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Permalink

<https://escholarship.org/uc/item/5m56k02w>

Journal

Journal of Allergy and Clinical Immunology, 134(2)

ISSN

0091-6749

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Publication Date

2014-08-01

DOI

10.1016/j.jaci.2013.08.055

Peer reviewed



Published in final edited form as:

J Allergy Clin Immunol. 2014 August ; 134(2): 295–305. doi:10.1016/j.jaci.2013.08.055.

GWAS and admixture mapping identify different asthma-associated loci in Latinos: The GALA II Study

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Abstract

Background—Asthma is a complex disease with both genetic and environmental causes. Genome-wide association studies of asthma have mostly involved European populations and replication of positive associations has been inconsistent.

Objective—To identify asthma-associated genes in a large Latino population with genome-wide association analysis and admixture mapping.

Methods—Latino children with asthma ($n = 1,893$) and healthy controls ($n = 1,881$) were recruited from five sites in the United States: Puerto Rico, New York, Chicago, Houston, and the San Francisco Bay Area. Subjects were genotyped on an Affymetrix World Array IV chip. We performed genome-wide association and admixture mapping to identify asthma-associated loci.

Results—We identified a significant association between ancestry and asthma at 6p21 (lowest p-value: rs2523924, $p < 5 \times 10^{-6}$). This association replicates in a meta-analysis of the EVE Asthma Consortium ($p = 0.01$). Fine mapping of the region in this study and the EVE Asthma Consortium suggests an association between *PSORS1C1* and asthma. We confirmed the strong allelic association between the 17q21 asthma in Latinos (*IKZF3*, lowest p-value: rs90792, OR: 0.67, 95% CI 0.61 – 0.75, $p = 6 \times 10^{-13}$) and replicated associations in several genes that had previously been associated with asthma in genome-wide association studies.

Conclusions—Admixture mapping and genome-wide association are complementary techniques that provide evidence for multiple asthma-associated loci in Latinos. Admixture mapping identifies a novel locus on 6p21 that replicates in a meta-analysis of several Latino populations, while genome-wide association confirms the previously identified locus on 17q21.

Keywords

Asthma; Latinos; Admixture Mapping; Genome-wide Association Study; Local Ancestry; 17q21; 6p21

Introduction

Asthma is the most common chronic disease among children. In the U.S., childhood asthma prevalence is highest among Puerto Ricans (18.4%), followed by Blacks (14.6%), Whites (8.2%) and Mexicans (4.8%).(1, 2) The discrepancy in asthma burden, as well as the paucity of studies of asthma in Latinos, has led the American Academy of Pediatrics to identify asthma among Latinos as an urgent priority for further research.(3)

Genetic variation between populations may account for some of these differences. Estimates of the heritability of asthma based on twin studies range from between 75%(4) and 92%(5). However, few genetic loci show consistent associations across studies.(6-8) Of the forty-eight genes reported to be associated with asthma in genome-wide studies,(9) only five have been identified in more than one GWAS.(10-16) This may be due to differences in study designs, differences in environment between study populations, and heterogeneity in asthma phenotypes.(17, 18) Our previous work has found that few genes associated with asthma replicate in Latino populations. Most that do replicate are consistent across Latino ethnic groups; however, five genes replicated in either Mexicans or Puerto Ricans (but not both) and showed significant heterogeneity in their association with asthma.(6) These observed differences in genetic variation may in part explain the discrepancy in asthma prevalence between the two groups.(19)

Most genome-wide association studies of asthma have been conducted in European and European American populations.(20) The recent identification of *PYHINI*, a gene associated with asthma in individuals of African descent but not in Latinos or European Americans(10) highlights the importance of studying diverse populations in genetic association analyses. Moreover, studies in non-European populations may help uncover some of the “missing heritability” in complex diseases and may provide insights into racial/ethnic disparities in asthma prevalence and severity.

Admixture mapping is a technique that can help identify asthma-associated loci in populations of mixed ancestry (such as African Americans and Latinos).(21) In these admixed populations, it is possible to use dense genotyping to estimate ancestry at a locus-specific (local) level.(22, 23) If the allele frequency of risk variants is higher in one ancestral population than others, there will be a correlation between ancestry at that locus and disease. Comparing local ancestry in individuals with disease to control subjects will show a deviation from the expected distribution of ancestry. Like genome-wide association studies, admixture mapping provides an unbiased method to screen for disease-associated loci. However, because admixture is a relatively recent phenomenon, ancestry blocks are significantly larger than haplotype blocks,(24, 25) so admixture-mapping offers increased coverage of genetic variation and a lower multiple testing burden than GWAS, though this also means that admixture mapping peaks cover an area in the hundreds of kilobases, which

makes narrowing down the causal variant more difficult. Admixture mapping has successfully identified disease-associated loci in breast(26) and prostate cancer,(27) renal disease,(28, 29) and white blood cell count,(30, 31) among others. Our own prior admixture mapping study demonstrated that admixture mapping peaks preferentially harbored asthma-associated genes,(32) while an admixture mapping study in African Americans and Puerto Ricans identified a locus on 6q14.1 that harbored a risk allele for asthma exclusively in individuals with local European admixture.(33)

In order to identify genetic risk factors for asthma we used genotype data from the Genes-environment & Admixture of Latino Americans (GALA II) study. GALA II is an ongoing, multicenter, case-control study to identify novel clinical and genetic risk factors associated with asthma and related phenotypes in Latino populations at five Sites in the United States: Puerto Rico, New York, Chicago, Houston, and the San Francisco Bay Area, which includes genome-wide genotype data on 3,774 participants. We hypothesize that by using a large population of Latino participants we would identify novel loci for asthma.

