HLA–DRB4 as a Genetic Risk Factor for Churg-Strauss Syndrome

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Objective. To explore the association between HLA alleles and Churg-Strauss syndrome (CSS), and to investigate the potential influence of HLA alleles on the clinical spectrum of the disease.

Methods. Low-resolution genotyping of HLA–A, HLA–B, and HLA–DR loci and genotyping of *TNFA* −238A/G and *TNFA* −308A/G single-nucleotide polymorphisms were performed in 48 consecutive CSS patients and 350 healthy controls.

Results. The frequency of the HLA–DRB1*07 allele was higher in the CSS patients than in controls (27.1% versus 13.3%; $\chi^2 = 12.64$, $P = 0.0003$, corrected P [P_{corr}] = 0.0042, odds ratio [OR] 2.42, 95% confidence interval [95% CI] 1.47–3.99). The HLA–DRB4 gene, present in subjects carrying either HLA–DRB1*04, HLA–DRB1*07, or HLA–DRB1*09 alleles, was also far more frequent in patients than in controls (38.5% versus 20.1%; $\chi^2 = 16.46$, $P = 0.000058$, $P_{corr} = 0.000232$, OR 2.49, 95% CI 1.58–3.09). Conversely, the frequency of the HLA–DRB3 gene was lower in patients than in controls (35.4% versus 50.4%; $\chi^2 = 7.62$, $P =$ 0.0057, $P_{corr} = 0.0228$, OR 0.54, 95% CI 0.35–0.84). CSS has 2 major clinical subsets, antineutrophil cytoplasmic antibody (ANCA)–positive, with features of small-vessel vasculitis, and ANCA-negative, in which organ damage is mainly mediated by tissue eosinophilic infiltration; analysis of HLA–DRB4 in patients categorized by different numbers of vasculitic manifestations (purpura, alveolar hemorrhage, mononeuritis multiplex, rapidly progressive glomerulonephritis, and constitutional symptoms) showed that its frequency strongly correlated with the number of vasculitis symptoms (P for trend = 0.001).

Conclusion. These findings indicate that HLA–DRB4 is a genetic risk factor for the development of CSS and increases the likelihood of development of vasculitic manifestations of the disease.

Churg-Strauss syndrome (CSS) is a rare vasculitic disease characterized by granulomatous and eosinophil-rich inflammation and systemic necrotizing vasculitis affecting small and medium-sized vessels (1,2). It usually occurs in patients with asthma and eosinophilia, and has a heterogeneous clinical spectrum that includes constitutional symptoms, sinusitis, pulmonary infiltration, peripheral neuropathy, and skin (e.g., purpura, nodules), renal (e.g., isolated urinary abnormalities, rapidly progressive glomerulonephritis [RPGN]), and gastrointestinal manifestations (3–6). Antineutrophil cytoplasmic antibodies (ANCAs) are present in ~40% of patients, usually in those developing clinical features resulting from active small-vessel vasculitis (e.g., RPGN, purpura) (7,8).

The pathogenesis of CSS has not been clearly elucidated. Eosinophils probably directly mediate organ damage, but T cells may also play a role, since serum interleukin-2 (IL-2) receptor levels are persistently high

in CSS patients (suggestive of T cell activation) (9), and CD4+ and CD8+ cells are abundant in CSS vasculitic lesions (10). Both Th1 and Th2 responses occur in CSS (11); the former may lead to granulomatous and vasculitic lesions as a result of interferon-γ production, whereas the latter may contribute to eosinophilia mediated by IL-4 and IL-13. T cell responses are usually due to an antigen-driven process; in CSS, as in other autoimmune diseases, several antigens potentially trigger the disease in a susceptible host (12).

HLA molecules are critically involved in antigen presentation and thymic deletion of autoreactive T cells, and this is basically why they are thought to contribute to the pathogenesis of immunologically mediated diseases (13). Additionally, some HLA alleles confer susceptibility to numerous immunopathologic conditions, such as giant cell arteritis, Behçet's syndrome, and rheumatoid arthritis (RA) (14–16).

