

HLA-DRB1*01 alleles are associated with sensitization to cockroach allergens

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Background: Sensitization to cockroach allergens is an important epidemiologic risk factor for asthma, particularly among African Americans living in urban environments. A recent genome screen in the Hutterites, a white founder population, identified a linkage between an HLA-linked marker and sensitization to cockroach allergens.

Objective: Our purpose was to determine whether alleles at one or more HLA loci are associated with sensitization to cockroach allergens in ethnically diverse populations.

Methods: Alleles at 14 HLA region loci were studied in the Hutterites. On the basis of these results, selected loci were examined in 54 African Americans with cockroach sensitization (cases) and 65 African Americans without cockroach sensitization (controls). Sensitivity to cockroach allergens was assessed in both samples by skin prick test to purified cockroach allergens (*Periplaneta americana* and *Blattella germanica*).

Results: Significant associations between cockroach allergies and DRB1*0101 ($P_{\text{corrected}} = .0066$), DQA1*0101 ($P_{\text{corrected}} = .0012$), and DQB1*0501 ($P_{\text{corrected}} = .00096$) were detected in the Hutterites. In the African American sample, the most significant association was with the DRB1*0102 allele ($P_{\text{corrected}} = .0088$, odds ratio 16.4, 95% confidence interval 2.0, 131). The DRB1*0101 allele was infrequent in the African American sample (frequency 0.06) and the DRB1*0102 allele was absent in the Hutterites. DRB1*0101 and DRB1*0102 are closely related alleles that differ from nearly all other DRB1 alleles at 3 amino acids in the 1 peptide binding domain of the HLA-DR molecule. **Conclusions:** The DRB1*0101 allele in the Hutterites and the DRB1*0102 allele in African Americans confer risk for cockroach sensitization. Elucidating this interaction at the molecular level may allow for more targeted treatment and prevention of atopic asthma in inner-city populations. (*J Allergy Clin Immunol* 2000;105:960-6.)

Key words: HLA, cockroach allergens, asthma, Hutterites, African Americans

A large number of potential environmental risk factors for asthma have been identified, such as respiratory viral infections,^{1,2} secondhand smoke,³ and indoor allergens.⁴

Abbreviations used

BHR:	Bronchial hyperresponsiveness
CI:	Confidence interval
CSGA:	Collaborative Study on the Genetics of Asthma
OR:	Odds ratio
SPT:	Skin prick test
TDT:	Transmission disequilibrium test

Among the latter, sensitization to cockroach allergen has been associated with asthma severity, particularly in urban areas⁵ and among African American populations.⁶⁻⁸ In a large epidemiologic study of asthma in inner-city children, asthma morbidity was highest among children with exposure to high bedroom levels of cockroach allergen and with cockroach allergy, measured by a skin prick test (SPT).⁷ Interestingly, not all children with high levels of exposure were allergic to cockroach, and exposure per se did not correlate with asthma severity, suggesting that genetic factors may also be important. Identifying the genes that influence sensitivity to cockroach allergens could allow for more targeted treatment and prevention of atopic asthma, particularly in inner-city populations.

A genome screen to identify loci that influence positive SPTs to 14 airborne allergens was recently completed in the Hutterites, a founder population of European origins.⁹ Founder populations are particularly useful for identifying genes for complex traits, such as asthma and atopy, because the small number of ancestral genomes present in these populations may result in a smaller number of susceptibility alleles at any particular locus and perhaps a smaller number of susceptibility loci. This reduced heterogeneity should facilitate the search for complex trait genes. A significant association between the microsatellite marker DQ.CAR and a positive SPT to cockroach was detected in the Hutterites.⁹ The DQ.CAR locus is located in the HLA class II region, between the *HLA-DQA1* and *HLA-DQB1* loci. Because of their role as immune response genes, the HLA loci were considered as positional candidate genes for the DQ.CAR-associated atopy-susceptibility locus. In this report we present results of studies of positive SPT to cockroach allergens and polymorphisms in 14 HLA region genes in the Hutterites. To further characterize the susceptibility locus and to determine whether the same susceptibility allele could influence cockroach allergies in an urban population, we studied selected HLA loci in 119 African American subjects who were evaluated by SPT to cockroach allergens.

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METHODS

The Hutterites

The Hutterites originated in the 1500s in the Tyrolean Alps and have been reproductively isolated for more than 200 years. As a result, the more than 35,000 North American Hutterites are descendants of fewer than 90 ancestors.^{10,11} The Hutterites live on communal farms of 10 to 15 families, which ensures that all Hutterites are exposed to a similar environment, including airborne allergens and other environmental and lifestyle factors that may influence susceptibility to asthma. Of particular relevance to these studies is that smoking is prohibited (and rare) in the population.