Methods

Recruitment and genotyping

Institutional review boards at UCSF and recruitment sites approved the study, and all participants/parents provided appropriate written assent/consent. Latino children were enrolled as a part of the ongoing GALA II case-control study. From July 2006 through June 2011, when genotyping began, a total of 4,045 children (1,976 participants with asthma and 2,065 healthy controls) were recruited from five centers (Chicago, Bronx, Houston, San Francisco Bay Area, and Puerto Rico) using a combination of community and clinic-based recruitment. Participants were eligible if they were 8-21 years of age and self-identified as Latino and had four Latino grandparents. Asthma cases were defined as participants with a history of physician diagnosed asthma and the presence of two or more symptoms of coughing, wheezing, or shortness of breath in the 2 years preceding enrollment. Participants were excluded if they reported any of the following: (1) 10 or more pack-years of smoking; (2) any smoking within 1 year of recruitment date; (3) history of lung diseases other than asthma (cases) or chronic illness (cases and controls); or (4) pregnancy in the third trimester. Details of recruitment are described elsewhere(34) and in the Online Repository.

Participants were genotyped at 818,154 SNPs on the Affymetrix Axiom World Array IV. (35) Details of individual and SNP quality control procedures are described in the Online Repository.

Ancestry was estimated using the program ADMIXTURE, with a three population model. (36) Details of the ancestral populations are described in the Online Repository. Local ancestry at all positions across the genome was estimated using the program LAMPLD(22), assuming three ancestral populations.

Statistical methods

We tested the associations between each SNP and asthma status using logistic regression, adjusting for potential confounders including global and local ancestry (for details, see the

Online Repository), using a threshold for significance of 5×10^{-8} . Admixture mapping was performed using a likelihood ratio test to compare a full model including two local ancestry estimates, and potential confounders to a restricted model excluding the local ancestry estimates. Adjustment for multiple comparisons was made using a permutation procedure (for details, see the Online Repository). Based on permutation testing, we used an adjusted significance threshold α^* of 9×10^{-6} , corresponding to 5278 effective independent comparisons. Analyses were performed using R (version 2.14.1). (37)

We searched for allelic associations within any admixture mapping peaks. A peak was defined as a contiguous region whose admixture mapping p-values were within $2 \log_{10}$ orders of the most significant p-value. We examined the p-values for significance, adjusting for multiple comparisons using both a Bonferroni correction for the number of SNPs within the peak and the effective number of comparisons accounting for linkage disequilibrium between SNPs measured via a spectral decomposition.(38, 39) The former correction resulted in a significance threshold α^* of 8×10^{-5} for 601 SNPs; however, accounting for the effective number of independent markers (~174 SNPs) within this region resulted in a less conservative significance threshold α^* of 2×10^{-4} .

Replication of admixture mapping results

We examined ancestry associations in each of the studies containing Latino participants in the EVE Asthma Consortium.(10) Details of the individual studies and estimation of ancestry are described elsewhere(32, 40-42) and in the Online Repository.

Admixture mapping of the region of interest was performed using logistic regression for case-control studies and the transmission disequilibrium test (TDT test) for trio-based studies. A meta-analysis of the five studies was performed using a fixed-effects model in PLINK.(43) A two-sided significance level of 0.05 was chosen.

Replication of prior GWAS and Admixture Mapping results

Using the NHGRI Catalog of Published GWAS studies,(9) we identified significant association(s) in prior genome-wide association studies for asthma (see Table E1 in the Online Repository). A two-sided significance level of 0.05 was chosen. We then examined the association between the reported SNPs and asthma in the GALA II study. SNPs that were not genotyped were imputed using Impute2.(44, 45) For the imputation, we used haplotypes from all available populations in the 1000 Genomes Project Phase I, integrated variant set release v3 as a reference panel, and we filtered our results to include only those variants imputed with an information score cutoff of > 0.3 .(46, 47)

We also investigated whether previously reported admixture mapping results in asthma (32, 33) replicated in the current study by examining the admixture mapping likelihood ratio test results underlying the peaks described in the two studies in Torgerson *et. al.* in Latinos and African Americans.

Results

The characteristics of the GALA II study population are shown in Table I. Nearly half of the subjects were Puerto Rican (47%) followed by Mexican (31-35%), and Latinos from other ethnicities (15-18%). The percentage of males was higher in cases than the controls (55% vs. 44%), and the cases had higher total IgE on average than controls (234 vs. 79 IU/ml). Differences in genomic ancestry between cases (11% African ancestry [AFR] and 15% Native American ancestry [NAM],) and controls (12% AFR and 15% NAM) were small, but statistically significant. Individual level ancestry proportions are shown in Figure 1 and highlight the variability between and within Latino ethnicities.

