

Concise Report

PTPN22 gene polymorphism in Takayasu's arteritis

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Objective. Takayasu's arteritis (TA) is a chronic, rare granulomatous panarteritis of unknown aetiology involving mainly the aorta and its major branches. In this study, genetic susceptibility to TA has been investigated by screening the functional single nucleotide polymorphism (SNP) of *PTPN22* gene encoding the lymphoid-specific protein tyrosine phosphatase.

Methods. Totally, 181 patients with TA and 177 healthy controls are genotyped by PCR-RFLP method for the SNP rs2476601 (A/G) of *PTPN22* gene. Polymorphic region was amplified by PCR and digested with *Xcm* I enzyme.

Results. Detected frequencies of heterozygous genotype (AG) were 5.1% (9/177) in control group and 3.8% (7/181) in TA group ($P=0.61$, odds ratio: 0.75, 95% CI: 0.3, 2.0). No association with angiographic type, vascular involvement or prognosis of TA was observed either.

Conclusion. The distribution of *PTPN22* polymorphism did not reveal any association with TA in Turkey.

KEY WORDS: Takayasu's arteritis, Single nucleotide polymorphism, *PTPN22*.

Introduction

Takayasu's arteritis (TA), also known as pulseless disease, is a chronic granulomatous panarteritis characterized by the involvement of large vessels, especially the aorta and its major branches [1]. Cellular immunity may play the major role in disease pathogenesis as involved vessels are infiltrated mainly by $\gamma\delta$ T cells, NK cells, cytotoxic T cells, Th1 cells and macrophages [2]. Previous studies showed an elevated ratio of CD4/CD8 T cells, increased number of HLA-DR+ circulating lymphocytes and markedly increased 65-kDa HSP expression in tissues along with the presence of $\gamma\delta$ T cells [3]. Infections, autoimmunity and genetic factors are implicated in the pathogenesis of TA. Evidences of genetic susceptibility to TA have also been demonstrated in several studies. Especially, associations of TA with HLA-B52 and -B39 have been reported previously from Japan, whereas HLA-B5 was associated with TA in India [4, 5].

Lymphoid-specific protein tyrosine phosphatase (LYP) is encoded on chromosome 1p13 at *PTPN22* gene and a single nucleotide polymorphism (SNP) of the gene has been demonstrated to be associated with several autoimmune disorders [6, 7]. LYP is involved in maintaining the resting phenotype of lymphocytes and in controlling signalling caused by an antigen, co-stimulation and cytokines [8]. The *PTPN22* gene polymorphism causing an amino acid change (R620W) at the proline-rich motif of LYP has been

shown to affect the protein–protein interaction with tyrosine kinase Csk in T-cell activation. Individuals carrying the variant allele of *PTPN22* are suggested to have changes in the threshold for thymic selection with increased numbers of auto-reactive T cells escaping negative selection and be prone to autoimmunity. However, the mechanism of action remains to be clarified, as both gain and loss of function mechanisms have been reported [7, 9, 10]. An association of *PTPN22* R620W polymorphism (rs2476601, A/G) was reported first with type 1 diabetes [11] and later also with myasthenia gravis [12], SLE [13] and RA [9]. However, there are also some inflammatory diseases such as IBD, psoriasis, multiple sclerosis, AS [6] and Behcet's disease (BD) [14] with no association of this polymorphism.

PTPN22 R620W polymorphism reveal a wide variation in allele frequencies among different populations, polymorphic allele being present highest in Scandinavia (15%), whereas is absent in Asian and African populations [15, 16]. Considering this spectrum of the diseases and variations among populations, we investigated the possible association of *PTPN22* polymorphism with TA in this study.

Materials and methods

The study was designed as a case–control study enrolling 181 patients with TA (19 men, 162 women; mean age: 37 ± 11.7 yrs). Patients were classified according to the 1990 ACR criteria for the classification of TA [17]. Angiographic classification is done as proposed by the International Cooperative Study on TA in 1997 [18]. According to this classification, 39% ($n=70$) of the patients had type 1 vessel involvement, 8.2% type 2a ($n=15$), 1.1% type 2b ($n=2$), 3.3% type 3 ($n=6$), 4.4% type 4 ($n=8$) and 44% type 5 ($n=80$). As controls, 177 healthy blood donors (86 men, 91 women; mean age: 40 ± 9.7 yrs) were recruited. All patients and controls were enrolled with local ethics committee approval and provided their informed consent.

