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ORIGINAL PAPER

The A946T polymorphism in the interferon induced helicase gene does not confer susceptibility to Graves' disease in Chinese population

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Abstract Genetic susceptibility plays a major role in the etiology of Graves' disease (GD). A recent study revealed that the A946T polymorphism (rs1990760) in interferon induced helicase (IFIH1) gene was a susceptible locus for GD. A case-control study in a Chinese population was undertaken, with 261 GD patients and 206 healthy subjects, to analyze the association of A946T polymorphism in IFIH1 gene with GD. In addition, the distribution of IFIH1 genotypes was investigated in subgroups according to the onset age and the Graves' ophthalmopathy (GO). No significant differences in the allele and genotype frequencies for A946T polymorphism were found between GD patients and healthy controls ($\chi^2 = 2.834$, $P = 0.242$; $\chi^2 = 1.127$, $P = 0.288$). The genotype–phenotype correlation was not identified either. Therefore we were unable to find the association of A946T polymorphism of the IFIH1 gene with the development of GD in a Chinese population.

Keywords IFIH1 · Graves' disease · Graves' ophthalmopathy · Polymorphism

Introduction

Graves' disease (GD) is an organ-specific autoimmune disease, characterized by hyperthyroidism, diffuse goitre, thyroid-specific autoantibodies, with or without Graves' ophthalmopathy (GO) and pretibial myxedema [1]. GD is generally accepted to be multifactorial. Although both genetic and environmental factors are involved in its pathogenesis [2], it seems that genetic factors play a major role in the development of GD. For example, data from Danish cohorts' twin studies showed higher concordance rate for GD in monozygotic as compared to dizygotic twins (0·35 *versus* 0·03), suggesting that 80% of the liability to the development of GD was attributable to genetic factors [3]. Genetic susceptibility to GD is a complex trait, and no single gene is known to cause the disease or to be responsible. A reproducible polygenic susceptibility has been reported including major histocompatibility complex (HLA) [4, 5], cytotoxic T lymphocyte antigen-4 (CTLA-4) [6, 7], lymphoid-specific protein tyrosine phosphatase nonreceptor type 22 (PTPN22) [8, 9], CD40 [10, 11], and thyrotropin receptor (TSHR) [12]. Such genes as HLA, CTLA-4, and PTPN22 are identified as risk factors in different autoimmune diseases.

Interferon induced helicase (IFIH1) gene is located at 2q24.3 and encodes a viral RNA activated apoptosis protein. The function of the IFIH1 protein is to detect viral double strand RNA (dsRNA) and result in the apoptosis of

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infected cells. Recently, a study reported a convincing statistical evidence for a novel nonsynonymous single nucleotide polymorphism, A946T in IFIH1, being the type 1 diabetes (T1D) susceptible locus [13]. Consequently, another case-control study confirmed that this polymorphism was also substantially associated with GD in a UK population [14]. As the susceptibility genes in GD may vary in different ethic groups, we investigated whether the association of A946T polymorphism in IFIH1 gene with GD existed in a Chinese population.

Subjects and method

Study populations

A total of 262 Chinese patients (194 female, 68 male; age range: 8–70 years; median age: 37.33 ± 13.11 years) with GD were included in our study. Patients were sequentially recruited from the Shanghai Clinical Center for Endocrine and Metabolic Diseases in Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine. The diagnosis of GD was based on diffuse goiter, hyperthyroidism, and the presence of TSH receptor antibodies. The severity of ophthalmopathy was assessed according to the NOSPECS classification. For the statistical analysis, patients with class II and higher were considered as having GO ($n = 129$). A total of 206 healthy Chinese volunteers, (162 female, 44 male; age range: 20–59 years; median age: 37.24 ± 8.66 years) without family history of GD or other autoimmune diseases, served as the control subjects. Informed consent was obtained from every participant and the study was approved by the ethics committee of Ruijin Hospital.

Genotype analysis

Genomic DNA was extracted from peripheral blood mononuclear cells, using a commercially available kit (Qiagen, Hilden, Germany) and according to the manufacturer's instruction. The polymorphism A946T (rs1990760) in IFIH1 gene was detected by single-based primer extension technology (Genomelab^{TM} SNPStart Primer Extension Kit, Beckman Coulter Inc., Fullerton, CA, USA). A 348 base-pair (bp) fragment containing the polymorphic site was amplified using IFIH1-specific primers (forward: 5′>CCTTTGATACTTATAGGGAAC T<3′; reverse: 5′>CAAGATTGGGAAATGTGAT<3′). PCR was performed with a volume of 50 μl mixture containing 1.5 mM $MgCl_2$, 10 mM dNTP, 50 ng genomic DNA, 20 μmol of each primer and 2.5 U Taq DNA polymerase (Sangon, Shanghai, China). Amplification was performed for 30 cycles with preheating at 95°C for 5 min, followed by denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 45 s. The polymorphism A946T was detected by Genomelab^{TM} SNPStart Primer Extension Kit (Beckman Coulter, Inc., Fullerton, CA, USA) with the interrogation primer (5′>CTTATA GGGAACTTTACATTGTAAGAGAAAACAAA<3′). The productions were identified on the CEQ^{TM} 8800 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, CA, USA) according to the manufacturer's instruction.