Most of the cases have either severe (43%) or moderate persistent (28%) asthma, based on medication/severity and symptoms/control classification.(48) The remaining cases are split between mild intermittent (17%) and mild persistent (12%) asthma.

Admixture mapping

We performed an admixture-mapping study of asthma in 3,774 US children of Latino ethnicity (Figure 2 and Figure E1 in the Online Repository). Our most significant admixture-mapping association was on chromosome 6p21 near the HLA region. It was centered on *MUC22*, where local ancestry was significantly associated with asthma ($p < 5 \times 10^{-6}$). The 0.05 genome-wide threshold established by permutation testing corresponds to a nominal p-value of 9×10^{-6} . The permutation-corrected p-value for the 6p21 peak was 0.02 adjusting for multiple testing. As expected with admixture mapping studies, the genome-wide significant region is broad, encompassing a genomic region of approximately 350 kb (Figure 2B). Because of this, there is slight genomic inflation ($\lambda = 1.1$, figure E-A in the Online Repository). However, if chromosome 6 is excluded from the analysis, there is no genomic inflation in the remainder of the genome ($\lambda = 0.99$, Figure E1B in the Online Repository). Within this genomic region, Native American ancestry is associated with lower odds of asthma (OR 0.87, 95% CI 0.82 – 0.93, $p = 1.7 \times 10^{-5}$).

We performed a meta-analysis of the association between Native American ancestry and asthma at this locus among five studies of Hispanic/Latino ethnicity from the EVE Asthma Consortium. Our initial results replicated in the same direction (OR 0.88, 95% CI 0.79 – 0.98, $p = 0.02$). A forest plot of the studies is shown in Figure 3.

We then searched for allelic associations within the peak, which we defined as the contiguous region whose admixture mapping p-values were within two log-orders of the most significant admixture association. This spanned a 354 kb region that included 13 genes and one open reading frame. We tested for allelic associations for the 601 SNPs identified within that region; the most significant association was between asthma and KG_6_31200283 (rs114235219), a SNP in *PSORS1C1* (OR 0.61, $p = 0.002$). Given that we tested for 601 allelic associations within the peak, the association did not meet the threshold for significance after adjusting for multiple comparisons, either using the within-peak Bonferroni correction ($\alpha^* = 8.3 \times 10^{-5}$), or adjusting for the effective number of comparisons accounting for linkage disequilibrium between SNPs ($\alpha^* = 2.9 \times 10^{-4}$; effective number of independent marker loci: 174).(38, 39)

We also tested for allelic associations within the peak in the EVE Asthma Consortium data. (10) There were 399 SNPs within the region in the EVE Asthma consortium dataset. Unfortunately, rs114235219 was not analyzed in the EVE Asthma consortium dataset. The most significant allelic association within the peak was rs3132550, which was also within *PSORS1C1* ($p = 3 \times 10^{-4}$). There were 307 SNPs common to both the GALA II and EVE datasets under the admixture-mapping peak. Two of these SNPs were significant in both the GALA II and EVE Asthma consortium datasets. Both SNPs were in the *PSORS1C1* gene, rs3094663 (GALA II OR 0.85, 95% CI 0.77 – 0.95, $p = 0.003$; EVE p-value 0.01) and rs3130564 (GALA II OR 1.30, 95% CI 1.07 – 1.58, $p = 0.009$; EVE p-value 0.02). In both cases, the direction of the association was consistent across the two studies (the “T” allele in rs2094663 and the “C” allele in rs3130564 were associated with increased asthma risk in both studies). A locus-zoom plot of this region is given in Figure E2 in the online repository.

Examining previously reported admixture-mapping peaks, (32, 33) we found that the peak reported on cytoband 8q12 in both Puerto Ricans and Mexicans replicated in this study (LR p-value 0.03); Native American ancestry was associated with increased risk of asthma (OR 1.08, 95% CI 1.02 – 1.15, $p = 0.01$) as in Puerto Ricans in the original study. There was no association with African ancestry at this locus ($p = 0.8$). We were unable to replicate the association between ancestry and asthma in the admixture-mapping peak in cytoband 6q15 found in Mexicans (LR p-value 0.3) or the association found in cytoband 6q14 in African Americans ($p = 0.2$). Of the 55 additional non-overlapping loci identified by Torgerson et al as being enriched for asthma peaks, we found 13 additional loci that were nominally associated (LR p-value < 0.05) with asthma in this study. The results are summarized in Table E3 in the Online Repository.

