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*HLA-DRB1**15:01 allele protects from asthma susceptibility

To the Editor:

Asthma is a chronic inflammatory disease associated with genetic and environmental factors. The HLA locus is the most polymorphic and gene-dense region of the human genome and has been associated with a large number of infectious and autoimmune diseases.[1] Before the advent of genome-wide association studies (GWAS), *HLA-DRB1* and *HLA-DQB1* genes were independently associated with asthma and related traits in several candidate gene association studies.[2] The importance of these genes in the pathogenesis of asthma has recently been corroborated by both individual and meta-analyzed GWAS.[3-5] In spite of the evidence, the interpretation of these associations can be problematic because of the complex relationship between the allele at single-nucleotide polymorphisms (SNPs) and the variation at classical HLA alleles. Interestingly, classic alleles associate with stronger effects than individual SNPs and constitute the most likely functional variants.[6] Here, we aimed to test the association of SNPs from *HLA-DRB1* and *HLA-DQB1* with asthma in Spanish samples, and to uncover the classic alleles that are involved in the susceptibility to the disease.

In the discovery stage, DNA samples from 574 physician-diagnosed asthmatic patients from the Genetics of Asthma (GOA) study in the Spanish population were compared with samples of 1186 nonasthmatic subjects obtained from the Spanish National DNA Biobank (www.bancoadn.org).[7] An independent sample of 568 asthma cases and 787 controls was used for replication. For further description of the study design, see Fig E1, Online Repository text, and Table E1 in this article's Online Repository at www.jacionline.org.

A total of 22 SNPs capable of predicting classic alleles from *HLA-DRB1* and *HLA-DQB1* in European populations were genotyped in the discovery sample using a combination of different methods (see Table E2 in this article's Online Repository at www.jacionline.org).[8] Their performance on predicting *HLA-DRB1* and *HLA-DQB1* classic alleles was first assessed by genotyping 313 DNA samples from healthy Spanish individuals with paired data for classic alleles at a 4-digit resolution (Luminex, Austin, Tex). To impute classic alleles, we used a reference data set with more than 2500 samples of European ancestry with dense SNP data and classical HLA allele typing.[6] We used a new probabilistic approach (HLP*IMP:02), which delivers increased accuracy on European samples, even under conditions of reduced SNP coverage.[6] The imputed alleles for the training sample were compared with the observed classic alleles at 2-digit and 4-digit resolution. Only those classic alleles that attained 80% or more sensitivity, specificity, and positive and negative predictive values and that were common in the training sample (frequency ≥5%) were tested for association.

The association of SNPs with asthma in the discovery sample was tested using logistic regression models and included previously obtained ancestry scores as covariates to adjust for population stratification.[7] In this stage, we performed multiple testing adjustments for SNPs and classic alleles by means of 10^5 permutations in which case-control labels were swapped while maintaining the haplotype structure.

Seventeen SNPs were successfully genotyped and passed quality control checks (see Online Repository text and Table E2). Four of them were associated with asthma in the discovery sample after multiple comparison adjustments ($1.24 \leq$ odds ratio [OR] ≤ 1.94, $2.8 \times 10^{-7} \leq P \leq .002$) (see Table E3 and Fig E2 in this article's Online Repository at www.jacionline.org) and 2 of them were nominally significant in replication samples ($P \leq .008$) (Table I). A meta-analysis confirmed the consistency of the effects for the association of rs3135388 and rs6457617 with asthma ($P = 7.8 \times 10^{-5}$ and 3.0×10^{-5}, respectively) (Table I), and conditional regression analysis in the overall sample revealed their independent association ($P = 1.3 \times 10^{-4}$ for rs3135388 and $P = 1.5 \times 10^{-4}$ for rs6457617), consistent with their weak linkage disequilibrium ($r^2 = 0.06$).

