An epigenome-wide association study of total serum IgE in Hispanic children

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Background: Total IgE is a therapeutic target in patients with allergic diseases. DNA methylation in white blood cells (WBCs) was associated with total IgE levels in an epigenome-wide association study of white subjects. Whether DNA methylation of eosinophils explains these findings is insufficiently understood. Methods: We tested for association between genome-wide DNA methylation in WBCs and total IgE levels in 2 studies of Hispanic children: the Puerto Rico Genetics of Asthma and Lifestyle Study (PR-GOAL; n = 306) and the Genes-environments and Admixture in Latino Americans (GALA II) study (n = 573). Whole-genome methylation of DNA from WBCs was measured by using the Illumina Infinium HumanMethylation450 BeadChip. Total IgE levels were measured by using the UniCAP 100 system. In PR-GOAL WBC types (ie, neutrophils, eosinophils, basophils, lymphocytes, and monocytes) in peripheral blood were measured by using Coulter Counter techniques. In the GALA II study WBC types were imputed. Multivariable linear regression was used for the analysis of DNA methylation and total IgE levels, which was first conducted separately for each cohort, and then results from the 2 cohorts were combined in a meta-analysis. Results: CpG sites in multiple genes, including novel findings and results previously reported in white subjects, were significantly associated with total IgE levels. However, adjustment for WBC types resulted in markedly fewer significant sites. Top findings from this adjusted meta-analysis were in the genes *ZFPM1* ($P = 1.5 \times 10^{-12}$), *ACOT7* ($P = 2.5 \times 10^{-11}$), and *MND1* ($P = 1.4 \times 10^{-9}$). Conclusions: In an epigenome-wide association study adjusted for WBC types (including eosinophils), methylation changes in genes enriched in pathways relevant to asthma and immune responses were associated with total IgE levels among Hispanic children. (J Allergy Clin Immunol 2017;140:571-7.)

Key words: *Epigenome-wide association study, total IgE, Hispanic, children*

Allergic diseases affect millions of persons worldwide. In patients with allergic diseases, secretion of IL-4 and IL-13 by T_H2 cells increases production of total IgE by B cells. Total IgE is a key intermediate phenotype and a therapeutic target in patients with allergic diseases, such as asthma and allergic rhinitis.[1]

Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) linked with total IgE levels, but their joint estimated effect accounts for a very small proportion of the trait's heritability.[2-5] Environmental exposures affect total IgE levels,[6,7] and thus epigenetic mechanisms, such as DNA methylation, might explain the "missing heritability" of total IgE.

A recent epigenome-wide association study (EWAS) using DNA from white blood cells (WBCs) reported that methylation in several genes is implicated in total IgE levels in white subjects,[8] with methylation of 3 genes (*LPCAT2*, *IL5RA*, and *ZNF22*) accounting for approximately 13% of total IgE variance.

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PR-GOAL was supported by grants HL079966 and HL117191 from the National Institutes of Health (NIH) and by the Heinz Endowments. The GALA II study was supported by grants HL088133, HL004464, HL117004, ES015794, ES24844, TRDRP 24RT 0025, MD006902, and GM007546 from the NIH. E.G.B. was also supported by the RWJF Amos Medical Faculty Development Award, the American Asthma Foundation, and the Sandler Foundation. E.F.'s contribution was supported by grant HL125666 from the NIH. C.L. is the chairholder of the Canada Research Chair in the Environment and Genetics of Respiratory Disorders and Allergy. E.P.-Y. was supported by the grant AC15/00015 by Instituto de Salud Carlos III within the ERACoSysMed 1st Joint Transnational Call (SysPharmPedia 99) from the European Union, under the Horizon 2020.

Disclosure of potential conflict of interest: M. Pino-Yanes's institution has received grant AC15/00015 from Instituto de Salud Carlos III and the European Union and has personally received lecture fees from Affymetrix. E. Forno's institution has received grant HL125666 from the National Institutes of Health (NIH) for the work under consideration. D. E. Weeks' institution has received grant R01HL117191 from the NIH/National Heart, Lung, and Blood Institute (NHLBI) for the work under consideration. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication July 15, 2016; revised October 26, 2016; accepted for publication November 24, 2016.

Available online January 6, 2017.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.11.030

Abbreviations used

ACOT7: Acyl-CoA thioesterase 7
eQTM: Expression quantitative trait methylation
ETV6: ETS variant 6
EWAS: Epigenome-wide association study
FDR: False discovery rate
GALA II: Genes-Environments and Admixture in Latino Americans Study
MND1: Meiotic nuclear division 1
MRCA: Medical Research Council Asthma
PAOX: Polyamine oxidase
PAPA: Poblogaeth Asthma Prifysgol Abertawe
PC: Principal component
PR-GOAL: Puerto Rico Genetics of Asthma and Lifestyle Study
PTGDR2: Prostaglandin D_2 receptor 2
QQ: Quantile-quantile
SLSJ: Saguenay-Lac-Saint-Jean
SNP: Single nucleotide polymorphism
SPRY2: Sprouty RTK signaling antagonist 2
WBC: White blood cell
ZFPM1: Zinc-finger protein multi-type 1

Whether those findings are explained by DNA methylation of eosinophils (which play a major role in allergic inflammation) or other WBCs is insufficiently understood.

In the United States, Puerto Ricans are heavily affected by allergic diseases, whereas Mexican Americans have a lower but nonnegligible burden from allergies.[9-11] We report the first EWAS of total IgE with adjustment for (percentage of) WBC types, as well as the first EWAS of total IgE in Hispanic subjects.

