

HLA-DRB1* and Allergy to *Parietaria*: Linkage and Association Analyses

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ABSTRACT: In this study, we used the affected sibling-pairs approach to investigate the linkage of HLA (human leukocyte antigen)-DRB1* with phenotypes related to allergy to *Parietaria*, the most common pollinosis in Mediterranean countries. The study population consisted of 51 nuclear families (235 subjects). Linkage was detected with *Parietaria* skin test positivity ($p < 0.01$), presence of IgG and IgE antibodies specific for the major allergen Par o 1 ($p < 0.020$ and $p < 0.025$, respectively), and absence of Par o 1-specific IgE ($p < 0.020$).

High levels of Par o 1-specific IgG were associated with DRB1*1101 and/or DRB1*1104 ($p < 0.0001$ and $p < 0.0119$, respectively) in parents and probands. High levels of Par o 1-specific IgE were associated with DRB1*1104 in parents ($p < 0.017$) and with DRB1*1101 in probands ($p < 0.0146$). When siblings were categorized according to high/low total IgE levels (≥ 125 IU/ml and < 125

IU/ml, respectively), high IgE antibody response was associated with DRB1*1104 in siblings with low total IgE ($p < 0.034$) and with DRB1*1101 in siblings with high total IgE ($p < 0.05$).

These results demonstrate that HLA-DRB1*, or genes in linkage disequilibrium, contributes to susceptibility to *Parietaria* allergy and that total IgE levels can discriminate population subsets where different alleles (at the HLA region or at loci in linkage disequilibrium) contribute to control allergen-specific IgE synthesis. *Human Immunology* 60, 1250–1258 (1999). © American Society for Histocompatibility and Immunogenetics, 1999. Published by Elsevier Science Inc.

KEYWORDS: allergy; genetic linkage; HLA-DRB1* association; *Parietaria*

ABBREVIATIONS

BBS	borate buffered saline
DARIA	double antibody radioimmunoassay
HLA	human leukocyte antigen
MHC	major histocompatibility complex
PCR	polymerase chain reaction

RAST	radioallergosorbent test
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SSO	sequence-specific oligonucleotides

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INTRODUCTION

The development of clinical allergy appears to be due to the interaction of susceptibility genes with the environment. Recent evidence supports the concept that the allergic phenotype of adults is irreversibly determined during the first months of life, and that early identification of genetically predisposed subjects is essential for effective prevention [1]. Multiple gene interaction and genetic heterogeneity hinder the identification of susceptibility genes for this and other common diseases. Linkage analysis, using intermediate, allergy-related pheno-

types (e.g., elevated total serum IgE level, atopy, and allergen-specific IgE antibody response), has shown that each phenotype is influenced by multiple genes, either in cooperation or independently [2, 3]. A strategy aimed at reducing genetic heterogeneity recruits a study population on the basis of a specific allergy. To this aim, we selected allergy to *Parietaria*—a disease of great epidemiological and clinical relevance in southern European and northern African countries bordering the Mediterranean, where it is a major cause of allergic rhinitis and asthma [4, 5]. The most studied species are *Parietaria judaica* and *Parietaria officinalis*. In Italy, the reported prevalence of this allergy in adults ranges from 36–77%. In areas of high exposure, 28–38% of subjects allergic to this pollen are monosensitized and 50–62% have asthma.

Previously, we reported that in Italian and Spanish allergy patients, the alleles HLA (human leukocyte antigen)-DRB1*1101 and/or DRB1*1104 were positively associated with IgG and IgE antibody response toward the major allergen of *P. officinalis*, Par o 1 ($p < 0.007$ and $p < 0.012$, respectively), whereas DRB1*03 was negatively associated with IgG and IgE antibody response to this allergen [6, 7]. These observations need to be confirmed in family-based studies, as the frequencies of these alleles are highly influenced by ethnic and geographic factors. The results of linkage and association analyses of nuclear families, recruited on the basis of having a *Parietaria* allergic sibling, are reported.