Asthma GWAS

Results of our genome-wide association study are summarized in Figure 4 and Table II. The genomic inflation factor (λ) for allelic association testing in this study is 1.03 (see Figure E3 in the Online Repository). Thirty-one SNPs, all in the 17q21 region, were significantly associated with asthma at a genome-wide corrected significance level of $< 5 \times 10^{-8}$. A locus zoom of the region is shown in Figure 4B. The most significantly associated SNP (rs907092) is a synonymous coding mutation in the *IKZF3* gene, which lies within a region of broad linkage disequilibrium that also includes *ORMDL3*, *GSDMB*, and *ZPBP2*. The minor allele of the *IKZF3* SNP conferred protection against asthma with an odds ratio of 0.67 (95% CI 0.61 – 0.75, $p = 6 \times 10^{-13}$). Conditioning on rs907092 removes virtually all associations within the 17q21 region, with only rs12450091, a non-synonymous coding mutation in *GSDMB* maintaining nominal significance (OR = 1.23, 95% CI 1.07 – 1.42, $p = 0.005$). Additionally, six regions had genotyped SNPs that showed a suggestive association with asthma at $p < 5 \times 10^{-6}$ (Table IIa). Examination of SNPs in each of these six regions in the EVE Asthma Consortium showed no significant associations with asthma in either the combined study ($p > 0.05$ for all SNPs), or in the subset of studies with individuals of Latino ethnicity ($p > 0.05$). Two additional regions showed suggestive associations with asthma at $p < 10^{-6}$ using data imputed with 1000 Genomes (Table IIb); neither of these replicated in EVE.

We also examined regions that had previously been associated with asthma in previously reported genome-wide association studies (Table E1 in the Online Repository). We identified twenty studies reporting results of genome-wide associations in asthma, including two that were meta-analyses of other reported studies. Twelve studies reported one or more associations, though only seven of these met strict genome-wide significance with $p < 5 \times 10^{-8}$. Thirty total SNPs were reported to be significant across the studies at genome-wide significance levels in at least one study. Twelve of these SNPs replicated in the current study (Table E4, in bold, Figure 5), though in four cases the direction of the observed effect was in the opposite direction than that of the original study (denoted with a minus sign). In five cases, the direction of the observed effect was in the same direction as that of the original study (denoted with an asterisk), and in three cases the original study did not report an odds ratio.

Discussion

We used the complimentary techniques of admixture mapping and allelic genome-wide association analysis to identify novel asthma-associated loci and confirm prior genetic associations. In the admixture mapping study, we found a genome-wide significant association between asthma and a peak at 6p21. This peak was centered on *MUC22* (likelihood ratio $p < 5 \times 10^{-6}$; permutation-adjusted p-value 0.02). Native American ancestry in this genomic region was associated with decreased risk of asthma (OR 0.87, 95% CI 0.82 – 0.93, $p = 1.7 \times 10^{-5}$). This association was replicated in a meta-analysis of five studies of Latinos in the EVE Asthma Consortium, where Native American ancestry was associated with a nearly identical protective effect (OR = 0.88, 95% CI: 0.79 – 0.98).

Admixture mapping and genome-wide association analysis depend on tagging causal variants through local ancestry blocks and haplotype blocks, respectively. Because admixture mapping relies on relatively large ancestry blocks to identify disease-associated regions of the genome, there are fewer effective comparisons and therefore a lower statistical penalty for multiple comparisons. In this case, permutation procedures lead to a significance threshold of 9×10^{-6} , suggesting that there are approximately 5,000 to 6,000 effective comparisons. In contrast, the traditional significance threshold for genome-wide association studies is 5×10^{-8} , which is equivalent to approximately 1,000,000 haplotype blocks. While the reduced multiple comparison penalty associated with admixture mapping make it an attractive technique for identifying regions associated with a disease, the drawback of the technique is that it results in a larger genomic region of interest, as in our study.

In the current study, the region was narrowed down to a 350 kB region centered on a cluster of mucin producing genes: *MUC21*, *MUC22*, *PBMUCL2* (see Figure 2), in the major histocompatibility complex region on chromosome 6. The genes in the mucin family are intriguing candidates for involvement in differential asthma susceptibility. These highly glycosylated macromolecules are broadly expressed in the airway epithelium, where they can be secreted or tethered to airway epithelial membranes, and play a critical role in innate immune defense.(49) The overproduction of mucin has been associated with chronic airway disease, including asthma.(50) A genetic polymorphism in a promoter in *MUC5B* has also

been strongly associated with idiopathic pulmonary fibrosis, suggesting a link between mucin genes and lung disease.(51) *MUC22* itself was identified by searching for genes in the region associated with diffuse panbronchiolitis,(52) which, like asthma, is an obstructive lung disease. The same study found that *MUC22* was expressed in the lung and in human bronchial epithelial cells differentiated at an air-liquid interface.

