

Letter to the Editor

Fine mapping of the myosin light chain kinase gene (*MYLK*) replicates the association with asthma in populations of Spanish descent

To the Editor:

Asthma is a chronic inflammatory disorder mainly characterized by reversible airflow obstruction, bronchial hyperresponsiveness, and dyspnea. The functional alterations in airway smooth muscle usually observed in asthmatic patients have been associated with increased expression of the smooth muscle myosin light chain kinase isoform (smMLCK).¹ This constitutes a key cytoskeleton effector of the smooth muscle contractile machinery and is encoded by the myosin light chain kinase gene (*MYLK*). Two *MYLK* single nucleotide polymorphisms (SNPs), which are common in Africans (>30%) but rare in Europeans ($\leq 1\%$), have been associated with asthma in African American and Afro-Caribbean subjects.^{2,3} However, none of the *MYLK* variants have attained the strict Bonferroni-corrected level of genome-wide significance in a genome-wide association study (GWAS) of asthma. This discrepancy might arise from the low coverage of this region on commercial arrays, the genetic specificities of the populations studied, or both because risk factors in one population might not generalize to another.⁴ Here we aimed to fine map the association of SNPs from the *MYLK* gene with asthma in case-control studies from Spanish ($n = 3219$) and Latino ($n = 4650$) populations.

In the discovery stage DNA samples from 606 patients with physician-diagnosed asthma from the Genetics of Asthma (GOA) study in the Spanish population (GOA I) were compared with 1258 nonasthmatic subjects. Top associated SNPs were then replicated in 2 independent Spanish studies: GOA II and the Genetics of Asthma study in the Spanish population from Malaga (GOAM). GOA II included 248 asthmatic cases and 537 control subjects genotyped with the Axiom Genome-Wide CEU 1 Array (Affymetrix, Santa Clara, Calif), whereas GOAM comprised 320 asthma cases and 250 control subjects genotyped by using TaqMan allelic discrimination assays (Life Technologies, Carlsbad, Calif). Further replication was assessed in 2 Latino studies using existing genome-wide genotyping data: the Genetics of Asthma in Latino Americans (GALA I) study,⁴ consisting of 529 cases and 347 control subjects, and the Genes-Environments & Admixture in Latino Americans (GALA II) study,⁵ including 1893 cases and 1881 control subjects. Detailed descriptions of the study design and sample characteristics can be found in Fig E1, Tables E1 and E2, and the Methods section in this article's Online Repository at www.jacionline.org.

In the discovery stage a total of 29 tagging SNPs (tSNPs) were selected from HapMap II to analyze variants with a minor allele frequency (MAF) of 5% or greater from the *MYLK* gene in European subjects. Genotyping was performed with the iPLEX Gold assay on the MassARRAY system (Sequenom, San Diego, Calif). A total of 26 SNPs passed quality control (after removing monomorphic SNPs or with a P value of 1.7×10^{-3} or less for Hardy-Weinberg equilibrium [HWE] in control subjects, see Table E3 in this article's Online Repository at www.jacionline.org). After imputation, 272 SNPs with MAFs of 5% or greater

and a squared correlation between imputed and true genotypes (R^2) of 0.3 or greater were kept for association testing by using logistic regression models (88% of the total variants with MAFs of $\geq 5\%$ in Europeans). Principal components were used as covariates to adjust for population stratification, as previously described.⁶

Three significantly associated SNPs were observed after Bonferroni correction ($P \leq 1.8 \times 10^{-4}$). Two intronic SNPs, rs77820417 and rs78442149, in perfect linkage disequilibrium ($LD; r^2 = 1$) and with MAFs of 9% showed the most significant associations (for both SNPs: odds ratio [OR] of 2.71 [95% CI, 1.79-4.11] for the minor allele, $P = 2.78 \times 10^{-6}$; Fig 1 and see Table E4 in this article's Online Repository at www.jacionline.org). We followed up SNP rs77820417 for replication in 4 independent studies. Association was significant in GOAM (OR, 2.32; 95% CI, 1.23-4.36; $P = 4.17 \times 10^{-3}$), GALA I (OR, 1.73; 95% CI, 1.03-2.93; $P = .040$), and GALA II (OR, 1.31; 95% CI, 1.05-1.64; $P = .019$) but not GOA II (OR, 0.88; 95% CI, 0.48-1.61; $P = .669$). Because of the heterogeneity of effects among studies ($P = .007$, Cochran Q test), a random-effects meta-analysis of the 7869 subjects was performed, confirming the strong association of rs77820417 with asthma susceptibility (OR, 1.66; 95% CI, 1.14-2.42; $P = 1.57 \times 10^{-7}$; Table 1). We further explored the association of rs77820417 with asthma exacerbations in the GALA II study, as defined by the presence of 1 or more asthma-related events (hospitalizations, emergency department visits, and oral steroid use) over the 12 months before recruitment and adjusting for the use of medication during the same period. The A allele, which is associated with asthma risk, was also associated with increased risk of asthma exacerbations (OR, 1.80; 95% CI, 1.08-2.99; $P = .023$). The associated SNP is located within the *MYLK* gene region encoding the smMLCK isoform, which participates in smooth muscle cell contractility. smMLCK activation is a critical step in cytoskeletal rearrangement, providing dynamic regulation of cell shape, cell motility, and adhesion, which are involved in the remodeling processes underlying asthma.⁷

