**Interleukin 1 beta (IL1β) rs16944 genetic variant as a genetic marker of severe renal manifestations and renal sequelae in Henoch-Schönlein purpura**

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**ABSTRACT**

**Objective.** Data from a small series suggested that the Interleukin 1 beta (IL1β) rs16944 polymorphism may be associated with severe renal involvement and persistent renal damage (renal sequelae) in Henoch-Schönlein purpura (HSP). To confirm this association, we assessed the largest cohort of Caucasian HSP patients ever considered for genetic studies.

**Methods.** 338 Spanish HSP patients and 635 sex and ethnically matched controls were recruited in this study. All patients were required to have had at least 6 months' follow-up. Patients and controls were genotyped for IL1β rs16944 by TaqMan genotyping assay.

**Results.** No differences between IL1β rs16944 genotype or allele frequencies were found either in the case/control study or when HSP patients were stratified according to the age at disease onset, presence of nephritis or gastrointestinal manifestations. Nevertheless, 4 (25%) of the 16 HSP patients who developed severe renal manifestations carried the TT genotype versus 29 (9%) of 322 who did not develop this complication (p=0.01, OR=5.48, 95% CI: 1.01-28.10). Accordingly, patients carrying the mutant T allele had an increased risk of developing severe nephropathy (p=0.016, OR=2.35, 95% CI: 1.09-5.07). Additionally, a significant increase of the TT genotype was observed in patients without this complication (25% versus 8.6%, respectively; p=0.0035, OR=4.90, 95% CI: 1.26-18.51). Moreover, renal sequelae were more common in patients carrying the mutant T allele (p=0.0076, OR=2.20, 95% CI: 1.17-4.14).

**Conclusion.** Our results support that the IL1β rs16944 polymorphism may be a potential marker of severe renal manifestations and renal sequelae in HSP.

**Introduction**

Henoch-Schönlein purpura (HSP), recently renamed Immunoglobulin-A (IgA) vasculitis (1), is a complex polygenic disease (2, 3). Although the causes of this vasculitis are largely unknown, epidemiologic studies have implicated both environmental and socioeconomic factors in HSP pathogenesis (2, 3). Furthermore, as suggested immunogenetic and familial case clusters studies, a genetic predisposition to HSP appears to influence the development and progression of this condition (3). However, the genetic background related to HSP has not been elucidated yet, since only a few studies have been carried out to address this issue (4-12). Replication studies for any novel genetic association are crucial, mainly if it has been described in a small sample size of individuals. Consequently, during the last year, much effort has been done to validate previous potential HSP susceptibility results in a well-powered cohort of HSP patients. In this regard, a recent human leukocyte antigen (HLA) typing analysis, performed in the largest series of Caucasian HSP patients ever recruited for genetic studies, has confirmed the previously suggested susceptibility effect of HLA-DRB1*01 to HSP (13). Even more, this study has identified HLA-DRB1*0103 as the main susceptibility HSP allele (13). In addition, an association between
HLA-B*41:02 and HSP susceptibility, irrespective of HLA-DRB1 status, has been disclosed in this large cohort of Caucasian HSP patients (14). In line with the above, cytokine pathway genes have been shown to represent a key component of the genetic network associated with immune-mediated pathologies and, specifically, polymorphisms located in those genes have been related to phenotype expression of HSP, in particular to the risk of nephritis (6–7). In this respect, a former study performed by Amoli et al. assessed the potential influence of the interleukin 1β (IL1β) rs16944 genetic variant (IL1β) promoter gene polymorphism that induces the -511 C/T change) in the HSP incidence and also its possible implication in HSP severe systemic complications in Caucasians (6). Interestingly, although no association between IL1β rs16944 and HSP susceptibility was disclosed, an influence of this genetic variant in HSP severity and outcome, in terms of severe nephritis and persistent renal damage (renal sequelae), was suggested (6). However, this study was performed in a small cohort of only 49 HSP Caucasian patients and it has not been validated yet. Taken together all these considerations, this study aimed at investigating whether the IL1β rs16944 polymorphism is actually involved in HSP pathogenesis, mainly in the risk of severe renal involvement and renal sequelae, in patients with HSP. For this purpose, we took advantage of the largest series of Caucasian HSP patients with this vasculitis ever assessed for genetic studies.

Patients and methods

Patients and Study Protocol

A set of 338 Spanish patients with cutaneous vasculitis who fulfilled the Michel et al. (15) classification criteria for HSP were included in the present study. According to them, they were classified as having HSP if they fulfilled 3 or more of the following characteristics: palpable purpura, bowel angina, gastrointestinal bleeding, macroscopic or microscopic haematuria, age at disease onset ≥20 years, and no previous history of medications prior to the onset of the disease. Also, all patients included in this series were required to fulfill the American College of Rheumatology classification criteria for HSP (16). Blood samples were obtained from patients recruited from Hospital Universitario Lucus Augusti (Lugo), Hospital Universitario Marqués de Valdecilla (Santander), Hospital Universitario La Princesa (Madrid), Hospital Universitario San Cecilio (Granada), Hospital Universitario Virgen del Rocio (Sevilla) and Hospital Universitario de Basurto (Bilbao).