MATERIALS AND METHODS

Study Population

Fifty one nuclear families (218 subjects) were recruited through probands attending allergy and asthma clinics in several regions of Italy (Calabria, Campania, Liguria, Lombardia, and Toscana). The following criteria were met for recruitment (1) one sibling (the proband) having clinical allergy, skin positive by skin prick test toward glycerinated *P. judaica* and *P. officinalis* and/or *Dermatophagoides pteronyssinus* extract; (2) at least a second sibling who was skin positive to these extracts; (3) two living parents, with at least one who was without clinical allergy; and (4) no previous desensitization therapy. For association analysis, 17 subjects from 10 uncompleted families (nine parents and eight probands) were included in the study population. All participants were informed volunteers.

Clinical History and Skin Prick Test

Clinical history was recorded by a physician using a standard questionnaire. Asthma was defined as a positive response to the following questions: (1) “Have you had an attack of asthma on more than one occasion?”; (2)

“Have you had attacks of shortness of breath with wheezing on more than one occasion?”; and (3) “Has asthma been diagnosed by a physician?”

Participants in the study group were analyzed for skin prick test reactivity towards 18 glycerinated extracts of common inhalant allergens (Hollister-Stier Laboratories). Skin test positivity was defined by a wheal diameter of >3 mm than the negative control. As the results of skin prick tests were always concordant between *P. judaica* and *P. officinalis* pollen extracts, participants in the study group were indicated as *Parietaria* skin test positive/negative. Information on skin prick test reactivity was not available for six parents.

Collection and Storage of Blood Samples

At least 25 ml of blood were drawn from each patient and stored in a solution of 0.1 M EDTA (pH 7.4). Cells were separated from plasma by centrifugation, aliquoted, and stored at -70°C . Sera were recovered from plasma by addition of 2 ml of a 2.4% solution of CaCl_2 to 1 ml of blood at 37°C ; afterward, sera were aliquoted and stored at -20°C .

Allergens

P. officinalis pollen was purchased from Allergon AB (Sweden). Par o 1 was purified from the pollen extract by Sephadex G75 and G50 gel filtration and DE52 ion exchange chromatography [6]. Five hundred milligrams of allergen were recovered from 1 g of pollen. This preparation was 95% homogeneous as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Radiolabeling

Forty milligrams of purified allergen were labeled using 2 mCi of ^{125}I (3.7 GBq/ml, Amersham, UK) by the chloramine T procedure [8]. One hundred milliliter aliquots of a solution containing 1 mg/ml ($10\text{--}20 \times 10^6$ cpm/ml) of radiolabeled protein in borate-buffered saline (BBS), pH 8.4 containing 5% bovine serum albumin (BSA) were stored at -20°C .

Immunoassays

The level of Par o 1-specific IgG and IgE antibodies was determined using the double antibody radioimmunoassay (DARIA). The assay was essentially performed as already described [9]. For each serum, IgG and IgE antibody levels were determined in parallel. For IgG antibody, 100 μl of 1/10 serum dilution in BBS were incubated for 4 h at room temperature with 100 μl of BBS, containing 1 ng of radiolabeled Par o 1 ($10\text{--}20$ cpm). Dilutions greater than 1/10 were in IgG carrier (1/10 control human serum). Antigen-antibody complexes were precipitated by adding the appropriate amount of