To date, GWASs have firmly identified susceptibility genes underlying asthma risk, although most of the studies were performed by using HapMap-based inferences, where the coverage for genetic variation is limited compared with the information provided by the 1000 Genomes Project (1KGP). In fact, neither the top hit observed in the current study nor its proxy (rs78442149) were tested for association in the GABRIEL (<http://www.cng.fr/gabriel/results.html>) or EVE consortia, the largest GWAS meta-analyses in asthma performed in Europeans⁸ and multiethnic groups.⁹ No other variants from 1KGP are in moderate LD with those 2 SNPs (highest $r^2 = 0.25$). However, 1 *MYLK* variant was associated with asthma in the GABRIEL consortium (rs7633133, $P = 1.01 \times 10^{-7}$; Fig 1). This SNP shows an MAF of 1% in 1KGP European subjects and therefore was not tested in our study. In the EVE consortium, although no *MYLK* SNP showed an outstanding significance (minimum $P = .01$, Fig 1), a 4-fold enrichment of significant associations was observed in Latino subjects ($P = 7.03 \times 10^{-5}$, Fisher exact test) but not in European or African American subjects ($P = .720$ and $P = 1.0$, respectively; see Table E5 and the Methods section in this article's Online Repository at www.jacionline.org).

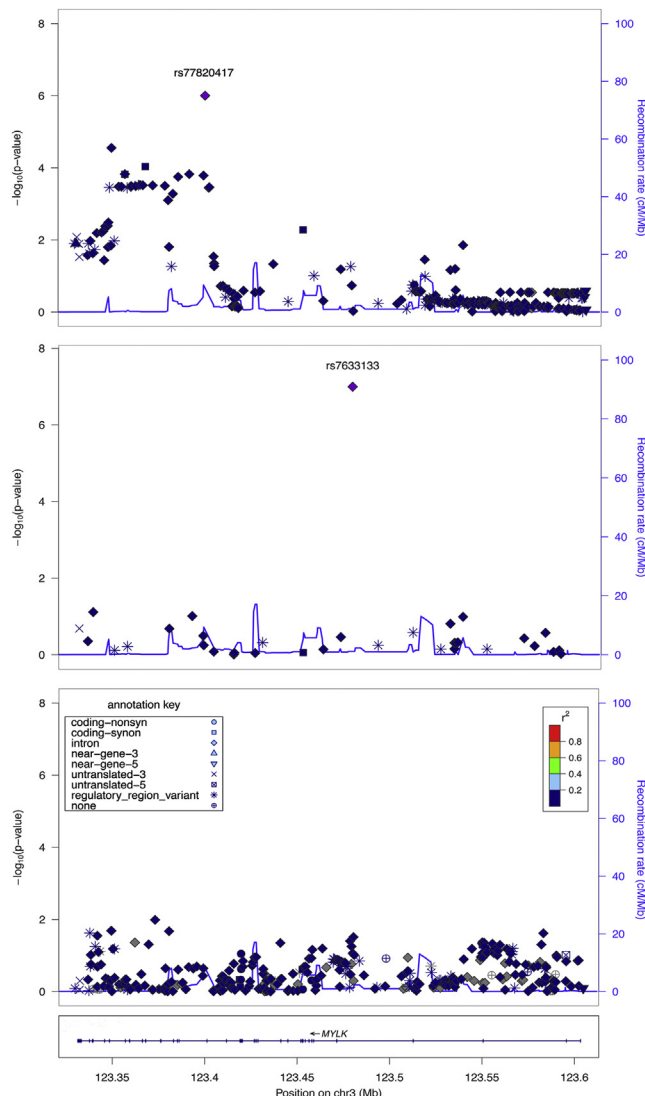


FIG 1. Regional plots of association results in the discovery sample (*upper panel*) and the GABRIEL (*middle panel*) and EVE (*lower panel*) consortia. The $-\log_{10}$ transformed P values for association tests are plotted as symbols according to their functional annotation. The SNP rs number indicated on the plot denotes the result for the most significantly associated SNP with asthma risk. The results for the remaining SNPs are color coded to reflect their degree of LD with the most significant SNP based on pairwise r^2 values from the European population of the 1KGP. Estimated recombination rates (*light blue line*) are plotted on the *right y-axis*.