In silico analysis of expression quantitative trait loci (eQTLs) revealed the role of associated SNPs as eQTLs for *HLA-DRB1* and/or *HLA-DRB5* in lymphoblastoid cells derived from European individuals (see Table E4 in this article's Online Repository at www.jacionline.org). The SNP rs3135388 was

TABLE I. Summary of association testing of *HLA-DQB1* and *HLA-DRB1* SNPs and classic alleles with asthma susceptibility

		Discovery sample (n = 1760)		Replication sample (n = 1355)		Meta-analysis (n = 3115)			
rs#/gene	Effect allele	OR (95% CI)	*P* value	OR (95% CI)	*P* value	Q test *P* value*	OR (95% CI)	FE *P* value	RE *P* value
rs2395175	A	1.94 (1.51-2.49)	2.8×10^{-7}†	0.99 (0.72-1.35)	.931	.001	1.39 (0.72-2.70)‡	6.6×10^{-5}	4.5×10^{-6}
rs3135388	A	0.66 (0.50-0.87)	.002†	0.63 (0.41-0.97)	.008†	.976	0.66 (0.54-0.81)§	7.8×10^{-5}†	9.4×10^{-5}
rs6457617	T	1.25 (1.08-1.44)	.003†	1.26 (1.07-1.48)	.005†	.947	1.25 (1.13-1.39)§	3.0×10^{-5}†	3.6×10^{-5}
rs7764856	A	1.24 (1.07-1.45)	.005†	1.00 (0.84-1.19)	.973	.063	1.13 (1.01-1.27)§	.029	.028
HLA-DQB1	*06	0.67 (0.56-0.80)	1.0×10^{-5}†	0.97 (0.91-1.02)	.249‖	1.3×10^{-4}	0.81 (0.57-1.16)‡	4.5×10^{-3}	1.2×10^{-4}
HLA-DRB1	*15	0.64 (0.49-0.84)	.001†	0.87 (0.80-0.95)	.001†‖	.026	0.77 (0.57-1.03)‡	1.2×10^{-4}	1.3×10^{-4}†
HLA-DRB1	*15:01	0.65 (0.49-0.86)	.002†	0.66 (0.49-0.90)	.008†	.918	0.66 (0.53-0.81)§	6.7×10^{-5}†	8.0×10^{-5}

FE, Fixed-effects; *RE*, random-effects.
*Cochran's Q test for heterogeneity.
†Significant *P* values in individual samples and those that revealed a consistent effect in the meta-analysis.
‡Effect and interval derived from the RE model as suggested by the Q test.
§Effect and interval derived from the FE model as suggested by the Q test.
‖Classical allele data were available only for a subset of 785 samples.

TABLE II. Summary of association testing of the SNP rs2395175 with allergic sensitization against dust mites, pollens, and animal epithelia

	Discovery sample			Replication sample			Meta-analysis				
Allergen	Sample size*	OR (95% CI)	*P* value	Sample size*	OR (95% CI)	*P* value	Sample size*	Q test *P* value†	OR (95% CI)	FE *P* value	RE *P* value
Dust mites	253:173	2.69 (1.58-4.59)	2.7×10^{-4}‡	437:110	1.18 (0.64-2.20)	.597	690:283	.048	1.81 (0.81-4.06)§	.002	.002
Pollens	163:262	0.46 (0.27-0.79)	.004‡	224:344	1.06 (0.64-1.76)	.830	387:606	.029	0.70 (0.31-1.57)§	.069	.042
Animal epithelia	128:282	2.07 (1.28-3.35)	.003‡	219:349	1.94 (1.20-3.12)	.007‡	347:631	.842	2.00 (1.43-2.81)‖	5.6×10^{-5}‡	6.7×10^{-5}‡

FE, Fixed-effects; *RE*, random-effects.
*Number of individuals with positive and negative sensitization (sensitized: nonsensitized).
†Cochran's Q test for heterogeneity.
‡Significant *P* values in individual samples and those that revealed a consistent effect in the meta-analysis.
§Effect and interval derived from the RE model as suggested by the Q test.
‖Effect and interval derived from the FE model as suggested by the Q test.

also located on an enhancer histone mark site in B-lymphocyte cells and a transcription factor binding site as demonstrated by empirical data from the Encyclopedia of DNA Elements (ENCODE) (www.genome.gov). In addition, our results for rs6457617 constitute a replication of a GWAS hit for asthma in Japanese.[3] Interestingly, the 2 associated SNPs showed consistent effects with those reported for lipid traits (see Table E5 in this article's Online Repository at www.jacionline.org).

