
Lack of association between interleukin-6 promoter polymorphism at position -174 and Henoch-Schönlein purpura

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ABSTRACT

Objective. To assess whether polymorphism of the interleukin (IL)-6 gene at the position -174 was implicated in the incidence of Henoch-Schönlein purpura (HSP). A further objective was to determine if any relationship existed with severe systemic complications of HSP, in particular with severe renal and gastrointestinal involvement

Methods. Unselected patients from Northwest Spain with primary cutaneous vasculitis classified as HSP according to proposed criteria were studied. All patients included in the present study were required to have had at least 2 year's follow-up. Patients and controls were genotyped for a single biallelic (G/C) nucleotide polymorphism in the promoter region at the position -174 of the IL-6 gene by a polymerase reaction chain-restriction fragment length polymorphism (PCR-RFLP) method.

Results. Forty-six Caucasian HSP patients and 124 healthy matched controls were studied. No allele or genotype differences between the whole group of HSP and controls were observed. This was also the case when HSP patients were stratified by the presence of gastrointestinal complications, nephritis, and permanent renal involvement (renal sequelae).

Conclusions. The polymorphism in IL-6 gene promoter (-174 G/C) does not appear to be a genetic risk factor for HSP in Northwest Spain.

Introduction

Henoch-Schönlein purpura (HSP) is the most common primary small-sized blood vessel vasculitis in children and a rare entity in adults (1). It is characterized by infiltration of the small blood vessels with polymorphonuclear leukocytes and the presence of leukocytoclasia (1). IgA-dominant immune deposits

in the walls of the small vessels and in the renal glomeruli are frequently observed (1). Palpable purpura, joint and gastrointestinal manifestations are typical of this condition. Renal manifestations constitute the most serious complications and long-term morbidity and mortality in HSP are mainly due to renal involvement (1).

The etiology of HSP remains unknown. Infections, in particular those from the upper respiratory tract, were reported to be a precipitating factor in at least 50% of the cases (2). Cytokines produced during the antigenic challenge may play a role in the susceptibility and severity of HSP (3, 4).

Genetic factors may determine the immunological and inflammatory response to unknown antigens in patients with this condition. In this regard, previous studies in patients with cutaneous vasculitis from Northwestern Spain have shown that different genes may influence the phenotype and the outcome of HSP (5-8).

Polymorphisms of inflammatory cytokine genes may mediate an abnormal inflammatory response that may promote the development of this vasculitis. In this regard, a functional polymorphism at the position -174 of the IL (interleukin) -6 gene has been associated with the development of autoimmune diseases (9). However, to the best of our knowledge, no information on this polymorphism in the pathogenesis of HSP has been reported. Due to this, we have conducted a study to assess the potential implication of the IL6 -174 promoter polymorphism in the susceptibility and severity of HSP in series of unselected patients from Northwest Spain.

Patients and methods

Patients were recruited from the Divi-

sions of Pediatrics and Rheumatology of the Hospital Xeral-Calde (Lugo, Northwest Spain). Ethnically and age-matched controls were also obtained from the same region of Lugo.

Inclusion criteria

Patients with primary cutaneous vasculitis who fulfilled the 1990 American College of Rheumatology classification criteria for hypersensitivity vasculitis (HV) or HSP were differentiated using the criteria proposed by Michel *et al.* (10). They were classified as having HSP if they fulfilled 3 or more of the following criteria: 1) palpable purpura, 2) bowel angina, 3) gastrointestinal bleeding, 4) macroscopic or microscopic hematuria, 5) age at disease onset \leq 20 years, and 6) no previous history of medications prior to the onset of the disease. Patients who met fewer than 3 criteria were classified as having HV and were excluded from the present study. Also, as previously reported (5-8), in all adults a skin biopsy showing pathologic signs of leukocytoclastic vasculitis was always required. In children, a diagnosis of cutaneous vasculitis was considered if they had typical non-thrombocytopenic symmetric palpable purpura involving the lower extremities. In these cases, other conditions that may present with cutaneous lesions such as connective-tissue diseases and infections had to be excluded. For the purpose of examining the outcome of

HSP, in this study, only patients with at least 2 year's follow-up were included.