One striking aspect of our study is the large effect sizes found for the association of rs77820417 with asthma susceptibility and exacerbations, which is only comparable with the effect reported for an SNP in *GSDMB* with early childhood asthma with severe exacerbations.¹⁰ However, this effect size might be confounded by the large sex and age differences among cases and control subjects in the discovery sample. Despite this, the validation across multiple studies of both children and adults with different sex balance suggests that the result from the discovery study is not a false-positive result. The catalog of asthma susceptibility genes could be more comprehensive if imputation based on 1KGP data were exploited to meta-analyze existing GWAS data. In addition, analysis of diverse populations also contributes with

TABLE I. Summary of association testing of rs77820417 with asthma susceptibility

Study	Sample size (cases/control subjects)	MAF	OR (95% CI)	P value
GOA I	1864 (606/1258)	0.090	2.71 (1.79-4.11)	2.78×10^{-6}
GOA II	785 (248/537)	0.084	0.88 (0.48-1.61)	.669
GOAM	570 (320/250)	0.052	2.32 (1.23-4.36)	4.17×10^{-3}
GALA I	876 (529/347)	0.073	1.73 (1.03-2.93)	.040
GALA II	3774 (1893/1881)	0.044	1.31 (1.05-1.64)	.019
Meta-analysis	7869 (3596/4273)	—	1.66 (1.14-2.42)	1.57×10^{-7}

P values of .05 or less, indicating statistical significance, are shown in boldface.

new susceptibility loci because many GWAS hits are not transferable to all populations.⁴ In fact, higher North African ancestry is detected in southwestern Europe and is decreased in northern latitudes.¹¹ Therefore novel susceptibility loci for asthma could be revealed in populations of Spanish descent.

In summary, we identified an *MYLK* SNP association with asthma in subjects of Spanish descent, showing suggestive genome-wide significance. Future studies will be needed to confirm its importance in other populations.

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METHODS

Study design

This study was conducted by using 5 case-control studies with unrelated subjects reporting at least 4 grandparents of Spanish or Latino origin. Demographic and clinical data are summarized in Tables E1 and E2 for the Spanish and Latino studies, respectively. All local institutional review boards and ethics committees approved the studies, and participants/parents provided written assent/consent, respectively.

GOA I

Sample description. This study included patients with 2 generations of Spanish descent. The group of cases included participants who fulfilled the Global Initiative for Asthma guidelines for diagnosis and classification of asthma (<http://www.ginasthma.com>) and were collected from respiratory medicine and allergy departments in hospitals from Spain as part of GOA I.^{E1} This study included 607 patients with a physician's diagnosis of asthma who were using specific medication for asthma symptoms and were older than 5 years. We recorded basic demographic data (age, sex, and smoking habits), age at diagnosis of the disease, asthma severity, family history of asthma and allergic diseases, allergic symptoms (rhinitis, atopic dermatitis, and food and drug allergy), basal pulmonary function measurements, and medication use for symptomatic treatment. Atopy was confirmed by the presence of a positive skin prick test response (a wheal with a diameter 3 mm greater than that elicited by the saline control) to one of 7 common allergens, including dust mite, epithelium, pollen, fungi, food, latex, and others or by specific serum IgE levels of greater than 0.35 IU/mL. Allergens evaluated for specific IgE were dust mite (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Glycyphagus* species, *Blomia tropicalis*, *Acarus siro*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, and *Euroglyphus maynei*), epithelia (*Felis domesticus*, *Canis familiaris*, *Equus caballus*, and *Oryctolagus cuniculus*), pollen (*Olea europaea*, *Salsola* species, *Lolium perenne*, *Artemisia vulgaris*, *Parietaria* species, *Platanus* species, *Chenopodium* species, *Plantago* species, *Rumex* species, and *Cupressus* species), fungi (*Penicillium notatum*, *Alternaria alternata*, *Aspergillus fumigatus*, and *Cladosporium herbarum*), food (cow's milk, hen's egg, peanut, soybean, wheat, fish, shrimp, crabs, lobster, clams, oysters, mussels, banana, chestnut, and kiwi), latex, and cockroach (*Blattella germanica*).

Control samples were obtained from the Spanish National DNA Biobank (<http://www.bancoadn.org>), a member of the Public Population Project in Genomics Consortium (<http://www.p3gobservatory.org>). After signing an informed consent form, donors self-declared general health status, physical activity, transportation and nutrition habits, employment and qualification, demographics, tobacco smoking history, alcohol consumption, genealogic information, residence, and mother's language of preference. In addition, personal and family histories of diseases, including infectious, cancerous, blood and circulatory, endocrine, mental and behavioral, respiratory (including asthma symptoms), immunologic (including allergies), bone, congenital, skin and digestive, and eye and hearing disorders, were documented. No information from medical records was incorporated or revised, and no medical testing was performed on donors. We minimized the presence of atopy among control subjects by excluding those participants self-reporting personal or family history of allergic or pulmonary diseases. We also minimized the differences in ancestry composition against cases by only including samples from patients with at least 4 grandparents born in Spain. A total of 1271 DNA samples from unrelated subjects were finally selected as controls for the discovery study. However, because the number of samples fulfilling these requirements was limited in the Spanish National DNA Biobank, the final design resulted in large differences in age and sex composition, with older age and higher proportion of male subjects among control subjects than among cases (see Table E1).

tSNP selection. Genotype data for SNPs with MAFs of 5% or greater for subjects with ancestry from northern and western Europe (CEU) were downloaded from the HapMap II project.^{E2} We selected the region in chromosome 3 comprising hg19 positions 123329143 to 123605149 to get the full transcript of *MYLK* (NM_053025) and to include 2 kb of its flanking

regions. Haplotypes of *MYLK* were reconstructed for the CEU data by using PHASE v2.1^{E3} under default parameters. The tSNP selection was performed on haplotypes showing a frequency of 1% or greater to provide a haplotype r^2 value of 0.85 or greater using the multiple-marker selection algorithm implemented in TagIT 3.03 software.^{E4} The final set of tSNPs consisted of 29 SNPs.