goat antihuman IgG for 16 h at 4°C. For IgE antibody, 50 µl of serum were mixed with 50 µl of the appropriate dilution of an IgE myeloma protein, prior to addition of the radiolabeled allergen. Antigen-antibody complexes were precipitated by adding the appropriate amount of goat antihuman IgE. Precipitates were collected on Whatman GF/A glass fiber paper filters. After washing (using BBS), the radioactivity retained on filters was measured by a gamma counter. Each experiment included a reference dose-response curve, obtained with twofold serial dilutions (1/10 to 1/2048) of a serum having a very high level of IgG antibody to Par o 1, and a negative control (a serum with an undetectable antibody to Par o 1 ratio). Par o 1 binding was considered statistically significant if the counts per minute bound by the tested sample were more than 1.3-fold the counts per minute bound by the negative control. IgG and IgE antibody levels were expressed as U/ml, by comparison with the reference curve, according to the following formula: $(a - b)/(B_{\max} - b)$, where b = background counts per minute bound by the negative control, a = counts per minute bound by the tested serum, and $B_{\max}$ = counts per minute bound by the reference serum at 1/10 dilution. Experiments were performed in duplicate and results were expressed as means. Determinations were repeated if the difference between duplicates was >11%. Background counts per minute represented $2.5 \pm 3.3\%$ and $1.9 \pm 2.5\%$ of $B_{\max}$ values for IgG and IgE DARIAs, respectively.

Determination of Total IgE

Total serum IgE level was determined by radioimmunoassay (Pharmacia CAP system, Pharmacia, Uppsala, Sweden) following the manufacturer's instructions. The measuring range was 2–2,000 kU/L.

HLA-DRB1* Typing

HLA-DRB1* typing was achieved combining dot-blot and DNA heteroduplex analysis [10, 11]. Several cell lines (most of which were from the 10th Histocompatibility Workshop) were used as negative and/or positive controls in every step of the procedure. Samples were classified as DRB1*01, DRB1*15/16, DRB1*04, DRB1*07, DRB1*09, DRB1*10, and DR52-DRB1 associated (DRB1*03, DRB1*11, DRB1*12, DRB1*13, DRB1*14, and DRB1*08) groups of alleles (following the 11th Histocompatibility Workshop DRB1* typing procedure), by amplification with DRB1 generic primers (DRBAMP-A and DRBAMP-B) and subsequent hybridizations with a series of sequence-specific oligonucleotides (SSOs) [10, 12]. DRB1*01-, DRB1*15/16-, and DR52-DRB1*-associated group positive individuals were then subjected to a second round of amplification for further characterization. The 5'-end-specific primers

TABLE 1 Number of sibling per family

	Number of siblings					Total
	1	2	3	4	5	
No. of families	2	35	12	1	1	51
No. of sibpairs	0	35	36	6	10	87

(DRBAMP-1, DRBAMP-2, and DRBAMP-3), in combination with DRBAMP-B, were used for DRB1*01-, DRB1*15/16-, and DR52-DRB1*-associated groups, respectively [12]. DRB1*15/16 alleles were distinguished via a previously described procedure [12], while DRB1*01 and DR52-DRB1* alleles were distinguished by hybridizing the samples separately with selected SSO probes selected from the 11th Workshop panel [13]. DR52-DRB1* data were refined, in some cases, by group-specific polymerase chain reactions and DNA heteroduplex analysis by an adaptation of the procedure reported by D'Amato et al. [11].

Statistical Analyses

Associations between HLA-DRB1* alleles and phenotypes were tested by nonparametric analysis (Yates-corrected χ^2 analysis and Mann-Whitney rank test). Linkage was tested by affected sibling-pairs analysis [14]. Only sibling pairs in which all four parental alleles could be unambiguously identified were counted (identity by descent). Sibling pairs from multiple sibships were considered as independent. The frequency of sharing of two, one, or no alleles was compared with that expected under the hypothesis of nonlinkage by χ^2 goodness-of-fit testing. The Transmission Disequilibrium Test was performed according to Spielman et al. [15].

RESULTS

Study Population

The number of siblings in pedigrees is reported in Table 1. For association analyses, the population was subdivided as reported in Table 2. Siblings were categorized according to low (<125 IU/ml, LIGE) or high ($\geq$ 125 IU/ml, HIGE) total serum IgE level. Mean total IgE levels were significantly different between these subgroups ($p < 0.0001$). Atopy was defined as skin prick test positive response to at least one of the tested allergen extracts. In atopics, the most prevalent sensitizations were to *Parietaria*, *Dermatophagoides pteronyssinus* and *Lolium perenne*.