Genotyping of the -174 IL-6 promoter polymorphism

DNA was extracted from anticoagulated blood collected in EDTA using a phenol-chloroform extraction method. The biallelic G/C polymorphism in the IL-6 promoter at the position -174 was examined using the following polymerase chain reaction (PCR) primers (11): Forward 5' TTG TCA AGA CAT GCC AAG TGC T 3'. Reverse 5' GCC TCA GAG ACA TCT CCA GTC C 3'. A total of 100 ng genomic DNA (5 μ l) was amplified in a 25 μ l PCR reaction containing 2.5 μ l buffer-NH4 (BiolineTM, London, UK), 1 μ l MgCl₂, 2.5 μ l dNTP's (BiolineTM), 0.25 μ l of each primer, 0.2 μ l Taq polymerase (Bioline) and 13.3 μ l distilled water. Thermal cycling was performed using a Hybaid OmniGene PCR machine. Cycles consisted of 10 minutes denaturation 95C followed by 35 rounds of 95C for 1 minute, 61C for 1 minute, 72C for 1 minute and a final extension at 72C for 10 minutes. The presence of product was verified on a 1% agarose gel stained with ethidium bromide. The IL-6 promoter polymorphism (G to C) at the position -174 in the 5' region creates a restriction site for *Nla*III. Due to this, PCR products were digested with *Nla*III in a 10 μ l final volume (11). The digest was incubated overnight at 37C

and the products of the digest were then visualized on a 4% agarose gel stained with ethidium bromide.

Patients and controls were included in this study after written informed consent. We obtained approval for the study from the local ethical committee.

Statistical analysis

Strength of association between patient groups and controls and alleles or genotypes of the IL-6 -174 (G/C) 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 with the statistical package Stata V6.

Results

Forty-six Caucasian HSP patients and 124 controls were studied. The main epidemiological and clinical data of the patients with HSP are shown in Table I. Hematuria with or without proteinuria and severe gastrointestinal manifestations were frequently observed in the group of patients with HSP. However, after a minimum of 2 year's follow-up (median 8 years) only 10 of the 46 patients had persistent renal involvement (renal sequelae), mainly hematuria.

In controls, no evidence of departure from Hardy-Weinberg equilibrium was found (p = N.S.).

Allele and genotype frequencies for the IL-6 -174 (G/C) gene polymorphism in HSP patients and controls are shown in Table II. However, no significant differences for this biallelic polymorphism were observed in patients with HSP compared to the controls (Table II).

The allele and genotype frequencies were also examined in HSP patients stratified by the presence of nephritis or renal sequelae during the course of the disease. Similarly, no statistically significant differences between HSP patients with or without renal manifestations or between patients with HSP nephritis and controls were observed Table II. This was also the case when HSP patients with severe gastrointestinal complications (gastro-intestinal

Table I. Main features of a series of 46 patients with Henoch-Schönlein purpura.

Children (age less than 21 years)/ adults		35/11
Male/female		22/24
Age at the onset of the disease (years)	Median	7
	Range	2- 62
Duration of follow-up (years)	Median	8
Palpable purpura and/or maculopapular rash		46 (100%)
Arthralgia and/or arthritis		34 (74%)
Gastrointestinal bleeding		20 (43%)
Bowel angina		38 (83%)
Renal manifestations		
Hematuria		31 (67%)
Proteinuria		18 (39%)
Nephrotic syndrome		5 (11%)
Renal insufficiency		1 (2%)
Renal sequelae (persistent renal involvement)		10 (22%)

Table II. Allele and genotype frequencies of the IL-6-174 (G/C) polymorphism at the promoter region in Henoch-Schönlein purpura (HSP) patients and controls from Northwestern Spain*.