Sample composition

Nine Hutterite communal farms (colonies) in South Dakota were visited in 1994 and 1996-1997 as part of our participation in the Collaborative Study on the Genetics of Asthma (CSGA).^{12,13} These colonies were selected because of a previously reported high prevalence of atopy¹⁴ and to represent different lines of colony descent.^{11,15} All members of the colonies who were 6 years old or older and home at the time of our visit were included in these studies. Our sample included 683 Hutterites, all of whom were derived from 64 Hutterite ancestors who lived in the early 1700s to the early 1800s.¹³

The African American subjects were recruited from asthma clinics in Chicago as part of the CSGA. This sample includes the following: 34 families that were ascertained through 2 asthmatic siblings (CSGA1)¹² and 34 children and 26 adults with asthma (CSGA2). In this study the asthmatic proband from the CSGA2 sample was classified as either a case (positive SPT to cockroach allergens) ($n = 35$) or a control (negative SPT to cockroach allergens) ($n = 25$). One case (positive SPT) was randomly selected from among the proband's sibship in each CSGA1 family ($n = 19$) and one or more unrelated controls (negative SPT) was selected from each CSGA1 family ($n = 40$). Our final sample included 54 unrelated cases and 65 unrelated controls.

Clinical evaluation

The details of the protocols used to evaluate subjects were reported previously.^{12,13} Of particular relevance to this study is that SPT was performed with the following 14 standardized allergens: house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), cockroaches (*Blattella germanica*, *Periplaneta americana*), molds (*Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*), pollens (*Lolium perenne*, *Ambrosia artemisiifolia*, *Artemisia vulgaris*, *Quercus alba*, *Betula verrucosa*), and animal dander (*Felix domesticus*, *Canis familiaris*). All tests included a negative (saline solution) and positive (histamine) control. A skin prick test was considered positive if the average wheal was at least 3 mm greater than the average wheal diameter of the negative control.

The diagnosis of asthma was based on the following criteria: (1) 2 of the following symptoms: cough, wheeze, or dyspnea; (2) either bronchial hyperresponsiveness (BHR) characterized by a fall from baseline FEV₁ of 20% at 25 mg/mL methacholine or bronchodilator reversibility defined by 15% increase from baseline FEV₁ after bronchodilator use; and (3) <3 pack-years of cigarette exposure.

Genotyping

Alleles at 14 loci in the HLA region were previously genotyped in the Hutterites: *HLA-A*, *HLA-C*, *HLA-B*, *TNF- α* , *Bf*, *C4A/C4B*, *DRB1*, *DQA1*, *DQB1*, *LMP2*, *TAP1*, *LMP7*, and *DPB1*.¹⁶ Genotyping methods are reported elsewhere.¹⁶ On the basis of the results in the Hutterites (described below), African American subjects were genotyped for *HLA-DRB1*, *-DQA1*, *-DQB1* alleles with use of the same methods.

Statistical analysis

The evidence for linkage between each HLA locus and the phenotype of a positive SPT to cockroach was evaluated in the Hutterites with the transmission disequilibrium test (TDT).^{17,18} The TDT is a test of both linkage and association and compares the number of transmissions of an allele from heterozygous parents to affected offspring with the expected (random) number of transmissions assuming no linkage (ie, 50:50). TDTs were performed for every allele with at least 10 transmission events at each locus. Differences in HLA frequencies between African American cases with positive SPTs and control subjects with negative SPTs were examined by chi-square tests. Odds ratios (OR) were calculated and 95% confidence intervals (CI) derived on the basis of the normal approximate to the distribution of log(OR).¹⁹ All *P* values were corrected for multiple comparisons by the Bonferroni correction (ie, multiplying the uncorrected *P* value by the number of comparisons at each locus).