Seventeen parents (15%) and 34 probands (59%) had asthma. In parents and probands, asthma was associated with high levels of total IgE ($p < 0.0073$ and $p < 0.0043$, respectively). In parents, asthma was also asso-

TABLE 2 Characteristics of parental and sibling study groups

Phenotype	Parents (<i>n</i> = 111)	Probands (<i>n</i> = 58)	Siblings ^a	
			HIGE (<i>n</i> = 37)	LIGE (<i>n</i> = 38)
Female/Male	58/53	22/36	17/20	14/24
Age ^c (years)	49 ± 9.2 (49 ^d)	21 ± 8 (20 ^d)	22 ± 7 (23 ^d)	27 ± 15 (26 ^d)
Atopy (%)	33 ^b	100	92	84
Skin test+ to <i>Parietaria</i> (%)	71 ^b	86	84	63
Skin test+ to <i>D. pteronyssinus</i> (%)	39 ^b	61	51	45
Rhinitis (%)	31	98	76	82
Asthma (%)	15	59	46	18
Skin test+ to <i>Parietaria</i> (%)	71	79	88	57
Skin test+ to <i>D. pteronyssinus</i> (%)	41	68	85	86
IgG antibody to Par o 1 (%)	67	94	89	76
IgE antibody to Par o 1 (%)	46	78	71	66
Total IgE (IU/ml)	117 ± 141 ^c (70 ^d)	291 ± 334 ^c (154 ^d)	424 ± 381 ^c (262 ^d)	47 ± 37 ^c (38 ^d)
IgG antibody (U/ml)	802 ± 1583 ^c (255 ^d)	1248 ± 1612 ^c (566 ^d)	1131 ± 1392 ^c (459 ^d)	1233 ± 1746 ^c (468 ^d)
IgE antibody (U/ml)	383 ± 908 ^c (129 ^d)	486 ± 882 ^c (222 ^d)	601 ± 1004 ^c (321 ^d)	286 ± 476 ^c (125 ^d)

Par o 1-specific IgG and IgE antibody levels were determined by DARIA, as described in Materials and Methods. Total IgE level was determined by PRIST (Pharmacia).

In atopics and asthmatics the proportion of subjects with skin test positivity (+) to *Parietaria* and *D. pteronyssinus* is reported.

^a Siblings categorized according to total IgE levels as follows: LIGE, total IgE < 125 U/ml; HIGE, total IgE ≥ 125 U/ml. Each group included no more than one sibling per family.

^b Skin test results available for 105 subjects.

^c Arithmetic mean.

^d Median.

ciated with high levels of Par o 1-specific IgG and IgE antibodies ($p < 0.0001$ and $p < 0.0001$, respectively, by Mann-Whitney analysis).

Linkage Analysis

The linkage of HLA-DRB1* locus with skin test positivity to *Parietaria* and with IgG and IgE antibody response to Par o 1 was tested using the affected sibling-pairs approach [19]. The frequency of sharing of two, one, or no alleles was compared with that expected under the null hypothesis of no linkage (χ^2 goodness-of-fit testing). Only sibling pairs in which all four parental alleles could be unambiguously distinguished were included in the analysis (identity by descent).

Table 3 reports the distribution of sibling pairs according to phenotypes and the number of HLA-DRB1* alleles shared between pairs, being either concordant for presence (+ +) or absence (− −) of phenotype or discordant (+ −). Sibling pairs that were concordant for skin test positivity to *Parietaria* and for the presence of serum Par o 1-specific IgG or IgE antibodies had a significant excess of shared alleles ($p < 0.01$, $p < 0.025$, and $p < 0.02$, respectively). Significant excess of sharing was also observed in sibling pairs concordant for the absence of detectable Par o 1-specific IgE antibody ($p < 0.02$). Nonconcordant sibling pairs did not show significant allele sharing.