Several studies have explored the role of HLA molecules in ANCA-associated vasculitides. Increased frequencies of HLA–B8 (17), HLA–DR2 (18), and HLA–DQ7 (19) and a decreased frequency of HLA–DR13 (20) have been found in Wegener's granulomatosis, and positive associations with HLA–DQ7 (19) and with the HLA–DRB1*0901;DQB1*0303 haplotype have been demonstrated in microscopic polyangiitis (21). However, subsequent studies have failed to confirm most of these associations (22). To our knowledge, the role of HLA in CSS has been investigated in only 2 studies, in which patients with CSS, Wegener's granulomatosis, and polyarteritis nodosa were pooled. Those studies, which included 14 CSS patients (18) and 7 CSS patients (23), respectively, did not show an association between HLA alleles and CSS, except for a (non–statistically significant) lower frequency of the HLA–DRB1*03 allele in CSS patients (23); however, the lack of significant associations might be explained by the inadequacy of the sample size and the genotyping methods.

The aim of the present study was to assess whether there is an association between HLA alleles and CSS, and whether this association differs according to the clinical pattern of the disease. We examined the alleles of genes belonging to HLA class I (HLA–A and B) and class II (HLA–DR); HLA class III also contains several polymorphic genes, such as *TNFA* (the gene for tumor necrosis factor α). We evaluated 2 single-nucleotide polymorphisms (SNPs) located in the promoter of the *TNFA* gene (*TNFA* −238A/G and *TNFA* −308A/G), since they have been demonstrated to modulate tumor necrosis factor α expression and increase susceptibility to autoimmune diseases (24).

PATIENTS AND METHODS

Patients and controls. We recruited 48 consecutive patients with CSS (30 women and 18 men, with a median age of 48 years [range 18–78]). The patients had been diagnosed at internal medicine departments (nephrology, clinical immunology, rheumatology, pulmonary medicine, and others) of general hospitals in Northern Italy. CSS was diagnosed based on the presence of asthma, hypereosinophilia (>10%, or >1,500 cells/mm^3), and clinical manifestations consistent with systemic vasculitis, with or without histologic confirmation (4). The absence of hypereosinophilia was not considered an exclusion criterion in patients receiving steroids for asthma if histologic evidence of vasculitis or extravascular eosinophils was available (8). In all patients, the diagnosis of CSS satisfied the American College of Rheumatology classification criteria (25) or the Chapel Hill Consensus Conference nomenclature (26).

All patients underwent a physical examination, routine laboratory testing, and appropriate imaging studies. The presence of ANCA was determined at the time of diagnosis, using indirect immunofluorescence on ethanol-fixed granulocytes, and antigen-specific proteinase 3 and myeloperoxidase (MPO) enzyme-linked immunosorbent assays (ELISAs). The different immunofluorescence patterns were characterized as previously reported (8). ANCAs were first tested in each local participating center, and the results were subsequently rechecked at the laboratory of San Carlo Borromeo Hospital, a participating institution in the European Commission/Community Bureau of Reference study for ANCA assay standardization (27).

Three hundred fifty healthy subjects (176 men and 174 women, with a median age of 32 years [range 20–55]) with no history of autoimmune/inflammatory disease served as controls. All of the cases and controls were white Italians; subjects from genetic isolates were not included. The frequencies of the analyzed alleles in our control subjects were concordant with those in other Italian control populations (28).

Written informed consent was obtained from all study participants. The study protocol was approved by the Ethics Committee of the University of Parma.

HLA genotyping. Genomic DNA was extracted from EDTA (5 ml)–treated peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) and stored at −20°C until use. Low-resolution genotyping for HLA–A, HLA–B, and HLA–DR loci was performed by polymerase chain reaction (PCR) using sequence-specific primers (One Lambda, Canoga Park, CA); 21 alleles in HLA–A genes, 35 in HLA–B genes, and 13 in HLA–DR genes were investigated. High-resolution genotyping for the HLA–DRB4 and HLA–DRB3 alleles was carried out, using the dideoxy chain termination method, on an automated sequencer (CEQ 2000XL DNA Analysis System; Beckman Coulter, Fullerton, CA). Sequence data were aligned using Seqman, version II (DNAStar, Madison, WI). The primer sequences have been described elsewhere (29).