Genotyping

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood mononuclear cells using standard methods. The IL1β rs16944 (C__1839943_10) polymorphism was genotyped by TaqMan single nucleotide polymorphism (SNP) genotyping assays in a 7900 HT Real-Time polymerase chain reaction (PCR) system, according to the conditions recommended by the manufacturer (Applied Biosystem, Foster City, CA, USA).

Negative controls and duplicate samples were included to check the accuracy of genotyping.

Statistical analysis

All genotypes data were checked for deviation from Hardy-Weinberg equilibrium (HWE) using http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. Both allelic and genotypic frequencies were calculated and compared by χ² or Fisher tests when necessary (expected values below 5). Strength of associations were estimated using odds ratios (OR) and 95% confidence intervals (CI). All analyses were performed with STATA statistical software 12/SE (Stata Corp., College Station, TX, USA).

Results

No divergence from Hardy-Weinberg equilibrium was observed either in controls or HSP cases. The genotyping success was greater than 99%.

First, we conducted a case/control association study. Accordingly, and as shown in Table II, no statistically significant differences in the genotype and allele frequencies of the IL1β rs16944 polymorphism between the whole group of HSP and controls were observed.

In a further step, to examine whether the IL1β rs16944 genetic variant might influence the clinical manifestations of the disease, HSP patients were stratified according to the age at disease onset, presence of nephritis or gastrointestinal manifestations. However, no statistically significant differences in the IL1β rs16944 genotype and allele fre-
quencies were detected between HSP in children (age ≤20 years) and adults (age >20 years) (Table II). Likewise, IL1β rs16944 genotype and allele frequencies did not differ when HSP patients were stratified by the presence of renal or gastrointestinal manifestations (Table II). Nevertheless, 4 (25%) of the 16 HSP patients who developed severe renal manifestations during the course of the disease carried the TT genotype versus 29 (9%) of 322 who did not develop this complication (p=0.0076, OR=2.20, 95% CI: (1.26-18.51)) (Table III). Moreover, renal sequelae were more commonly observed in HSP patients carrying the mutant T allele (p=0.0076, OR=2.20, 95% CI: (1.17-4.14)) (Table III).

Discussion

HSP is generally considered a benign and self-limited disorder (17). However, renal involvement may be a serious complications and the major cause of long-term morbidity and mortality in patients with vasculitis (18-19). In this respect, cytokine pathway genes have been shown to represent a key component of the genetic network associated with the risk of nephritis in HSP (6-7).

IL-1β is a proinflammatory pleiotropic cytokine that possesses the ability to stimulate the expression of genes associated with immune response and to increase the expression on endothelial adhesion molecules (20) playing a key role in the pathogenesis of inflammatory diseases (21). Accordingly, high IL-1β expression has been observed in the skin biopsy specimens of patients with HSP (22). Furthermore, high IL-1β serum concentration was also observed in HSP patients with nephritis (23). Regarding genetic studies, a potential association between IL1β rs16944 and HSP severity and outcome was suggested (6). Interestingly, this polymorphism seems to influence IL-1β production and it has been related to several diseases (24). However, in the former study (6), a potential false positive association could not be excluded due to the small size of the series of HSP patients analysed. Because of that, we have performed a genetic study to establish whether IL1β rs16944 is actually involved in HSP pathogenesis, in particular in the risk of severe renal involvement and persistent renal damage. For this purpose we took advantage of the largest series of HSP Caucasian patients ever considered for genetic studies. Interestingly, our findings revealed a genetic association between the IL1β rs16944 TT genotype and both severe renal involvement and persistent renal damage in HSP Caucasian patients. Our positive results were found in a