Association of HLA-DRB1* Alleles With ST Positivity to *Parietaria* and Antibody Response to Par o 1

In an earlier study of Italian allergic subjects, we reported that HLA-DRB1*1101 and/or DRB1*1104 were positively associated with the presence of Par o 1-specific IgG antibody and with high levels of Par o 1-specific IgE antibody response ($p < 0.007$ and $p < 0.012$, respectively). We also reported the negative association of HLA-DRB1*03 with IgE antibody response to Par o 1 [7].

These observations were tested in the parental and proband subgroups. In parents, the prevalence of DRB1*1101 and/or DRB1*1104 was increased in subjects that were skin test positive to *Parietaria* (64%) as compared with those that were skin test negative (45%). For the latter group, the results were similar to the expected prevalence of these alleles in the Italian population (39%, 11th International HLA Workshop Data Analysis Book II Addendum).

The frequency of selected DRB1* alleles in parents and probands categorized according to presence/absence of Par o 1-specific antibody (responders/nonresponders, respectively) is reported in Table 4. There was a significant excess of HLA-DRB1*1101 and/or DRB1*1104 in IgG antibody responder parents and probands as compared with nonresponder parents ($p < 0.03$ and $p <$

TABLE 3 Affected sibling-pairs analysis sharing of HLA-DRB1* alleles in pairs concordant/discordant for phenotypes

Phenotype	No. of pairs	Alleles shared			<i>p</i>
		2	1	0	
<i>Parietaria</i> skin test					
+/+	5	15	15	5	<0.01
+/-	29	10	9	10	NS
-/-	11	3	6	2	NS
IgE antibody to Par o 1					
+/+	32	15	13	4	<0.025
+/-	27	7	6	14	NS
-/-	22	8	13	1	<0.02
IgG antibody to Par o 1					
+/+	49	20	18	12	<0.02
+/-	20	8	7	5	NS
-/-	11	3	7	1	NS

Pairs were subdivided into concordant for presence (+/+) or absence (-/-) of phenotype or discordant (+/-). Only sibling-pairs in which all four parental alleles could be unambiguously identified were counted (identity by descent). Sibling pairs from multiple sibships were considered as independent. The frequency of sharing of two, one, or no alleles was compared with that expected under the hypothesis of nonlinkage by χ^2 goodness-of-fit testing.

0.018, respectively). HLA-DRB1*03 was decreased in responders as compared with nonresponders, where it was similar to the reported prevalence of this allele in the Italian population (20%, 11th International HLA Workshop Data Analysis Book II Addendum).

Mann-Whitney Analysis for the Association of HLA-DRB1*1101, DRB1*1104, and DRB1*03 With Par o 1-Specific Antibody Level

The association of HLA-DRB1* alleles with the magnitude of antibody response to Par o 1 was analyzed using the Mann-Whitney nonparametric rank test (Table 5). Twenty-three parents (21%) and 11 probands (21%) had HLA-DRB1*1101 and 39 parents (35%) and 20 probands (35%) had DRB1*1104. The phenotype HLA-DRB1*1101 and/or DRB1*1104 was associated with high levels of Par o 1-specific IgG antibody in parents ($p < 0.0001$) and in probands ($p < 0.0119$). In contrast, a high level of Par o 1-specific IgE antibody was associated with HLA-DRB1*1104 in parents ($p < 0.017$) and with HLA-DRB1*1101 in probands ($p < 0.0146$).

Prompted by these observations, we investigated the association of alleles in siblings categorized according to low (<125 IU/ml, LIGE) or high (≥ 125 IU/ml, HIGE) total serum IgE levels. In siblings with a low total IgE level, high IgE antibody response to Par o 1 was associated with DRB1*1104 ($p < 0.034$); whereas, in siblings with high total IgE level, this response was associated with DRB1*1101 ($p < 0.053$). In both sibling subgroups, high IgG antibody response to Par o 1 was associated with DRB1*1101 and/or DRB1*1104 ($p < 0.0125$ and $p < 0.0188$, respectively).

