BRIEF REPORT

Association of HLA–DRB1*01 With IgA Vasculitis (Henoch-Schönlein)

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Objective. IgA vasculitis (Henoch-Schönlein) (IgAV), formerly called Henoch-Schönlein purpura, is the most common vasculitis in children, but it is not rare in adults. Increased familial occurrence supports a genetic predisposition to IgAV. In this context, an

association with the HLA–DRB1*01 phenotype has been suggested in Caucasian individuals with IgAV. However, data on the potential association of IgAV with HLA–DRB1*01 were based on small case series. We undertook this study to further investigate this potential association by performing HLA–DRB1 genotyping in the largest series of IgAV patients ever assessed for genetic studies in Caucasians.

Methods. We assessed 342 Spanish patients with IgAV as well as 303 controls matched for sex and ethnicity. IgAV patients were required to fulfill the classification criteria described by Michel et al as well as the American College of Rheumatology 1990 classification criteria. HLA–DRB1 alleles were determined using the polymerase chain reaction–sequence-specific oligonucleotide probe method.

Results. We found a statistically significant increase in the frequency of the HLA–DRB1*01 phenotype in IgAV patients compared with controls (43% versus 27%; P < 0.001) (odds ratio 2.03 [95% confidence interval 1.43–2.87]). This was due to the increased frequency of the HLA–DRB1*01 allele in IgAV patients compared with controls (14.3% versus 2.0%; P < 0.001) (odds ratio 8.27 [95% confidence interval 3.46–23.9]). These results remained statistically significant after Bonferroni adjustment. In contrast, a statistically significant decrease in the frequency of the HLA–DRB1*01 allele in IgAV patients compared with controls (14.3% versus 2.0%; P < 0.001) (odds ratio 0.26 [95% confidence interval 0.14–0.47]), even after Bonferroni adjustment. No association of HLA–DRB1 with specific features of the disease was found.

Conclusion. Our study confirms an association of IgAV with HLA–DRB1*01 in Caucasians. There also appears to be a protective effect against the development of IgAV in Caucasians carrying the HLA–DRB1*01 phenotype.
IgA vasculitis (Henoch-Schönlein) (IgAV), formerly called Henoch-Schönlein purpura, is the most common type of primary small vessel leukocytoclastic vasculitis in children, but it is not rare in adults (1). The classic clinical triad of IgAV consists of palpable purpura involving predominantly the lower extremities, joint pain (arthritis), 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 IgAV is linked to the presence of glomerulonephritis that in some cases, mainly in adults, may lead to chronic renal failure.

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

The HLA region includes a group of genes located on chromosome 6 (6p21) that encode 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). A few studies performed in IgAV patients suggest a potential association between HLA class II region genes and this pathology in Caucasian individuals (5,6). However, these studies were performed in small series of IgAV patients who were generally assessed in tertiary referral centers. Based on these considerations, we aimed to determine whether the HLA–DRB1 locus is actually involved in susceptibility to IgAV by performing HLA–DRB1 genotyping in the largest series of Caucasian patients with this vasculitis ever assessed for genetic studies.

PATIENTS AND METHODS

Subjects and study protocol. A total of 342 Spanish patients with cutaneous vasculitis who fulfilled the classification criteria for IgAV described by Michel et al (7) were included in the present study. According to these criteria, patients were classified as having IgAV if they fulfilled ≥3 of the following criteria: palpable purpura, bowel angina, gastrointestinal bleeding, macroscopic or microscopic hematuria, age at disease onset ≥20 years, and no history of drug treatment prior to the onset of the disease. All patients included in this series were also required to fulfill the American College of Rheumatology 1990 classification criteria for IgAV (8). Blood samples were obtained from patients recruited from Hospital Universitario Lucus Augusti (Lugo, Spain), Hospital Universitario Marqués de Valdecilla (Santander, Spain), Hospital Universitario La Princesa (Madrid, Spain), Hospital Universitario San Cecilio (Granada, Spain), Hospital Universitario Virgen del Rocio (Seville, Spain), and Hospital Universitario de Basurto (Bilbao, Spain). Information on the main features of the 342 Spanish IgAV patients recruited for this study is shown in Table 1. Clinical definitions of IgAV 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 the last followup visit.

The study also included 303 controls matched for sex and ethnicity without a history of cutaneous vasculitis or any other autoimmune disease. Controls were represented by blood donor samples from the National DNA Bank Repository (Salamanca, Spain).

All subjects provided written consent according to the Declaration of Helsinki. The study was approved by the Ethics Committees of Galicia for Hospital Universitario Lucus Augusti, Cantabria for Hospital Universitario Marqués de Valdecilla, Madrid for Hospital Universitario La Princesa, Andalucía for Hospital Universitario San Cecilio and Hospital Universitario Virgen del Rocio, and País Vasco for Hospital Universitario de Basurto.

