Original Article

PTPN22 1858 C/T Exon Polymorphism is not Associated with Graves' Disease in Kashmiri population

Faheem Shehjar, Dil-Afroze[1], Riaz A. Misgar, Sajad A. Malik, Bashir A. Laway

Departments of Endocrinology and [1]Immunology and Molecular Medicine, Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, Jammu and Kashmir, India

Abstract

Background: Graves' disease (GD) is a multifactorial autoimmune disease with contribution from both genetic and epigenetic factors in its causation. Association of genetic factors and GD has been extensively studied. Gene "protein tyrosine phosphatase nonreceptor 22" (*PTPN22*) is an important immunoregulatory gene preventing hyper responsiveness of T cells by negatively regulating their signal transduction. Association of single-nucleotide polymorphism (SNP) 1858 C/T within *PTPN22* with some autoimmune diseases has been described. **Methods:** We aimed to analyze whether 1858 C/T SNP of *PTPN22* gene has any association with GD in Kashmiri population. Polymerase chain reaction-restriction fragment length polymorphism was performed for genotyping 1858 C/T SNP in 135 patients with GD and 150 age- and gender-matched healthy controls. **Results:** Among the patients with GD, the frequencies of *PTPN22* 1858 CC, CT, and TT genotypes were 97.7, 2.2, and 0%, respectively, whereas in healthy controls the frequencies of CC, CT genotypes were 100 and 0%, respectively. No significant association was found between *PTPN22* 1858 C/T SNP and patients with GD. **Conclusion:** GD is not associated with *PTPN22* 1858 C/T SNP in Kashmiri population. Furthermore, 1858 C/T SNP in *PTPN22* gene could be a part of variation in different ethnic populations across the globe.

Keywords: Graves' disease, protein tyrosine phosphatase nonreceptor 22, restriction fragment length polymorphism, single-nucleotide polymorphism

Introduction

Graves' disease (GD) is an autoimmune disorder, clinically presenting as hyperthyroidism, diffuse thyroid enlargement with or without ophthalmopathy and occasionally dermopathy.[1] Cause of GD is multifactorial with contribution of genetic and environmental factors.[2-4] Protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) gene helps to prevent hyperresponsiveness of T cells via a protein it encodes [known as lymphoid tyrosine phosphatase (LYP) protein], which causes negative T cell regulation.[5] Among several single-nucleotide polymorphisms (SNPs) in *PTPN22* gene, 1858 C/T (Arg620Trp) is an important SNP. Initial studies showed Type 1 diabetes be associated with *PTPN22* Arg620Trp SNP, where T allele acted as a risk allele.[6] Later, T allele of this SNP was shown to be associated with many other autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus in addition to GD.[7-13] Ichimura *et al*. did not find an association between *PTPN22* 1858 C/T SNP and GD susceptibility in Japanese population, suggesting that it does not play a causal role for its development.[14] Variations in the results from different studies could predominantly be due to distinct ethnicities of the studied populations. It would be useful to study populations having distinct ethnic background. As no study on the role of *PTPN22* 1858 C/T SNP in GD predisposition has been conducted till date in our population, using a conventional polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, we studied the association between 1858 C/T polymorphism and GD in Kashmiri population.

Materials and Methods

A total of 135 patients with GD (33 men and 102 women) between the age of 14 and 68 years (average = 37.97 years) were enrolled in this study. Diagnosis of GD was established

Access this article online

Quick Response Code:

Website: www.ijem.in

DOI: 10.4103/ijem.IJEM_105_18

Address for correspondence: Dr. Bashir A Laway, Department of Endocrinology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India.
E-mail: drlaway@gmail.com

How to cite this article: Shehjar F, DA, Misgar RA, Malik SA, Laway BA. PTPN22 1858 C/T exon polymorphism is not associated with Graves' disease in Kashmiri population. Indian J Endocr Metab 2018;22:457-60.

on the basis of clinical parameters of thyrotoxicosis (history of marked weight loss, presence of diffuse goiter, presence of skin or eye changes) that diffusely increased Technetium^{99m} pertechnetate uptake on thyroid scintigraphy and positive thyroid stimulating hormone (TSH) receptor antibody.

