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Association of PTPN22 single nucleotide polymorphisms with chronic spontaneous urticaria

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Received 7 August 2020; Accepted 12 September 2020
Available online 1 March 2021

KEYWORDS
urticaria;
gene polymorphism;
PTPN22;
chronic spontaneous urticaria

Abstract
Introduction and objectives: Chronic spontaneous urticaria (CSU) is thought to be an autoimmune disease in a subpopulation of patients. Protein tyrosine phosphatase-22 (PTPN22) polymorphisms are considered to be one of the strongest contributing factors to autoimmune diseases. In this study, we aimed to investigate the potential association of several PTPN22 single nucleotide polymorphisms (SNPs) with CSU in an Iranian population.
Material and methods: A total of 93 CSU patients and 100 healthy individuals were included in this study. Five SNPs within the PTPN22 gene were analyzed using TaqMan genotyping assays. The frequency of alleles, genotypes, and haplotypes of PTPN22 SNPs (rs12760457, rs2476601, rs1310182, rs1217414, and rs33996649) was investigated.
Results: A significantly higher prevalence of the rs1310182 T allele was observed among patients compared with controls [OR = 1.75 (95% CI: 1.17-2.63); P = 0.007]. In addition, the rs1310182 CC genotype and TT genotype were 0.47 and 2.06 times more common in patients, respectively (P = 0.03). Moreover, haplotype analysis demonstrated that CGCGC, CGTGC, and TGCGC (P < 0.001) were significantly associated with CSU. No significant differences were observed between the patients and controls in the other analyzed PTPN22 SNPs.
Conclusions: Polymorphisms of the PTPN22 gene are associated with an increased susceptibility to CSU in the studied Iranian population.
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https://doi.org/10.15586/aei.v49i2.53
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Introduction

Urticaria, generally known as hives, is a common mast-cell driven disease that is characterized by the development of wheals, angioedema, or both. Urticaria is classified by its duration and the involvement of eliciting factors; chronic spontaneous urticaria (CSU) is defined as the presence of wheals, angioedema, or both for at least 6 weeks that is not provoked by any specific factor.[1] Urticaria affects between 15 and 25% of the population at some point during their life, which is most often acute.[2,3]. Chronic urticaria has a prevalence of 0.1–3% among the general population and is associated with a marked decrease in the quality of life of patients.[4-7] This condition tends to be more common among adults and is more commonly observed in women than in men.[8] Although an exogenous trigger is often found in patients with inducible urticaria (such as cold, heat, delayed pressure, etc.), the etiopathogenesis of spontaneous urticaria is not well understood.[9] Currently, autoimmunity is thought to play an important role in the etiology of chronic spontaneous urticaria.[3,10,11] Studies have reported that in approximately 30–50% of patients with chronic urticaria it has an autoimmune origin, with IgE autoantibodies against auto-allergens or functional IgG autoantibodies against IgE or the high-affinity IgE receptor FcεRIα detected in patients' sera.[3,12] Other possible causes of CSU that have been proposed in different studies include pseudo-allergy (non-allergic hypersensitivity reactions) to foods or drugs, and acute or chronic infections.[1,13,14]

A relationship between CSU and autoimmune diseases has been established[9]; a considerable percentage of CSU patients have antithyroid, antimitochondrial, and antinuclear antibodies. Other autoimmune diseases associated with chronic urticaria include vitiligo, pernicious anemia, and insulin-dependent diabetes mellitus.[11,15,16] In addition, one study reported that chronic urticaria may have an increased prevalence among those with a positive family history,[17] suggesting a possible role for genetic factors.[18,19]

Protein tyrosine phosphatase N22 (PTPN22) encoded by the PTPN22 gene, located on chromosome 1p13.3-13.1, is a lymphoid-specific phosphatase (Lyp) that regulates T-cell activation. It is currently considered as one of the strongest contributing factors to autoimmunity, along with major histocompatibility complex (MHC). Several studies have shown that polymorphisms of PTPN22 are linked to the development of autoimmune diseases such as Graves' disease, Hashimoto's thyroiditis (HT), rheumatoid arthritis (RA), type 1 diabetes mellitus (T1DM), systemic lupus erythematosus (SLE), vitiligo, and progressive systemic sclerosis.[20-25] The 1858 C/T variant, which is the main autoimmune-related polymorphism, causes a change in the function of Lyp and thus encodes a protein that cannot bind to the protein tyrosine kinase Csk.[24]