Although *MUC22* is an intriguing candidate for association with asthma, we cannot exclude other genes under the admixture mapping peak, including *HLA-C* and *HLA-B*. *HLA-B* has been evaluated as a candidate gene for asthma in two studies with mixed results. In a study of Croatian children with atopic asthma, the HLA-B8 antigen was associated with asthma, (53) while an earlier study in Greek children with allergic asthma found no specific HLA-B alleles associated with asthma.(54)

We attempted to narrow the region of interest down by searching for allelic associations under the admixture-mapping peak. We found no allelic associations that met region-wide significance criteria ($\alpha^* = 8.3 \times 10^{-5}$, adjusting for 601 SNPs evaluated) in the GALA II study. However, the most significant allelic association within the region in GALA II, (rs114235219, KG_6_31200283, $p = 0.002$), and the most significant SNP in the EVE asthma consortium study (rs3132550, $p = 3 \times 10^{-4}$) were both located on *PSORSIC1*, a psoriasis susceptibility candidate,(55) as were the two SNPs that achieved nominal significance in both studies (rs3094663 and rs3130564). *PSORSIC1* was identified as a risk locus for systemic sclerosis,(56) Stevens-Johnson Syndrome,(57, 58), Crohn's disease,(50) COPD biomarkers,(59) and Behçet's disease(60) in GWAS. A recent study showed that SNPs associated with both asthma and autoimmune diseases have opposite effects on immunopathogenesis.(61)

Although the evidence suggests that variation within *PSORSIC1* is associated with asthma, it may be necessary to sequence the region to identify the causal variant or variants. The lack of a definitive allelic association within 6p21 may be due to one of several reasons. It is possible that the genotyping platform includes SNPs that poorly tag the causal variants. Although the World Array IV was specifically designed to maximize coverage in Latinos, its coverage of variation in Mexicans in Los Angeles (MXL) from the 1000 Genomes project is still imperfect, especially for uncommon and rare variants. Approximately 80% of SNPs with a minor allele frequency between 0.05 and 0.1 were tagged with an $r^2 > 0.7$, and for rarer alleles, coverage was less than 70%. The Eve Asthma Consortium study was genotyped on a variety of older platforms, which may not have captured variation in non-European populations. Furthermore, the MHC region is highly variable, which increases the probability that the admixture-mapping signal was driven by untyped variation. Under these circumstances, admixture mapping has an advantage over allelic and imputed associations because the breadth of ancestry blocks allows for nearly complete coverage across the genome. As long as the allele frequency of the causal variant or variants differs between the ancestral populations, an admixture mapping association may be detectable even if the untyped causal SNP is in poor linkage disequilibrium with genotyped SNPs. Admixture mapping can also find associations driven by rare variants unlikely to be identified through conventional means. Finally, the lack of allelic association could also be due to a number of

variants with small effects clustered in the region, which would not be surprising in the MHC region given its highly variable nature.

We were also able to replicate the admixture-mapping association between 8q12 and asthma, as well as a substantial minority (13 of 55, See Table E3 in the Online Repository) of the additional admixture-mapping peaks previously reported as being enriched for asthma genes.(32) This provides additional evidence that admixture mapping is able to uncover novel disease-associated regions.

Our genome-wide association analysis replicated the previously described association between the 17q21 region and asthma. Our reported odds ratio of 0.67 (95% CI 0.61 - 0.75) is consistent with the previously reported effect magnitudes at this site. Although our most statistically significant SNP was in *IKZF3* (approximately 50 kb upstream from reported associations in *ORMDL3* and *GSDMB*),(15) there is a high degree of linkage disequilibrium in this region, and our statistically significant results extended across all 3 genes in the region. Moreover, functional work by Verlaan *et al.* has shown that genetic variants in this region are associated with domain-wide *cis*-regulatory effects on expression of multiple genes in the region, likely through their effect on chromatin states.(62)

Our evaluation of the previous genome-wide association studies described in table E1 in the online repository found that nearly half of the associations with asthma nominally replicated in our study. This contrasts with our earlier evaluation of principally candidate gene studies, where we found that only 17 genes contained SNPs that replicated in at least one Latino population out of 124 genes that had been previously associated with asthma.(6) This may be partially explained by the fact that this study, which included nearly 4000 individuals, was better powered to detect associations than our prior study, which included nearly 700 trios. However, it also seems likely that GWAS findings may reflect stronger effect sizes than candidate gene studies, may be less likely to reflect Type I error, and may be more likely to confer universal risk across ethnic groups.

Our prior study also showed that for a small number of genes, there was an ethnic-specific replication pattern with significant statistical heterogeneity between Mexicans and Puerto Ricans. In this study, we found no genes with a statistically significant gene by ethnicity interaction. This suggests that associations with asthma uncovered through genome-wide studies are more universal than those from candidate gene studies, which may also explain why they are easier to replicate.

We do note that in four out of 25 of the associations for which the original direction of effect was reported, the direction of replication was in the opposite direction. Such “flip flop” associations have been previously reported in an asthma GWAS where a protective minor allele in European subjects was associated with increased risk in African American participants.(63) Such a finding was ascribed to differences in the underlying genomic architecture between the two study populations, as could occur when the genotyped SNP was correlated through linkage disequilibrium or interaction with a causal variant but the direction of the correlation varies between populations, resulting in an observed SNP that tags opposite alleles of the causal variant.(64, 65)

It should be noted that our case definition of asthma was based on physician diagnosis and self-report of symptoms. Although our cases, on average had evidence of airway obstruction, with significantly lower FEV₁ and FEF₂₅₋₇₅ than controls (Table I), and many had significant response to albuterol, participants who did not have evidence of bronchodilator response were not required to undergo methacholine provocation. The lack of objective confirmation of the asthma diagnosis may have resulted in some misclassification of some participants. Such misclassification bias, which would be expected to be non-differential with respect to the predictor, biases the results towards the null, understating our results. Nonetheless, our finding of a strong association at the 17q21 region with comparable if not larger effect magnitudes than the original study suggests that this effect was likely to have been small.