Information was collected on the patient's age at onset, history of smoking, size of thyroid gland, presence of thyroid eye disease, serum thyroid hormone levels and antibody titers of TSH receptor antibodies. A group of 150 age- and sex-matched healthy volunteers (42 men and 108 women), who were euthyroid and had no personal or family history of Grave's or other autoimmune diseases, served as controls. Informed consent was obtained from each individual who participated in the study. The frequency distribution, demographics, and risk factors in patients with GD and controls are shown in Table 1.

Sample collection and molecular analysis

Five milliliters of peripheral blood were collected from GD patients and healthy controls in ethylenediaminetetraacetic acid containing tubes. DNA was isolated using HiPurA™ Blood Genomic DNA Miniprep Purification Kit (HiMedia Laboratories Pvt. Ltd., Mumbai, India).

Amplification of the exon 1858 C/T *PTPN22* gene

PCR amplification was carried out using a single set of the primer pair (Forward = ACTGATAATGTTGCTTCAACGG; Reverse = TCACCAGCTTCCTCAACCAC) that amplified the exonic region *PTPN22* gene containing the 1858 C/T polymorphic site. The amplicon size was 218bp. PCR was carried out in a final volume of 25 µl containing 50 ng genomic DNA template, 1X PCR buffer (Biotools, B and M Labs, S.A. Madrid-Spain) with 2 mM $MgCl_2$, 0.4 mM of each primer (Sigma-Aldrich Co. LLC, USA), 50 mM dNTPs (Biotools, B and M Labs, S.A. Madrid-Spain), and 1U DNA polymerase (Biotools, B and M Labs, S.A. Madrid-Spain). For PCR amplification, the standard program was used as follows: one initial denaturation step at 94°C for 5 min, followed by 30 denaturation cycles of 30 s at 94°C, 30 s of annealing at 62°C, and 60 s of extension at 72°C, followed by a final elongation cycle at 72°C for 5 min.

Table 1: Frequency distribution of demographic factors, genotypic and allelic frequencies of PTPN22 1858 C/T polymorphism in patients with GD and controls

Variables	Cases *n*=135 (%)	Controls *n*=150 (%)	*P*
Age ≤40 yrs	99 (73.25)	106 (70.66)	-
Age >40 yrs	36 (26.75)	44 (29.34)	0.69
Female	102 (75.56)	108 (72)	-
Male	33 (24.44)	42 (28)	0.50
Rural dwelling	99 (73.33)	105 (70)	-
Urban dwelling	36 (26.67)	45 (30)	0.60
PTPN22 1858 genotype			
CC*	132 (97.78)	150 (100)	-
CT*	03 (2.22)	0 (0)	0.10
PTPN22 1858 allele (2n)	270	300	
C	267 (98.89)	300 (100)	-
T	03 (1.11)	0 (0)	0.10

*CC, Homozygous wild genotype; *CT, Heterozygous genotype

Genotypic analysis of PTPN22 1858 C/T polymorphism by RFLP

The PCR product of 218bp [Figure 1] containing the 1858 C/T restriction site was digested by enzyme *RsaI* (Fermentas Thermo Fisher Scientific Inc., Massachusetts, USA). Products were resolved on 3% Agarose gel. *PTPN22* 1858C allele if present is recognized as a cleavage site by endonuclease *RsaI*. The 1858T allele is not digested and yields one fragment of 218 bp, while the 1858C allele is digested and yields two fragments of 176 and 46 bp and the heterozygous C/T gives three bands of 218, 176, and 46 bp [Figure 2].

Laboratory tests

Blood samples were collected from each patient in the fasting state. Serum concentrations of TSH, total T3, and total T4 were measured by commercial chemiluminescent immunoassays (Beckman Coulter Unicel DXI 800 Access Immunoassay System, Brea, California). TSH receptor antibody was measured by enzyme-linked immunosorbent assay (Elisa RSR™ TRAb second-generation kit; RSR, Cardiff, UK). The normal values were as follows: TSH, 0.5–6.5 mIU/ml; total T3, 0.70–2.50 ng/ml; total T4, 4.0–13.0 mcg/dl, and TRAb ≤ 1U/l.