### Table I. Main clinical features of a series of 338 Spanish patients with HSP.

<table>
<thead>
<tr>
<th>Main characteristics</th>
<th>% (n/N)</th>
</tr>
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<tbody>
<tr>
<td>Children (age ≤20 years)/ adults (age &gt;20 years)</td>
<td>278/60</td>
</tr>
<tr>
<td>Male/ female</td>
<td>173/165</td>
</tr>
<tr>
<td>Age at the onset of the disease (years, median [IQR])</td>
<td>7 [5-17]</td>
</tr>
<tr>
<td>Duration of follow-up (years, median [IQR])</td>
<td>1.5 [1-4]</td>
</tr>
<tr>
<td>Palpable purpura and/or maculopapular rash</td>
<td>100 (338/338)</td>
</tr>
<tr>
<td>Arthralgia and/or arthritis</td>
<td>56.2 (190/338)</td>
</tr>
<tr>
<td>Gastrointestinal manifestations (if any of the following characteristics)</td>
<td>53.2 (180/338)</td>
</tr>
<tr>
<td>a) Bowel angina</td>
<td>51.8 (175/338)</td>
</tr>
<tr>
<td>b) Gastrointestinal bleeding</td>
<td>16.5 (56/338)</td>
</tr>
<tr>
<td>Renal manifestations (if any of the following characteristics)</td>
<td>35.5 (120/338)</td>
</tr>
<tr>
<td>a) Haematuria</td>
<td>34.6 (117/338)</td>
</tr>
<tr>
<td>b) Proteinuria</td>
<td>32.8 (111/338)</td>
</tr>
<tr>
<td>Severe renal manifestations (if any of the following characteristics)</td>
<td>4.7 (16/338)</td>
</tr>
<tr>
<td>a) Nephrotic syndrome</td>
<td>4.1 (14/338)</td>
</tr>
<tr>
<td>b) Nephritic syndrome</td>
<td>1.8 (6/338)</td>
</tr>
<tr>
<td>Persistent renal damage (renal sequelae)*</td>
<td>7.1 (24/338)</td>
</tr>
</tbody>
</table>

HSP: Henoch-Schönlein purpura; IQR: interquartile range. *At the end of the study (at least 6 months’ follow-up).

### Table II. Frequency of IL1β rs16944 in controls and HSP patients stratified according to the age at disease onset and the presence/absence of renal and GI manifestations.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Controls (n=635)</th>
<th>HSP (n=338)</th>
<th>Children (age ≤20 years)</th>
<th>HSP with renal manifestations*</th>
<th>HSP with GI manifestationsb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=278)</td>
<td>No (n=60)</td>
<td>Yes (n=120)</td>
<td>No (n=218)</td>
<td>Yes (n=180)</td>
</tr>
<tr>
<td><strong>IL1β rs16944</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>280 (44.1)</td>
<td>163 (48.2)</td>
<td>125 (44.9)</td>
<td>27 (45.0)</td>
<td>62 (51.7)</td>
</tr>
<tr>
<td>CT</td>
<td>281 (44.3)</td>
<td>142 (42.0)</td>
<td>111 (39.9)</td>
<td>24 (40.0)</td>
<td>45 (37.5)</td>
</tr>
<tr>
<td>TT</td>
<td>74 (11.7)</td>
<td>33 (9.8)</td>
<td>42 (15.2)</td>
<td>9 (15.0)</td>
<td>13 (10.8)</td>
</tr>
<tr>
<td>C</td>
<td>841 (66.2)</td>
<td>468 (69.2)</td>
<td>361 (64.9)</td>
<td>78 (65.0)</td>
<td>169 (70.4)</td>
</tr>
<tr>
<td>T</td>
<td>429 (33.8)</td>
<td>208 (30.8)</td>
<td>195 (35.1)</td>
<td>42 (35.0)</td>
<td>71 (29.6)</td>
</tr>
</tbody>
</table>

Results are shown as n (%). HSP: Henoch-Schönlein purpura; GI: gastrointestinal; SNP: single nucleotide polymorphism.

* If haematuria with or without proteinuria was present at the onset or during the clinical course of the disease.

b If bowel angina and/or gastrointestinal bleeding was present at the onset or during the clinical course of the disease.
small group of HSP patients and, therefore, the reliability of this finding could be considered somehow low. Nevertheless, it is important to highlight that our report may be considered as a genetic replication study whose results are in keeping with those previously described by Amoli et al. and further evidence that \( \text{IL1} \beta \) rs16944 may actually be a potential genetic marker of severe and persistent renal damage in HSP Caucasian patients.

In accordance with our findings, \( \text{IL1} \beta \) polymorphisms have been related to different types of primary systemic vasculitis (suggesting the relevant role of this cytokine in the pathogenesis of these conditions). In accordance with that, \( \text{IL1} \beta \) rs16944 was involved in Takayasu (a large-sized blood vessel vasculitis) disease predisposition in the Mexican population (25). Additionally, \( \text{IL1} \beta \) polymorphisms increase the susceptibility to Behcet’s disease in different populations (26-27).

The results obtained in the present study provide additional evidence for the potential role that genetic factors may play in the pathogenesis of HSP. Furthermore, they may have potential clinical implication as they may help to better identify HSP individuals at risk for severe renal involvement and persistent renal damage.

In conclusion, our results support that the \( \text{IL1} \beta \) rs16944 polymorphism may be a potential marker of severe renal manifestations and renal sequelae in HSP.

**Acknowledgements**

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