Genome-wide association study of the age of onset of childhood asthma

Erick Forno, MD, MPH,[a,c,d] Jessica Lasky-Su, ScD,[a,d] Blanca Himes, PhD,[a,d] Judie Howrylak, MD,[a] Clare Ramsey, MD, MPH,[e] John Brehm, MD, MPH,[a,b,d] Barbara Klanderman, PhD,[a] John Ziniti, BA,[a] Erik Melén, MD, PhD,[a,f,g] Goran Pershagen, MD, PhD,[f] Magnus Wickman, MD, PhD,[f,h] Fernando Martinez, MD,[i] Dave Mauger, PhD,[j] Christine Sorkness, PharmD,[k] Kelan Tantisira, MD,[a,b,d] Benjamin A. Raby, MD, MPH,[a,b,d] Scott T. Weiss, MD, MS,[a,d] and Juan C. Celedón, MD, DrPH[l] *Boston, Mass, Miami, Fla, Winnipeg, Manitoba, Canada, Stockholm, Sweden, Tucson, Ariz, Hershey and Pittsburgh, Pa, and Madison, Wis*

Background: Childhood asthma is a complex disease with known heritability and phenotypic diversity. Although an earlier onset has been associated with more severe disease, there has been no genome-wide association study of the age of onset of asthma in children.

Objective: We sought to identify genetic variants associated with earlier onset of childhood asthma.

Methods: We conducted the first genome-wide association study of the age of onset of childhood asthma among participants in the Childhood Asthma Management Program (CAMP) and used 3 independent cohorts from North America, Costa Rica, and Sweden for replication.

Results: Two single nucleotide polymorphisms (SNPs) were associated with earlier onset of asthma in the combined analysis of CAMP and the replication cohorts: rs9815663 (Fisher $P = 2.31 \times 10^{-8}$) and rs7927044 ($P = 6.54 \times 10^{-9}$). Of these 2 SNPs, rs9815663 was also significantly associated with earlier asthma onset in an analysis including only the replication cohorts. Ten SNPs in linkage disequilibrium with rs9815663 were also associated with earlier asthma onset ($2.24 \times 10^{-7} < P < 8.22 \times 10^{-6}$). Having 1 or more risk alleles of the 2 SNPs of interest (rs9815663 and rs7927044) was associated with lower lung function and higher asthma medication use during 4 years of follow-up in CAMP.

Conclusions: We have identified 2 SNPs associated with earlier onset of childhood asthma in 4 independent cohorts. (J Allergy Clin Immunol 2012;130:83-90.)

Key words: *Asthma, pediatrics, age of onset, asthma genetics,* C1orf100*, genome-wide association study, pediatric asthma*

Asthma is a complex disease affecting approximately 7 million children in the United States.[1] Variants in more than 40 genes have been associated with asthma.[2,3] Of these potential asthma susceptibility genes, a handful (eg, *ORMDL3*, *PDE4D*, and *DENND1B*) have been identified by using genome-wide association studies (GWASs).[4-7] Recently, a large-scale GWAS confirmed *ORMDL3* and identified several other variants, including *IL1RL1/IL18R1*, *HLA-DQ*, *IL-33*, and *SMAD3*.[8]

Childhood asthma has significant phenotypic heterogeneity. The age of onset of asthma has important phenotypic and prognostic implications,[9,10] and an earlier age of onset is associated with increased severity of asthma in children with symptoms persisting into school age and adolescence.[11,12]

In recent years, 2 studies looking at variants of *ORMDL3* found them to be strongly associated with asthma only among those whose symptoms started before 4 to 5 years of age.[13,14] However, there have been no genome-wide studies directly assessing the genetic determinants of the age of onset of asthma in children. We present the results of a GWAS of the age of onset of asthma in a cohort of North American children enrolled in the Childhood Asthma Management Program (CAMP), followed by replication studies in 3 independent cohorts of asthmatic children from Latin America, North America, and Europe.

From [a]the Channing Laboratory and [b]the Division of Pulmonary/Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Boston; [c]the Division of Pediatric Pulmonology, Department of Pediatrics, University of Miami; [d]Harvard Medical School, Boston; [e]the Department of Medicine, University of Manitoba, Winnipeg; [f]the Institute of Environmental Medicine, Karolinska Institutet, Stockholm; [g]Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm; [h]Sachs Children's Hospital, Stockholm; [i]the Department of Pediatrics, University of Arizona, Tucson; [j]the Penn State Hershey College of Medicine, Hershey; [k]the University of Wisconsin, Madison; and [l]the Division of Pediatric Pulmonary Medicine, Allergy and Immunology, Department of Pediatrics, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh School of Medicine.

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Corresponding author: Erick Forno, MD, MPH, Department of Pediatrics, University of Miami, 1580 NW 10th Ave #125, Miami, FL 33136. E-mail: eforno@med.miami.edu.

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Abbreviations used

BAMSE: Children, Allergy, Milieu, Stockholm, Epidemiological Survey
CAMP: Childhood Asthma Management Program
CAMPCS/2: CAMP Continuation Study, Part 2
C1orf100: Chromosome 1 open-reading frame 100
ETS: Environmental tobacco smoke
FBAT: Family-based association testing
GACRS: Genetics of Asthma in Costa Rica Study
GWAS: Genome-wide association study
LD: Linkage disequilibrium
PACT: Pediatric Asthma Controller Trial
SNP: Single nucleotide polymorphism

METHODS

Population for GWAS

CAMP was a multicenter clinical trial of the effects of anti-inflammatory medications in children with mild-to-moderate asthma aged 5 to 12 years at enrollment. Study protocol and subject recruitment have been described in detail.[15,16] Of the 1024 children in CAMP, we included 573 genotyped non-Hispanic white children (413 index children in nuclear families and 160 singletons) in our analysis. Further details can be found in the Methods section in this article's Online Repository at www.jacionline.org. CAMP was approved by the Institutional Review Boards of Brigham and Women's Hospital and the other participating centers.

