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Lack of association of a functional single nucleotide polymorphism of *PTPN22*, encoding lymphoid protein phosphatase, with susceptibility to Henoch-Schönlein purpura

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ABSTRACT

Objective. *To assess the possible association between the* PTPN22 *gene 1858C→T polymorphism and the susceptibility to Henoch-Schönlein purpura (HSP) and determine if this polymorphism is implicated in the severity of this systemic vasculitis.*

Patients and methods. *Fifty-seven unselected patients from Northwest Spain with primary systemic vasculitis, classified as HSP according to previously proposed criteria, with a follow-up of at least 2 years and 229 healthy controls, were included in this study. All the individuals were of Spanish Caucasian origin. Genotyping of the* PTPN22 *gene 1858C→T polymorphism was performed by real time PCR technology, using TaqMan 5' allelic discrimination assay..*

Results. *No significant differences in allele or genotype distribution frequencies for the* PTPN22 *gene polymorphism were observed between HSP patients and controls. It was also the case when HSP patients were stratified for the presence of severe gastrointestinal complications (n = 46), nephritis (n= 37) or permanent renal involvement (renal sequelae) (n = 12).*

Conclusions. *Our results do not support a potential implication of the* PTPN22 *gene polymorphism in the susceptibility to and clinical expression of HSP.*

Introduction

Henoch-Schönlein purpura (HSP) is the most common primary small-sized blood systemic vasculitis in children (1) and rare in adults (2). HSP is characterized by IgA-dominant immune deposits and infiltration of the small blood vessels with polymorphonuclear leukocytes and the presence of leukocytoclasia (3). Palpable purpura, joint, gastrointestinal and renal manifestations are typical in HSP patients. Long-term morbidity and mortality in HSP are mainly due to renal involvement (1, 2).

HSP is a polygenic disease. Although other gene polymorphisms implicated in the pathogenesis of autoimmune diseases were not found to be associated with HSP Northwestern Spain (4, 5), some other genes influence the phenotype and the outcome of HSP (6-11).

Protein tyrosine phosphatases (PTPs) are critical regulators of T-cell signal transduction. PTPs regulate the reversible phosphorylation of tyrosine residues and play important roles in different aspects of T cell physiology (12). T-cells displaying dysregulated tyrosine phosphorylation would be expected to mediate the pathological process in inflammatory-immune diseases. The *PTPN22* (protein tyrosine phosphatase non-receptor 22) gene, located on chromosome 1p13, encodes a lymphoid-specific phosphatase (Lyp). Lyp is an intracellular PTP 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 signalling requires their physical association (12). A *PTPN22* SNP (1858C→T; rs2476601; R620W), located at the P1 motif, disrupts the interaction between Lyp and Csk, avoiding the formation of the complex and the suppression of the T-cell activation (13). The T variant of this polymorphism has been associated with type 1 diabetes mellitus (13) and a number of autoimmune diseases (14)

We have recently observed an association between the functional 1858C→T polymorphism of the *PTPN22* gene and the susceptibility to rheumatoid arthritis (RA) and systemic lupus erythematosus in a large Spanish cohort that included patients from Northwest Spain (15). Taken together all these considerations, in the present study we sought to determine the potential role of the *PTPN22* 1858C→T gene polymorphism in the predisposition and clinical expression of HSP patients.

Patients and methods

Patients (n = 57) were recruited from the Divisions of Pediatrics and Rheumatology of the Hospital Xeral-Calde (Lugo, Northwest Spain). Healthy controls (n = 229), matched by ethnicity, age and sex, were also obtained from the same geographic area. Patients and controls were included in this study after written informed consent given by them or their parents. We obtained approval for the study from the local ethical Committee.

Inclusion criteria

Patients with primary systemic vasculitis who fulfilled the 1990 American

College of Rheumatology (ACR) classification criteria for hypersensitivity vasculitis or HSP were differentiated using the criteria proposed by Michel *et al*. (16). For the purpose of the present study only those patients who fulfilled classification criteria for HSP we studied. Although an age at disease onset less than or equal to 20 years is one of the ACR criteria put forward by Mills *et al*. (17) and also by Michel *et al*. (16) to classify patients as having HSP, 12 individuals older than 20 years met additional criteria proposed by Mills *et al*. and also by Michel *et al*. (16, 17) and, due to this, they were classified as having HSP. In these individuals other primary systemic vasculitides involving small blood vessels, such as microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome or mixed cryoglobulinemia were excluded. All patients required to have had at least a 2-year follow-up.

Table I. Main features of a series of 57 patients with Henoch-Schönlein purpura from Lugo, Northwestern Spain.

	No.	
Patients	57	
Children (age less than 21 years)	45	(79%)
Adults	12	(21%)
Male/female	28/29	
Age at the onset of the disease (years)		
Median	7	
Range	2 – 62	
Duration of follow-up (years)		
Median	8	
Range	2 – 20	
Palpable purpura and/or maculopapular rash	57	(100%)
Arthralgia and/or arthritis	39	(68%)
Gastrointestinal manifestations	46	(81%)
Gastrointestinal bleeding	24	(42%)
Bowel angina	43	(75%)
Renal manifestations	37	(65%)
Hematuria	37	(65%)
Proteinuria	19	(33%)
Nephrotic syndrome	7	(12%)
Renal insufficiency	2	(4%)
Renal sequelae (persistent renal involvement)	12	(21%)