Genotyping and statistical methods. DNA extracted from blood (GFX kit; GE Healthcare, Little Chalfont, United Kingdom) or saliva (Oragene DNA; DNA Genotek Inc, Ottawa, Ontario, Canada) was whole genome amplified with the Illustra *GenomiPhi*V2 DNA Amplification Kit (GE Healthcare, Pittsburgh, Pa). The concentration was quantified by using a SYBR Green I-based DNA quantification method (Molecular Probes, Eugene, Ore). Genotyping was performed with the iPLEX Gold assay on MassARRAY at the University of Chicago Sequencing Facility and was blind to disease status. We included duplicated samples (approximately 6%) to monitor genotyping quality. Genotype calls were performed automatically by using TYPER 3.4 software (Sequenom, San Diego, Calif). Summary quality control measures were generated by using SNPing,^{E5} and monomorphic SNPs and those that deviated from HWE within control subjects after multiple comparison adjustment ($P \leq 1.7 \times 10^{-3}$ [α -corrected = .05/29]) were excluded from the analyses (see Table E2). After removing samples with genotype call rates of less than 80%, 606 cases and 1258 control subjects were retained for association testing.

Ninety-three ancestry informative markers of population structure within Europe were also genotyped with the iPLEX Gold assay to reduce the risk for false-positive results, allowing correcting for major population stratification effects among European and Spanish populations.^{E6,E7} Principal component analysis with EIGENSOFT 4.2^{E8} was used to derive the scores for the first principal component to be used as ancestry estimates in cases and control subjects.

MaCH 1.0^{E9} was used for SNP imputation by using as reference the Phase I data for European samples (May 2011) deposited in 1KGP.^{E10} Association testing was performed by using Mach2dat^{E9} for SNPs showing MAFs of 5% or greater, ensuring that tested SNPs had good-quality imputation scores ($R_{sq} > 0.3$). Imputed variants with MAFs of less than 5% were filtered out for several reasons: (1) the selection of tSNPs was based on HapMap II information to cover variants with frequencies of 5% or greater but not rarer variants; (2) observations in diverse populations suggest that genotype imputation errors produce the greatest power reduction for rarer variants^{E11}; and (3) the discovery sample has low statistical power to detect associations of low-frequency variants (<5%). For example, by using Quanto (<http://biostats.usc.edu/Quanto.html>) and assuming an OR of 2 and a Bonferroni-corrected P value of 1.8×10^{-4} , the statistical power of the discovery study was 55% for MAFs of 3%, whereas it was 87% for MAFs of 5%.

Representation of association results was then performed with LocusZoom 1.1^{E12} based on LD data from hg19 deposited in 1KGP. Functional annotation on the plots was performed with UCSC Genome Browser data for dbSNP build 142. Regulatory variants were assessed with the Variant Annotation Integrator tool based on ChIP-seq experiments^{E13} for 161 factors from the ENCODE Project. Multiple comparison adjustment was performed by using Bonferroni correction for the number of SNPs tested ($P < 1.8 \times 10^{-4}$ [α -corrected = .05/272]). SNPs with the most significant association after multiple comparisons adjustment were followed up in replication studies.

GOA II

Sample description. GOA II included a total of 248 asthma samples collected during 2011 and 2012 from the same centers as in GOA I. Recruitment protocols of asthma cases in GOA II and GOA I were the same (including that the diagnosis of asthma was made by a physician, patients had an active use of specific medication for asthma symptoms according to severity, and their age was >5 years), and the clinical characterization of asthma and atopy was similar.^{E14}

Control subjects included a total of 537 DNA samples from subjects who self-reported having at least 2 generations of ancestors born in Spain and without personal or family history of chronic diseases. These subjects were

obtained from the Spanish National Genotyping Center, Santiago de Compostela Node (CeGen, <http://www.cegen.org>), consisting of unrelated healthy adult subjects collected from hospitals across Spain by Fundacion Publica Galega de Medicina Xenomica (FPGMX, <http://fundacion.xenomica.org>). After signing an informed consent form, donors self-declared general health status, demographics, residence, and personal and family history of infectious, cancerous, mental, cardiovascular, or immunologic diseases were documented. No information from medical records was incorporated or revised, and no medical testing was performed on these control subjects.

