)		

Table 2b: rs1053874 gene polymorphisms in GD patients and healthy controls between male and female.

Gender	Allele	Control(%)	GD(%)	OR(95%CI)	χ^2	P
Male	A	72(51.43)	69(46.00)	0.81		
	G	68(48.57)	81(54.00)	(0.51-1.28)	0.85	0.36
Female	A	141(53.01%)	175(41.87)	0.64		
	G	125(46.99)	243(58.13)	(0.47-0.87)	8.12	0

Table 2c: Genotype distribution rs1053874 in GD patients and healthy controls between male and female.

Genotype frequencies	Male (%)	Female (%)	χ^2	P
A/A	23(30.67)	45(21.53)	3.39	0.18
A/G	23(30.67)	85(40.67)		
G/G	29(38.66)	79(37.80)		
Allele frequencies				
A	57(46.00)	175(41.87)	0.77	0.38
G	71(54.00)	243(58.13)		

Table 2d: Genotype distribution and allele frequencies ofrs1053874in GD patients between male and female.

		Control (%)	GO (%)	NO-GO (%)	χ^2	P
Genotype frequencies	A/A	60(29.56)	8(36.36)	60(29.56)	[a]9.83	[a] 0.00
	A/G	93(45.81)	3(13.64)	105(51.72)	[b]6.17	[b]0.046
	G/G	50(24.63)	11(50.00)	97(47.78)	[c]8.40	[c]0.02
Allele frequencies	A	213(52.46)	19(43.18)	225(42.94)	8.59	0.01
	G	193(47.54)	25(56.82)	299(57.06)	[c]8.33	[c]0.00

Note: There were significant differences in the genotype among the three groups. χ 2 =15.60, P=0.00)
[a]GO patients compared with controls;
[b]NO-GD patients compared with GO patients;
[c]NO-GD patients compared with controls; GO: GD patients with ophthalmopathy.

Table 2e: Genotype distribution and allele frequencies of rs1053874 in GD patients with or without ophthalmopathy and healthy controls.

distributions were observed (χ^2 = 3.19 and 2.82, respectively; P = 0.20 and 0.25, respectively). There were significant differences among these three groups with respect to the overall frequencies of the A and G alleles (χ^2 = 8.66, P = 0.01). In the GD groups with a family history and the GD groups of without family history ,the major allele was the G allele, which was found at frequencies of 56.71% and 57.39%, respectively; these frequencies were higher than the frequency of the G allele in the control groups (47.54%) (in comparisons with the control groups, χ^2= 3.45 and 7.90, respectively; P = 0.06 and 0.00, respectively). There was not significantly differ with respect to allele frequency in the GD groups with or without a family history (χ^2 = 0.07, P = 0.79) (Table 2F).

Stratification by history of relapse: The frequencies of the A/A, A/G, and G/G genotypes were 17.14%, 14.29%, and 68.57%, respectively, in the relapsed GD patients and 24.90%, 41.37%, and 33.73%, respectively, in the newly diagnosed GD patients (with no history of relapse). There were significant differences in the overall distribution of genotypes between the control groups, the relapsed-GD groups, and the newly diagnosed GD groups (χ^2 = 27.34, P = 0.00). With respect to genotype distribution, the control groups significantly differed from the relapsed-GD groups (χ^2 = 27.40, P = 0.00) but did not significantly differ from the newly diagnosed GD groups (χ^2 = 4.54, P = 0.10). There was a significant difference in genotype distribution between the groups of GD patients with a history of relapse and the newly diagnosed GD groups (χ^2 = 16.48, P = 0.00). The three aforementioned groups exhibited significantly different frequencies of the A and G alleles (χ^2 = 19.77, P = 0.00). In the relapsed-GD patients and the newly diagnosed patients, the major allele was the G allele, which was found at frequencies of 75.71% and 54.42%, respectively; these frequencies were higher than the frequency of the G allele in the control groups (47.54%). Compared with the control groups, both the relapsed GD patients and the relapsed-GD patients GD patients exhibited significant differences in allele frequency (χ^2 = 18.98 and 4.24, respectively, P = 0.00 and 0.04, respectively). There was also a significant difference in allele frequency between the relapsed-GD patients and the newly-diagnosed patients (χ^2 = 11.36, P = 0.00) (Table 2G).

Discussion

GD is an organ-specific autoimmune disease that is influenced by interactions among genetic and environmental factors. No prior research has examined the associations between a polymorphism in the DNASE1 gene and GD in the Han Chinese population. This study utilized a case control approach. PCR-RFLP and direct sequencing techniques were used to comprehensively analyze the association between the rs1053874 polymorphism in the DNASE1 gene and GD in Han Chinese populations.