RESULTS

Clinical characteristics of study samples

The clinical characteristics of the Hutterite and African American samples are shown in Tables I and II. The differences in clinical characteristics between the 2 samples reflect, in part, differences in sampling strategies. The Hutterite sample was population based and participants were unselected with respect to asthma or associated phenotypes; the African American families were selected through one or more individuals with asthma. In these samples, 45% of African Americans and 22% of Hutterites were positive to German (*B germanica*) or American (*P americana*) cockroach. Among the individuals who had a positive SPT to cockroach allergens, 65% of Hutterites and 63% of African Americans were reactive to both German and American cockroach. The remaining Hutterites with a positive SPT were nearly equally reactive to only American cockroach or only German cockroach. In contrast, 15% of African American cases with a positive SPT were reactive to American cockroach only, whereas 22% were reactive to German cockroach only. The greater prevalence of reactivity to German cockroach has been reported for other urban populations,²⁰ and the small difference in allergen specificity between the African American and Hutterite samples likely reflects differences in exposures in the urban Chicago and rural South Dakota environments. Among individuals who had a negative SPT to cockroach allergens, only 29% (158/536) of Hutterites but 46% (30/65) of African Americans had a positive SPT to one or more other allergens ($P = .006$). The proportion of subjects with asthma or with BHR did not differ among those sensitized to *P americana* only, *B germanica* only, or to both in either sample ($P > .15$ for all comparisons) (Table III).

Results of genetic analyses in the Hutterites

The results of the TDT analyses in the Hutterites for all alleles at 14 loci are shown in Appendix I. Only alleles at the class II loci, *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* were significantly associated with positive SPT to cockroach allergens after adjustment for multiple

TABLE I. Clinical characteristics of Hutterite subjects who were positive and negative to cockroach allergens

	Positive SPT to cockroach allergens	Negative SPT to cockroach allergens
Sample size	147	536
Mean age (y) (SD)	28.1 (15.9)	30.4 (17.3)
Percent with asthma*	9.5	9.7
Percent BHR*	25.2	20.3
Mean percent predicted FEV ₁	100.3	100.9
Sex ratio (male/female)	87:60	229:307
Percent positive SPT to <i>P americana</i> only (No.)	19.0	0
Percent positive SPT to <i>B germanica</i> only (No.)	15.6	0
Percent positive SPT to <i>P americana</i> and <i>B germanica</i> (No.)	65.3	0
Percent positive SPT other allergens (No.)†	67.3	29.5

For more detailed descriptions of the clinical characteristics of the samples, see references 9 and 13.

*Diagnosis based on CSGA criteria.¹²

†See Methods.

TABLE II. Clinical characteristics of African American subjects who were positive and negative to cockroach allergens

	Positive SPT to cockroach allergens	Negative SPT to cockroach allergens
Sample size	54	65
Mean age (y) (SD)	23.3 (19.0)	31.0 (18.0)
Percent with asthma*	94.4	64.6
Percent BHR*	96.3	67.7
Mean percent predicted FEV ₁	66.2	74.1
Sex ratio (male/female)	29:25	29:36
Percent positive SPT to <i>P americana</i> only	15.0	0
Percent positive SPT to <i>B germanica</i> only	22.2	0
Percent positive SPT to <i>P americana</i> and <i>B germanica</i>	63.0	0
Percent positive SPT other allergens†	83.3	46.1

For more detailed descriptions of the clinical characteristics of the samples, see reference 12.

*Diagnosis based on CSGA criteria.¹²

†See Methods.

TABLE III. Clinical characteristics of samples by cockroach specificities

	Total No.	No. asthmatic (%)	No. BHR (%)
Hutterites	683		
Positive SPT to <i>P americana</i> only	28	3 (10.7)	10 (35.7)
Positive SPT to <i>B germanica</i> only	23	2 (8.7)	2 (8.7)
Positive SPT to both	96	9 (9.4)	25 (26.0)
Negative SPT to both	536	52 (9.7)	109 (20.3)
African Americans	119		
Positive SPT to <i>P americana</i> only	8	8 (100)	8 (100)
Positive SPT to <i>B germanica</i> only	12	12 (100)	12 (100)
Positive SPT to both	34	31 (91.2)	32 (94.1)
Negative SPT to both	65	42 (64.6)	44 (67.7)

comparisons. The HLA-DRB1*0101 allele was transmitted to 66 and not transmitted to 32 affected offspring of 98 heterozygous parents (expected 49:49; $P_{uncorrected} = .00059$; $P_{corrected} = .0066$), the HLA-DQA1*0101 allele was transmitted to 70 and not transmitted to 32 affected offspring of 102 heterozygous parents (expected 51:51; $P_{uncorrected} = .00017$; $P_{corrected} = .0012$), and the

DQB1*0501 allele was transmitted to 71 and not transmitted to 32 affected offspring of 103 heterozygous parents (expected 51.5:51.5; $P_{uncorrected} = .00012$; $P_{corrected} = .00096$). No alleles at any of the other loci examined were associated with this phenotype ($P_{corrected} > .10$ for all alleles at other loci).