Statistical analysis

For statistical analysis, the genotype and allelic frequency distributions of polymorphisms in the control and GD patient groups were compared using the χ^2-test. When the assumption of the χ^2-test was violated (i.e., when one cell had an expected count of <1, or >20% of the cells had an expected count of <5), Fisher's exact test was used. Odds ratios (ORs) with 95% confidence intervals (CIs) were determined for the disease susceptibility of specific genotypes and alleles. Results were considered statistically significant when the probability of findings occurring by chance was <5% ($P < 0.05$).

Results

A total of 285 individuals (135 GD cases and 150 healthy controls) were included in this study. The distribution of *PTPN22* 1858 genotypic and allelic frequencies in cases and controls is given in Table 1. Among the patients with GD, the frequencies of *PTPN22* 1858 CC, CT and TT genotypes were 132 (97.78%), 3 (2.22%), and 0 (0%), respectively, whereas every healthy control had a positive CC genotypes. None among controls was positive for CT and TT genotypes. Statistical analysis indicated that genotypic and allelic frequencies of the *PTPN22* 1858 polymorphism in cases and controls did not differ significantly ($P > 0.05$).

Furthermore, the association between *PTPN22* 1858 polymorphism with that of the clinicopathological parameters was also analyzed. No significant association was seen in any of the parameters ($P > 0.05$) with the said polymorphism [Table 2].

Discussion

Apart from Major Histocompatibility Complex, PTPN22 locus is a potent risk factor associated with several autoimmune diseases. LYP protein encoded by *PTPN22* gene is expressed exclusively by the cells of immune system.[15] In the coding region of this gene, a single-base change within a polyproline binding motif (from amino acid arginine to tryptophan) has been associated with Type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosis, Hashimoto's thyroiditis, and GD.[16-22]

In this case–control study, we investigated the association between *PTPN22* gene 1858 C>T polymorphism and GD in Kashmiri population. Among patients with GD, the frequencies of *PTPN22* 1858 CC and CT genotypes were 97.78 and 2.22%, respectively, and TT genotype seen in none. Among healthy controls, *PTPN22* 1858 CC genotype was seen in all and none had CT and TT genotypes. Genotypic and allelic frequencies of the *PTPN22* 1858 polymorphism in cases and controls were not significantly different ($P > 0.05$) [Table 1]. The individuals harboring T allele of the *PTPN22* C1858T gene SNP have been shown to be more susceptible to development of GD in British and Polish Caucasians.[11,12,16,23] Further, a meta-analysis showed association of *PTPN22* 1858T allele with rheumatoid arthritis, systemic lupus erythematosus, GD, and type 1 diabetes in Caucasians.[24] It has been proposed that the disease-associated T allele encodes a protein that does not bind to the protein tyrosine kinase Csk and may therefore cause general hyperresponsiveness of T cells.[6] In our study only, 3 out of 135 GD cases had CT genotype, while T allele was completely absent in controls. Our results are consistent with the study in Japanese populations by Ichimura *et al*.,[14] documenting an absence of the T allele in all the patients and healthy controls. Also, several other reports have shown that *PTPN22* T variant is absent in patients with GD, type 1 diabetes, rheumatoid arthritis as was in healthy population,[25-27] implying that *PTPN22* gene polymorphism shows ethnic differences. The results of the present study suggest that 1858 C/T SNP may be of little importance in our patient population with GD.

Table 2: Association between PTPN22 1858 C/T polymorphism and various clinical parameters of patients with GD

Parameter	Cases (n=135) PTPN22 1858 C/T		
	CC (132)	CT (3)	*P*
Age			
≤40 yrs (99)	96 (96.97)	3 (3.03)	
>40 yrs (36)	36 (100)	0 (0)	0.56
Gender			
Females (102)	99 (97.06)	3 (2.94)	
Males (33)	33 (100)	0 (0)	0.57
Family history			
Positive (31)	31 (100)	00 (00.00)	
Negative (104)	101 (97.12)	3 (2.88)	0.58
Orbitopathy			
Positive (22)	22 (100)	00 (0)	
Negative (113)	110 (97.35)	3 (2.65)	1
Smoking			
Yes (20)	20 (100)	0 (00.00)	
No (115)	112 (97.39)	3 (2.61)	1
Dwelling			
Rural (99)	97 (97.98)	2 (2.02)	
Urban (36)	35 (97.22)	1 (2.78)	1