Eighteen parents (16%) and seven probands (12%) were DRB1*03. This allele was associated with low levels of Par o 1-specific IgG antibody in parents, when four subjects which were also DRB1*1104 were excluded ($p < 0.021$), and in probands ($p < 0.0212$). There

TABLE 4 HLA-DRB1* allelic frequencies in parents and probands with and without antibody to Par o 1

No. of subjects with allele (%)	HLA-DRB1* allele								
	B1*01	B1*02 B1*15 B1*16	B1*03	B1*04	B1*1101	B1*1104	B1*1101 and/or B1*1104	B1*13 B1*1102 B1*1103	B1*07 B1*09
Parents									
With IgG antibody (<i>n</i> = 74)	10 (14)	14 (19)	9 (12)	12 (16)	17 (23)	31 (42)	41 (55)	21 (28)	19 (26)
With IgE antibody (<i>n</i> = 51)	4 (8)	10 (20)	6 (12)	8 (16)	11 (22)	24 (47)	32 (63)	16 (31)	13 (26)
Without IgG antibody (<i>n</i> = 60)	7 (19)	7 (19)	9 (24)	8 (22)	6 (16)	8 (22)	12 (27)	5 (14)	11 (30)
Without IgE antibody (<i>n</i> = 37)	13 (22)	11 (18)	12 (20)	12 (20)	12 (20)	15 (25)	22 (37)	10 (18)	17 (28)
Probands									
With IgG antibody (<i>n</i> = 50)	7 (14)	9 (18)	3 (6)	5 (10)	10 (20)	21 (42)	30 (60)	7 (14)	17 (34)
With IgE antibody (<i>n</i> = 41)	6 (15)	10 (24)	4 (10)	6 (15)	10 (24)	14 (34)	23 (56)	5 (12)	14 (34)

Subjects were categorized according to the presence of Par o 1-specific antibody, as determined by DARIA. Yates-corrected χ^2 .

TABLE 5 Association between HLA-DRB1* haplotype and levels of anti-Par o 1 antibody and of total serum IgE (Mann-Whitney rank test)

HLA-DRB1*	Phenotype											
	IgG antibody (U/ml)				IgE antibody (U/ml)				Total IgE (IU/ml)			
	a	b	Z'	p	a	b	Z'	p	a	b	Z'	p
Parents												
B1*1101 (<i>n</i> = 23)	64	54	-1.416	NS	55	56	-.23	NS	53	57	-.517	NS
B1*1104 (<i>n</i> = 39)	67	50	-2.767	<0.0028	64	52	-2.12	<0.017	55	57	-.309	NS
B1*1101 a/o 1104 ^a (<i>n</i> = 54)	67	46	-3.424	<0.0001	61	52	-1.627	<0.050	55	57	-.307	NS
B1*03 (<i>n</i> = 18)	46	58	-1.508	NS	51	57	-.85	NS	68	54	-1.792	<0.036
B1*03 ^b (<i>n</i> = 14)	40	58	-2.055	<0.021 (-)	44	55	-1.314	NS	72	54	-1.963	<0.025
Probands												
B1*1101 (<i>n</i> = 11)	35	28	-1.222	NS	39	27	-2.175	<0.0146	30	28	-.4	NS
B1*1104 (<i>n</i> = 20) ^a	35	27	-1.647	<0.0495	28	31	-.606	NS	24	30	-1.4	NS
B1*1101 a/o 1104 (<i>n</i> = 30)	34	24	-2.206	<0.0119	31	28	-.679	NS	26	31	-1.199	NS
B1*03 (<i>n</i> = 7)	17	31	-2.034	<0.0212 (-)	21	31	-1.527	NS	24	29	-.631	NS
Siblings (total IgE < 125 IU/ml)												
B1*1101 (<i>n</i> = 10)	24	18	-1.624	<0.053	22	19	-.812	NS	21	19	-.564	NS
B1*1104 (<i>n</i> = 12) ^a	23	18	-1.236	NS	24	17	-1.827	<0.034	16	21	-1.429	NS
B1*1101 a/o 1104 (<i>n</i> = 21)	23	15	-2.238	<0.0125	21	17	-1.154	NS	19	21	-.617	NS
B1*03 (<i>n</i> = 7)	8	22	-3.079	<0.001 (-)	16	20	-.922	NS	22	19	-.527	NS
Siblings (total IgE ≥ 125 IU/ml)												
B1*1101 (<i>n</i> = 8)	23	18	-1.274	NS	25	18	-1.623	<0.053	18	19	-.351	NS
B1*1104 (<i>n</i> = 15)	22	17	-1.285	NS	18	20	-.614	NS	17	21	-1.098	NS
B1*1101 a/o 1104 ^a (<i>n</i> = 21)	22	15	-2.085	<0.0188	20	19	-.593	NS	18	21	-.889	NS
B1*03 (<i>n</i> = 4)	11	20	-1.517	NS	13	20	-1.219	NS	20	19	-.196	NS