Replication cohorts

Genetics of Asthma in Costa Rica Study (GACRS). The protocols for subject recruitment and data collection for the GACRS have been previously described.[17-19] A total of 591 children (aged 6-14 years) were included in the replication analysis.

BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiological Survey). BAMSE is a study of Swedish children recruited at birth between 1994 and 1996 and followed prospectively. BAMSE protocols have been previously described.[20,21] A total of 107 genotyped children with physician-diagnosed asthma and persistent wheezing at age 8 years were included in the analysis.

Pediatric Asthma Controller Trial (PACT). The PACT compared the effectiveness of different controller regimens for asthmatic children aged 6 to 14 years with mild-to-moderate persistent asthma and documented bronchial reversibility, methacholine sensitivity, or both.[22] A total of 233 genotyped children were included in the analysis. Data from PACT were available through the Single-nucleotide Polymorphism Health Association Asthma Resource Project (see the acknowledgments section). All studies (GACRS, BAMSE, and PACT) were approved by the respective institutional review boards, ethics committees, or both of the participating institutions.

Phenotyping

The age of onset of asthma was obtained in CAMP, GACRS, and PACT by means of parental report through a questionnaire at the beginning of each study. The age of onset was analyzed as a continuous variable; any age of onset reported to be less than 6 months was considered 0.5 years. In BAMSE questionnaires were mailed to parents when participating children were approximately 1, 2, 4, and 8 years of age. For our analysis, the age of onset was assigned to be the midpoint of each time interval: 0.5, 1.5, 3, 5, or 8 years. Details on phenotyping for other variables can be found in the Methods section in this article's Online Repository.

Gene expression

Gene expression profiling was performed on $CD4^+$ lymphocytes collected from 299 subjects participating in the CAMP Continuation Study, Part 2 (CAMPCS/2). Details can be found in the Methods section in this article's Online Repository.

Genotyping and quality control

Genome-wide single nucleotide polymorphism (SNP) genotyping was performed by Illumina, Inc (San Diego, Calif), on the HumanHap550v3 BeadChip for CAMP subjects and their parents. After stringent quality control (see the Methods section, Table E1, and Fig E1 in this article's Online Repository at www.jacionline.org for details), 512,296 SNPs remained for analysis. Genotyping details for replication cohorts can be found in the Methods section in this article's Online Repository. When SNPs selected for replication from CAMP were not available in PACT (genotyping done with a different platform), we performed imputation for the original SNPs using data from HapMap and the 1000 Genomes Project (see the Methods section in this article's Online Repository for details).

Statistical methods

The population-based GWAS of the age of onset of asthma in CAMP was performed by using survival analysis in R software with PLINK.[23] We used an additive model adjusted for age at enrollment, sex, and environmental tobacco smoke (ETS) exposure during infancy. The main eigenvectors describing the population substructure, as identified by using EIGENSTRAT,[24] were included as covariates to correct for population stratification. We also performed family-based association testing (FBAT; a generalization of the transmission disequilibrium test to test association with any phenotype, sampling structure, and pattern of missing marker information) in the 403 index children in nuclear families.[25,26] SNPs with the lowest *P* values in the population-based survival analysis that also had FBAT *P* values of less than .20 were considered for replication.

SNPs were tested in GACRS by using the same adjusted survival and FBAT analyses and in BAMSE and PACT by using adjusted survival analysis. Replication of the original finding was defined as a nominal 1-sided *P* value of less than .05 with an effect in the same direction as in the GWAS. Fisher combined *P* values for all cohorts and for the replication cohorts only were calculated by using the population-based *P* values from CAMP, BAMSE, and PACT and the FBAT *P* value from GACRS (only the *P* value from the FABT is presented to adjust for potential population stratification because genome-wide genotypic data to estimate eigenvectors were not available for this cohort). Bonferroni correction was used as a reference to control for multiple tests; the significance threshold was a *P* value of less than 9.8×10^{-8} (0.05/512,296) for the GWAS and the analysis of all cohorts and a *P* value of less than .0036 (0.05/14) for the analysis including only the replication cohorts.

RESULTS

Table I summarizes the baseline characteristics of all cohorts. Compared with children in CAMP, those in GACRS had earlier onset of asthma, higher lung function and eosinophil counts, and lower total IgE levels and were less likely to have been exposed to ETS; children in BAMSE had similar age of onset, lower frequency of exposure to ETS, and higher lung function; and children in PACT were older and had higher lung function, higher frequency of exposure to ETS, and lower total IgE levels.

GWAS *P* values are shown in Fig 1. Two SNPs (rs7927044 [chromosome 11q24] and rs10521233 [on 17p12]) were significantly associated with the age of onset of asthma after the Bonferroni correction. These 2 SNPs, as well as the 12 SNPs with the next-lowest *P* values, were carried forward for replication in GACRS, BAMSE, and PACT.

Three SNPs were significantly associated with asthma onset in at least 1 of the replication cohorts in the same direction (earlier onset) as in the GWAS (Table II). SNP rs9815663 (3p26) showed significant association in CAMP BAMSE and