In summary, our genome-wide association study confirmed significant associations at 17q21 region in Latinos, and our admixture mapping study identified a novel asthma-associated locus at 6p21 centered on the *MUC22* gene near the MHC region. Fine mapping in this region points to the involvement of the gene *PSORS1C1* in the pathogenesis of asthma. This confirms the advantages of performing admixture mapping studies in diverse populations to identify novel asthma-associated genes.⁽⁶⁶⁾ Follow up of results from admixture mapping and GWAS with sequencing and functional studies in minority populations carried out in large multiethnic cohorts could help to relate the significance of such findings to disparities in asthma prevalence and severity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge the families and patients for their participation and thank the numerous health care providers and community clinics for their support and participation in GALA II. In particular, the authors thank study coordinator Sandra Salazar; the recruiters who obtained the data: Duanny Alva, MD, Gaby Ayala-Rodriguez, Lisa Caine, Elizabeth Castellanos, Jaime Colon, Denise DeJesus, Blanca Lopez, Brenda Lopez, MD, Louis Martos, Vivian Medina, Juana Olivo, Mario Peralta, Esther Pomares, MD, Jihan Quraishi, Johanna Rodriguez, Shahdad Saeedi, Dean Soto, Ana Taveras. The authors would also like to acknowledge the EVE Asthma Consortium for providing summary data for replication of suggestive associations, including the following Eve Principal Investigators: Carole Ober, PhD, Dan Nicolae, PhD, Scott Weiss, MD, Benjamin Raby, MD, Keoki Williams, MD, MPH, Kathleen Barnes, PhD, Deborah Meyers, PhD, Eugene Bleecker, MD, and Jim Gauderman, PhD. The authors would like to thank Amy Markowitch, JD for her editorial comments and assistance preparing this manuscript. Some computations in this manuscript were performed using the UCSF Biostatistics High Performance Computing System.

Sources of funding:

This research was supported in part by National Institutes of Health (R01 ES015794, R01 HL088133, M01 RR000083, R01 HL078885, R01 HL104608, P60 MD006902, U19 AI077439, M01 RR00188); ARRA grant RC2 HL101651; EGB was supported in part through grants from the Flight Attendant Medical Research Institute (FAMRI), the Sandler Foundation, the American Asthma Foundation and NIH (K23 HL004464); JMG was supported in part by NIH Training Grant T32 (GM007546) and career development awards from the NHLBI K23 (K23HL111636) and NCATS KL2 (KL-TR000143) as well as the Hewett Fellowship; CRG was supported in part by NIH Training Grant T32 (GM007175) and the UCSF Chancellor's Research Fellowship and Dissertation Year Fellowship; RK was supported with a career development award from the NHLBI (K23HL093023); HJF was supported in part by the GCRC (RR00188); PCA was supported in part by the Ernest S. Bazley Grant. SJL was supported in part by the Division of Intramural Research, National Institute of Environmental Health Sciences (ZIA ES49019). This publication was supported by various institutes within the National Institutes of Health. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Abbreviations

CARE	Childhood Asthma Research and Education network
CHS	Children's Health Study
FEV1	Forced expiratory volume in 1 sec
FVC	Forced vital capacity
GALA I	Genetics of Asthma in Latino Americans
GALA II	Genes-environments & Admixture in Latino Americans
GWAS	Genome-wide association study
IgE	Immunoglobulin E
MCCAS	Mexico City Childhood Asthma Study
SNP	Single nucleotide polymorphism
TDT	transmission disequilibrium test

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Capsule Summary

In this study, we use the complementary techniques of admixture mapping and genome-wide association in Latinos to identify novel asthma-associated loci.

Key Messages

- Admixture mapping of asthma in nearly 4000 Latinos identifies a novel asthma locus on 6p21 that replicates in a meta-analysis of several additional studies of Latinos
- Fine mapping of this region suggests involvement of the *PSORS1C1* gene with asthma.
- Genome-wide association of asthma confirms the previously identified asthma locus on 17q21 and replicates several genes that had previously been associated with asthma in GWA studies.