(-) indicates association with low levels. Par o 1 specific IgG and IgE determined by DARIA. Total IgE level determined by PRIST (Pharmacia).

^aSubjects DRB1*1101 and/or 1104.

^bSubjects DRB1*03 but not DRB1*1104.

was a trend toward an excess of DRB1*03 also in non-responder siblings which reached statistical significance (for IgG antibody) in the low total IgE subgroup (Table 5; $p < 0.001$). In parents, HLA-DRB1*03 had a significant association with high levels of total IgE ($p < 0.036$).

TDT Analysis

The Transmission Disequilibrium Test was introduced by Spielman as a test for linkage between a complex disease and a genetic marker and is particularly useful in cases where an association of the disease with a locus allele has already been found [15]. In the study group, 20 families had at least one parent DRB1*1101, and 27 families had at least one parent DRB1*1104 (40 families had at least one parent DRB1*1101 and/or DRB1*1104). In 20 informative families, DRB1*1104 was transmitted in 35 meiotic cycles (18 paternal and 17 maternal) and not transmitted in 32 meiotic cycles (19 paternal and 13 maternal). DRB1*1101 was both transmitted and not transmitted in 14 meiotic cycles each.

DISCUSSION

The results of this study demonstrate the linkage between the locus HLA-DRB1* and phenotypes related to allergy to *Parietaria*. Linkage was defined by significant excess of allele sharing (identity by descent) in affected sibling pairs. The phenotypes in linkage with DRB1* were the following: (1) skin test positivity to *Parietaria* ($p < 0.01$); (2) IgE and IgG antibody response to the major allergen Par o 1 ($p < 0.025$ and <0.020 , respectively); and (3) absence of detectable IgE antibody response to Par o 1 ($p < 0.020$).

The effect of HLA II class allelic variation on the ability to mount antibody response to certain allergens has been investigated in a series of classic genetic-epidemiologic studies [16]. Few of the reported associations have been tested for linkage, although this is an essential test, as allelic frequencies are influenced by ethnic and geographic factors. The HLA region has been reported to be in linkage with asthma, in a sibling-pairs-based study of 28 Columbian families recruited through asthmatic siblings allergic to mites ($p < 0.0001$) [17], and with antibody response to the allergens Alt a 1 and Fel d 1, in a larger study on 83 English families ($p < 0.05$).

and <0.02 , respectively) [18]. Recently, in two genomic searches, evidence has been obtained for the linkage of HLA region markers with serum total IgE levels (80 British families, $p < 0.05$) [19] and with IgE antibodies specific for the mite allergen Der p 1 (45 Australian families, $p < 0.0064$) [20].