Among individuals who had a positive SPT to cockroach allergens, there was no difference in allele frequencies between individuals sensitized to different cockroach allergens. For example, 14 of the 28 (50%) subjects who were positive to American cockroach only, 12 of 23 (50%) who were positive to German cockroach only, and 44 of 96 (45%) who were positive to both cockroaches carried the DRB1*0101 alleles ($P > .50$). The overall frequency of this allele in the Hutterite population is 0.25.¹⁶

DRB1*0101, DQA1*0101, and DQB1*0501 occur on 8 Hutterite haplotypes (or chromosomes) (Table IV). A more detailed description of these haplotypes and their relationship to other Hutterite haplotypes is reported in Weitkamp and Ober.¹⁶ The DQA1*0101 and DQB1*0501 alleles are always present on the same haplotypes and occur together with DRB1*0101 on 7 haplotypes and with DRB1*1001 on a single haplotype. The DRB1*1001 was not significantly overtransmitted to affected offspring, but there were few informative transmissions for this allele (18 transmitted and 12 nontransmitted). All

TABLE IV. Hutterite haplotypes with HLA-DRB1*0101, DQA1*0101, or DQB1*0501

Haplotype	HLA loci							Haplotype frequency	TR:NT
	A	C	B	DRB1	DQA1	DQB1	DPB1		
1017	2	0401	62	0101	0101	0501	0402	0.033	16:6*
1019	3	0202	27	0101	0101	0501	0201	0.035	22:15
1023	11	0401	35	0101	0101	0501	0301	0.017	8:4
1026	24	0501	44	0101	0101	0501	0301	0.016	6:4
1027	24	1401	51	1001	0101	0501	0501	0.052	18:12
1080	2	0304	60	0101	0101	0501	0201	0.001	1:0
1088	3	0401	35	0101	0101	0501	0401	0.018	7:6
1089	3	0401	35	0101	0101	0501	0402	0.056	16:7
Pooled								0.228	94:54†

See reference 16 for complete description of haplotypes. TR:NT, Number of haplotypes transmitted and not transmitted from heterozygote parent to child with positive SPT to cockroach allergens.

* $P_{\text{uncorrected}} = .033$.

† $P_{\text{uncorrected}} = .001$.

these haplotypes were overtransmitted to offspring with a positive SPT to cockroach, but the deviation from random expectations was only marginally significant for one haplotype (haplotype 1017, $P_{\text{uncorrected}} = .033$). These data suggest that it is not a haplotype per se that is associated with a positive SPT to cockroach allergens in the Hutterites but rather that the DRB1*0101, *DQA1*0101, or DQB1*0501 alleles acting alone or together confer risk.

Because the three associated alleles occur together on the same haplotypes in the Hutterites, it was not possible to evaluate the individual effects of each locus or allele on sensitivity to cockroach allergens in this population. Therefore we extended these studies to include a distantly related population of African Americans. The relatively long evolutionary time separating Europeans and Africans (40,000 to 100,000 years)²¹ increases the likelihood that HLA alleles are present on different haplotype backgrounds in these 2 populations. On the basis of the results of our studies in the Hutterites, the African American sample was genotyped for alleles at the *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* loci.

Results of genetic analyses in African Americans

There were significantly more DRB1*0102-positive cases compared with controls in the African American sample ($P_{\text{corrected}} = .0088$; OR 16.4, 95% CI 2.0, 131) (Table V). In addition, there were fewer DRB1*1101-positive cases than controls, which was not significant after adjustment for multiple comparisons ($P_{\text{corrected}} = .086$), and a small excess of DRB1*1302 cases compared with controls, which was only nominally significant after adjustment for multiple comparisons ($P_{\text{corrected}} = .039$) (Table V). The DRB1*0101 allele, which was associated with a positive SPT to cockroach allergens in the Hutterites, and the DRB1*1001 allele, which was present on one Hutterite haplotype carrying DQA1*0101 and DQB1*0501 alleles, were infrequent in the sample (frequency *0101 = 0.059, *1001 = 0.008). None of the 8 subjects who were positive to American cockroach only, 4 of

the 12 (33.3%) subjects positive to German cockroach only, and 7 of the 34 (20.6%) subjects positive to both cockroaches carried the DRB1*0102 allele ($P_{\text{exact}} > .50$).