Genotyping. High molecular weight genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer’s instructions. All DNA samples were stored at −20°C until the HLA analysis. DNA-based HLA class II typing was performed by the poly-

Table 1. Main characteristics of the 342 Spanish patients with IgA vasculitis (Henoch-Schönlein)*

| Characteristic                                      | No. of patients |
|----------------------------------------------------|-----------------|
| No. of children (age <20 years)/no. of adults (age >20 years) | 279/63          |
| No. male/no. female                                | 177/165         |
| Age at disease onset, years                        | 14.7 ± 17.8     |
| Mean ± SD                                          | 7 (5–16)        |
| Median (IQR)                                       | 2.4 ± 1.7       |
| Duration of followup, mean ± SD years              | 342 (100)       |
| Palpable purpura and/or maculopapular rash         | 194 (56.7)      |
| Arthralgia and/or arthritis                        | 183 (53.5)      |
| Gastrointestinal manifestations                    | 178 (52.0)      |
| Bowel angina and/or gastrointestinal bleeding       | 54 (15.8)       |
| Renal manifestations                               | 121 (35.4)      |
| Any renal manifestation                            | 118 (34.5)      |
| Hematuria                                          | 112 (32.7)      |
| Proteinuria                                        | 12 (3.5)        |
| Nephrotic syndrome                                 | 24 (7.0)        |

* Except where indicated otherwise, values are the number (%) of patients. IQR = interquartile range.
† At last followup visit.
merase chain reaction–sequence-specific oligonucleotide probe method using a Luminex 100 system (Luminex) and Lifecodes HLA typing kits (Gen-Probe) in accordance with the manufacturers’ instructions. Negative and positive controls and duplicate samples were included to check the accuracy of genotyping.

**Statistical analysis.** Continuous data are described as the mean ± SD, and categorical variables are described as percentages. The strength of association between IgAV and HLA–DRB1 phenotypes was estimated using odds ratios (ORs) and 95% confidence intervals (95% CIs). Levels of significance were determined using contingency tables and either chi-square test or Fisher’s exact test (expected values <5). Results were subjected to Bonferroni adjustment. In order to obtain an internal validation of the associations that had not been previously reported, we carried out a bootstrap test with 1,000 replications. All analyses were performed with Stata statistical software version 12/SE (StataCorp).

### RESULTS

Table 2 shows the HLA–DRB1 allele frequencies in the whole cohort of IgAV patients and controls. When IgAV 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 IgAV (5,6), the frequency of the HLA–DRB1*0103 allele (14.3% in IgAV patients versus 2.0% in controls; \( P < 0.001 \)) (OR 8.27 [95% CI 3.46–23.9]) (Table 2).

| Phenotype, allele | IgAV patients (n = 342) | Controls (n = 303) | \( P \) | OR (95% CI) |
|------------------|-------------------------|--------------------|-------|-------------|
| HLA–DRB1*01      |                         |                    |       |             |
| *0101            | 66 (19.3)               | 40 (13.2)          | 0.037 | 1.57 (1.00–2.47) |
| *0102            | 32 (9.4)                | 36 (11.9)          | 0.29  | 0.76 (0.45–1.30) |
| *0103            | 49 (14.3)               | 6 (2.0)            | <0.001 | 8.27 (3.46–23.9) |
| HLA–DRB1*03      |                         |                    |       |             |
| *0301            | 19 (5.6)                | 55 (18.2)          | <0.001 | 0.26 (0.14–0.47) |
| HLA–DRB1*04      |                         |                    |       |             |
| *0401            | 18 (5.3)                | 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 (2.0)                 | 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) |
| HLA–DRB1*11      |                         |                    |       |             |
| *1101            | 50 (14.6)               | 45 (14.9)          | 0.93  | 0.98 (0.62–1.56) |
| *1102            | 6 (1.8)                 | 6 (2.0)            | 0.83  | 0.88 (0.23–3.34) |
| *1103            | 3 (0.9)                 | 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) |
| HLA–DRB1*12      |                         |                    |       |             |
| *1201            | 13 (3.8)                | 9 (3.0)            | 0.56  | 1.29 (0.50–3.47) |
| HLA–DRB1*13      |                         |                    |       |             |
| *1301            | 45 (13.2)               | 27 (8.9)           | 0.09  | 1.55 (0.91–2.67) |
| *1302            | 19 (5.6)                | 23 (7.6)           | 0.29  | 0.72 (0.30–1.40) |
| *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) |
| HLA–DRB1*14      |                         |                    |       |             |
| *1404            | 7 (2.0)                 | 15 (5.0)           | 0.04  | 0.40 (0.14–1.06) |
| HLA–DRB1*07      |                         |                    |       |             |
| *0701            | 84 (24.6)               | 78 (25.7)          | 0.73  | 0.94 (0.64–1.36) |
| HLA–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) |
| HLA–DRB1*09      |                         |                    |       |             |
| *0901            | 7 (2.0)                 | 5 (1.7)            | 0.71  | 1.24 (0.34–5.02) |
| HLA–DRB1*10      |                         |                    |       |             |
| *10              | 10 (2.9)                | 11 (3.6)           | 0.61  | 0.80 (0.29–2.01) |

