Lack of Association of a Functional Single Nucleotide Polymorphism of *PTPN22*, Encoding Lymphoid Protein Phosphatase, with Susceptibility to Biopsy-Proven Giant Cell Arteritis

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ABSTRACT. ***Objective.*** To assess the possible association between *PTPN22* 1858C→T polymorphism and susceptibility to giant cell arteritis (GCA) and to determine if this polymorphism is implicated in the clinical expression of this vasculitis.

Methods. Ninety-six patients with biopsy-proven GCA and 229 ethnically matched controls from the Lugo region of Northwest Spain were studied using molecular methods. All individuals were of Spanish Caucasian origin. Genotyping of *PTPN22* gene 1858C→T polymorphism was performed by real time polymerase chain reaction technology, using TaqMan 5' allelic discrimination assay.

Results. No significant differences in allele or genotype frequencies for *PTPN22* polymorphism were observed between patients with GCA and controls or when patients were stratified by presence of polymyalgia rheumatica (n = 38) or severe ischemic manifestations (n = 47).

Conclusion. Our results do not support potential involvement of *PTPN22* gene polymorphism in the susceptibility or clinical expression of GCA. (J Rheumatol 2005;32:1510–2)

Key Indexing Terms:
GIANT CELL (TEMPORAL) ARTERITIS
SEVERE ISCHEMIC MANIFESTATIONS
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SUSCEPTIBILITY
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POLYMORPHISM

Giant cell arteritis (GCA) is the most common systemic vasculitis in people over the age of 50 years in Western countries, in particular in those with Northern European ancestry[1]. GCA has proved to be a polygenic disease, and different genes may influence the phenotype and outcome of this condition.

Protein tyrosine phosphatases (PTP) are critical regulators of T cell signal transduction. In conjunction with protein tyrosine kinases (PTK), PTP regulate the reversible phosphorylation of tyrosine residues and play important roles in different aspects of T cell physiology[2]. Thus, T cells displaying dysregulated tyrosine phosphorylation would be expected to mediate the pathological process in inflammatory immune diseases. There is evidence that GCA is caused by a pathogenic immune response that is ultimately controlled by T cells[3]. Interestingly, interferon-γ-producing, activated T cells and macrophages are implicated in the immunopathogenesis of arterial damage observed in GCA[3].

The *PTPN22* (protein tyrosine phosphatase non-receptor 22) gene, located on chromosome 1p13, encodes a lymphoid-specific phosphatase (Lyp). Lyp is an intracellular PTP with a molecular weight of 110 kDa containing an N-terminal catalytic domain and a noncatalytic C terminus with 4 proline-rich domains. Lyp is physically bound through one proline-rich motif (referred to as P1) to the SH3 domain of the Csk kinase. The ability of Csk and Lyp to inhibit T cell receptor signaling requires their physical association[4]. A *PTPN22* single nucleotide polymorphism (SNP) (1858C→T; rs2476601; R620W) located at the P1 motif disrupts the interaction between Lyp and Csk, preventing formation of the complex and suppression of T cell activation[5]. The T variant of this polymorphism has been associated with type 1 diabetes mellitus[5]. Interestingly, *PTPN22* 1858 C→T is associated with a number of autoimmune diseases[6,7].

We recently observed an association between the functional 1858C→T polymorphism of *PTPN22* and susceptibility to rheumatoid arthritis (RA) in a large Spanish cohort

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that included patients from Northwest Spain[8]. Considering these observations, we sought to determine the potential role of 1858C→T polymorphism in *PTPN22* in the predisposition and clinical expression of patients with biopsy-proven GCA.

MATERIALS AND METHODS

Study population. The study group comprised patients diagnosed with biopsy-proven GCA (n = 96) at the Division of Rheumatology of the Hospital Xeral-Calde (Lugo, Northwest Spain). All patients fulfilled the 1990 American College of Rheumatology criteria for the classification of GCA[9]. Ethnically matched controls (n = 229) were recruited from the same area of Lugo.