Genotype Frequencies	Control (%)	GD-Family History (%)	GD- No-Family History (%)	χ^2	P
A/A	60 (29.56)	17 (20.99)	51 (25.12)	[a]3.19	[a] 0.20
A/G	93 (45.81)	37 (45.68)	71 (34.98)	[b]2.82	[b]0.25
G/G	50 (24.63)	27 (33.33)	81 (39.9)	[c]11.02	[c]0.00
Allele frequencies					
A	213 (52.46)	71 (43.18)	173 (42.61)	8.66	0.01
G	193 (47.54)	91 (56.17)	233 (57.39)	[c]7.90	[c]0.00

Note: There were significant differences in the genotype among the three groups. (χ^2 =12.46, P=0.01).
[a]GD with family history compared with controls;
[b]GD with family history compared with GD with-out family history;
[c]GD without family history compared with controls.

Table 2f: Genotype distribution and allele frequencies of rs1053874 in GD patients with or without family history and healthy controls.

Genotype frequencies	Control (%)	Relapsed GD (%)	GD-newly diagnosed (%)	χ^2	P
A/A	60 (29.56)	6 (17.14)	62 (24.90)	[a]27.40	[a]0.00
A/G	93 (45.81)	5 (14.29)	103 (41.37)	[b]4.54	[b]0.10
G/G	50 (24.63)	24 (68.57)	84 (33.73)	[c]16.48	[c]0.00
Allele frequencies					
A	213 (52.46)	17 (24.29)	227 (45.58)	19.77	0
G	193 (47.51)	53 (75.71)	271 (54.42)	[a]18.98	[a]0.00
				[b]4.24	[b]0.04
				[c]11.36	[b]0.00

Note: *There were significant differences in the genotype among the three groups. (χ^2 =37.34 P=0.00)
[a]GD patients with history of relapse compared to healthy controls;
[b]Newly diagnosed GD patients compared to controls;
[c]GD patients with history of relapse compared to newly diagnosed GD patients.

Table 2g: Genotype distribution and allele frequencies of rs1053874 in GD patients with or without history of relapse and healthy controls.

The study results revealed that the DNASE1 gene may be a GD susceptibility gene in the Han Chinese population of Guangdong Province. The G allele at the rs1053874 SNP is a predisposing factor for GD in this population. This allele did not appear to be correlated with gender, family history of GD, or the presence of ophthalmopathy, although it may be associated with disease relapse. The allele frequencies determined for the rs1053874 polymorphism did not significantly differ from those reported in a previous investigation of a Han Chinese population (9)(P > 0.05). In the examined Han Chinese population, the frequencies of the A allele (the major allele) and the G allele of the rs1053874 polymorphism were 0.52 and 0.48, respectively; comparisons of these results with the corresponding findings for other populations reveal that the allele frequencies determined in this study are similar to the allele frequencies for other Asian populations, such as Japanese, Mongolian, and Korean populations (P > 0.05), but significantly different from the allele frequencies in Turkish, Namibian, and German populations (P < 0.05) [9,10].

We selected only one SNP locus in DNASE1 (rs1053874, which is associated with DNASE1*1 and DNASE1*2) for examination in this study. This approach was utilized for the following reasons. First, after the data for DNASE1 in the Han Chinese population were downloaded from HapMap (http://snp.cshl.org/) and introduced into Haploview for computational analysis, it was found that the minor allele frequency (MAF) was significantly lower for other SNPs than for rs1053874. Moreover, the alleles associated with these other DNASE1 SNPs occur at extremely low frequencies. Takeshita et al. [11] reported that in the Japanese population, the frequencies of the four other alleles were 0.0074 for DNASE1*3, 0.0009 for DNASE1*4, 0.0002 for DNASE1*5, and 0.0002 for DNASE1*6. Furthermore, these four alleles have not been detected in German, Turkish, or Namibian populations [12]. Moreover, the sys- tematic selection of SNP loci should account for mutations in the conserved regions of genes. Yasuda et al. [3] considered the DNASE1*1 allele to be the original allele for DNase I in mammalian species, and the DNASE1*2 allele can arise from the DNASE1*1 allele via a point mutation in exon 8 of the DNASE1 gene. Based on the two major reasons above, we selected the rs1053874 polymorphism as our target site, ignoring the distributions of other alleles, thereby avoiding complicated examination procedures and greatly improving screening efficiency for large populations.