The DQA1*0101 was increased in the cases compared with controls, but this difference was not significant after adjustment for multiple comparisons ($P_{\text{corrected}} = .091$). The DQB1*0501 allele was also increased in the cases compared with controls, and this difference remained nominally significant after adjustment for multiple comparisons ($P_{\text{corrected}} = .029$) (Table V). The small increase in frequency of the DQA1*0101 and DQB1*0501 alleles in the African American cases reflects the fact that *all* individuals with the DRB1*0102 allele also carried the DQA1*0101 and DQB1*0501 alleles. However, unlike in the Hutterites, many other individuals with DQA1*0101 or DQB1*0501 alleles carried alleles other than DRB1*0102 at the *HLA-DRB1* locus. For example, among the non-DRB1*0102 positive individuals, there were 11 cases and 12 controls positive for DQA1*0101 and 11 cases and 9 controls positive for DQB1*0501.

DISCUSSION

These studies demonstrate that HLA class II DRB1*01 alleles confer susceptibility to cockroach sensitivity in 2 distantly related populations. Sensitization to cockroach allergens was significantly associated with DRB1*0102 in African Americans ($P_{\text{corrected}} = .0068$; OR 16.4, 95% CI 2.0-131) and with DRB1*0101 in the Hutterites (TDT $P_{\text{corrected}} = .0066$). The DQA1*0101 and DQB1*0501 alleles also showed evidence of association with a positive SPT to cockroach allergens in the Hutterites. However, the associations with DQA1*0101 and DQB1*0501 were likely due to linkage disequilibrium with the DRB1*0101. As we hypothesized, the DQA1*0101 and DQB1*0501 alleles were on different haplotype backgrounds in the African Americans than in the Hutterites, as evidenced by the weak association with DQA1*0101 and DQB1*0501 in this population. These data indicate that the primary association with cockroach allergies is with DRB1*01 alleles. In fact, associations

TABLE V. The number (frequency) of cases and controls who were positive for each allele

Alleles	Cases (n = 54)	Controls (n = 65)
<i>HLA-DRB1</i>		
0102*	11 (0.20)	1 (0.02)
0301	9 (0.17)	10 (0.15)
0302	3 (0.06)	11 (0.17)
0701	6 (0.11)	10 (0.15)
0804	7 (0.12)	8 (0.12)
1101†	1 (0.02)	11 (0.17)
1201	7 (0.12)	1 (0.02)
1301	3 (0.06)	8 (0.12)
1302‡	7 (0.12)	0 (0.00)
1303	7 (0.12)	6 (0.09)
1501	6 (0.11)	12 (0.18)
1503	10 (0.19)	18 (0.28)
Other§	33 (0.61)	37 (0.57)
<i>HLA-DQA1</i>		
0101	22 (0.41)	13 (0.20)
0102	29 (0.54)	30 (0.46)
0103	0	5 (0.08)
0201	7 (0.13)	10 (0.15)
0301	14 (0.26)	17 (0.26)
0401	7 (0.13)	17 (0.26)
0501	18 (0.33)	30 (0.46)
<i>HLA-DQB1</i>		
0201	18 (0.33)	25 (0.38)
0301	14 (0.26)	26 (0.40)
0302	10 (0.19)	9 (0.14)
0402	6 (0.11)	11 (0.17)
0501#	21 (0.38)	10 (0.15)
0502	6 (0.11)	3 (0.05)
0602	17 (0.31)	26 (0.40)
Other**	10 (0.19)	10 (0.15)

Alleles in 5 or fewer individuals in both samples were pooled into "other."

* $P_{\text{uncorrected}} = .00068$; $P_{\text{corrected}} = .0088$.

† $P_{\text{uncorrected}} = .0066$; $P_{\text{corrected}} = .086$.

‡ $P_{\text{uncorrected}} = .003$; $P_{\text{corrected}} = .039$.

§Includes alleles 0101, 0401, 0402, 0403, 0404, 0405, 1001, 1102, 1101, 1103, 1304, 1401, 1402, 1601, 1602, and 1603.

|| $P_{\text{uncorrected}} = .013$; $P_{\text{corrected}} = .091$.

$P_{\text{uncorrected}} = .0036$; $P_{\text{corrected}} = .029$.

**Includes alleles 03032, 0601, 0603, 0604, and 0605.

between HLA-DR antigens have been reported for a number of allergens, such as associations between DR2 and ragweed,^{22,23} DR5 and rye grass,²⁴ DR3 and American fever few pollen,²⁵ DRw52 and birch pollen,²⁶ DR4 and molds and DR1 and cat dander,²⁷ and DRB1*1101 and house dust mite.²⁸ However, this is the first report of an HLA association with cockroach allergy (defined as a positive SPT) and identification of a genetic locus influencing sensitivity to cockroach allergens.