TNFA −238A/G and *TNFA* −308A/G SNPs were genotyped using sequence-specific primers and a Cyclerplate Cytokines 2 System, according to the protocol recommended

by the manufacturer (Protrans, Ketsch, Germany). The PCR products were resolved on 2.5% (weight/volume) agarose gels stained with ethidium bromide. Quality measures were adopted as recommended by consensus conferences of the Centers for Disease Control and Prevention and the National Institutes of Health (30,31). Each patient and healthy subject was assigned a code. The samples were handled by 2 investigators, who were unaware of the subjects' clinical status. The degree of reproducibility between quality control replicates was 100%. The laboratory in which the HLA genotyping was performed is certified by the European Foundation for Immunogenetics.

Statistical analysis. Differences in allele frequencies between CSS patients and controls were analyzed by Pearson's chi-square test. The direction and strength of these differences were assessed by calculating odds ratios (ORs). Two-sided P values less than 0.05 were considered significant. Nominal P values for allele associations were corrected by the Bonferroni method to allow for multiple testing. The power of these tests was estimated using the Power for Association With Errors program (32), with the test allele as the first allele and the other alleles as the second allele, under the assumption of an error rate of 1%. The patient and control groups were in Hardy-Weinberg equilibrium for all alleles analyzed.

Because CSS has different disease patterns, we used 2 approaches to explore the clinical manifestations associated with the HLA–DRB4 gene. First, we examined whether the prevalence of each clinical manifestation differed between HLA–DRB4–positive and HLA–DRB4–negative patients, using Fisher's exact test and the Mann-Whitney U test. Second, in order to achieve a more powerful analysis, we performed trend tests of the proportion of DRB4-positive patients across different categories defined by increasing numbers of symptoms characterizing the "vasculitic subset" of CSS (33), i.e., purpura, alveolar hemorrhage, mononeuritis multiplex, RPGN, and constitutional symptoms (7,8,33). The chi-square test for trend in proportions was used for this analysis.

RESULTS

Clinical characteristics and laboratory findings. Clinical and laboratory characteristics of the CSS patients are shown in Table 1. All of the patients had bronchial asthma. Peripheral neuropathy was another major clinical manifestation, affecting 32 patients (67%), 17 of whom had mononeuritis multiplex. Constitutional symptoms (e.g., fatigue, fever, anorexia, weight loss, diffuse myalgias, and arthralgias) were found in 30 patients (63%). Among the otolaryngologic manifestations, sinusitis occurred in 27 patients (56%), but other features were also found, such as nasal polyps (23 cases [48%]) and sensorineural hearing loss (8 cases [17%]). Fifty percent of the patients exhibited lung involvement: pulmonary infiltrates were common (13 cases [27%]), but some patients also presented with nodules (6 cases [13%]), pleural effusions (4 cases [8%]), and alveolar hemorrhage (4 cases [8%]). Skin manifestations were

Table 1. Demographic, clinical, and laboratory characteristics of the 48 patients with CSS*

No. male/no. female	18/30
Age, median (range) years	48 (18–78)
Asthma	48 (100)
Peripheral neuropathy	32 (66.7)
Constitutional symptoms†	30 (62.5)
Sinusitis	27 (56.2)
Lung involvement	24 (50.0)
Renal involvement	22 (45.8)
Skin involvement	21 (43.7)
Gastrointestinal involvement	13 (27.1)
Cardiac involvement	7 (14.6)
CNS involvement	3 (6.2)
Eosinophilia, median (range) cells/mm^3	2,913 (63–28,815)
ANCA positivity‡	21 (47.7)

* Except where indicated otherwise, values are the number (%) of patients. CSS = Churg-Strauss syndrome; CNS = central nervous system; ANCA = antineutrophil cytoplasmic antibody.
† Fatigue, fever, anorexia, weight loss, diffuse myalgias, and arthralgias.
‡ Data available on 44 patients.

found in 21 patients (44%), with purpura (6 cases [13%]), maculopapular rash (5 cases [10%]), and nodules (4 cases [8%]) being the most frequent lesions.

Twenty-two patients (46%) exhibited renal involvement: isolated urinary abnormalities (i.e., microscopic hematuria and proteinuria) were found in 17 cases (36%); 5 patients (10%) had renal insufficiency (serum creatinine >1.4 mg/dl), 3 of whom had RPGN. Gastrointestinal manifestations (e.g., abdominal pain, gastrointestinal bleeding), cardiac manifestations (e.g., pericarditis, acute coronary syndrome), and central nervous system manifestations (e.g., stroke, meningitis) occurred in 13 patients (27%), 7 patients (15%), and 3 patients (6%), respectively.