Genotyping, quality control, and statistical methods. Samples were genome-wide genotyped on the Axiom Genome-Wide Human CEU Array (Affymetrix, Santa Clara, Calif) by using the services provided by the National Genotyping Center (CeGen), Universidad de Santiago de Compostela Node, as described elsewhere.^{E14} Genotype calls were obtained simultaneously for all cases and control subjects by using the Axiom GT1 algorithm on the Affymetrix Genotyping Console (GTC), according to the manufacturer's recommendations. Quality of data was assessed for dish quality control and the metrics recommended by the manufacturer, call rate ($\geq 95\%$), MAF of 1% or greater, genotype missing rate differences between cases and control subjects ($P > 1 \times 10^{-5}$), HWE P values of greater than 1×10^{-6} in control subjects, concordance between genetically predicted and reported sex, and showing no relatedness ($PI_HAT < 0.2$). A total of 484,597 variants passed all quality control filters and had 99.3% of mean genotype concordance among duplicated samples. The genomic inflation factor of this study was 1.038 adjusting for the 2 first principal components EIGENSOFT.^{E8}

GIANT v.3 haplotypes from the integrated Phase I release of 1KGP^{E10} were used as references to impute untyped SNPs by using Minimac^{E15} for the entirety of chromosome 3, which was previously phased with SHAPEIT version 2.^{E16} The SNP rs77820417 showed an imputation quality score (Rsq) of 0.39. Association testing was performed by means of logistic regression models using Mach2dat.^{E9}

GOAM

Sample description. GOAM consisted of 320 asthmatic cases collected from allergy departments in 2 Spanish centers from the Canary Islands and Malaga fulfilling the Global Initiative for Asthma guidelines for diagnosis and classification of asthma and 250 control subjects collected from the Department of Allergy in the Carlos Haya Hospital (Malaga, Spain).^{E14} Asthmatic patients had physician-diagnosed disease with active specific medication for asthmatic symptoms according to severity and were 5 years or older. Patients were demographically and clinically characterized as in GOA I and GOA II. Atopy was confirmed by evaluating allergens that are common in Malaga and included dust mites (*D pteronyssinus* and *L destructor*), epithelia (*F domesticus* and *C familiaris*), pollen (*O europaea*, *Salvia* species, *L perenne*, *Phleum pratense*, *A vulgaris*, *Parietaria* species, *Platanus* species, *Chenopodium* species, *Plantago* species, and *Cupressus* species), fungi (*A alternata* and *A fumigatus*), and latex.

Control subjects were ascertained for asthma and atopy while accompanying the patients at routine visits to the allergy department. These subjects were unrelated to cases and were demographically and clinically characterized for the presence of a personal or family history of asthma and atopy. Although none of these control subjects were given a diagnosis of asthma at the time of enrollment, 130 of them reported having a personal or family history of atopy.

Genotyping and statistical methods. Genotyping of rs77820417 was assessed by using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, Calif), with automated calls generated by using a 7500 Fast Real-Time PCR System (Life Technologies) based on discriminating plots with 95% confidence. Genotyping was blind to disease status, and duplicated samples (5%) were included to monitor genotyping quality. HWE assessment was done with SNPing.^{E5} Association was tested under an additive model by means of regression analysis with SNPassoc.^{E17}

GALA I

Sample description. The GALA I sample included 529 patients who had physician-diagnosed mild to moderate-to-severe asthma, had experienced 2 or more symptoms in the previous 2 years at the time of recruitment (wheezing, coughing, and/or shortness of breath), and self-identified all 4 grandparents as Puerto Rican or Mexican.^{E18,E19} Healthy control subjects ($n = 347$) who reported all 4 grandparents as Puerto Rican or Mexican were recruited from the same sites as asthmatic patients in the San Francisco Bay Area and Puerto Rico.

Genotyping and statistical methods. Genotyping was performed with the Affymetrix 6.0 GeneChip.^{E19} Given that the SNP of interest (rs77820417) was not genotyped in the Affymetrix 6.0 GeneChip, imputation was performed. For that, genotyped SNPs were first phased by using the software SHAPE-IT,^{E20} and then imputed genotypes were assessed with the program Impute2^{E21} by using the haplotypes from all the populations available in the 1KGP (Phase I, version 3) as a reference.^{E10} The SNP rs77820417 was accurately imputed both in Puerto Ricans and Mexicans from the GALA I study (info score was 0.68 and 0.55, respectively). Association testing was performed with PLINK, including ethnicity and the estimates of African and Native American ancestry obtained in a previous study as covariates.^{E19}

GALA II

Sample description. The GALA II study is an ongoing multicenter case-control study of asthma in Latino children and adolescents.^{E22} Cases and healthy control subjects were recruited by using a combination of community and clinic-based recruitment from 5 urban study centers in Chicago, the Bronx, Houston, the San Francisco Bay Area, and Puerto Rico. Subjects were eligible if they were 8 to 21 years of age, self-identified all 4 grandparents as Latino, and had a smoking history of less than 10 packs per year. Subjects were classified into 3 Latino ethnicity categories according to the self-reported ethnicity of their 4 grandparents: Puerto Rican, Mexican American, and other Latino (including South American, Central American, non-Puerto Rican Caribbean, or mixed Latino).

Asthma was defined based on a physician's diagnosis and report of symptoms and medication use within the last 2 years. Control subjects had no reported history of asthma or allergies and no reported symptoms of wheezing or shortness of breath during their lifetimes. Detailed clinical measurements and demographic and general health data were recorded for each subject. Asthma exacerbation was defined as a binary variable (yes/no) based on the presence of 1 or more of the following asthma-related self-reported events over the 12 months before recruitment: hospitalizations, emergency department visits, and oral steroid use.