A previous study that investigated the rs2488457C, rs1310182T, and rs3811021T polymorphisms in a Polish population with CSU revealed that the CTT haplotype was significantly associated with this disease.[9] Herein, we aimed to investigate the potential association of several single nucleotide polymorphisms (SNPs) of the PTPN22 gene with chronic spontaneous urticaria in an Iranian population.

Material and methods

Study population

A total of 93 patients diagnosed with CSU, who had been referred to the Children's Medical Center, the Pediatrics Center of Excellence in Tehran, Iran, were enrolled in the study.

The diagnosis of CSU was made based on the EAACI/GA²LEN/EDF/WAO guidelines of urticaria[1] after taking a detailed medical history, performing physical examination, and, when indicated, performing diagnostic serological tests. Other possible differential diagnoses, such as inducible urticaria, urticarial vasculitis, urticaria pigmentosa, and Schnitzler's syndrome were excluded with the consideration of this diagnostic workup. Patients who had any other underlying disease were not included in the study. The control group comprised 100 healthy individuals with no known history of any autoimmune or systemic disease. The racial background of all included participants was Iranian.

This study was approved by the Ethics Committee of Tehran University of Medical Sciences, and all subjects were given written informed consent before participation in the study.

Blood sampling and genotyping of PTPN22 SNPs

Blood samples were obtained from all patients and controls in EDTA-coated tubes, and stored at -20°C until further processing. Genomic DNA from 3–5 mL of peripheral blood was extracted by using the phenol-chloroform protocol.[26] SNP genotyping was performed by real-time polymerase chain reaction (RT-PCR) using allelic discrimination TaqMan genotyping assays (ABI Applied Biosystems, 7300 Real-Time PCR System, USA) based on the manufacturer's protocol. According to pre-designed primers (Table 1), the following PTPN22 SNPs (rs12760457, rs2476601, rs1310182, rs1217414, and rs33996649) were investigated and recorded.

Statistical analysis

Allele, genotype, and haplotype frequencies were reported as number (percentage), and the differences between cases and controls were assessed via chi-square test. The odds ratio (OR) and 95% confidence interval (CI) were measured for each allele and genotype. All statistical analyses were performed using the Epi-Info statistical software (version 7.2.0.1, World Health Organization, Geneva, Switzerland). P-value of less than 0.05 was considered to be statistically significant.

Results

Allele and genotype frequencies

A significantly higher prevalence of the rs1310182 T allele was observed among patients compared with controls [OR = 1.75 (95% CI: 1.17–2.63); P = 0.007], as demonstrated in Table 2. In addition, the rs1310182 CC genotype and TT genotype were 0.47 and 2.06 times more common in

Table 1 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
rs33996649	Chr1: 113852067	TGAAGGCCTCTGTGTCCGCATTTCC[C/T] GGATCAAACTGAAAACACTGAAGTT	Intron variant, missense

Table 2 PTPN22 gene allele and genotype polymorphisms in patients with chronic spontaneous urticaria and controls.

