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Original Article

Association Of PTPN22 Gene Polymorphisms in Patients with Graves' Disease in Iranian Papulation

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Abstract

Background: Graves' disease (GD) is an autoimmune disease that is associated with increased thyroid gland irritation and, consequently, hyperthyroidism. Autoimmune diseases are common in the general population and are influenced by genetic and environmental factors. PTPN22, which was reported as a susceptible locus for GD in several populations, acts as a negative regulator for activation of primary T-cells, and LYP polymorphism could potentially increase susceptibility to Graves' disease, which may play a role in other autoimmune conditions as well. In this study, we investigated the association of several PTPN22 single nucleotide polymorphisms (SNPs) with Graves patients.

Methods: After DNA extraction from peripheral blood cells, SNP Genotyping was performed through real-time PCR with allelic discrimination TaqMan genotyping assays (ABI Applied Biosystems, 7300 Real-Time PCR System, USA) based on manufacturer protocols. The frequencies of alleles and genotypes of PTPN22 SNPs (rs12760457, rs2476601, rs1310182, and rs1217414) were recorded.

Results: In our study, the rs1310182 was significantly more frequent in patients with GD than in healthy individuals. While the C allele of rs1310182 was 1.78 times more frequent in GD patients (95%CI: 1.18-2.69, P=0.005), the T allele was more frequent in healthy subjects (OR=0.56, 95% CI: 0.37-0.84, P=0.005). In addition, the CC genotype of this SNP was 1.86 times more common in patients (P=0.05). No significant differences were observed between the other SNPs of this gene in case and control.

Conclusion: The results demonstrate that one SNP (rs1310182) of the PTPN22 gene is associated with susceptibility to GD in an Iranian population. Further studies, including functional analyses, are required.

Keywords: Autoimmune Disease, Graves' Disease, PTPN22 Gene, Polymorphisms

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Introduction

Graves' disease (GD) is an autoimmune disease that is associated with increased thyroid gland irritation and, consequently, hyperthyroidism. Autoimmune diseases are common in the general population and are influenced by genetic and environmental factors. (1).

Thyroid autoimmune diseases (AITD), including Graves' disease and Hashimoto's thyroiditis, are the most common autoimmune diseases. Hyperthyroidism, disseminated goiter, and the presence of thyrotropin receptor antibodies are the common symptoms of Graves' disease. Graves' disease is caused by the production of antibodies binding to TSHR, which stimulate hyperthyroidism and enlargement of the thyroid gland. Genetic and family studies have proposed the genetic risk factors responsible for over 79% susceptibility to Graves' disease (2, 3). These genes include HLA, CTLA4, FCRL3, T2 (RANTES), secretoglobin, TSHR, and TG, which have been widely associated with Graves' disease in different ethnicities (2, 4). PTPN22, located on chromosome 1, which codes LYP (a lymphoid tyrosine phosphatase protein), acts as a negative regulator for the activation of pre-T-cells. The signal transduction acts by binding to the CSK protein, and this LYP polymorphism is a susceptible allele for Grave's disease. Moreover, it may also play a role in other autoimmune conditions, which increase the risk of grave disease. The gene variant, R620W, in the +1858 position, was first identified as a susceptible locus for type 1 diabetes in the European population. This variant is also associated with several other autoimmune diseases (AIDs), such as rheumatoid arthritis, autoimmune thyroid disease, and SLE. Previous studies have suggested that the subtypes of Gravies' disease may be caused by various significant predisposing genes or different variants of a susceptible gene (5, 6). PTPN22 was previously reported as a susceptible locus for Europe's Graves disease.

T-helper cells mediate the innate immune system and include four subtypes such as Th1, Th2, Th17, and Treg. Despite the lower concentration of Th17 in human blood in comparison with other T helpers, it plays a vital role in chronic inflammation as well as the pathology of several autoimmune diseases (3). Differentiation of pre-T CD4 cells to Th17 occurs by activating the STAT3 pathway and the Th17 transcription factor. IL17A and IL17F are two members of the cytokine family IL17, which are responsible for the pathological activity of Th17 cells (2). Recently, one study has identified the relationship between autoimmune thyroid disease and SNPs of IL17A and IL17F. While the IL17F rs763780 polymorphism might be a potential risk factor for AITD, the IL17A rs3819025 polymorphism could be considered a protective factor against Graves' disease in the Chinese population (3).

Therefore, it is essential to identify the disposed genes and the position of those that facilitate the diagnosis, as well as the prevention and treatment of the disease.