Statistical analysis

Distribution of alleles and genotypes among the studied groups were analyzed by χ^2 test for 2 × 2 or 2 × 3 tables. Statistical significance was defined as $P < 0.05$. Deviation from the Hardy–Weinberg equilibrium was tested using the Pearson's χ^2 test statistic (SPSS software version, 11.5 SPSS Inc., Chicago, IL, USA). QUANTO software (http://www.hydra.usc.edu/gxe) was used to calculate the power of association studies.

Results

Association between the A946T polymorphism in IFIH1 gene and GD

The genotype frequencies of both patients and control subjects are all compatible with the Hardy–Weinberg law. However, we did not find a significant difference for the frequencies of alleles or genotypes between the GD patients and the control subjects. (Tables 1, 2)

Association between the A946T polymorphism in IFIH1 gene with the age of onset in Graves' disease

Regarding the onset age, there were no significant differences in the allele and genotype frequencies between the patients who developed GD after 40-years-old and those with an earlier onset age. In addition, we found no significant difference between patients with an onset age of less than 40-years-old and control subjects. (Tables 1, 2)

Association between the A946T polymorphism in IFIH1 gene and GO

There were no significant differences in genotype or allele frequencies of A946T polymorphism in IFIH1 gene between the patients with evident ophthalmopathy

Table 1 Frequency of alleles of A946T polymorphism in IFIH1

	GD	GO		GD onset		Controls
	($n = 262$)	With ($n = 129$)	Without ($n = 133$)	<40 yr ($n = 144$)	≥40 yr ($n = 118$)	($n = 206$)
C	417[a] (79.6)	200[b,c] (77.5)	217 (81.6)	223[d,e] (77.4)	194 (82.2)	316 (76.7)
T	107 (20.4)	58 (22.5)	49 (18.4)	65 (22.6)	42 (17.8)	96 (23.3)

Values given are the number of alleles, with the percentage in *parentheses*

[a] The difference in the frequencies of alleles between GD patients and controls was not statistically significant ($\chi^2 = 1.127$, $P = 0.288$)

[b] The difference in the frequencies of alleles between GD patients with ophthalmopathy and the controls was not statistically significant ($\chi^2 = 0.06$, $P = 0.806$)

[c] The difference in the frequencies of alleles between GD patients with and without ophthalmopathy was not statistically significant ($\chi^2 = 1.328$, $P = 0.249$)

[d] The difference in the frequencies of alleles between subjects with the onset of GD at less than 40 year and controls was not statistically significant ($\chi^2 = 0.051$, $P = 0.821$)

[e] The difference in the frequencies of alleles between subjects with the onset of GD at less than 40 year compared with those with later onset was not statistically significant ($\chi^2 = 1.818$, $P = 0.178$)

Table 2 Frequency of genotypes of A946T polymorphism in IFIH1

	GD	GO		GD onset		Controls
	($n = 262$)	With ($n = 129$)	Without ($n = 133$)	<40 yr ($n = 144$)	≥40 yr ($n = 118$)	($n = 262$)
CC	171[a] (65.3)	80[b,c] (62.0)	91 (68.4)	90[d,e] (62.5)	81 (68.6)	171[a] (65.3)
TC	75 (28.6)	40 (31.0)	35 (26.3)	43 (29.9)	32 (27.1)	75 (28.6)
TT	16 (6.1)	9 (7.0)	7 (5.2)	11 (7.6)	5 (4.2)	16 (6.1)

Values given are the number of genotypes, with the percentage in *parentheses*

[a] The difference in the frequencies of genotypes between GD patients and controls was not statistically significant ($\chi^2 = 2.834$, $P = 0.242$)

[b] The difference in the frequencies of genotypes between GD patients with ophthalmopathy and the controls was not statistically significant ($\chi^2 = 1.061$, $P = 0.588$)

[c] The difference in the frequencies of genotypes between GD patients with and without ophthalmopathy was not statistically significant ($\chi^2 = 1.23$, $P = 0.541$)

[d] The difference in the frequencies of genotypes between subjects with the onset of GD at less than 40 year and controls was not statistically significant ($\chi^2 = 1.843$, $P = 0.398$)

[e] The difference in the frequencies of genotypes between subjects with the onset of GD at less than 40 year compared with those with a later onset was not statistically significant ($\chi^2 = 1.774$, $P = 0.412$)

(NOSPECS class II or higher) and those without (NOSPECS class 0, I) or control subjects (Tables 1, 2).