METHODS

Subject recruitment and study procedures

Puerto Rico genetics of asthma and Lifestyle Study (PR-GOAL). From March 2009 to June 2010, 678 children with and without asthma (defined as physician-diagnosed asthma and ≥1 episode of wheeze in the prior year) were recruited in San Juan, Puerto Rico, as described elsewhere.[12] Eligibility criteria included age of 6 to 14 years and having 4 Puerto Rican grandparents. Of the 562 subjects who had blood samples with sufficient DNA for genome-wide genotyping, 306 had additional data (including WBC count, total IgE level measurement, and a genome-wide study of DNA methylation) and were thus included in this analysis. Total serum IgE levels were measured by using the UniCAP 100 system (Pharmacia & Upjohn, Kalamazoo, Mich), and total WBC counts and percentages of the 5 possible cell types (ie, neutrophils, eosinophils, basophils, lymphocytes, and monocytes) in peripheral blood were measured by using Coulter Counter techniques. There were no significant differences in age, sex, asthma status, or total IgE levels between subjects with available DNA who were (n = 306) and were not (n = 256) included in the current analysis.

After bisulfite conversion of DNA from WBCs by using the EZ DNA Methylation Kit (Zymo Research, Irvine, Calif), whole-genome DNA methylation levels were measured by using the HumanMethylation450K BeadChip system (Illumina, San Diego, Calif). In brief, 200 ng of bisulfite-converted DNA was whole-genome amplified for 23 hours, followed by end point fragmentation. The fragmented DNA was precipitated, denatured, and hybridized to the BeadChips for 18 hours at 48°C. The BeadChips were then washed, and the hybridized primers were extended and labeled before scanning the BeadChips with the Illumina iScan system.

Principal components (PCs) were calculated from genome-wide SNP data, which were genotyped with the HumanOmni2.5 BeadChip platform (Illumina), as previously described.[12] Genome-wide gene expression in a subset of 121 whole-blood samples was measured at the University of Pittsburgh Genomics and Proteomics Core Laboratories by using the Illumina HumanHT-12 v4 Expression BeadChip, as described in our previous work.[13]

Written parental consent was obtained for participating children, from whom written assent was also obtained. The study was approved by the Institutional Review Boards of the University of Puerto Rico (San Juan, Puerto Rico), Brigham and Women's Hospital (Boston, Mass), and the University of Pittsburgh (Pittsburgh, Pa).

Genes-environments & Admixture in Latino Americans (GALA II) study. The GALA II study is a multicenter case-control study of asthma in 1858 Latino children and adolescents.[14] Cases and healthy control subjects were recruited from 5 centers throughout the United States (Chicago, the Bronx, Houston, the San Francisco Bay Area, and Puerto Rico). Subjects were eligible if they were 8 to 22 years of age, self-identified all 4 grandparents as Latino, and had a smoking history of less than 10 pack-years. Asthma was defined as physician-diagnosed asthma and report of symptoms and medication use in the previous 2 years. Participants were classified into 3 Latino subgroups (Puerto Rican, Mexican, and other Latino), according to the self-reported ancestry of their 4 grandparents. All participants were genotyped on the Axiom LAT1 array (World Array 4; Affymetrix, Santa Clara, Calif), as described elsewhere.[15] A subset of 573 participants (219 Puerto Ricans, 269 Mexicans, and 85 other Latinos) with data for genome-wide DNA methylation and total IgE levels were included in the current analysis; there were no significant differences in age, sex, asthma status, or total IgE levels between subjects who were (n = 573) and were not (n = 1285) included in the analysis. Total serum IgE levels were measured with the ImmunoCAP 100 system (Phadia, Kalamazoo, Mich).[4] WBC DNA was bisulfite converted and hybridized with Infinium HumanMethylation450K BeadChips to measure methylation levels.

The study was approved by the Institutional Review Boards of the University of California at San Francisco and all other participating centers. All children and their parents provided written informed assent and consent, respectively.

Preprocessing and quality control for 450K DNA methylation data

We performed the same preprocessing and quality control for 450K DNA methylation data in PR-GOAL and the GALA II study. We read methylation data from the raw IDAT files by using the R package *methylumi* and calculated the β-value for each CpG as follows:

$$\beta = M/(M + U + \alpha),$$

where M and U represent methylated and unmethylated signal intensities at the specific site and α is an arbitrary offset (usually 100) intended to stabilize β-values where fluorescent intensities are low. Next, the methylation data were preprocessed by means of color balance adjustment, background correction, and quantile normalization with the *lumi* R package and followed by Beta-Mixture Quantile normalization with the *wateRmelon* package. This normalization strategy first quantile normalized the intensities of methylation signals among all arrays and then used Beta-Mixture Quantile dilation to normalize the β-values within each array.[16-18]

In each data set we removed poor-quality probes with detection P values of greater than .01 in at least 20% of the samples. We also removed 11,656 probes with CpG loci located on sex chromosomes and 19,344 probes that had rs* names or located at 0 distance to known SNPs, according to the Illumina product annotation of Infinium HD Methylation SNP List. This quality control process left 454,552 CpGs in both data sets. We then filtered CpG sites with an overall mean β-value of greater than 0.9 or less than 0.1 (considering extreme values as noise signals and to reduce multiple tests), which left 188,368 CpGs for data analysis.

Comparison with previous findings in white subjects

For further information, see the Methods section in this article's Online Repository. We compared our results in Hispanic children with those of Liang et al[8] in white subjects. The comparison sample consists of 3 cohorts of white subjects (see Table E1 in this article's Online Repository at www.jacionline.org), as