SNP	Allele/ Genotype	Cases (n = 93) N (%)	Controls (n = 100) N (%)	P-value	OR	95% CI
rs12760457	C	130 (70.65)	143 (71.5)	0.73	Reference	Reference
	T	54 (29.34)	54 (27)	0.73	1.10	0.70-1.71
	CC	48 (52.17)	54 (54)	0.77	0.90	0.51-1.60
	CT	34 (36.95)	36 (36)	1.00	1.02	0.56-1.84
	TT	10 (10.86)	9 (9)	0.80	1.21	0.47-3.15
rs2476601	A	1 (0.54)	0	0.47	-	-
	G	183 (99.45)	200 (100)	0.47	Reference	Reference
	AA	0	0	1.00	0.00	-
	AG	1 (1.08)	0	0.47	-	-
	GG	91 (98.91)	100 (100)	0.47	0.00	-
rs1310182	**C**	**80 (43.47)**	**115 (57.5)**	**0.007**	Reference	Reference
	T	**104 (56.52)**	**85 (42.5)**	**0.007**	**1.75**	**1.17-2.63**
	CC	**18 (19.56)**	**34 (34)**	**0.03**	**0.47**	**0.24-0.91**
	CT	44 (47.82)	47 (47)	1.00	1.03	0.58-1.82
	TT	**30 (32.60)**	**19 (19)**	**0.03**	**2.06**	**1.06-4.00**
rs1217414	A	50 (27.17)	61 (30.5)	0.49	Reference	Reference
	G	134 (72.82)	137 (68.5)	0.49	1.19	0.76-1.85
	AA	7 (7.60)	9 (9)	0.79	0.82	0.29-2.30
	AG	36 (39.13)	43 (43)	0.56	0.83	0.47-1.49
	GG	49 (53.26)	47 (47)	0.12	1.57	0.90-2.74
rs33996649	C	183 (99.45)	198 (99)	1.00	1.84	0.16-20.5
	T	1 (0.54)	2 (1)	1.00	Reference	Reference
	CC	91 (98.91)	98 (98)	1.00	1.85	0.16-20.8
	CT	1 (1.08)	2 (2)	1.00	0.54	0.04-6.76
	TT	0	0	0.49	0.00	-

OR: odds ratio; CI: confidence interval.

patients, respectively (P = 0.03). However, the allele and genotype frequencies of the other four investigated PTPN22 SNPs did not differ significantly between the patients and controls.

Haplotype analysis

Haplotype analysis demonstrated that CGCGC, CGTGC, and TGCGC (P < 0.001) were significantly associated with CSU (Table 3). Other haplotypes did not significantly increase susceptibility to chronic spontaneous urticaria.

Discussion

This study investigated five SNPs within the PTPN22 gene in Iranian patients with chronic spontaneous urticaria. Although the underlying mechanisms of chronic spontaneous urticaria are not well understood, genetic factors may be associated with its pathogenesis.[17-19] The major genetic markers identified in chronic urticaria are genes related to mast cell activation and histamine and also those related to the arachidonic acid (AA) pathway, as well as other contributing genes such as PTPN22.[18] Nevertheless, data on the role of the PTPN22 gene in

Table 3 PTPN22 gene haplotype in patients with chronic spontaneous urticaria and controls.

Haplotypea	Cases (n = 93) N (%)	Controls (n = 100) N (%)	P-value	OR	95% CI
CGCGC	+56 (60.2)	+24 (24)	**<0.001**	**4.79**	**2.58–8.89**
CGCAC	+22 (23.6)	+33 (33)	0.15	1.58	0.84–2.99
CGTAC	+28 (30.1)	+28 (28)	0.75	1.1	0.59–2.06
CGTGC	+23 (24.7)	0 (0)	**<0.001**	-	-
TGTGC	+53 (56.9)	+56 (56)	1.00	1.04	0.58–1.84
CGTGT	+1 (1.07)	0 (0)	1.00	-	-
TGCGC	0 (0)	+53 (53)	**<0.001**	-	-
CGCGT	0 (0)	+2 (2)	1.00	-	--

[a]rs12760457, rs2476601, rs1310182, rs1217414, rs33996649.
OR: odds ratio; CI: confidence interval.

patients with urticaria is very scarce. Hence, we examined whether polymorphisms in this gene may contribute to CSU development. This study approved an association of the rs1310182 PTPN22 polymorphism with chronic spontaneous urticaria. We observed that patients with CSU had a higher risk of rs1310182 T allele carriage compared with controls. Moreover, three out of eight sequenced haplotypes were significantly associated with CSU (CGCGC, CGTGC, and TGCGC). In 2011, Brzoza et al.[24] found that PTPN22 1858C/T did not increase susceptibility to chronic urticaria in a Polish population. However, 1 year later, the same authors reported that PTPN22 polymorphisms may contribute to CSU, after investigating three other SNPs (rs2488457C, rs1310182T, and rs3811021T).[9]

