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HLA-DRB1 association with Henoch-Schonlein purpura

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Abstract

Objective: Henoch-Schönlein purpura (HSP) is the most common vasculitis in children but it is not exceptional in adults. Increased familial occurrence supports a genetic predisposition for HSP. In this context, an association with the human leukocyte antigen-*HLA-DRB1*01* phenotype has been suggested in Caucasian individuals with HSP. However, data on the potential association of HSP with *HLA-DRB1*01* were based on small case series. To further investigate this issue, we performed *HLA-DRB1* genotyping of the largest series of HSP patients ever assessed for genetic studies in Caucasians.

Methods: 342 Spanish patients diagnosed with HSP fulfilling the American College of Rheumatology and the Michel *et al* classification criteria, and 303 sex and ethnically matched controls were assessed. *HLA-DRB1* alleles were determined using a PCR–Sequence-Specific-Oligonucleotide Probe (PCR-SSOP) method.

Results: A statistically significant increase of *HLA-DRB1*01* in HSP patients when compared with controls was found (43% *vs* 7%, respectively; p<0.001; odds ratio-OR=2.03 [1.43-2.87]). It was due to the increased frequency of *HLA-DRB1*0103* phenotype in HSP (14% *vs* 2%; p<0.001; OR=8.27 [3.46-23.9]). These results remained statistically significant after adjusting for Bonferroni correction. In contrast, a statistically significant decreased frequency of the *HLA-DRB1*0301* phenotype was observed in patients compared to controls (5.6% *vs* 18.1%, respectively; p<0.001, OR=0.26 [0.14-0.47]), even after adjustment for Bonferroni correction. No *HLA-DRB1* association with specific features of the disease was found.

Conclusion: Our study confirms an association of HSP with *HLA-DRB1*01* in Caucasians. Also, a protective effect against the development of HSP appears to exist in Caucasians carrying the *HLA-DRB1*03* phenotype.

Introduction

Henoch-Schönlein purpura (HSP), recently renamed immunoglobulin A (IgA) vasculitis, is the most common type of primary small-sized blood vessel leukocytoclastic vasculitis in children but it is not exceptional in adults (1). The classic clinical triad of HSP consists of palpable purpura, involving predominantly the lower extremities, joint and gastrointestinal manifestations. However, renal complications may also be observed in patients with this condition (2, 3). In this regard, the outcome of patients with HSP is linked to the presence of glomerulonephritis that in some cases, mainly in adults, may lead to chronic renal failure.

The etiology of HSP remains unknown, although some pieces of evidence support immunopathological mechanisms (3). Besides environmental and socioeconomical factors, a genetic preposition to HSP is suggested by both familial case clusters and immunogenetic studies (3). In this respect, it is plausible to think that, as with many other immune-mediated disorders, the genetic basis of susceptibility to HSP may be conferred by different genes, including those located in the human leukocyte antigen (HLA) region.

HLA region includes a group of genes located in chromosome 6 (6p21) that encodes the most polymorphic human proteins, the class I and class II antigen-presenting molecules (4). HLA is involved not only in the immune response against infectious pathogens, but also in the response against self-antigens. Accordingly, HLA is the main genetic factor implicated in inflammatory immune-mediated pathologies, being associated with more diseases than any other region of the human genome (4). In this

way, a few studies performed in HSP patients suggest a potential association between HLA genes class II region and this pathology in Caucasian individuals (5, 6). However, these studies were performed in small series of HSP patients that were generally assessed in tertiary referral centers.

Taken together these considerations, we aimed to establish whether the *HLA-DRB1* locus is actually involved in the susceptibility to HSP in the largest series of Caucasian patients with this vasculitis ever assessed for genetic studies.

Patients and Methods

Patients and Study Protocol

A set of 342 Spanish patients with cutaneous vasculitis who fulfilled Michel *et al*. (7) classification criteria for HSP were included in the present study. According to these criteria, 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 hematuria, 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 (8). 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 Rocío (Sevilla) and Hospital Universitario de Basurto (Bilbao). Information on the main features of the whole series

of 342 HSP Spanish patients recruited in this study is shown in **Table 1**. Clinical definitions of HSP features were reported elsewhere (2, 5). Hematuria with or without proteinuria and severe gastrointestinal manifestations were frequently observed in these patients. However, only 24 of the 342 patients (7%) had persistent renal involvement (renal sequelae) at last follow-up.

A set of 303, sex and ethnically matched controls without history of cutaneous vasculitis or any other autoimmune disease constituted by blood donors from National DNA Bank Repository (Salamanca, Spain), was also included in the study.