Eosinophilia was found in all but 5 patients (who were receiving oral corticosteroids for asthma). ANCAs were positive by immunofluorescence in 21 patients (44%), negative in 23 (48%), and undetermined in 4 (8%); this rate of ANCA positivity was similar to that reported by other investigators (4,7,8). Sixteen of the 21 ANCA-positive patients (76%) had a perinuclear ANCA pattern; ELISA findings were specific for MPO in 14 of these cases but negative in the remaining 2. A cytoplasmic ANCA (cANCA) pattern was found in 2 of 21 patients (10%); 1 was negative by ELISA whereas the other, whose immunofluorescence pattern was "cANCA atypical" (8), tested positive for MPO. Finally, 3 of 21 cases (14%) had an atypical immunofluorescence pattern (cytoplasmic plus perinuclear), and all of them were positive for MPO.

In accordance with previous findings (7,8), the

Table 2. Main HLA findings in the 48 CSS patients and 350 healthy controls*

Allele/gene	Patients	Controls	χ^2	P	P_{corr}	OR	95% CI
DRB1*04	10/96 (10.4)	44/700 (6.3)	2.278	0.1312	–	1.74	0.84–3.57
DRB1*07	26/96 (27.1)	93/700 (13.3)	12.64	0.0003	0.0042	2.42	1.47–3.99
DRB1*09	1/96 (1.0)	4/700 (0.6)	0.299	0.5844	–	3.78	0.20–16.56
DRB1*13	3/96 (3.1)	87/700 (12.4)	7.287	0.0069	0.0966	0.23	0.07–0.73
DRB4	37/96 (38.5)	141/700 (20.1)	16.461	0.000058	0.000232	2.49	1.58–3.09
DRB3	34/96 (35.4)	353/700 (50.4)	7.616	0.0057	0.0228	0.54	0.35–0.84

* Values are the number positive/2× number of subjects (%). CSS = Churg-Strauss syndrome; P_{corr} = corrected P (after Bonferroni adjustment for multiple testing); OR = odds ratio; 95% CI = 95% confidence interval.

ANCA-positive patients showed a higher frequency of manifestations of small-vessel vasculitis, such as alveolar hemorrhage and purpura, whereas the ANCA-negative patients more frequently exhibited cardiac, gastrointestinal, or lung involvement (other than alveolar hemorrhage) (data not shown). One or more tissue biopsies were performed in 29 patients (60.4%), and in each case, findings in at least 1 biopsy sample were suggestive of CSS.

HLA findings. No statistically significant differences were found in the frequencies of HLA–A and HLA–B alleles between CSS patients and controls (data not shown). Table 2 illustrates the frequencies of the genes or alleles that showed a significant (positive or negative) association with CSS, as well as the frequencies of other alleles of the HLA–DRB4 haplotype.

The HLA–DRB1*07 allele was present in 24 of 48 CSS patients (50.0%) and in 87 of 350 controls (24.9%), and its allele frequency in the 2 groups, respectively, was 26 of 96 (27.1%) and 93 of 700 (13.3%) (P = 0.0003, P corrected for multiple comparisons [P_{corr}] = 0.0042, OR 2.42, uncorrected 95% confidence interval [95% CI 1.47–3.99]). We also examined the allele frequencies of HLA–DRB1*04 and HLA–DRB1*09 (which, like DRB1*07, belong to the HLA–DRB4 haplotype), but they were not found to differ significantly between CSS patients and controls (P = 0.13 and P = 0.58, respectively).

The HLA–DRB4 gene, which encodes the supertypic HLA–DR53 antigen, is in strong linkage disequilibrium with the HLA–DRB1*04, *07, and *09 alleles; therefore, we calculated the HLA–DRB4 gene frequency by summing the frequencies of the 3 alleles and then confirmed these findings using high-resolution genotyping by HLA–DRB4 gene sequencing. The DRB4 gene was present in 31 of 48 patients (64.6%) and in 124 of 350 controls (35.4%), whereas its allelic frequency was 37 of 96 (38.5%) and 141 of 700 (20.1%) in patients and controls, respectively (P = 0.000058, P_{corr} = 0.000232, OR 2.49, uncorrected 95% CI 1.58–

3.09). The power value for allelic tests for the HLA–DRB4 gene was 98.2%.