For association analysis, the study population was subdivided into parents and siblings as reported in Table 1. Although this was not a study on asthma, the observation that in parents this clinical phenotype was strongly associated with high levels of IgE and IgG antibodies to Par o 1 (Table 3; $p < 0.0001$) is interesting, as it indicates that sensitization to *Parietaria* pollen is a key initiating event and does not support the hypothesis that only indoor allergens are a direct environmental cause of asthma [21, 22].

Previously, we reported that Par o 1-specific IgG and IgE antibody responses were positively associated with DRB1*1101 and/or DRB1*1104 (these alleles were not discriminated) and negatively associated with DRB1*03 [11]. In the present study, these associations were essentially confirmed by Mann-Whitney analysis (Table 5). In parents and probands, the magnitude of IgG antibody response to Par o 1 was positively associated with DRB1*1101 and/or DRB1*1104 ($p < 0.0001$ and <0.0019 , respectively) and negatively associated with DRB1*03 ($p < 0.021$ and <0.0212 , respectively); however, the allele associated with high IgE antibody response was DRB1*1104 in parents ($p < 0.017$) and DRB1*1101 in probands ($p < 0.0146$). This differential association of Par o 1-specific IgE antibody response was reproduced between siblings categorized according to low (<125 IU/ml) and high (≥ 125 IU/ml) total serum IgE levels. In siblings with low total IgE levels, the allele associated with high IgE antibody response was DRB1*1104 ($p < 0.034$), whereas, in siblings with high total IgE levels, it was DRB1*1101 ($p < 0.053$).

These observations indicate that DRB1*1101 and DRB1*1104 have a differential influence on IgG and IgE antibody synthesis, and that HLA-dependent genetic regulation of IgE antibody synthesis differs between subjects with high and low total IgE levels. The notion—derived from studies in animal models—that the binding affinity of peptides for major histocompatibility complex products is a factor regulating TH1/TH2-like T helper cells' response, and therefore, B cells' IgE synthesis, could be relevant to the first observation [23–25]. DRB1*1104 and DRB1*1101 differ in a single amino acid substitution at position 86—a position known to influence the specificity of peptide binding [26]. Allergen-specific IgE is a parameter influenced by allergen specificity and by allergen independent metabolic pathways [27, 28]. The differential DRB1* association observed in subjects with high and low total IgE levels

could reflect the interaction between HLA genes, controlling immune recognition, and genes at closely linked loci, controlling allergen-independent metabolic pathways leading to IgE synthesis. The DRB1* genotype could help to discriminate subsets of patients where differential therapeutic/preventive strategies should be applied. A gene closely linked to the HLA region, associated with asthma and increased total IgE level, is the tumor necrosis factor gene [29, 30].

DRB1*03 was associated with low levels of IgG antibody response to Par o 1 (Table 5). In parents, this allele was associated with high total IgE levels ($p < 0.025$). Possible explanations include sensitization to nonidentified allergens and influence of alleles at closely linked loci, including the tumor necrosis factor gene [29].

DRB1*11 and DRB1*03 are associated with responsiveness to other allergens and with certain diseases. DRB1*11 is positively associated with skin test monosensitization to wasp venom [31], protection against severe hepatitis virus infection [32], and Mediterranean Kaposi's sarcoma [33]. DRB1*03 is positively associated with specific IgE to inhaled anhydrides [34] and the birch pollen allergen Bet v 1 [35]. It contributes to susceptibility to myasthenia gravis [36], low responsiveness to hepatitis B vaccines [37], and protection against a subset of Crohn's disease [38].

The results of this study confirm that HLA-DRB1* genes (or genes in linkage disequilibrium) contribute to susceptibility to (or to protection against) allergy to *Parietaria*, and suggest that total IgE level is a parameter which allows for the definition of subsets of genetically predisposed subjects. The results favor the hypothesis that different pathogenetic mechanisms, based on allergen-specific and allergen-independent metabolic pathways, lead to the clinical syndrome of allergy. The results also suggest that a definition of phenotypes, taking into account the specificity of allergic sensitization, can contribute to increased resolution power of molecular genetic analysis.

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