A subject's written consent was obtained according to the declaration of Helsinki, and the study was approved by the Ethics Committees of Galicia for Hospital Universitario Lucus Augusti, of Cantabria for Hospital Universitario Marqués de Valdecilla, of Madrid for Hospital Universitario La Princesa, of Andalucía for Hospital Universitario San Cecilio and Hospital Universitario Virgen del Rocío, and of País Vasco for Hospital Universitario de Basurto.

Genotyping

High-molecular-weight genomic deoxyribonucleic acid (DNA) was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

All DNA samples were stored at -20ºC until the HLA analysis. DNA-based HLA class II typing was performed by PCR–Sequence-Specific Oligonucleotide Probe (PCR-SSOP) using the Luminex 100 system (Luminex, Austin, TX, USA) and the Lifecodes HLA typing Kits (Gen-Probe Inc., San Diego, CA, USA) following the manufacturer's instructions.

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

Statistical analysis

Continuous data were described as mean and standard deviation (mean ± SD) and categorical variables as percentages.

The strength of association between HSP and *HLA-DRB1* phenotypes was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either chi-square test or Fisher exact (expected values below 5) analysis. Results were adjusted for Bonferroni correction.

In order to obtain an internal validation of the associations that had never been reported before, we carried out a bootstrap test with 1000 replications.

All analyses were performed with STATA statistical software 12/SE (Stata Corp., College Station, TX, USA).

Results

Table 2 describes the *HLA-DRB1* phenotype frequencies in the whole cohort of HSP patients and controls.

When HSP patients were compared with matched controls, some differences in *HLA-DRB1* phenotype frequencies were observed. In this regard, in keeping with data previously reported in small series of Caucasian individuals with HSP (5, 6), *HLA-DRB1*01* phenotype was significantly increased in HSP patients compared to controls (43% *versus* 27%, respectively; p<0.001; OR=2.03 [1.43-2.87]). This association is

mainly due to *HLA-DRB1*0103* allele (14% in HSP patients *versus* 2% in controls; p<0.001; OR=8.27 [3.46-23.9]) (**Table 2**). These results remained statistically significant after adjusting for Bonferroni correction. In contrast, a statistically significant decrease in the frequency of the *HLA-DRB1*03* phenotype, due to the presence of *HLA-DRB1*0301* allele, was observed in HSP patients compared to controls (5.6% *versus* 18.1%, respectively; p<0.001, OR=0.26 [0.14-0.47]) (**Table 2**). These results remained statistically significant after adjusting for Bonferroni correction. Since information on the potential protective effect of *HLA-DRB1*03* on HSP had not been previously reported, we carried out a bootstrapping procedure that confirmed the protective effect on HSP susceptibility associated with *HLA-DRB1*03* (OR=0.22; 95% CI: 0.13-0.40). However, no statistically significant results were observed regarding other *HLA-DRB1* phenotypes and HSP susceptibility (**Table 2**).

No *HLA-DRB1* phenotype differences were observed when patients were stratified according to specific features of the disease, such as age at onset before and after 20 years, presence of joint or gastrointestinal manifestations. It was also the case when HSP patients who experienced nephritis or had renal sequelae at last follow-up were compared with those patients who did not suffer these renal complications (data not shown).

Discussion

The vasculitides constitute a heterogeneous group of diseases characterized by a primary process of inflammation and damage of the blood vessel wall (9). These disorders often have overlapping clinical and pathological manifestations (9). A

number of studies have highlighted the relevant role of a genetic component in the susceptibility and severity of these conditions, being HLA the main genetic factor related to these pathologies. However, there is scarce information on the implication of HLA genes in HSP. In fact, only a few studies performed in small cohorts of patients have been carried out to evaluate the potential association between HLA class II genes and HSP (5, 6). Because of that, we have performed a study to establish whether the *HLA-DRB1* gene is actually involved in the susceptibility to HSP in the largest series of Caucasian patients with this vasculitis ever assessed for genetic studies. In this context, our findings support the role of the *HLA-DRB1*01* phenotype in HSP as a susceptibility marker of this disease (5, 6). Additionally, we also observed a protective effect against the development of HSP in individuals carrying the *HLA-DRB1*03* phenotype which was overall less frequently observed compared to the *HLA-DRB1*01* phenotype in both HSP patients and controls.

HLA-class II molecules have been associated with different types of primary systemic vasculitis. With respect to this, an association of *HLA-DRB1*04* with giant cell arteritis, a large-sized blood vessel vasculitis, has been observed (10). An intergenic region between *HLA-DQB2* and *HLA-DOB* was associated with Kawasaki disease, a vasculitis involving medium-sized blood vessels (11). Additionally, *HLA-DP* and *HLA-DRB1*04* has been shown to be related to small-sized vessel anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides, which includes granulomatosis with polyangiitis, microscopic polyangiitis and eosinophilic granulomatosis with polyangiitis (12). Furthermore, *HLA-DQw7, HLA-DRB1*03, HLA-DRB1*01*, *HLA-DRB1*09* and *HLA-DRB1*04* have also been involved in the susceptibility to granulomatosis with polyangiitis (13).