While PTPN22 has been reported as a susceptible locus for Graves' disease in the European population, its role is not clearly understood in the Asian population (5, 6)

Therefore, this study aimed to investigate the single nucleotide polymorphisms (SNP) of the PTPN22 gene, including rs12760457, rs1310182, rs1217414, and rs2476601, in patients with Graves' disease, compared to the control group.

Material and Methods

Study population

In this study, 150 patients were recruited who have been referred to the Molecular Immunology Research Center at the Children's Medical Center Hospital, Tehran, Iran. Complete patients' history confirmed diagnosis of GD in addition to clinical records and laboratory findings of GD. The control group was comprised of 93 healthy individuals with no current or previous history of any autoimmune disease. This study was approved by the Ethics Committee of Tehran University of Medical Sciences, and all subjects were requested to fill out the written informed consent before sampling.

Blood Sampling and Genotyping of PTPN22 Single nucleotide polymorphism (SNP)

A total amount of 3-5 cc peripheral blood was obtained from all patients and controls in EDTA-covered tubes in order to avoid coagulation. Genomic DNA was extracted using the Phenol-Chloroform method as described elsewhere (7). SNP genotyping was performed by real-time

polymerase chain reaction (PCR) using allelic discrimination TaqMan genotyping assays (ABI Applied Biosystems 7300 Real-Time PCR System, USA) based on manufacturer protocols. Using pre-designed primers (**Table 1**), the allele and genotype frequencies of PTPN22 SNPs (rs12760457, rs2476601, rs1310182, and rs1217414) were investigated and recorded.

Statistical analysis

Allele and genotype frequencies were reported in percentage, and the comparison of their frequencies between cases and controls was assessed by Epi-Info statistical software version 7.2.0.1 (health organization Geneva, Switzerland) and the chi-square test. The odds ratio (OR) and 95 % confidence interval (CI) were evaluated for each allele and genotype. A P-value of less than 0.05 was considered to be statistically significant.

Result

Allele and Genotype Frequencies of PTPN22 Single Nucleotide Polymorphisms

The allele and genotype frequencies of five

Table1. PTPN22 SNPs and primers used in this study

PTPN22 gene	Location	Context sequence	Snp type
RS12760457	Chr.1: 113847126	TTTTCAATTCATTAATTCTTTTTTC[C/T]GTACCCTCTATTCTGCTGTTGAACC	Intron, Transition Substitution, Intragenic
RS2476601	Ch1: 113834946	ACCACAATAAATGATTCAGGTGTCC[A/G]TACAGGAAGTGGAGGGGGGATTTCA	Intron, Miss-sense Mutation, Transition Substitution, Intragenic
RS1310182	Chr.1: 113830881	TAAACAAACCCAATGACCAATGACA[C/G/T]GTGAACCTCTTGTACTTTACTGCAT	Transition Substitution, Intron, UTR 3, Intragenic
RS1217414	Ch1: 113870045	AAACCTTTGCCTCCTGGAGCTAACA[C/T]TCTGGTACCGGATAGCAGATGTGAA	Intron, Transition Substitution, Intragenic

PTPN22 SNPs were compared between patients and control groups (**Table 2**). The rs1310182 was found to be significantly more frequent in patients with GD compared to healthy individuals. While the "C" allele of rs1310182 was 1.78 times more frequent in patients (95% CI: 1.18-2.69, P=0.005), the "T" allele was more common in healthy controls. Interestingly, the "CC" genotype

of this SNP was also 1.86 times more prevalent in patients (95%CI: 0.98-3.54, P=0.05), suggesting that the "C" allele of this SNP could be considered as the risk allele for GD in this population.

No significant differences were observed between the other SNPs of this gene in the case and control (**Table 2**).

Table2. PTPN22 gene allele and genotype polymorphisms in patients with GD and controls