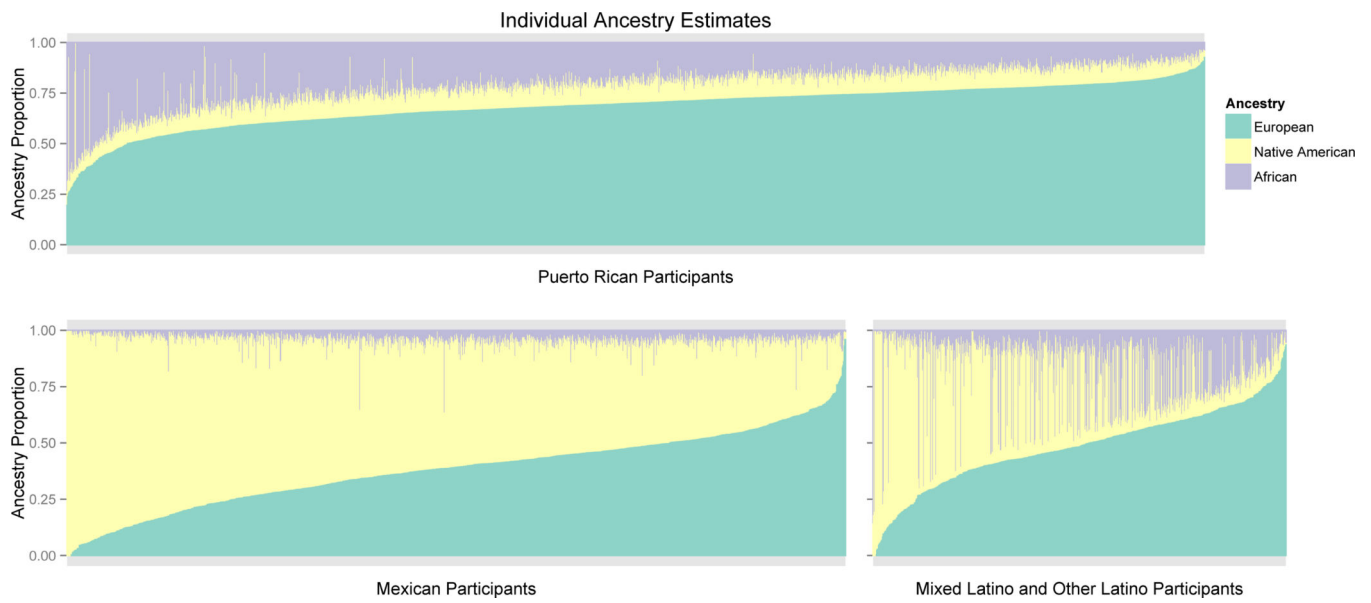


Figure 1. Proportions of individual Native American, European, and African ancestry in the GALA II participants. Each bar represents one individual. There is substantial variability in individual ancestry both within and between Latino ethnic sub-groups.