Genotyping and statistical methods. GALA II subjects were genotyped with the Axiom LAT1 array (World Array 4, Affymetrix),^{E23} which was specifically designed to capture genetic variation in Latino populations.^{E24} SNPs that failed quality control procedures were removed from the analyses based on the following criteria: failing Axiom quality controls, less than 95% call rate, or showing deviation from HWE ($P < 10^{-6}$) in control subjects within each defined population (Puerto Rican, Mexican, and other Latino). Subjects were filtered based on 97% call rates, discrepancy between genetic sex and reported sex, cryptic relatedness (identity by descent [$PI_HAT > 0.3$]), and standard Affymetrix Axiom manufacturer's recommendations. After quality controls, a total of 3774 samples with high-quality genotype data were available for association testing. Estimates of African, European, and Native American ancestries were obtained by using an unsupervised analysis in ADMIXTURE^{E25} assuming 3 ancestral populations (African, European, and Native American), as described elsewhere.^{E23} Association testing was performed with PLINK, adjusting by ethnicity and the estimates of African and Native American ancestries and as covariates. Association testing with asthma exacerbations was also adjusted by medication use during the 12-month period before recruitment.

Meta-analysis

A meta-analysis was performed with the software METASOFT (v.2.0.1) to estimate the overall effect size of rs77820417 across the 5 studies by using the Han and Eskin random effects model.^{E26}

Enrichment analysis of association signals in the EVE consortium

We extracted summary data results for the region containing the *MYLK* gene from the EVE consortium for the 3 population groups: African American, European American, and Latino subjects. For each subgroup, we calculated the independent number of tested and associated SNPs in the region using SNPSpD software (<http://gump.qimr.edu.au/general/daledN/SNPSpD/>) based on the LD information from 1KGP. The independent observed number of associations was compared with the expected number, assuming a level of significance of a *P* value of .05 and a Fisher exact test applied to assess the existence of enrichment in association signals (Table E5).

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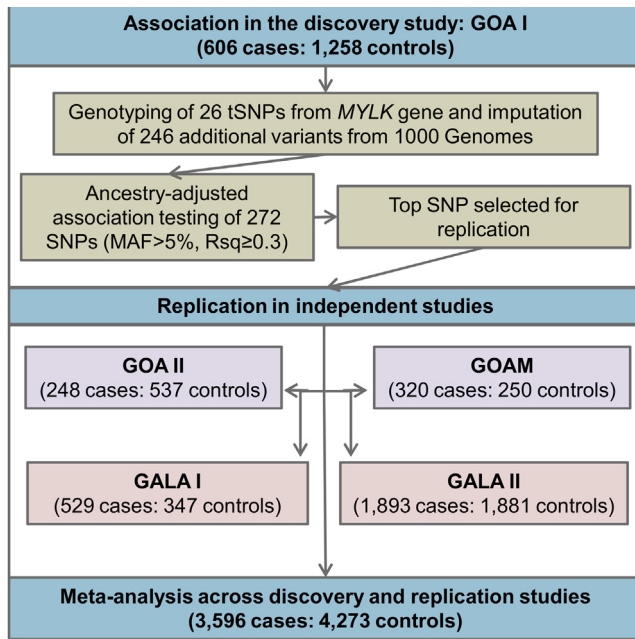


FIG E1. Flow chart providing an overall description of the study design. *GALA I*, Genetics of Asthma in Latino Americans; *GALA II*, Genes-Environments & Admixture in Latino Americans; *GOA I*, Genetics of Asthma study in the Spanish population; *GOA II*, Genetics of Asthma study in the Spanish population II; *GOAM*, Genetics of Asthma study in the Spanish population from Malaga.

TABLE E1. Summary of demographic and clinical features of study samples from Spain

Variable	Discovery sample			Replication samples					
	GOA I (n = 1864)			GOA II (n = 785)			GOAM (n = 570)		
	Cases (n = 606)	Control subjects (n = 1258)	P value	Cases (n = 248)	Control subjects (n = 537)	P value	Cases (n = 320)	Control subjects (n = 250)	P value
Male sex (%)	34.2	60.2	<.001	40.7	51.2	.008	35.9	38.8	<.001
Age (y)	35 (25-44)	40 (32-48)	<.001	28 (19-38.5)	54 (46-62)	<.001	30 (20-42)	45 (32-54)	<.001
Smoking habits (ever smokers [%])	27.1	47.6	<.001	24.1	NA	NA	28.1	NA	NA
Clinical features of asthma cases									
Total IgE levels (IU/mL)	202.0 (89.3-394.5)	NA	NA	310 (124-749)	NA	NA	224.5 (104.8-585.3)	NA	NA
Age at diagnosis (y)	26 (14-40)	NA	NA	9 (4-17)	NA	NA	15 (5-25)	NA	NA
FEV ₁ (% predicted)	83.0 (68.0-95.0)	NA	NA	89 (79-99)	NA	NA	89.5 (80.3-97)	NA	NA
Treatment									
Inhaled corticoids (%)	85.5	NA	NA	72.6	NA	NA	84.5	NA	NA
Oral corticoids (%)	4.0	NA	NA	7.3	NA	NA	16.9	NA	NA
Long-acting β_2 -agonists (%)	52.8	NA	NA	52.0	NA	NA	64.8	NA	NA
Short-acting β_2 -agonists (%)	81.7	NA	NA	57.7	NA	NA	56.3	NA	NA
Body mass index (kg/m ²)	24.2 (21.1-27.5)	NA	NA	24.1 (20.8-28.5)	NA	NA	24.6 (22.2-28.4)	NA	NA

For continuous variables, means and interquartile ranges are displayed, and Mann-Whitney *U* test *P* values are presented for comparison of cases versus control subjects. For categorical variables, the proportion of subjects in each category is shown, and χ^2 test *P* values are presented for comparison of cases versus control subjects.