Studies have indicated that in a significant portion of patients with CSU it has an autoimmune origin.[3,12] This hypothesis is supported by the evidence that IgG autoantibodies against IgE or the high-affinity IgE receptor FcεRIα are detected in the serum of up to 50% of patients with CSU.[27,28] In addition, an association between CSU and autoimmune diseases has been established. Recent studies have shown that about 25, 27, and 29% of CSU patients have antithyroid, antimicrosomal, and antinuclear antibodies, respectively.[9,11,15,16,29] On the other hand, many studies have confirmed that several specific polymorphisms of PTPN22 increase patients' susceptibility to autoimmune diseases, including RA, SLE, T1DM, Graves' disease, HT, vitiligo, and sclerosis.[30–35] A study by Umemura et al.[20] showed that polymorphisms in PTPN22 may have a protective role in susceptibility to autoimmune liver diseases in the Japanese population. Recently, Houcken et al.[25] reported that the rs2476601 PTPN22 polymorphism is significantly associated with autoimmune polyglandular syndromes (APS), including T1DM and Graves' disease.

PTPN22 is a protein tyrosine phosphatase that causes inactivation of T-cells by dephosphorylating the protein tyrosine kinases involved in T-cell activation.[36] Polymorphisms in this gene result in functional changes of Lyp, and thus lead to the overactivation of T-cells. However, some researchers have stated that the autoimmunity associated with PTPN22 polymorphisms is due to the increased threshold of T-cell activation, and subsequently, the survival of autoreactive T-cells in the thymus.[37–39] While mast cells play the main role in chronic urticaria, there is evidence that CD4+ T-cells are highly activated in these patients.[40] Although the relationship between T-cell activation and mast cell degranulation in CSU is not fully understood, the interaction of these immune cells residing in urticarial lesions may lead to degranulation, cytokine release, and secretion of matrix metalloproteinase (MMP)-9 by mast cells.[41,42] MMP-9 is known to be involved in the transmigration of lymphocytes, eosinophils, and neutrophils to the skin.[40] Furthermore, the production of IgG autoantibodies requires the initial activation of autoantigen-primed T-cells.[43] This evidence further supports the findings of our study, which suggest a potential association of PTPN22 SNPs in patients afflicted with chronic spontaneous urticaria.

Currently, the mainstay of treatment in chronic urticaria is second-generation antihistamines. It is widely accepted that CSU patients of an autoimmune origin are more likely to be resistant to conventional treatments. Based on the literature, at least 50% of patients with autoimmune urticaria do not respond well to treatment with antihistamines and thus, require alternative therapies, including omalizumab, cyclosporine, corticosteroids, and plasmapheresis.[1,44] Therefore, identifying such patients will help physicians in predicting patients' response to therapy and in the earlier prescription of alternative therapies, which would have a great impact on the quality of life of patients, as well as reducing the burden on health care systems.

One of the limitations of this study is that we did not assess the association between PTPN22 SNPs and disease activity in our patients. Another limitation is that IgG autoantibodies were not measured, so we cannot estimate the percentage of patients with a likely autoimmune origin. Although our study demonstrated a significant relationship between specific PTPN22 polymorphisms and chronic spontaneous urticaria, it is important to note that this study was conducted in a relatively small number of patients who were all from an Iranian racial background. Thus, the contribution of these polymorphisms in the development of CSU should also be investigated among other ethnicities and races. So far, autoimmunity-related serological tests are not implemented in the routine diagnostic workup of patients with CSU; however, if further comprehensive studies confirm the autoimmune origin of this disease,

measuring the level of specific autoantibodies in patients' sera could be considered.

Conclusively, our study showed that polymorphisms of the PTPN22 gene are associated with an increased susceptibility to CSU in the Iranian population. Understanding the molecular mechanisms contributing to the development and progression of autoimmune diseases through genetic association studies will provide a platform for introducing new personalized diagnostic and therapeutic approaches.

Declaration of interest

All the authors declare that they have no conflict(s) of interest.

Funding

This work was supported by Tehran University of Medical Sciences [grant number 96-03-93-36505].

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