Snp	Alleles	Genotype	Case (n= 150) N(%)	Control (n= 93) N(%)	*P*-value	Or(95%CI)	Range of OR
	C		139(67)	136 (73.1)	0.17	0.74	0.47-1.14
	T		69 (33.1)	50 (26.9)	0.17	1.35	0.87-2.08
		CC	45 (43.2)	51 (54.8)	0.34	0.78	0.48-1.28
		TC	49 (47.1)	34 (33.6)	0.33	1.28	0.76-2.16
RS 12760457		TT	10(9.6)	8 (8.6)	0.82	1.11	0.42-2.95
	A		6 (2.0)	0 (0)	0.08	_	_
	G		292(98.0)	186 (100)	0.08	_	_
		AA	0	0 (0)	_	_	_
		AG	6 (4.0)	0	0.084	_	_
RS2476601		GG	143 (96)	93 (100)	0.084	0.00	_
	T		**78 (41.4)**	**104 (55.9)**	**0.005**	**0.56**	**0.37-0.84**
	C		**110(58.5)**	**82 (44.1)**	**0.005**	**1.78**	**1.18-2.69**
RS1310182		TT	18 (19.1)	29 (31.2)	0.14	0.61	0.3-1.18
		TC	42 (44.6)	46 (49.5)	0.69	0.9	0.54-1.55
		CC	**34 (36.1)**	**18 (19.4)**	**0.05**	**1.86**	**0.98-3.54**
	A		74 (25.5)	56 (30.1)	0.27	0. 8	0.52-1.98
	G		216 (74.4)	130 (69.9)	0.27	1.26	0.83-1.87
RS1217414		AA	13 (9.6)	8 (8.6)	0.80	1.12	0.44-2.8
		AG	38 (28.1)	40 (43)	0.10	0.65	0.38-1.9
		GG	84 (62.2)	45 (48.4)	0.27	1.28	0.82-2.01

The bold values indicate statistical significance at the α = 0.05 level.

Discussion

Our study showed the association of SNPs of the PTPN22 gene, including rs12760457, rs1310182, rs1217414, and rs2476601, in Graves' Disease as one of the autoimmune thyroid diseases and compared with healthy controls. The results of this case–control study demonstrated that individuals with the "CC" genotype and "C," "T"

alleles of PTPN22 rs1310182 might be susceptible to an autoimmune disease.

On the other hand, we found one SNP of PTPN22, which is significant in patients compared to the control group. So, those alleles and genotypes might be the most important SNP responsible for the development of GD among these SNPs in Iranian patients.

The PTPN22 gene is located at chromosome 1 and encodes the LYP as an intracellular tyrosine phosphatase with a negative regulatory influence on the signal transduction of the early activation of T-cells(8). Immune disorders and polymorphisms of the PTPN22 gene have been investigated as a risk factor in autoimmune diseases. This kind of autoimmunity resulted from the creation of immune responses by the interaction of environmental factors with genetically background individuals (9). Several studies have shown that certain autoimmune disorders have similar susceptible genetic factors. The specific role of PTPN22 in T-cell stimulation has appeared in PTPN22 murine homologue knockout, which caused a reduction in the T-cell receptor signaling threshold in these animals (10, 11). Several studies showed that various SNPs of PTPN22 are considered a susceptible locus that may play an important role in Graves Patients(12).

The other alleles and genotypes of PTPN22 gene polymorphisms in our study (rs12760457 C/T and rs1217414 A/G) were not associated with GD. Other studies demonstrated different results in several populations; for example, a common variant of PTPN22 (rs2476601, C1858T), which is associated with RA and GD in Caucasians, was not found in Japanese GD patients, whereas rs3789604 is against the protective Graves' disease(2). Wawrusiewicz-Kurylonek et al. showed a relation between rs2476601 and Graves' disease predisposition in the adult northeastern Polish population. Patients have shown more occurrences of the TT allele rather than the occurrence of the T allele(13), and yang wang et al. showed Heterogeneity exists in the relationship between excessive fluoride and PTPN22 (rs3765598) polymorphisms in the Chinese population (14).

On the opposite, The SNP37 of the PTPN22 gene is associated with susceptibility to GD in the Japanese population (15). In the study by Ban et al., 334 patients with AITD and 179 Japanese controls reported that PTPN22 polymorphism had no role in the AITD. On the opposite, the PTPN22 polymorphism is a predisposing cause for Graves' disease, which may play a role in many other autoimmune conditions(3) Dose of the gene-dependent effect of the PTPN22 of Graves' disease has been reported in the British population (16).

Our study showed that rs1310182 is significantly more frequent in Graves patients compared to healthy individuals. Also, this SNP (rs1310182) was significant in our previous study on ulcerative colitis and chronic spontaneous urticaria (17, 18). It seems this polymorphism of the ptpn22 gene has an important role in the Iranian population.

Although in our study, the rs2476601 PTPN22 gene is more frequent in AIDTs, statistically, it was not found significant; it's more frequent (p=0.08), and it might be significant in a larger sample size.

Conclusion

The results demonstrate that one SNP (rs1310182) of the PTPN22 gene is associated with susceptibility to GD in an Iranian population. Further studies, including functional analyses, are required.

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Conflict of interest

There is no conflict of interest.

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