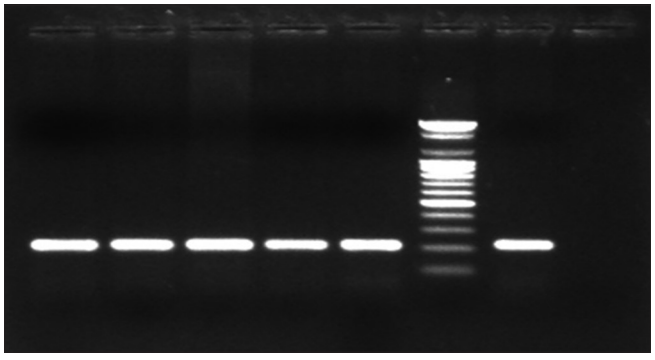

Figure 1: Representative gel picture showing PTPN22 1858 amplicon.

Limitations

Small sample size might be a limitation in our study. We suggest more such studies with a larger sample size to be conducted because of rarity of *PTPN22* 1858 T allele in Kashmiri population.

Conclusion

PTPN22 1858 C/T polymorphism does not show an association with GD in Kashmiri population.

Acknowledgement

We are grateful to the head and Staff of Department of Endocrinology, SKIMS, who helped us in the sample procurement and hormone assays.

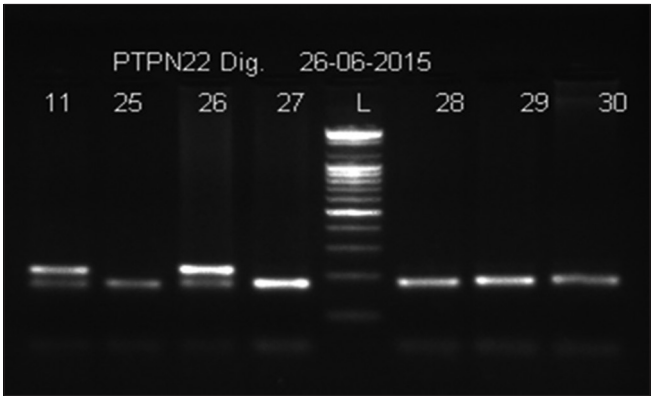

Figure 2: Representative gel picture showing RFLP analysis of PTPN22 1858 PCR product

Financial support and sponsorship

The study was supported by the research grant received from SKIMS.

Conflicts of interest

There are no conflict of interest.