Definitions

Following former studies, patients older than 20 years were considered adults and those younger than this age as children (2, 6, 16, 17). For gastrointestinal manifestations, bowel angina was considered to be present if there was diffuse abdominal pain that worsened after meals or bowel ischemia usually with bloody diarrhea. Gastrointestinal bleeding was defined as the presence of melena, hematochezia or a positive test for occult blood in the stool (2, 6, 16). Nephritis was defined as previously reported: hematuria (≥ 5 red blood cells/hpf), proteinuria (>300 mg/24 hours), nephrotic syndrome (1g/day/m^2 body surface area or > 3.5 g/day proteinuria with plasma albumin < 25 g/l, with or without edema) (6). Renal insufficiency was considered if the plasma creatinine concentration was above 125% the upper limit of normal (2, 6). Persistent renal damage, defined as renal sequelae, is an important matter of concern as it is considered to be the most common long-term complication of this disease. The presence of persistent renal damage was considered to be present if, after a minimum of 2 year's follow-up, patients had any of the renal complications described above.

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) as previously reported (15).

Statistical analysis

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 exact analysis. Statistical significance was defined as p equal or less than 0.05. Calculations were performed using the statistical package Stata V6.

Results

Fifty-seven HSP patients and 229 controls were studied. The main epidemiological and clinical data of the patients with HSP are shown in Table I. Patients included in this study required at least 2 years follow-up. Also, 12 (21%) of them were adults, and it is known that HSP in adults has been associated with more severe disease and more common sequelae than in children (2). These facts may have led to some kind of selection bias due to disease severity. It may explain that over the course of the disease severe gastrointestinal manifestations were observed in 46 (81%), renal involvement (in all cases hematuria with or without proteinuria) in 37 (65%) and nephrotic syndrome in 7 (12%) of the patients from this series (Table I). However, over the extended follow-up only 2 (4%) experienced renal insufficiency and at last followup (median 8 years; range 2 to 20 years) 12 (21%) had persistent renal involvement (renal sequelae), mainly microscopic hematuria.

Table II shows the *PTPN22* 1858C→T allele and genotype frequencies in HSP patients and matched subjects. No evidence of departure from Hardy-Weinberg equilibrium was found in patients or controls. No statistically significant differences in the allele or genotype distribution between HSP patients and controls were found.

Also, the *PTPN22* 1858 variation failed to discriminate HSP patients according to the specific characteristics of the disease (Table II).

Table II. Frequency of *PTPN22* 1858C→T allele and genotype distribution among 57 patients with Henoch-Schönlein purpura (HSP) and 229 healthy controls*.

PTPN22 1858C/T	Controls	HSP	HSP with renal manifestations		HSP with renal sequelae		HSP with severe gastrointestinal manifestations	
			Yes	No	Yes	No	Yes	No
No. patients	229 (%)	57 (%)	37 (%)	20 (%)	12 (%)	45 (%)	46 (%)	11 (%)
Genotype								
CC	188 (82)	49 (86)	32 (86.5)	17 (85)	10 (83)	39 (87)	41 (89)	8 (73)
CT	39 (17)	8 (14)	5 (13.5)	3 (15)	2 (17)	6 (13)	5 (11)	3 (27)
TT	2 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Allele (2N)	458 (%)	114 (%)	74 (%)	40 (%)	24 (%)	90 (%)	92 (%)	22 (%)
C	415 (91)	106 (93)	69 (93)	37 (92.5)	22 (92)	84 (93.5)	87 (95)	19 (86)
T	43 (9)	8 (7)	5 (7)	3 (7.5)	2 (8)	6 (6.5)	5 (5)	3 (14)

*No statistically significant differences between patients and controls were found.

Discussion

A recent meta-analysis of the functional 1858C→T polymorphism of the *PTPN22* gene confirmed that the *PTPN22* gene plays an important role in the pathogenesis of a subgroup of autoimmune diseases (14). However, these common autoimmune alleles may not be shared in some other groups of autoimmune conditions. In this regard, despite being the first attempt to determine the potential implication of the 1858C→T polymorphism of the *PTPN22* gene in the susceptibility to patients with small-sized blood vessel systemic vasculitis who fulfilled classification criteria for HSP, our results do not support the role of this polymorphism in the susceptibility to or clinical expression of patients with HSP from Northwestern Spain.

A few years ago, Begovich *et al*. described an association of the *PTPN22* gene 1858C→T polymorphism with the susceptibility to RA in North American individuals (18). They observed a role of this biallelic polymorphism in disease severity, manifested by the association between the *PTPN22* SNP and the presence of rheumatoid factor positive (18). They also confirmed the functional effect of the *PTPN22* 1858 variation in the binding of Lyp to Csk previously reported by Bottini *et al*. (13), suggesting that the association of this polymorphism with autoimmunity may be due to the role of the *PTPN22* gene in the negative regulation of T-cell activation (13, 18). Interestingly, this association was also observed in RA patients from the Lugo region of Northwestern Spain (15).

However, as described for HSP patients, the *PTPN22* SNP polymorphism was not found to be implicated in the susceptibility to or the clinical expression of giant cell arteritis (19) and ankylosing spondylitis in the Lugo population (20).

The different results in terms of *PTPN22* SNP association between different autoimmune diseases observed in a well-defined population like this from the Lugo region of Northwest Spain support the notion that different pathogenic mechanisms are involved in the development of polygenic diseases. However, since ethnicity may also explain differences in terms of genetic susceptibility to autoimmune diseases in different parts of the world, which might imply possible different pathogenic mechanisms for the development of systemic vasculitis, and in particular of HSP, in different populations, additional studies in HSP patients with different genetic backgrounds are required to fully exclude the potential role of this polymorphism in the pathogenesis of this vasculitis.

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