The results of this study demonstrated that in the Han Chinese population, the rs1053874 SNP of the DNASE1 gene is associated with GD. Carriers of the G allele of this polymorphism had a higher risk of GD than non-carriers of this allele (odds ratio (OR) = 0.65, 95% confidence interval (CI): 0.49–0.86). There were no significant differences among the three examined genotypes with respect to the age of GD onset. Analyses after stratifying by gender indicated that the G/G genotype and the G allele were significantly more frequent in the male GD patients and the female GD patients than in the controls. However, there were no significant differences in genotype and allele frequencies between the male and female GD patients ($P > 0.05$). Analyses after stratifying by family history revealed no significant differences in allele frequency between the GD patients with a family history and the GD patients without family history. Analyses after stratifying by ophthalmopathy found no significant differences between the patients with GO and the patients without GO. However, we found differences in G allele frequencies between the No-GO group and control group are significant, and differences of the same allele frequencies between the GO group and control group are not significant. For this result, we think there are two reasons. Firstly, maybe not the SNP rs1053874 its own polymorphism associated with GD, but with a chain polymorphism loci in the coding and show the relevance to GD. Secondly, individual differences exist from patient to patient, and the number of GO patients is low; we need sufficient number of patients to achieve a sufficient effect to find a better results in the further. Analyses after stratifying by a history of relapse demonstrated that the relapsed-patients and the newly diagnosed patients significantly differed with respect to genotype distributions and allele frequencies. These results suggest that the DNASE1 gene is unrelated to the age of onset, gender, family history, or presence of ophthalmopathy in GD patients but could be associated with the relapse of GD. The stratified analyses of this study demonstrated that although the G/G genotype frequencies in the subgroups of male GD patients and the subgroups of GD patients with a family history did not significantly differ from the G/G genotype frequency of the control groups, the other subgroups of GD patients exhibited higher G/G genotype frequencies than the control groups. These results suggested that the G/G genotype and the G allele may be associated with the pathogenesis of GD. The two aforementioned subgroups and the control groups exhibited no significant differences with respect to G/G genotype frequency. It may be related to the reduction of the sample size after the stratification. After stratificated by gender, the number of GD male groups is much less than the female groups, so only the female's differences were significant in our study. Additionally, possible reason is that GD is not a monogenic disease; it is an autoimmune disease in which multiple genetic factors are suspected to play an important role. Though only a few risk factors for these diseases have been identified. Susceptibility seems to be stronger in women, pointing toward a possible role for genes related to sex steroid action or mechanisms related to genes on the X-chromosome [13].

So in the further more research is required to explore the complex interactions that relate to the development of GD, which include interactions between genes, between genetic and environmental factors, and between genes and organs. Dittmar et al. [14] demonstrated that the enzymatic activity of DNase I was significantly lower in patients with monoglandular or polyglandular autoimmune syndromes than in normal individuals. In particular, DNase activity was reduced by 54%, 31%, and 24% in patients with a monoglandular autoimmune disease, patients with a polyglandular autoimmune disease, and the healthy relatives of individuals with autoimmune diseases, respectively. In addition, AITD patients exhibited reduced mRNA expression of the DNASE1 gene [15]. Fujihara et al. [16] found that DNASE1 genotype affected the serum activity of this enzyme; in particular, relative to the A/A genotype, the G/G and A/G genotypes were associated with higher serum activity but decreased thermal stability of DNase I. In vitro experiments by Kumamoto et al. [17] revealed that an A to G substitution at the rs1053874 locus leads to the replacement of a glutamine (Gln) with an arginine (Arg) in the resulting protein, which appeared to significantly reduce the enzymatic activity of DNase I. This result was inconsistent with Fujihara's findings. Dittmar et al. [6] found that a G to A substitution at position 1218 in exon 5 of the DNASE1 gene could reduce DNase I activity. However, this reduction was only observed for DNASE1*1 that is, the effect was only observed for the A allele of the rs1053874 locus. The aforementioned substitution caused no change in DNase I activity for the G allele of the rs1053874 locus, suggesting that DNase I activity is not only associated with the genotype of the rs1053874 locus but may also be affected by other SNP genotypes. The mechanisms involved in the associations between DNase I and susceptibility to GD require additional study. A possible mechanism is that a reduction in DNase I activity leads to cellular apoptosis and secondary necrosis, causing the exposure of chromatin fragments; the recognition of these fragments by Toll-like receptor 9 (TLR9) could trigger autoimmune diseases [18]. Leadbetter et al. [19] found that after anti-DNA B cell antigen receptors (BCRs) bind hypomethylated CpG DNA, this DNA can be internalized or endocytosed into B cells. At a later stage, TLR9 combines with CpG DNA to form complexes in lysosomes and endosomes, eventually activating B cells. Although unmethylated CpG DNA is rarely found in the body under normal conditions, self DNA from apoptotic cells or necrotic tissues may combine with antibodies and TLR9 to form immune complexes that disrupt the tolerance status of autoreactive B cells and can thereby act as potential predisposing factors of autoimmune diseases. In addition, under certain circumstances, immune complexes containing self DNA from apoptotic cells or necrotic tissues have the potential to self activate. Anti-DNA B cells can bind DNA through BCRs and TLR9 to directly activate hypomethylated CpG DNA without involving the immune complexes described above. Thus, irrespective of whether this DNA is in these immune complexes, it can facilitate the disruption of the tolerance status of autoreactive B-cells, thereby initiating or promoting the development and progression of autoimmune diseases [19-21].

Conclusion

This study confirmed that the DNASE1 gene may be a GD susceptibility gene in thein the Southern Chinese Han population. The G allele at the rs1053874 SNP would be a direct genetic risk for GD in this population. This allele did not appear to be correlated with gender, family history of GD, or the presence of ophthalmopathy among GD patients, although it may be associated with disease relapse. The results contribute to the accumulation of data for population genetics and anthropological research and helping to establish a genetic database for the Han population.

Conflicts of Interest

None of the authors have any conflicts of interest to declare in connection with this manuscript.

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