NA, Not available.

TABLE E2. Summary of demographic and clinical features of Latino studies

Variable	GALA I (n = 876)		P value	GALA II (n = 3774)		P value
	Cases (n = 529)	Control subjects (n = 347)		Cases (n = 1893)	Control subjects (n = 1881)	
Male sex (%)	54.7	27.0	<.001	55.3	43.7	<.001
Age (y)	12 (9-16)	25 (20-32)	<.001	12 (10-15)	13 (11-16)	<.001
Ethnicity						
Mexican, no. (%)	253 (47.8)	158 (45.5)	NA	618 (32.6)	667 (35.5)	NA
Puerto Rican, no. (%)	276 (52.2)	189 (54.5)	NA	910 (48.1)	890 (47.3)	NA
Other Latino, no. (%)	NA	NA	NA	365 (19.3)	324 (17.2)	NA
Total IgE levels (IU/mL)	253 (92-632)	NA	NA	232 (68-650)	80 (26-248)	<.001
FEV ₁ (% predicted)	90.8 (81.3-100.6)	NA	NA	90.8 (81.3-100.6)	98.8 (89.8-107.1)	<.001
Age at diagnosis (y)	3 (1-8)	NA	NA	3 (1-6)	NA	NA

For continuous variables, means and interquartile ranges are displayed, and Mann-Whitney *U* test *P* values are presented for comparison of cases versus control subjects. For categorical variables, the proportion of subjects in each category is shown, and χ^2 test *P* values are presented for comparison of cases versus control subjects.

NA, Not available.

TABLE E3. Summary of quality control measures for the 29 *MYLK* genotyped tagging SNPs

rs No.	Position	Alleles	CR	HWE <i>P</i> value in control subjects
rs2682211	123552138	A/T	99.9%	.802
rs36025624	123541404	C/G	99.5%	.007
rs4678062	123529108	C/T	97.6%	.759
rs28497577	123512627	G/T	97.6%	.481
rs34261801	123479785	C/T	99.4%	.651
rs11718105	123463708	C/T	99.0%	.506
rs4678047	123452838	A/G	97.5%	.038
rs820336	123415781	A/G	99.0%	.298
rs33264	123409788	C/G	99.1%	.404
rs820325	123385677	A/G	93.3%	5.64×10^{-6}
rs820447	123348179	C/T	96.5%	.355
rs860224	123337414	C/G	99.2%	.519
rs11714297	123519579	C/T	99.5%	.768
rs9867910	123493190	A/G	96.1%	.043
rs9846863	123513478	A/G	99.7%	.483
rs7613712	123522605	C/T	100.0%	.221
rs11712609	123530145	A/G	99.1%	.416
rs10511417	123535336	A/G	99.8%	1.000
rs3911406	123538843	C/T	99.7%	1.000
rs2682206	123555676	C/G	99.6%	.801
rs9837215	123473110	C/T	100.0%	.650
rs1378812	123478426	A/G	99.5%	1.000
rs7641248	123426984	C/T	98.7%	6.27×10^{-7}
rs2332589	123438187	C/T	Monomorphic	Monomorphic
rs820329	123444785	A/T	96.7%	.816
rs7622739	123349491	A/G	91.6%	.054
rs820323	123382083	G/T	99.2%	.510
rs820368	123402420	A/T	99.0%	.052
rs3732485	123419782	C/T	98.1%	1.000

SNPs that were filtered out because of deviation from HWE or monomorphic in control subjects are shown in boldface.

CR, Call rate.

TABLE E4. Summary of association testing results for the 53 SNPs nominally associated with asthma in the discovery stage