References

1. Weetman AP. Graves' disease. N Engl J Med 2000;343:1236-48.
2. Brix TH, Kyvik KO, Christensen K, Hegedüs L. Evidence for a major role of heredity in Graves' disease: A population-based study of two Danish twin cohorts. J Clin Endocrinol Metab 2001;86:930-4.
3. Tomer Y. Mechanisms of autoimmune thyroid diseases: From genetics to epigenetics. Annu Rev Pathol Mech Dis 2014;9:147-56.
4. Brix TH, Hegedüs L. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease. Clin Endocrinol (Oxf) 2012;76:457-64.
5. Burn GL, Svensson L, Sanchez-Blanco C, Saini M, Cope AP. Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? FEBS Lett 2011;585:3689-98.
6. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, *et al*. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet 2004;36:337-8.
7. Begovich AB, Carlton VEH, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, *et al*. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75:330-7.
8. Carlton VEH, Hu X, Chokkalingam AP, Schrodi SJ, Brandon R, Alexander HC, *et al*. PTPN22 genetic variation: Evidence for multiple variants associated with rheumatoid arthritis. Am J Hum Genet 2005;77:567-81.
9. Kyogoku C, Ortmann WA, Lee A, Selby S, Carlton VEH, Chang M, *et al*. Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. Am J Hum Genet 2004;75:504-7.
10. Orozco G, Sánchez E, González-Gay MA, López-Nevot MA, Torres B, Cáliz R, *et al*. Association of a functional single-nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with rheumatoid arthritis and systemic lupus erythematosus. Arthritis Rheum 2005;52:219-24.
11. Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JMM, *et al*. Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. Diabetes 2004;53:3020-3.
12. Velaga MR, Wilson V, Jennings CE, Owen CJ, Herington S, Donaldson PT, *et al*. The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. J Clin Endocrinol Metab 2004;89:5862-5.
13. Zhebrun D, Kudryashova Y, Babenko A, Maslyansky A, Kunitskaya N, Popcova D, *et al*. Association of PTPN22 1858T/T genotype with type 1 diabetes, Graves' disease but not with rheumatoid arthritis in Russian population. Aging 2011;3:368-73.
14. Ichimura M, Kaku H, Fukutani T, Koga H, Mukai T, Miyake I, *et al*. Associations of protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) gene polymorphisms with susceptibility to Graves' disease in a Japanese population. Thyroid 2008;18:625-30.
15. Bottini N, Peterson EJ. Tyrosine phosphatase PTPN22: Multifunctional regulator of immune signaling, development, and disease. Annu Rev Immunol 2014;32:83-119.
16. Heward JM, Brand OJ, Barrett JC, Carr-Smith JD, Franklyn JA, Gough SC. Association of PTPN22 haplotypes with Graves' disease. J Clin Endocrinol Metab 2007;92:685-90.
17. Orrú V, Tsai SJ, Rueda B, Fiorillo E, Stanford SM, Dasgupta J, *et al*. A loss-of-function variant of PTPN22 is associated with reduced risk of systemic lupus erythematosus. Hum Mol Genet 2009;18:569-79.
18. Rodríguez-Rodríguez L, Taib WRW, Topless R, Steer S, González-Escribano MF, Balsa A, *et al*. The PTPN22 R263Q polymorphism is a risk factor for rheumatoid arthritis in Caucasian case-control samples. Arthritis Rheum 2011;63:365-72.
19. Steck A, Baschal E, Jasinski J, Boehm B, Bottini N, Concannon P, *et al*. rs2476601 T allele (R620W) defines high-risk PTPN22 type I diabetes-associated haplotypes with preliminary evidence for an additional protective haplotype. Genes Immun 2009;10:S21-6.
20. Wesoly J, Hu X, Thabet MM, Chang M, Uh H, Allaart CF, *et al*. The 620W allele is the PTPN22 genetic variant conferring susceptibility to RA in a Dutch population. Rheumatol Oxf Engl 2007;46:617-21.
21. Yu N, Rymar O, Maximov V, Simonova G, Zankina M, Mustafina S, *et al*. Association of PTPN22 haplotypes with Hashimotos thyroiditis in population of Novosibirsk. Clin Exp Thyroidol 2009;5:47-52.
22. Zoledziewska M, Perra C, Orrù V, Moi L, Frongia P, Congia M, *et al*. Further evidence of a primary, causal association of the PTPN22 620W variant with type 1 diabetes. Diabetes 2008;57:229-34.
23. Skorka A, Bednarczuk T, Bar-Andziak E, Nauman J, Ploski R. Lymphoid tyrosine phosphatase (PTPN22/LYP) variant and Graves' disease in a Polish population: Association and gene dose-dependent correlation with age of onset. Clin Endocrinol (Oxf) 2005;62:679-82.
24. Lee YH, Rho YH, Choi SJ, Ji JD, Song GG, Nath SK, *et al*. The PTPN22 C1858T functional polymorphism and autoimmune diseases—a meta-analysis. Rheumatology 2007;46:49-56.
25. Ban Y, Tozaki T, Taniyama M, Tomita M, Ban Y. The codon 620 single nucleotide polymorphism of the protein tyrosine phosphatase-22 gene does not contribute to autoimmune thyroid disease susceptibility in the Japanese. Thyroid 2005;15:1115-8.
26. Kawasaki E, Awata T, Ikegami H, Kobayashi T, Maruyama T, Nakanishi K, *et al.* Systematic search for single nucleotide polymorphisms in a lymphoid tyrosine phosphatase gene (PTPN22): Association between a promoter polymorphism and type 1 diabetes in Asian populations. Am J Med Genet A 2006;140A: 586-93.
27. Ikari K, Momohara S, Inoue E, Tomatsu T, Hara M, Yamanaka H, *et al*. Haplotype analysis revealed no association between the PTPN22 gene and RA in a Japanese population. Rheumatol Oxf Engl 2006;45:1345-8.