Frequent overlap between vasculitides, mainly in those involving the skin blood vessels, often occurs. It is especially true in cases of adults with small vessel vasculitis presenting with palpable purpura. In this regard, in a former study of our group we suggested that, unlike HSP patients, those with isolated cutaneous leukocytoclastic vasculitis are not associated with any specific *HLA-DRB1* pattern of disease susceptibility. However, that study was based on a small series of cases (14). Interestingly, Cacoub *et al* reported an influence of *HLA-DRB1* locus on the risk of hepatitis C virus (HCV)-associated mixed cryoglobulinemia (MC) (15). Like HSP, MC is often associated with small-sized blood vessel vasculitis, and palpable purpura is also a typical presenting feature of the cryoglobulinemic vasculitis. These authors found that *HLA-DRB1*11* was significantly more frequent in patients with type II MC than in those without MC, regardless of the presence of vasculitis accompanying the MC. In contrast, *HLA-DRB1*07* was less frequent in HCV-infected patients with MC than in those without MC, with a particularly lower frequency in those with type II vasculitis.

Although in the present study we also found the association with *HLA-DRB1*01* shown in previous reports and, because of that, it may be considered in itself as a confirmatory study, there are a number of potential limitations that need to be addressed. First, we observed a protective effect against HSP susceptibility mediated by *HLA-DRB1*03*. This result needs to be confirmed using an independent cohort of Caucasian individuals with HSP. Moreover, an analysis focused on the potential implication of HLA-class I molecules is also warranted to elucidate the implication of HLA region in the susceptibility to HSP.

In conclusion, our study supports an association of HSP with *HLA-DRB1*. These results may have clinical implication as they may help to better identify patients with this vasculitis.

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References

1. Gonzalez-Gay MA, Garcia-Porrua C. Epidemiology of the vasculitides. Rheum Dis Clin North Am 2001;27:729-49.

2. Calvo-Río V, Loricera J, Mata C, Martín L, Ortiz-Sanjuán F, Alvarez L, et al. Henoch-Schönlein purpura in northern Spain: clinical spectrum of the disease in 417 patients from a single center. Medicine (Baltimore) 2014;93:106-13.

3. Wyatt RJ, Julian BA. IgA Nephropathy. N Engl J Med 2013;368:2402-14.

4. Beck S, Geraghty D, Inoko H, Rowen L. The MHC sequencing consortium. Complete sequence and gene map of a human major histocompatibility complex. Nature 1999;401:921-3.

5. Amoli MM, Thomson W, Hajeer AH, Calviño MC, Garcia-Porrua C, Ollier WE, et al. *HLA-DRB1*01* Association with Henoch-Schönlein Purpura in Patients from Northwest Spain. J Rheumatol 2001;28:1266-70.

6. Amoroso A, Berrino M, Canale L, Coppo R, Cornaglia M, Guarrera S, et al. Immunogenetics of Henoch-Schönlein disease. Eur J Immunogenet 1997;24:323-33.

7. Michel BA, Hunder GG, Bloch DA, Calabrese LH. Hypersensitivity vasculitis and Henoch-Schonlein purpura: a comparison between the 2 disorders. J Rheumatol 1992;19:721-8.

8. Mills JA, Michel BA, Bloch DA, Calabrese LH, Hunder GG, Arend WP, et al. The American College of Rheumatology 1990 criteria for the classification of Henoch-Schönlein purpura. Arthritis Rheum 1990;33:1114-21.

9. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013;65:1-11.

10. González-Gay MA, Amoli MM, Garcia-Porrua C, Ollier WE. Genetic markers of disease susceptibility and severity in giant cell arteritis and polymyalgia rheumatica. Semin Arthritis Rheum 2003;33:38-48.

11. Onouchi Y, Ozaki K, Burns JC, Shimizu C, Terai M, Hamada H, et al. A genome-wide association study identifies three new risk loci for Kawasaki disease. Nat genet 2012;44:517-21.

12. Lyons PA, Rayner TF, Trivedi S, Holle JU, Watts RA, Jayne DR, et al. Genetically distinct subsets within ANCA-associated vasculitis. N Engl J Med 2012;367:214-23.

13. Heckmann M, Holle JU, Arning L, Knaup S, Hellmich B, Nothnagel M, et al. The Wegener's granulomatosis quantitative trait locus on chromosome 6p21.3 as characterised by tagSNP genotyping. Ann Rheum Dis 2008;67:972-9.

14. Amoli MM, Thomson W, Hajeer AH, Calviño MC, Garcia-Porrua C, Ollier WE, et al. Henoch-Schönlein purpura and cutaneous leukocytoclastic angiitis exhibit different HLA-DRB1 associations. J Rheumatol 2002;29:945-7.