rs No.	Minor allele	Frequency	Rsq	OR (95% CI)*	P value
rs2626028	C	0.20	0.96	1.24 (1.04-1.47)	1.41×10^{-2}
rs2720313	C	0.20	0.97	1.24 (1.04-1.47)	1.42×10^{-2}
rs6438804	C	0.16	0.79	1.31 (1.07-1.61)	9.29×10^{-3}
rs9422	T	0.20	0.97	1.24 (1.04-1.47)	1.47×10^{-2}
rs6438805	G	0.14	0.74	1.28 (1.03-1.60)	2.94×10^{-2}
rs848145	A	0.07	0.52	1.45 (1.01-2.09)	4.53×10^{-2}
rs860224	G	0.20	NA	1.23 (1.04-1.46)	1.41×10^{-2}
rs820459	G	0.06	0.42	1.63 (1.05-2.56)	3.23×10^{-2}
rs820458	C	0.20	0.99	1.24 (1.05-1.47)	1.22×10^{-2}
rs3845915	G	0.14	0.75	1.29 (1.04-1.61)	2.39×10^{-2}
rs820455	G	0.06	0.42	1.67 (1.07-2.62)	2.60×10^{-2}
rs2697525	A	0.20	0.97	1.26 (1.06-1.50)	7.62×10^{-3}
rs820452	C	0.20	0.97	1.26 (1.06-1.50)	7.35×10^{-3}
rs820451	C	0.20	0.96	1.27 (1.07-1.50)	6.92×10^{-3}
rs41271431	T	0.21	0.34	0.72 (0.53-0.98)	3.35×10^{-2}
rs820450	T	0.20	0.96	1.28 (1.08-1.52)	5.13×10^{-3}
rs820448	G	0.20	0.95	1.29 (1.08-1.53)	4.82×10^{-3}
rs79040346	C	0.13	0.68	1.37 (1.07-1.76)	1.28×10^{-2}
rs820447	G	0.20	NA	1.30 (1.09-1.55)	4.00×10^{-3}
rs820446	C	0.11	0.70	1.55 (1.20-2.01)	9.73×10^{-4}
rs7622739	G	0.45	NA	1.19 (1.02-1.40)	2.73×10^{-2}
rs9844173	G	0.07	0.52	2.07 (1.43-2.99)	1.18×10^{-4}
rs1254397	T	0.07	0.49	1.55 (1.07-2.25)	2.06×10^{-2}
rs1254401	C	0.11	0.68	1.56 (1.20-2.03)	9.22×10^{-4}
rs820460	C	0.11	0.68	1.56 (1.20-2.03)	9.23×10^{-4}
rs820463	G	0.10	0.64	1.67 (1.26-2.21)	3.75×10^{-4}
rs820464	C	0.10	0.64	1.67 (1.26-2.21)	3.71×10^{-4}
rs1254403	C	0.12	0.67	1.54 (1.19-2.00)	9.92×10^{-4}
rs878594	T	0.11	0.67	1.56 (1.20-2.01)	9.18×10^{-4}
rs72968592	C	0.11	0.67	1.55 (1.20-2.01)	9.03×10^{-4}
rs820470	C	0.11	0.67	1.56 (1.20-2.02)	8.93×10^{-4}
rs820472	C	0.11	0.63	1.58 (1.21-2.06)	8.56×10^{-4}
rs820473	G	0.13	0.56	1.58 (1.21-2.07)	8.57×10^{-4}
rs820475	A	0.11	0.63	1.58 (1.21-2.06)	8.56×10^{-4}
rs1254392	G	0.11	0.60	1.69 (1.28-2.23)	2.44×10^{-4}
rs702032	T	0.11	0.63	1.58 (1.21-2.06)	8.37×10^{-4}
rs39639	C	0.11	0.63	1.58 (1.21-2.06)	8.66×10^{-4}
rs42765	G	0.10	0.58	1.61 (1.20-2.17)	1.71×10^{-3}
rs11709947	C	0.48	0.60	1.23 (1.02-1.48)	3.10×10^{-2}
rs820323	T	0.38	NA	1.18 (1.01-1.38)	3.56×10^{-2}
rs820324	A	0.11	0.35	1.81 (1.26-2.61)	1.38×10^{-3}
rs820325	G	0.15	NA	1.78 (1.27-2.48)	7.30×10^{-4}
rs820327	C	0.15	0.32	1.80 (1.29-2.51)	6.20×10^{-4}
rs820363	A	0.15	0.32	1.81 (1.28-2.54)	7.06×10^{-4}
rs78442149	T	0.09	0.32	2.72 (1.79-4.13)	2.78×10^{-6}
rs77820417	A	0.09	0.32	2.71 (1.79-4.11)	2.78×10^{-6}
rs820367	G	0.27	0.88	1.31 (1.11-1.56)	2.04×10^{-3}
rs820368	A	0.27	NA	1.31 (1.11-1.56)	1.90×10^{-3}
rs4678047	G	0.31	NA	1.25 (1.06-1.48)	8.45×10^{-3}
rs6783507	C	0.08	0.68	0.67 (0.48-0.92)	1.28×10^{-2}
rs10511418	T	0.06	0.81	0.70 (0.49-0.99)	4.17×10^{-2}
rs16834774	A	0.06	0.82	0.69 (0.49-0.99)	3.84×10^{-2}
rs3911406	C	0.08	NA	0.66 (0.49-0.88)	4.28×10^{-3}

Significant SNPs after Bonferroni correction ($P \leq 1.8 \times 10^{-4}$ [.05/272]) are shown in boldface.

NA, Not available; *Rsq*, squared correlation between imputed and observed genotypes not available for tagging SNPs.

*Estimated for the minor allele.

TABLE E5. Enrichment analysis of association signals in the EVE consortium summary data

	Latino subjects	European American subjects	African American subjects
No. of tested SNPs	318	181	319
No. of associated SNPs	61	9	30
No. of independent tested SNPs	168	92	188
No. of independent associated SNPs	32	3	9
Expected independent associated SNPs at $P = .05$	8	5	9
Fisher test P value	7.03×10^{-5}	.720	1.0