The fact that 2 different DRB1*01 alleles are associated with cockroach sensitivity in different populations may provide some clues to the molecular basis of cockroach allergies. The DRB1*0101 and DRB1*0102 are closely related alleles that differ from each other at only 2 amino acid sites. DRB1*0101 has a valine at position 85 and a glycine at position 86, whereas DRB1*0102 has an alanine at position 85 and a valine at position 86. The fact that both alleles are associated with a positive SPT to

cockroach allergen suggests that amino acids 85 and 86 may not be critical in binding peptides derived from cockroach proteins. On the other hand, the DRB1*0101 and DRB1*0102 alleles differ from all other DRB1 alleles (except DRB1*1001 and DRB1*1130) at amino acids 11, 13, and 31, which are within the peptide-binding 1 domain of the HLA-DR molecule. These alleles encode a leucine at position 11, a phenylalanine at position 13, and an isoleucine at position 31. In all other DRB1 alleles, position 31 is a phenylalanine but positions 11 and 13 are highly variable and close to the P4 and P6 peptide binding pocket.²⁹ Because the DRB1*1001 allele is a low-frequency allele that was present on only a single Hutterite haplotype and in a single individual in the African American sample, and the DRB1*1130 allele was absent in both samples, the relationship between these alleles and a positive SPT to cockroach allergies could not be assessed in this study. However, the associations between cockroach allergies and DRB1*0101 in the Hutterites and DRB1*0102 in the African Americans suggest that a leucine at amino acid 11, an isoleucine at amino acid 13, and a phenylalanine at amino acid 31 act alone or together in binding cockroach antigens and eliciting an immune response. Molecular characterization of cockroach antigen binding by DRB1*0101 or DRB1*0102 molecules may suggest pharmaceutical interventions in genetically susceptible individuals. Further, differential binding affinities between these 2 related alleles that differ at amino acids 85 and 86 may influence the strength of the response to cockroach antigen and contribute differentially to cockroach-related asthma risk in whites and African Americans.

Although sensitization to *P americana* was less common than sensitization to *B germanica* in the African American sample and although none of the 8 subjects with a positive SPT to *P americana* only were DRB1*0102+, the sample size was too small to draw conclusions about differential susceptibility to the American versus German cockroach. In the Hutterites, however, 14 of the 28 (50%) subjects with a positive SPT to *P americana* only and 12 of the 23 (50%) subjects with a positive SPT to *B germanica* only were DRB1*0101 positive, suggesting that the DRB1*0101 allele is associated with sensitization to both the American and German cockroach.

It is noteworthy that, although DRB1*01 alleles are significantly associated with sensitivity to cockroach allergens both in a rural white farming population and an urban African American sample, cockroach sensitivity per se is not associated with asthma and BHR in Hutterites (Table III; asthma, $P > .50$; BHR, $P > .05$). Because of the way in which the African American sample was selected, it is not possible to draw conclusions regarding cockroach sensitivity and asthma in this study. However, previous epidemiologic studies in US cities, including Chicago, have identified cockroach sensitization as a major risk factor for asthma.⁵⁻⁸ The fact that cockroach sensitivity is associated with asthma in urban African Americans but not in the Hutterites could be due to differences in environmental or genetic risk factors. In

particular, environmental differences with respect to the timing or levels of exposure to cockroach allergen may be important. For example, the Hutterites live communally and all meals are prepared and eaten in a central kitchen. As a result, cockroach exposure in the homes may be quite low. Hutterite children under the age of 5 years do not eat in the communal dining room or spend time in the barns, another potential source of cockroach exposure. Thus exposure to cockroach allergens before the age of 5 years may be low among the Hutterites. In contrast, exposure to cockroach allergens among urban African Americans likely begins shortly after birth. Such differences in early life exposures can have a significant impact on subsequent risks for asthma.³⁰⁻³² Furthermore, the levels of cockroach allergen may be much lower overall on Hutterite farms than in city homes and may not provide a sufficient "trigger" for asthma symptoms and BHR. Alternatively, differences between the 2 samples may reflect differences in the DRB1 susceptibility alleles in each population. For example, the DRB1*0102 allele may have a higher affinity for cockroach allergens and elicit a stronger response to bound antigens than the DRB1*0101 allele, as discussed above. Such gene-environment interactions are likely to be common risk factors for complex disorders such as asthma. Identifying susceptibility loci and environmental risk factors, such as the DRB1*01 association with cockroach sensitization identified in this study, is the first step in elucidating the more complex pathways involved in the pathogenesis of atopy and asthma.