* Values are the number (%) of subjects. IgAV = IgA vasculitis (Henoch-Schönlein); OR = odds ratio; 95% CI = 95% confidence interval.
† Remained statistically significant after Bonferroni adjustment.
These results remained statistically significant after Bonferroni adjustment. In contrast, a statistically significant decrease in the frequency of the HLA–DRB1*03 phenotype, due to the presence of the HLA–DRB1*0301 allele, was observed in IgAV patients compared with controls (5.6% versus 18.2%; P < 0.001) (OR 0.26 [95% CI 0.14–0.47]) (Table 2). These results remained statistically significant after Bonferroni adjustment. Since information on the potential protective effect of HLA–DRB1*03 against IgAV had not been previously reported, we carried out a bootstrapping procedure that confirmed the protective effect against IgAV 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 IgAV susceptibility (Table 2).

No HLA–DRB1 phenotype differences were observed when patients were stratified according to specific features of the disease, such as disease onset before or after age 20 years or the presence of joint or gastrointestinal manifestations. This was also the case when IgAV patients who experienced nephritis or had renal sequelae at the last followup visit were compared with IgAV patients who did not have 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 pathologic manifestations (9). A number of studies have highlighted the relevant role of a genetic component in the susceptibility to and severity of these conditions, with HLA being the main genetic factor related to these pathologies. However, information is scarce regarding the implications of HLA genes in IgAV. In fact, only a few studies in small cohorts of patients have been performed to evaluate the potential association between HLA class II genes and IgAV (5,6). Because of that, we performed a study in the largest series of Caucasian patients with this vasculitis ever assessed for genetic studies, to determine whether the HLA–DRB1 gene is actually involved in susceptibility to IgAV. In this context, our findings support the role of the HLA–DRB1*01 phenotype in IgAV as a marker of susceptibility to this disease (5,6). We also observed a protective effect against the development of IgAV in individuals carrying the HLA–DRB1*03 phenotype, which overall was observed less frequently than the HLA–DRB1*01 phenotype in both IgAV 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 vessel vasculitis, has been observed (10). An intergenic region between HLA–DQB2 and HLA–DOB was associated with Kawasaki disease, a medium vessel vasculitis (11). Additionally, HLA–DP and HLA–DRB1*04 have been shown to be related to small vessel anti–neutrophil cytoplasmic antibody–associated vasculitides, which include 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 susceptibility to granulomatosis with polyangiitis (13).

Frequent overlap between vasculitides often occurs, mainly in those involving the skin blood vessels. This is especially true in adults with small vessel vasculitis who present with palpable purpura. In this regard, our group previously suggested that, unlike the case in patients with IgAV, patients with isolated cutaneous leukocytoclastic vasculitis do not have any specific HLA–DRB1–associated susceptibility to their disease. However, that study was based on a small series of cases (14). Interestingly, Cacoub et al reported an influence of the HLA–DRB1 locus on the risk of hepatitis C virus–associated mixed cryoglobulinemia (HCV-MC) (15). Like IgAV, MC is often associated with small vessel vasculitis, and palpable purpura is also a typical presenting feature of cryoglobulinemic vasculitis. Those authors found that HLA–DRB1*11 was significantly more frequent in patients with type II MC than in those without MC, regardless of whether vasculitis accompanied 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 we found the previously reported association with HLA–DRB1*01, and our study may therefore be considered a confirmatory study, a couple of items still need to be addressed. First, we observed a protective effect against IgAV susceptibility mediated by HLA–DRB1*03. This result needs to be confirmed in an independent cohort of Caucasian individuals with IgAV. Moreover, an analysis focused on the potential implications of HLA class I molecules is warranted to elucidate the involvement of the HLA region in susceptibility to IgAV.
In conclusion, our study supports an association of IgAV with HLA–DRB1. These results may have clinical relevance, as they may enhance the identification of patients with this vasculitis.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. González-Gay had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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