Power calculation

At the 0.05 level of significance with the two-sided test for the A946T polymorphism, the present study has 91.2% power to detect an effect at a relative risk of 2.0 in the groups of GD patients and control subjects.

Discussion

Interferon induced helicase, also known as the melanoma differentiation-associated 5 (MDA-5), is a viral RNA activated apoptosis protein. It detects viral dsRNA via a helicase domain, resulting in the activation of a caspase recruitment domain (CARD), which triggers a chain of signaling events in immune response [15, 16]. Recently, a large-scale candidate variant study revealed the A946T SNP in IFIH1 gene as a novel susceptible locus for T1D. The A946T SNP leads to an Alanine for Threonine substitution and the C allele was found to be a risk factor for T1D [13]. The genetic association of IFIH1 with T1D may be explained by the link with preceding viral infections. Although the A946T SNP does not reside in either the helicase or CARD, it does lie in a region that is highly conserved between mammals, suggesting functional importance [17]. A more recent study by Sutherland et al. confirmed that this polymorphism in IFIH1 gene is also associated with GD, but not with autoimmune Addison's

disease [14]. Although the role of viral infection in precipitating GD remains largely hypothetical [18], several studies suggested that retrovirus might be involved in the pathogenesis of GD, such as human foamy virus [19] and human T cell leukemia virus [20]. At the same time, interferon-α, a cytokine produced by leukocytes and dendritic cells in response to viral infections, is proved to be associated with the development of autoimmune thyroid disease, including GD [21].

Nevertheless, our data did not show significant differences in allele or genotype frequencies for A946T polymorphism between GD patients and healthy control subjects. This was incompatible with the result from Sutherland et al., in which the positive association ($P = 1.9 \times 10^{-5}$, OR = 1.47) with A946T polymorphism in IFIH1 gene was found in 602 GD patients and 446 health subjects. Furthermore, with stratification of onset age, we found no significant difference between GD patients with an onset age of less than 40-years-old and those with a later onset. Some explanations could be: (1) Distribution of polymorphisms appears to vary in different racial population [22]. In our study, the distribution of A946T polymorphism in healthy Chinese (0.23/0.77) was different from that in Newcastle population (0.57/0.43). Interestingly, differences for distribution of polymorphisms were also found in some other susceptible genes, such as PTPN22 [23]. The PTPN22 R620W polymorphism, a recently identified functional missense single nucleotide polymorphism and a risk factor for some autoimmune diseases, including GD, does not exist in Asian population [23]. Thus, it is possible that A946T polymorphism in IFIH1 gene is associated with GD in UK patients, but not in those from other ethnic groups, including the Chinese population. (2) Environmental factors also play some roles in the presence of GD. Multifactorial disease susceptibility is dependent on both the prevalence of the polymorphism in the population and exposure to environmental factors. Therefore, certain susceptible genes may have distinct effects in distinct populations. (3) The number of patients or control subjects included in our study was relatively small. So, we maybe having no efficient statistical power to detect a weak genetic effect, although the present analysis showed a greater than 90% power to detect a disease association assuming an odds ratio of 2.0.

In the present study, we also made the correlation study between the A946T polymorphism and GO. However, we did not find significant differences for the frequencies of alleles or genotypes between the patients with evident ophthalmopathy and those without or control subjects. Prevalence of clinical ophthalmopathy in GD is about 25–50% [24, 25]. In the present study, patients with NOSPECS class II and higher were considered GO for the statistical analysis. Although GO appears within 18 months after the diagnosis of thyroid disease in most cases [26, 27], it can be actually present or worse at any time during the course of the disease. Thus, some non-ophthalmopathy patients in our study could develop GO in the subsequent months or years that could affect our results.

In conclusion, we found no association of A946T polymorphisms in the IFIH1gene with the development of GD in a Chinese population, contrasting with that observed in the UK population. We also found no significant differences regarding ophthalmopathy or onset age in GD. With the limitation of our small sample size, more further appropriately powered studies are needed to elucidate the role of IFIH1gene in GD.

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