Quality assessment of the imputation of the 76 classic alleles detected in the training sample (see Table E6 in this article's Online Repository at www.jacionline.org) revealed that 14 of the 25 common alleles passed our quality criteria and were followed-up for association analysis using logistic regression models. Three classic alleles were associated with asthma in the discovery sample after multiple testing adjustments (see Table E7 in this article's Online Repository at www.jacionline.org): *HLA-DQB1**06, *HLA-DRB1**15, and *HLA-DRB1**15:01 ($.001 \leq P \leq 1.0 \times 10^{-5}$). However, only 2 of them replicated at nominal significance: *HLA-DRB1**15 and *HLA-DRB1**15:01 ($P = .001$ and .008, respectively). A meta-analysis confirmed a consistent protective effect of *HLA-DRB1**15:01 for asthma across all the samples (OR, 0.66; 95% CI, 0.53-0.81; $P = 6.7 \times 10^{-5}$). Interestingly, *HLA-DRB1**15:01 has previously been associated with an increased risk for multiple sclerosis and narcolepsy. This result is consistent with other susceptibility genes that have been shown to have opposite effects in asthma and autoimmune diseases (Table E5).[9,10] However, this study is the first revealing such an effect for a classic HLA allele.

Because a previous study has found an association between SNPs from the HLA region and sensitization to specific allergens,[11] we performed a *post hoc* analysis for sensitization to the 3 most common specific allergens within the asthma cases (see Online Repository text), and identified SNP rs2395175 as associated with the presence of sensitization to dust mites, pollens, and animal epithelia in the discovery sample ($P \leq .004$), with both harmful and protective associations, depending on the allergen (see Table E8 in this article's Online Repository at www.jacionline.org). However, only the association with animal epithelia sensitization was replicated in independent samples ($P \leq .003$), showing a large effect size in the meta-analysis (OR, 2.00; 95% CI; 1.43-2.81; $P = 5.6 \times 10^{-5}$) (Table II). This SNP was also an eQTL for *HLA-DRB1* and/or *HLA-DRB5* in lymphoblastoid cells (Table E4).

Our study has 2 main limitations. First, it had more than 80% statistical power for variants with an OR of more than 1.3, but not for smaller effect sizes (see Fig E3 in this article's Online Repository at www.jacionline.org). Second, association testing of the full spectrum of classic alleles at these 2 genes was impracticable, both because of sample size limitations and because of the imperfect predictive ability of the SNPs retained

for classic allele imputation. Consequently, we analyzed only 56% of common classic alleles in this population (see Online Repository text). It is possible that we missed other interesting associations, as suggested by the fact that a weighted score including the 2 SNPs associated with asthma explained slightly higher phenotypic variance than did a model including the classic alleles (Nagelkerke's $R^2 = 0.14$ vs 0.10, respectively).

In summary, a deeper examination of HLA genes has revealed a classic allele that shows pleiotropic effects for asthma and other immune-related diseases and likely constitutes a putative causal variant. Analysis of SNPs also revealed shared genetic risk factors between asthma and lipid levels. Finally, we provided evidence supporting the role of HLA polymorphisms in specific allergic sensitization.

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Circulating endothelial cells as markers of endothelial dysfunction during hematopoietic stem cell transplantation for pediatric primary immunodeficiency

To the Editor:

Endothelial damage is the pathological hallmark of numerous acute complications occurring during hematopoietic stem cell transplantation (HSCT): sinusoidal obstruction syndrome (SOS), transplantation-associated thrombotic microangiopathy (TA-TMA), capillary leak syndrome (CLS), and pulmonary arterial hypertension (PAH).[1] These disorders remain an important cause of morbidity and mortality, despite improvement in their prevention and their therapy. Many factors are thought to induce endothelial dysfunction: the underlying disease, the conditioning regimen, the immunosuppressive therapy, the intercurrent infections, and graft-versus-host disease (GVHD).[1-3] Circulating endothelial cells (CECs) are specific and sensitive markers of endothelial dysfunction. They have been used in a variety of pathological conditions to monitor the occurrence of vascular damage.[4] Because vascular complications impact the outcome of HSCT for several types of primary immunodeficiency (PID), we decided to monitor endothelial dysfunction during HSCT for PID through the measurement of CECs.

Thirty-four pediatric patients treated in the department of Pediatric Immuno-Hematology at the Necker-Enfants Malades Hospital who received allogeneic HSCT after full-intensity conditioning regimen for PID were enrolled between April 2011 and December 2013. Informed consent for participation in the study was obtained from the children's parents in accordance