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REFERENCES

- Busse WW. The relationship between viral infections and the onset of allergic disease in asthma. *Clin Exp Allergy* 1989;19:1-9.
- Martinez FD. Viral infections and the development of asthma. *Am J Respir Crit Care Med* 1995;151:1644-8.
- Neuspiel DR, Rush D, Butler NR, Golding J, Bijur PE, Kuron M. Parental smoking and post-infancy wheezing in children: a prospective study. *Am J Public Health* 1989;79:168-71.
- Platts-Mills TA, Ward GW, Sporik R, Gelber LE, Chapman MD, Heyman PW. Epidemiology of the relationship between exposure to indoor allergens and asthma. *Int Arch Allergy Appl Immunol* 1991;94:339-45.
- Kang BC, Johnson J, Veres-Thorner C. Atopic profile of inner-city asthma with a comparative analysis on the cockroach sensitive and ragweed-sensitive subgroups. *J Allergy Clin Immunol* 1993;92:393-401.
- Sarpong SB, Hamilton RG, Eggleston PA, Adkinson NF. Socioeconomic status and race as risk factors for cockroach allergen exposure and sensitization in children with asthma. *J Allergy Clin Immunol* 1996;97:1393-401.
- Rosenstreich DL, Eggleston P, Kattan M, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med* 1997;336:1356-63.
- Togias A, Horowitz E, Joyner DJL, Malveaux F. Evaluating the factors that relate to asthma severity in adolescents. *Intern Arch Allergy Immunol* 1997;113:87-95.
- Ober C, Tselenko A, Willadsen SA, et al. Genome-wide screen for atopy susceptibility alleles in the Hutterites. *Clin Exp Allergy* 1999;4:11-5.
- Martin AO. The founder effect in a human isolate: evolutionary implications. *Am J Phys Anthropol* 1970;32:351-68.
- Ober C, Weitkamp LR, Cox N, Dytch H, Kostyu D, Elias S. HLA and mate choice in humans. *Am J Hum Genet* 1997;61:497-504.
- CSGA. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nature Genet* 1997;15:389-92.
- Ober C, Cox N, Parry R, et al. Genome-wide search for asthma susceptibility loci in a founder population. *Hum Mol Genet* 1998;7:1393-8.
- Schlenker EH, Lenardson G, McClain C, Barnes E, Parry R. Respiratory symptoms among farmers and ranchers in southeastern South Dakota. *South Dakota J Med* 1985;38:5-9.
- Mange AP. Growth and inbreeding of a human isolate. *Hum Biol* 1964;36:104-33.
- Weitkamp LR, Ober C. Ancestral and recombinant 16-locus HLA haplotypes in the Hutterites. *Immunogenetics* 1999;49:491-7.
- Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993;52:506-16.
- Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet* 1996;59:983-9.
- Breslow NE, Day NE. Statistical methods in cancer research, Vol 1: the analysis of case control studies. Lyon (France): IARC Scientific Publications, 1980. Publication No.: 32.
- Pope AM, Patterson R, Burge H. Indoor allergens: assessing and controlling adverse health effects. Washington (DC): National Academy Press, 1993.
- Jorde LB, Bamshad M, Rogers AR. Using mitochondrial and nuclear DNA markers to reconstruct human evolution. *Bioessays* 1998;20:126-36.
- Marsh DG, Meyers DA, Freidhoff LR, et al. HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5.II: response after ragweed immunotherapy. *J Exp Med* 1982;155:1452-63.
- Marsh DG, Freidhoff LR, Ehrlich-Dautsky E, Bias WB, Roebber M. Immune responsiveness to *Ambrosia artemisiifolia* (short ragweed) pollen allergen Amb a VI (Ra6) is associated with HLA-DR5 in allergic humans. *Immunogenetics* 1987;26:230-6.
- Freidhoff LR, Ehrlich-Kautsky E, Meyers DA, Ansari AA, Bias WB, Marsh DG. Association of HLA-DR3 with human response to Lol p I and Lol p II allergens in allergic subjects. *Tissue Antigens* 1988;31:211-9.
- Sriramarao P, Sevlakumar B, Damodaran C, Subba Rao BS, Prakash O, Subba Rao PV. Immediate hypersensitivity to *Parthenium* rhinitis. I: association of HLA antigens and *Parthenium* rhinitis. *Clin Exp Allergy* 1990;20:555-60.
- Fischer GF, Pickl WF, Faé I, et al. Association between IgE response against Bet v I, the major allergen of birch pollen, and HLA-DRB alleles. *Hum Immunol* 1992;33:259-65.
- Young RP, Dekker JW, Wordsworth BP, et al. HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. *Clin Exp Allergy* 1994;24:431-9.
- Lara-Marquez ML, Yunis JJ, Layrisse Z, et al. Immunogenetics of atopic asthma: association of DRB1*1101 DQA1*0501 DQB1*0301 haplotype with *Dermatophagoides* spp.-sensitive asthma in a sample of the Venezuelan population. *Clin Exp Allergy* 1999;29:60-71.
- Jardetzky T. Crystal structures of the MHC class I and class II molecules. In: Browning M, McMichael A, editors. HLA and MHC: genes, molecules and function. Oxford: BIOS Scientific Publishers; 1996. 249-76.
- Weiss ST, Tager IB, Munoz A, Speizer FE. The relationship of respiratory infections in early childhood to the occurrence of increased levels of bronchial responsiveness and atopy. *Am Rev Respir Dis* 1985;131:573-8.
- Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332:133-8.
- Warner JA, Jones AC, Miles EA, Colwell BM, Warner JO. Prenatal origins of asthma and allergy. In: Chadwick DJ, Cardew G, editors. The rising trends in asthma. Chichester: John Wiley; 1997. Ciba Foundation Symposium 206.