	Controls	HSP	HSP with renal manifestations		HSP with renal sequelae		HSP with severe gastrointestinal manifestations	
			Yes	No	Yes	No	Yes	No
No. patients	124 (%)	46 (%)	31 (%)	15 (%)	10 (%)	36 (%)	39 (%)	7 (%)
Genotype								
GG	56 (45)	20 (43)	12 (39)	8 (53)	4 (40)	16 (44)	15 (38)	5 (71)
GC	54 (44)	21 (46)	17 (55)	4 (27)	5 (50)	16 (44)	19 (49)	2 (29)
CC	14 (11)	5 (11)	2 (6)	3 (20)	1 (10)	4 (11)	5 (13)	0 (0)
Allele (2N)								
G	166 (67)	61 (66)	41 (66)	20 (67)	13 (65)	48 (67)	49 (63)	12 (86)
C	82 (33)	31 (34)	21 (34)	10 (33)	7 (35)	24 (33)	29 (37)	2 (14)

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

bleeding or bowel angina) were compared with those without these manifestations or controls Table II.

Discussion

There is increasing evidence showing that polymorphisms in cytokine genes may play an important role in modifying the immune response. Genetic factors are thought to determine immunological and inflammatory response to unknown antigens in HSP. We have previously shown the involvement of different inflammatory mediators in the development of HSP. A biallelic (-511 C/T) polymorphism in the IL-1 β gene was directly implicated in the severity and outcome but not in the susceptibility of unselected patients with cutaneous vasculitis (12). Association between an IL-1 receptor antagonist variable number of tandem repeat gene polymorphism with the development of severe renal manifestations and renal sequelae in HSP was also observed in our population (13).

IL-6 is a cytokine that has been implicated in a number of immune-mediated diseases. Association of the -174 (G/C) polymorphism at the promoter region of the IL-6 have been observed in rheumatic diseases. The IL-6 genotype was found to influence the age at disease onset in rheumatoid arthritis (RA) patients from Southern Spain (14). Also, Polish patients with RA carrying the GG genotype had higher disease activity score, erythrocyte sedimentation rate and number of swollen and tender joints

than the remaining RA patients (9).

In a previous study we described that in Northwestern Spanish individuals with giant cell arteritis (GCA), a disease involving mainly large and medium-sized blood vessels, the distribution of the G/C 174 genotype was similar to that observed in ethnically matched controls (11). However, we observed an increased frequency of CC genotype in biopsy-proven GCA who had polymyalgia rheumatica (PMR) manifestations compared with the remaining biopsy-proven GCA patients without PMR features (11). In addition, the frequency of allele C in the group of biopsy-proven GCA HLA-DRB1*04 negative patients with PMR was significantly increased compared with the HLA-DRB1*04 positive ones (11). However, this biallelic polymorphism was not associated with a higher risk of ischemic events in Spanish GCA patients (11). Likewise, in a recent study on biopsy-proven GCA patients from Reggio Emilia, Italy, the distribution of the G/C 174 genotype was similar to that found in controls (15). Also, in Italians with biopsy-proven GCA this polymorphism was not associated with the presence of PMR or with severe ischemic complications of this vasculitis (15).

The present report constitutes the first study aimed to determine the potential implication of the IL-6 -174 (G/C) polymorphism in the susceptibility to cutaneous vasculitis. In keeping with the results observed in GCA patients

from Reggio Emilia, our results do not support the role of this polymorphism in the susceptibility and/or severity of cutaneous vasculitis in Northwestern Spain.

Different pathogenic mechanisms between vasculitides such as GCA or cutaneous vasculitis and other autoimmune diseases may explain the different results in terms of association of the IL-6 -174 (G/C) polymorphism. Furthermore, the different ethnic origins of the world populations may explain differences in terms of genetic susceptibility to autoimmune diseases. This might imply possible different pathogenic mechanisms for the development of cutaneous vasculitis, and in particular of HSP, in different populations. Due to this, additional studies in patients with different genetic background are required to fully elucidate the potential role of the IL-6 -174 (G/C) polymorphism in the pathogenesis of HSP.

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