15. Cacoub P, Renou C, Kerr G, Hüe S, Rosenthal E, Cohen P, et al. Influence of HLA-DR phenotype on the risk of hepatitis C virus-associated mixed cryoglobulinemia. Arthritis Rheum 2001;44:2118-24.

Table 1. Main features of a series of 342 Spanish patients with HSP.

Main characteristics	% (n/N)
Children (age ≤20 years)/ adults (age >20 years)	279/63
Male/ female	176/165
Age at the onset of the disease (years)	
mean ± SD	14.7 ± 17.8
median [IQR]	7 [5-16]
Duration of follow-up (years, mean ± SD)	2.4 ± 1.7
Palpable purpura and/or maculopapular rash	100 (342/342)
Arthralgia and/or arthritis	56.7 (194/342)
Gastrointestinal manifestations (if “a” and/or “b”)	53.5 (183/342)
a) Bowel angina	52.0 (178/342)
b) Gastrointestinal bleeding	15.7 (54/342)
Renal manifestations (if any of the following characteristics)	35.3 (121/342)
a) Hematuria	34.5 (118/342)
b) Proteinuria	32.7 (112/342)
c) Nephrotic syndrome	3.5 (12/342)
d) Renal sequelae (persistent renal involvement)*	7.0 (24/342)

HSP: Henoch-Schönlein purpura; SD: standard deviation; IQR: Interquartile Range.
*At last follow-up.

Table 2. *HLA-DRB1* phenotype frequencies in patients with HSP and controls.

HLA-DRB1		**Patients with HSP** (n=342)	**Controls** (n=303)	**P**	**OR [95% CI]**
*DRB1*01*	0101	66 (19.3)	40 (13.2)	0.037	1.57 [1.00-2.47]
	0102	32 (9.3)	36 (12.2)	0.29	0.76 [0.45-1.30]
	0103	**49 (14.3)**	**6 (1.9)**	**<0.001**	**8.27 [3.46-23.9]**
*DRB1*03*	**0301**	**19 (5.6)**	**55 (18.1)**	**<0.001**	**0.26 [0.14-0.47]**
*DRB1*04*	0401	18 (5.2)	10 (3.3)	0.22	1.63 [0.69-4.01]
	0402	7 (2.0)	4 (1.3)	0.47	1.56 [0.39-7.34]
	0403	24 (7.0)	19 (6.3)	0.70	1.13 [0.58-2.23]
	0404	15 (4.4)	18 (5.9)	0.37	0.73 [0.33-1.56]
	0405	20 (5.8)	16 (5.3)	0.75	1.11 [0.54-2.34]
	0407	7 (2.04)	2 (0.7)	0.13	3.14 [0.59-31.2]
	0408	9 (2.6)	1 (0.3)	0.02	8.16 [1.12-358.8]
*DRB1*11*	1101	50 (14.6)	45 (14.8)	0.93	0.98 [0.62-1.56]
	1102	6 (1.7)	6 (1.9)	0.83	0.88 [0.23-3.34]
	1103	3 (0.6)	2 (0.7)	0.75	1.33 [0.15-16.0]
	1104	9 (2.6)	7 (2.3)	0.79	1.14 [0.37-3.65]
*DRB1*12*	1201	13 (3.8)	9 (2.9)	0.56	1.29 [0.50-3.47]
*DRB1*13*	1301	45 (13.1)	27 (8.9)	0.09	1.55 [0.91-2.67]
	1302	19 (5.6)	23 (7.6)	0.29	0.72 [0.36-1.40]
	1303	8 (2.3)	11 (3.6)	0.33	0.63 [0.22-1.76]
	1305	4 (1.2)	4 (1.3)	0.86	0.88 [0.16-4.79]
*DRB1*14*	1404	7 (2.0)	15 (4.9)	0.04	0.40 [0.14-1.06]
*DRB1*07*	0701	84 (24.6)	78 (25.7)	0.73	0.94 [0.64-1.36]
*DRB1*08*	0801	19 (5.6)	11 (3.6)	0.25	1.56 [0.69-3.69]
	0804	2 (0.6)	2 (0.7)	0.90	0.88 [0.06-12.28]
*DRB1*09*	0901	7 (2.05)	5 (1.6)	0.71	1.24 [0.34-5.02]
*DRB1*10*	10	10 (2.9)	11 (3.6)	0.61	0.80 [0.29-2.01]

HLA: Human leukocyte phenotype; HSP: Henoch- Schönlein purpura; OR: odds ratio; CI: confidence interval.

Values are expressed as n (percentages, %). The results that remained statistically significant after adjusting for Bonferroni correction are highlighted in **bold**.