Only patients with a positive temporal artery biopsy showing disruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without giant cells were included.

Patients with GCA were considered to have an associated polymyalgia rheumatica (PMR) if they had severe bilateral pain and aching involving at least 2 of the 3 regions (neck, shoulder, and pelvic girdles) associated with morning stiffness. Patients were considered to have severe ischemic manifestations if they suffered visual manifestations (transient visual loss including amaurosis fugax, permanent visual loss, or diplopia), cerebrovascular accidents (stroke and/or transient ischemic attacks), or jaw claudication.

Prior to participation in the genetic studies patients and controls gave informed consent, which was approved by the local institutional committee.

Genotyping. DNA from patients and controls was obtained from peripheral blood using standard methods. Samples were genotyped for *PTPN22* 1858C→T variants using a TaqMan 5' allelic discrimination Assay-By-Design method (Applied Biosystems, Foster City, CA, USA). The primer sequences were 5´- CCA GCT TCC TCA ACC ACA ATA AATG (forward) and 5´- CAA CTG CTC CAA GGA TAG ATG ATGA (reverse), and the TaqMan MGB probe sequences were 5´-CAG GTG TCC ATA CAGG, and 5´-CAG GTG TCC GTA CAGG; the probes were labeled with fluorescent dyes VIC and FAM, respectively. Polymerase chain reaction (PCR) was carried out in a total reaction volume of 12.5 µl with the following amplification protocol: denaturation at 95ºC for 10 min, followed by 40 cycles of denaturation at 92ºC for 15 s and annealing and extension at 60ºC for 1 min. Post-PCR, the genotype of each sample was attributed automatically by measuring the allelic specific fluorescence on the ABI PRIM 7000 Sequence Detection Systems using the SDS 1.1 software for allelic discrimination (Applied Biosystems). To confirm the genotyping obtained by the TaqMan 5´ allelic discrimination assay, PCR restriction fragment length polymorphism (RFLP) was performed on representative samples from each genotype as described[8]. We used the forward primer 5´-TCA CCA GCT TCC TCA ACC ACA-3´ and the reverse primer 5´-GAT AAT GTT GCT TCA ACG GAA TTTA-3´. Genotypes were identified by *Xcm* I restriction endonuclease digestion, which recognizes its target sequence only when the *PTPN22* 1858 T allele is present. Digestion products were resolved on 3% agarose gels.

Statistical analysis. Allelic and genotypic frequencies of *PTPN22* 1858 C→T polymorphism were obtained by direct counting. Strength of association between patient groups and controls and alleles or genotypes of this polymorphism was estimated using odds ratios and 95% confidence intervals. Levels of significance were determined using contingency tables by either chi-square or Fisher's exact analysis. Statistical significance was defined as $p \leq 0.05$. Calculations were performed using the statistical package Stata V6.

RESULTS

Clinical characteristics of the patients with GCA. The main clinical features of the patients with biopsy-proven GCA are summarized in Table 1. This series of patients comprised 54 women and 42 men (median age at disease diagnosis 75 years; range: 60-92 years). Between onset of GCA symptoms and 1 month after onset of steroid therapy, 38 had PMR and 47 developed severe ischemic manifestations. Among them, 37 had jaw claudication, 23 visual ischemic manifestations, and 1 stroke. In all cases the erythrocyte sedimentation rate (ESR Westergren) at disease diagnosis was greater than 40 mm/h.

PTPN22 1858C→T genotypic and allelic frequencies in patients with GCA and healthy controls. Table 2 shows the allele and genotype frequencies in patients with biopsy-proven GCA and healthy subjects. No statistically significant differences between patients and controls were found. Next, we stratified patients with GCA according to presence of PMR or severe ischemic manifestations. However, *PTPN22* 1858C→T polymorphism failed to discriminate GCA patients according to specific disease characteristics (Table 2). Also, no statistically significant differences in the allelic or genotypic frequencies were found when patients with GCA were stratified according to gender or smoking history (data not shown).