Genotyping

For genotyping, cellular DNA was isolated from 10 ml of peripheral blood using standard procedures. For the determination of *PTPN22* alleles, PCR-based restriction fragment length polymorphism (RFLP) analysis was performed. DNA was amplified using the forward primer 5'GGC CTC AAT GAA CTC CTC AA 3' and reverse primer 5'AAT GTT GCT TCA

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ACG GAA TTT 3'. PCR amplification was carried out in $1 \times (\text{NH}_4)$ buffer with 2 mM MgCl_2 , 200 μM dNTP, 0.4 μM of each primer, 100 ng of genomic DNA and 0.75 IU of *Taq* polymerase within a final volume of 30 μl . The cycling parameters were as follows: initial denaturation step of 2 min at 95°C; 30 cycles of 30 s at 95°C, 45 s at 59°C, 45 s at 72°C and final extension step of 2 min at 72°C.

The polymorphism was identified by *XcmI* digestion for 4 h at 37°C. Variant allele (A) was cut into 163 and 166 bp fragments and the digest (329 bp) was run on 3% agarose gel stained with ethidium bromide.

Statistical analysis

Genotype frequencies were compared between patients and control groups by chi-square test. The *post hoc* power analysis was performed using the statistical program PowerSampleSize assuming $\alpha = 0.05$ and small effect size of 0.1 (<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>).

Results

When the distribution of the polymorphism is evaluated in the healthy population, genotype frequencies were in Hardy–Weinberg equilibrium. The frequency of heterozygous genotype (AG) was 5% (9/177) in the control group and this was corresponding to an allele frequency of 0.025. The homozygous AA genotype was not present in this control group. In TA patients, polymorphic A allele was only detected in 7 out of 181 patients as a heterozygote genotype AG (3.8%). There was no statistically significant difference between TA and control group according to the frequency of heterozygote genotype ($P = 0.61$, odds ratio: 0.75, 95%CI: 0.3, 2.0). AG genotype was not associated with angiographic type, vascular involvement or prognosis of TA. *Post hoc* analysis revealed a power of 83% with the assumed low effect size in this sample.

Discussion

Pathophysiology of TA is not exactly known but is thought to be multifactorial involving infectious agents (*Mycobacterium tuberculosis*, different types of viruses), autoimmunity and genetic influences [2]. Since cell-dependent autoimmunity may play a major role in pathogenesis, we investigated polymorphism of *PTPN22* gene which is repeatedly shown to be associated with different autoimmune diseases and considered as one of the most common markers of susceptibility to autoimmunity [6]. However, in our study, no significant difference between TA and control group is observed, suggesting that LYP does not influence the pathogenesis of TA.

Our previous study in BD, another systemic disorder with autoimmune features, did also not reveal any association with *PTPN22* gene polymorphism [14]. An important factor in genetic studies is the ethnic heterogeneity and there are ethnic differences regarding the frequencies of *PTPN22* polymorphism in different populations. In Turkey, it is somewhat lower compared with the other Caucasian populations (5%). Considering previous findings on similarities of the Turkish population with the other European data, this was unexpected. However, low frequencies of this polymorphism have also been reported in populations such as Japanese and Mexican. Absence of a TA-*PTPN22* association may also be related to this low prevalence and may not rule out an association in other, especially Caucasian, populations.

Another explanation for our results may be the lack of autoimmune features in TA. Although clinical spectrum of TA such as low-grade, continuous inflammation causing a long, generally unremitting disease course and tissue-infiltrating cell types such as T cells suggest the involvement of cell-mediated immunity, TA lacks some other autoimmune phenomena such as the presence of autoantibodies (ANA) and co-existence with other

autoimmune disorders such as multiple sclerosis or type 1 diabetes. Granulomatous panarteritis is also uncommon in autoimmune diseases such as SLE. In this respect, it is interesting to note that *PTPN22* is found to be associated with WG, but not with GCA [19, 20]. Although both disorders are accepted to be granulomatous vasculitides, WG patients also have ANCA and have medium-size vessel involvement with neutrophil infiltrations, whereas GCA have more resemblance to TA such as large-vessel-confining vasculitis without classical autoantibodies.

As a conclusion, a role of *PTPN22* R620W polymorphism could not be demonstrated in TA pathogenesis. Low prevalence of this polymorphism in Turkey or pathogenetic differences with the classic autoimmune disorders may account for this result.

Rheumatology key message

- *PTPN22* R620W polymorphism is found to be unassociated with TA in Turkey.

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