High-resolution genotyping by sequencing was also performed to investigate whether a particular HLA–DRB4 allele was associated with CSS. Only 2 alleles (HLA–DRB4*0101 and HLA–DRB4*0103) were present with similar frequencies (P = 0.62) in the CSS patients and controls.

Unlike the HLA–DRB1*07 allele and the HLA–DRB4 gene, the HLA–DRB1*13 allele was less frequent in the CSS patients (allelic frequency 3 of 96 [3.1%]) than in controls (87 of 700 [12.4%]) (P = 0.0069), although the significance was lost after correction for multiple comparisons (P_{corr} = 0.0966). In addition, the frequency of the HLA–DRB1*03 allele was lower in the CSS patients (4 of 96 [4.2%]) than in controls (64 of 700 [9.1%]), but, in accordance with previous findings (23), this difference did not reach statistical significance (P = 0.10).

Because the HLA–DRB3 gene, which encodes the HLA–DR52 antigen, is in strong linkage disequilibrium with the HLA–DRB1*03, *11, *12, *13, and *14 alleles, the frequencies of these alleles were summed to estimate HLA–DRB3 gene frequency. The HLA–DRB3 gene was significantly less frequent in the CSS patients (27 of 48 [56.2%]) than in controls (258 of 350 [73.7%]); its allelic frequency was 34 of 96 (35.4%) in the patients and 353 of 700 (50.4%) in the controls (P = 0.0057, P_{corr} = 0.0228, OR 0.54, uncorrected 95% CI 0.35–0.84). High-resolution genotyping with HLA–DRB3 gene sequencing confirmed these results. The power value for allelic tests for the HLA–DRB3 gene was 78%.

The frequencies of HLA–DRB1*07, HLA–DRB4, and HLA–DRB3 in the subgroups of patients with and those without biopsy confirmation of the diagnosis of CSS were not significantly different (data not shown). Finally, we found no difference in *TNFA* −238A and *TNFA* −308A allele frequencies between the CSS patients and controls (P = 0.48 and P = 0.13, respectively).

Table 3. Comparison of the main clinical and laboratory characteristics of the HLA–DRB4–positive and HLA–DRB4–negative CSS patients*

	DRB4-positive (n = 31)	DRB4-negative (n = 17)
Mononeuritis multiplex	13 (41.9)	4 (23.5)
Constitutional symptoms†	24 (77.4)‡	6 (35.3)
Sinusitis	20 (64.5)	7 (41.2)
Lung involvement (all kinds)	14 (45.2)	10 (58.8)
Alveolar hemorrhage	4 (12.9)	0 (0.0)
Kidney involvement (all kinds)	16 (51.6)	6 (35.3)
RPGN	2 (6.5)	1 (5.9)
Purpura	6 (19.4)	0 (0.0)
Gastrointestinal involvement	6 (19.4)	7 (41.2)
Cardiac involvement	5 (16.1)	2 (11.8)
Eosinophilia, median (range) cells/mm^3	3,168 (291–28,815)	2,399 (63–22,903)
ANCA positivity§	17 (58.6)	4 (26.7)

* Except where indicated otherwise, values are the number (%) of patients. CSS = Churg-Strauss syndrome; RPGN = rapidly progressive glomerulonephritis; ANCA = antineutrophil cytoplasmic antibody.
† Fatigue, fever, anorexia, weight loss, diffuse myalgias, and arthralgias.
‡ $P = 0.006$ versus DRB4-negative patients, by Fisher's exact test.
§ Data available on 44 patients.

HLA–DRB4 and vasculitic manifestations of CSS. Comparison of the main clinical and laboratory findings in the HLA–DRB4–positive versus the HLA–DRB4–negative CSS patients showed that the former group had significantly more frequent constitutional symptoms (Table 3). In addition, they exhibited a trend, though not statistically significant, toward a higher prevalence of "vasculitis symptoms" that identify the ANCA-positive subset of CSS, i.e., purpura, alveolar hemorrhage, and mononeuritis multiplex (7,8,33).