APPENDIX I. Results of TDT with HLA and positive SPT to cockroach allergens in Hutterites

	TR:NT	Chi-square		TR:NT	Chi-square
HLA-A			C4		
1	48:39	1.16	A3	24:28	0.31
2	58:47	1.15	B1	17:14	0.29
3	52:35	3.32	BQ0	17:17	0
11	10:6	1	Other‡	22:21	0.02
24	34:51	3.4	DRB1		
26	6:9	0.6	0101	66:32	11.8
30	4:7	0.82	0301	17:38	8.02
31	9:13	0.73	0401	27:32	0.42
32	8:16	2.67	0404	11:14	0.36
Other*	4:11	3.3	0701	40:23	4.59
HLA-C			1001	18:13	0.81
0102	10:12	0.18	1101	18:33	4.41
0202	28:26	0.07	1301	27:35	1.03
0302	3:5	0.5	1501	11:15	0.62
0303	7:7	0	8032	12:9	0.43
0304	20:31	2.37	Other§	4:7	0.82
0401	45:24	6.39	DQA1		
0501	15:21	1	0101	70:32	14.16
0602	43:28	3.17	0102	13:20	1.48
0701	7:15	2.91	0103	27:35	1.03
0702	7:17	4.17	0201	40:23	4.59
1203	20:29	1.65	0301	35:43	0.82
1402	20:14	1.06	0401	12:9	0.43
1601	20:16	0.44	0501	30:65	12.89
HLA-B			DQB1		
7	2:8	3.6	0201	29:52	6.53
8	12:31	8.4	0301	31:44	2.25
13	10:15	1	0302	14:21	1.4
18	4:10	2.57	0303	37:19	5.79
27	36:30	0.55	0402	17:18	0.03
35	32:28	0.27	0501	71:32	14.77
44	6:4	0.4	0502	2:4	0.67
51	44:37	0.6	0602	12:15	0.33
57	37:19	5.79	0603	27:35	1.03
60	12:16	0.57	LMP7		
62	28:25	0.17	1	67:83	1.71
Other†	8:11	0.47	2	83:67	1.71
TNF-α			TAP1		
99	5:8	0.69	190	81:60	3.13
101	43:38	0.31	192	66:68	0.03
103	6:7	0.08	202	16:17	0.03
107	36:49	1.99	206	16:34	6.48
108	35:25	1.67	LMP2		
111	21:23	0.09	1	50:64	1.72
115	2:2	0	2	64:50	1.72
117	43:36	0.62	DPB1		
119	6:15	3.86	0101	7:13	1.8
121	3:7	1.6	0201	45:67	4.32
123	16:6	4.55	0202	5:8	0.69
Bf			0301	19:19	0
F	68:64	0.12	0401	65:68	0.07
F1	5:8	0.69	0402	32:15	6.15
S	67:68	0.01	0501	18:13	0.81
			1301	31:18	3.45
			1701	2:3	0.2

TR:NT, The number of haplotypes transmitted and not transmitted from heterozygote parent to child with positive SPT to cockroach allergens.

*33, 34.

†38, 50, 56.

‡A2, 2; A2, 3; A3, 3; A4; A6; AQ0; B1; B1, 2; B3.

§0405, 1601.