DISCUSSION

Begovich, *et al* recently observed an association of *PTPN22* 1858C→T polymorphism with susceptibility to RA in a North American population[10]. These authors also found this polymorphism plays a role in disease severity, manifested by the association between the *PTPN22* SNP and the presence of rheumatoid factor positive disease[10]. In addition, they confirmed the functional effect of the *PTPN22* 1858 variation in the binding of Lyp to Csk previously reported by Bottini, *et al*[5], suggesting that the association of this

Table 1. Main clinical features of 96 patients with biopsy-proven GCA from Lugo (Northwest Spain). Values in parentheses indicate the percentage of patients with a particular variable.

Variable	
Age, yrs	
Mean age ± SD	74.5 ± 6.2
Median	75
Range	60–92
Men:Women	42:54
Women, %	56.3
Headache	82 (85)
Abnormal temporal artery on physical examination	72 (75)
Polymyalgia rheumatica	38 (40)
Jaw claudication	37 (39)
Visual manifestations*	23 (24)
Stroke	1 (1)
Severe ischemic manifestations**	47 (49)
ESR > 40 mm/h	96 (100)

* Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia. ** Visual ischemic manifestations, cerebrovascular accidents (stroke and/or transient ischemic attacks), or jaw claudication.

Table 2. Frequency of *PTPN22* 1858C→T allele and genotype distribution among 96 patients with biopsy-proven GCA and 229 healthy controls. No statistically significant differences were found between patients with GCA and controls and between patients with or without polymyalgia rheumatica (PMR) or severe ischemic manifestations. Frequencies are presented as number (%).

	Controls	GCA	GCA with PMR		GCA with Severe Ischemic Manifestations	
			Yes	No	Yes	No
Allele (2N)						
C	415 (90.6)	175 (91.1)	67 (88.2)	108 (93.1)	84 (89.4)	91 (92.9)
T	43 (9.4)	17 (8.9)	9 (11.8)	8 (6.9)	10 (10.6)	7 (7.1)
Gemotype						
CC	188 (82.1)	79 (82.3)	29 (76.3)	50 (86.2)	37 (78.7)	42 (85.7)
CT	39 (17.0)	17 (17.7)	9 (23.7)	8 (13.8)	10 (21.3)	7 (14.3)
TT	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

polymorphism with autoimmunity may be due to the role of PTPN22 in the negative regulation of T cell activation[5,10].

Due to its functional relevance as a negative regulator of T cell activation, the important role of T cells in GCA development, and the *PTPN22* association with RA in individuals from Northwestern Spain, we assessed the influence of *PTPN22* 1858C→T polymorphism in the development of GCA in a large series of patients with biopsy-proven GCA. However, in our study the *PTPN22* SNP polymorphism was not found to be associated with either susceptibility or clinical expression of the disease. Taking into account the frequency of this vasculitis and the number of patients assessed in our study, we may exclude a potential role of this polymorphism in susceptibility to GCA in individuals from Northwestern Spain.

It has been proposed that inflammatory/autoimmune diseases share a common genetic background, and several autoimmune diseases have been shown to be associated with the *PTPN22* 1858 T allele[6,7]. However, no evidence of its association with multiple sclerosis has been found[11,12]. Similar contradictory findings have also been reported concerning to the influence of *CTLA-4* gene in autoimmune diseases: while the *CTLA-4*-CT60 marker was found to be associated with type 1 diabetes, Graves' disease, thyroiditis, and systemic lupus erythematosus[13,14], no linkage was observed with RA[15].

The different results of *PTPN22* SNP association between patients with RA and those with biopsy-proven GCA from Northwest Spain support the notion that different pathogenic mechanisms are involved in the development of polygenic diseases. Alternatively, it is possible that other genes, such as those related to the aging process, may lead to the development of GCA due to loss of immune-homeostasis.

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