This phenomenon could be better distinguished after the patients were divided into categories defined on the basis of the number of vasculitis symptoms and the proportion of HLA–DRB4–positive patients was computed for each of these categories (Figure 1). This analysis showed that the higher the number of vasculitis symptoms, the greater the probability that the CSS patients carried the HLA–DRB4 gene. In fact, HLA–DRB4 was found in 3 of 10 patients with no vasculitic manifestations (30.0%), 13 of 22 patients with 1 vasculitic manifestation (59.1%), 10 of 11 patients with 2 vasculitic manifestations (90.9%), and 5 of 5 patients with ≥3 vasculitic manifestations (100%) (P for trend = 0.001) (Figure 1A). When ANCA positivity was included among the vasculitic manifestations, we found that HLA–DRB4 was positive in 2 of 8 patients with no vasculitic manifestations (25.0%), 10 of 17 with 1 vasculitic manifestation (58.8%), 7 of 11 with 2 vasculitic manifestations (63.6%), and 12 of 12 with ≥3 vasculitic manifestations (100%) (P for trend = 0.0015) (Figure 1B).
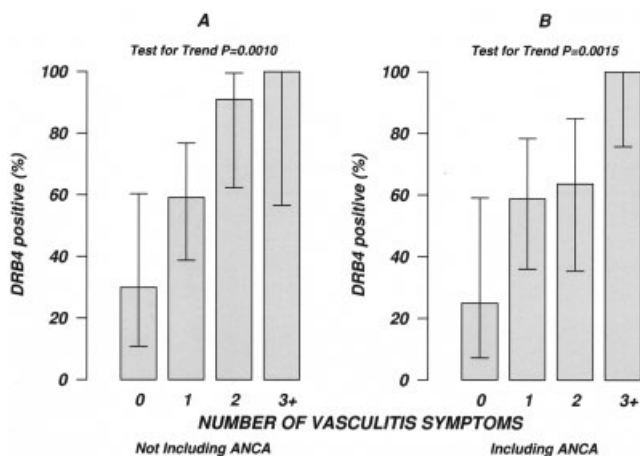
Figure 1. Proportion of Churg-Strauss syndrome (CSS) patients carrying the HLA–DRB4 gene, according to the number of vasculitis symptoms identifying the antineutrophil cytoplasmic antibody (ANCA)–positive subset of CSS (i.e., purpura, alveolar hemorrhage, mononeuritis multiplex, rapidly progressive glomerulonephritis, and constitutional symptoms) (8,33). In **A,** ANCA positivity is not included among the vasculitis symptoms counted; in **B,** it is included. Values are the percent of patients who were DRB4 positive and the 95% confidence interval, among all 48 CSS patients in **A** and among the 44 in whom ANCA testing was performed in **B**. P values were determined by chi-square test for trend.

DISCUSSION

The results of this study provide evidence that there is an association between HLA genotype and CSS. In comparison with healthy controls, the frequencies of

the HLA–DRB4 gene and the HLA–DRB1*07 allele were significantly higher in our CSS patients, and the frequency of the HLA–DRB3 gene was lower. Furthermore, the association between HLA–DRB4 and CSS was stronger in patients with full-blown vasculitic manifestations.

However, the study has some limitations. As in the case of all association studies, the findings do not necessarily indicate causation, but might also be explained by the presence of another susceptibility locus within or near the HLA region, or by an artifact caused by population admixture. They should therefore be confirmed by replication studies in other populations. Additionally, our study of the HLA class III alleles assessed only 2 SNPs of the *TNFA* gene, but a number of other candidate genes located in this region warrant analysis. Finally, some associations might have been missed, as a result of use of the Bonferroni multiple comparison adjustment procedure, which has the disadvantage of being very conservative.

Nevertheless, the apparent close association between HLA and CSS has potential implications with regard to pathogenesis. HLA molecules are critical in the dialogue between T cells and antigen-presenting cells, since the former recognize antigenic epitopes only when they are displayed by antigen-presenting cells in association with HLA molecules (14). The finding that CSS patients have a restricted HLA repertoire supports the hypothesis that only selected antigenic determinants may be involved in CSS, and simultaneously highlights the pathogenetic role of T cells. In accordance with this view, clonal T cell expansion has been demonstrated in CSS patients, and, because the T cell clones had similarly specific T cell receptors, they could recognize only a limited number of antigens (34). This finding, together with the present results, supports the notion that CSS is an antigen-driven disease.

The HLA–DRB4 gene encodes the supertypical HLA–DR53 antigen and exists only on haplotypes possessing the HLA–DRB1*04, DRB1*07, and DRB1*09 alleles. A number of diseases are linked to these specificities, some of which also have clinical and pathogenetic features relevant to CSS. Among autoimmune conditions, RA is associated with HLA–DRB1*04 (35), and HLA–DRB7 has been found to correlate with the occurrence of anticardiolipin antibodies in systemic lupus erythematosus (36). Some vasculitides are also associated with such HLA alleles: giant cell arteritis is significantly associated with HLA–DRB1*04 (14). HLA–DRB1*04 and (to a lesser extent) HLA–DRB1*07 have been found to be associated with atopy

(37,38), but these results were not corroborated in other investigations (39). Finally, an HLA profile strikingly similar to that observed in CSS (i.e., increased HLA–DRB4 and reduced HLA–DRB3 frequency) has been found in childhood acute lymphoblastic leukemia (40).

The observation that CSS and lymphoproliferative diseases may have a common immunogenetic background suggests that lymphocyte clones may play a role in CSS. Abnormal T cell clones producing high levels of IL-5 are involved in the pathogenesis of idiopathic hypereosinophilic syndrome (HES) (41), a disease whose clinical manifestations often overlap those of CSS since the latter also has hallmark features of idiopathic eosinophilia and eosinophilic tissue infiltration (33).

In some conditions, not only does HLA play a role in disease susceptibility, but it also influences the spectrum of clinical characteristics. For instance, the HLA–DRB1*0401 allele is closely associated with extraarticular manifestations in RA (42), and HLA–C3 is associated with rheumatoid vasculitis (16).

CSS has traditionally been regarded as a single disease entity, but its clinical manifestations are heterogeneous. Recently, 2 independent studies showed that ANCA positivity in CSS patients was strongly associated with renal involvement and, in particular, RPGN; furthermore, ANCAs were more frequent in patients with alveolar hemorrhage, mononeuritis multiplex, and purpura, which are considered to be features of small-vessel vasculitis (7,8). In addition, constitutional symptoms suggesting systemic rather than localized vasculitic disease were far more frequent in the ANCA-positive group (8), whereas the ANCA-negative group was characterized by a higher prevalence of cardiac and lung involvement (other than alveolar hemorrhage), which are more often due to eosinophilic tissue infiltration and subsequent fibrotic organ damage (7,8,33). On the basis of these findings, it has been postulated that there are 2 distinct subsets of CSS: one that is ANCA positive and has the features of systemic necrotizing vasculitis, and one that is ANCA negative and more related to eosinophilic tissue infiltration, and may also follow pathways comparable with those underlying other eosinophilic disorders such as HES (33).

After identifying the association between HLA–DRB4 and CSS, we compared the main clinical and laboratory findings in HLA–DRB4–positive and HLA–DRB4–negative patients and observed that the former not only had constitutional symptoms significantly more frequently, but also more frequently exhibited vasculitic features, including purpura, alveolar hemorrhage, mononeuritis multiplex, and ANCAs. These latter dif-

ferences were not statistically significant, possibly because of the sample size and, in some cases, the relative infrequency of the clinical features (e.g., alveolar hemorrhage), but they prompted us to evaluate the prevalence of HLA–DRB4 across groups of patients with different degrees of vasculitic manifestations. There was a statistically significant trend in the association between the "vasculitic phenotype" and HLA–DRB4: in particular, all of the patients with 3 or more vasculitis symptoms were positive for HLA–DRB4.

Our data therefore not only reinforce the view that CSS has 2 separate forms, but also provide preliminary evidence that immunogenetic factors play a role in determining this clinical dichotomy. These findings require confirmation in larger populations of CSS patients, and mechanistic studies to clarify the underlying pathophysiologic mechanisms are warranted.

In conclusion, the results of the present study show that HLA–DRB4 can be a genetic risk factor for the development of CSS. Furthermore, they indicate that DRB4 positivity may be associated with a disease subset that is characterized by features of small-vessel vasculitis.

AUTHOR CONTRIBUTIONS

Dr. Vaglio 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.
Study design. Vaglio, Martorana, Neri.
Acquisition of data. Martorana, Grasselli, Zanetti, Pesci, Garini, Manganelli, Bottero, Tumiati, Sinico, Savi, Buzio.
Analysis and interpretation of data. Vaglio, Martorana, Maggiore, Savi, Buzio, Neri.
Manuscript preparation. Vaglio, Neri.
Statistical analysis. Martorana, Maggiore, Neri.

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