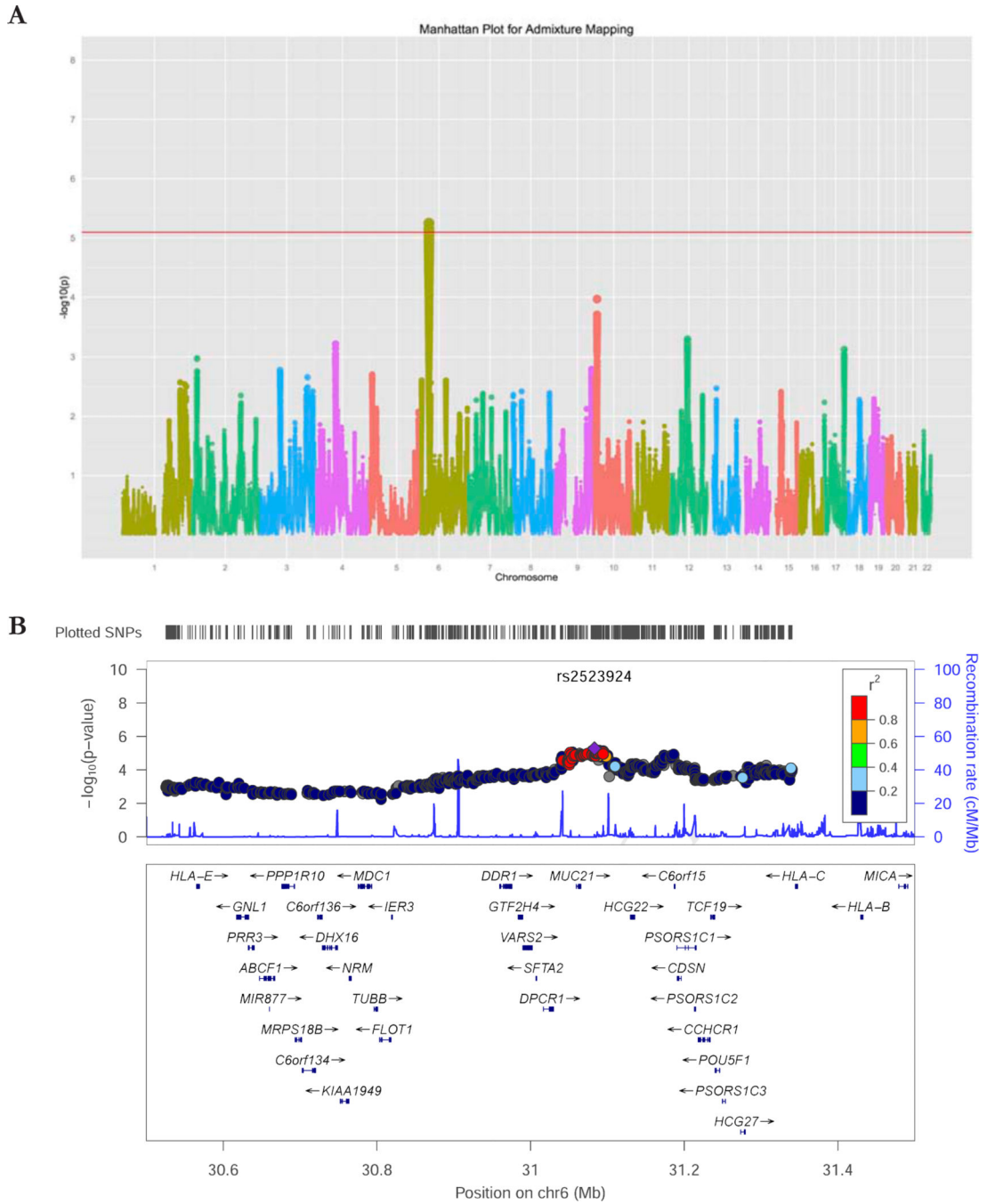


Figure 2. Admixture mapping results. A. Manhattan plot of the admixture mapping study, showing a significant peak in chromosome 6. The red line represents the fifth percentile of the lowest p3 value in 10,000 permutations. B. Locus-zoom plot of the admixture mapping peak, which is centered in the *MUC21* and *MUC22* gene cluster, upstream of *HLA-B* and *HLA-C* genes.

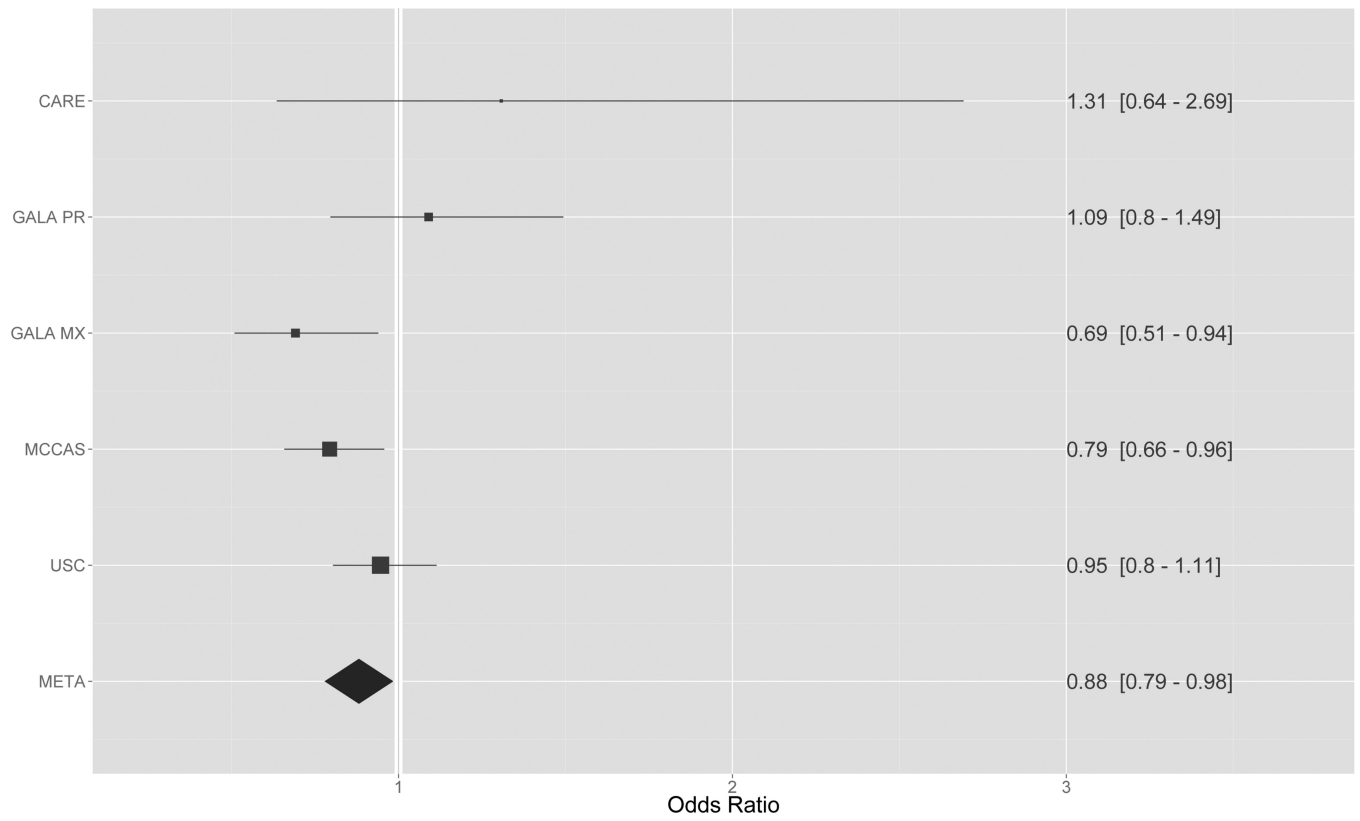


Figure 3. Forest plot of the meta-analysis of the effect of Native American ancestry on asthma in the Latino studies within the EVE consortium. The odds ratio and [95% confidence interval] are listed next to each study.

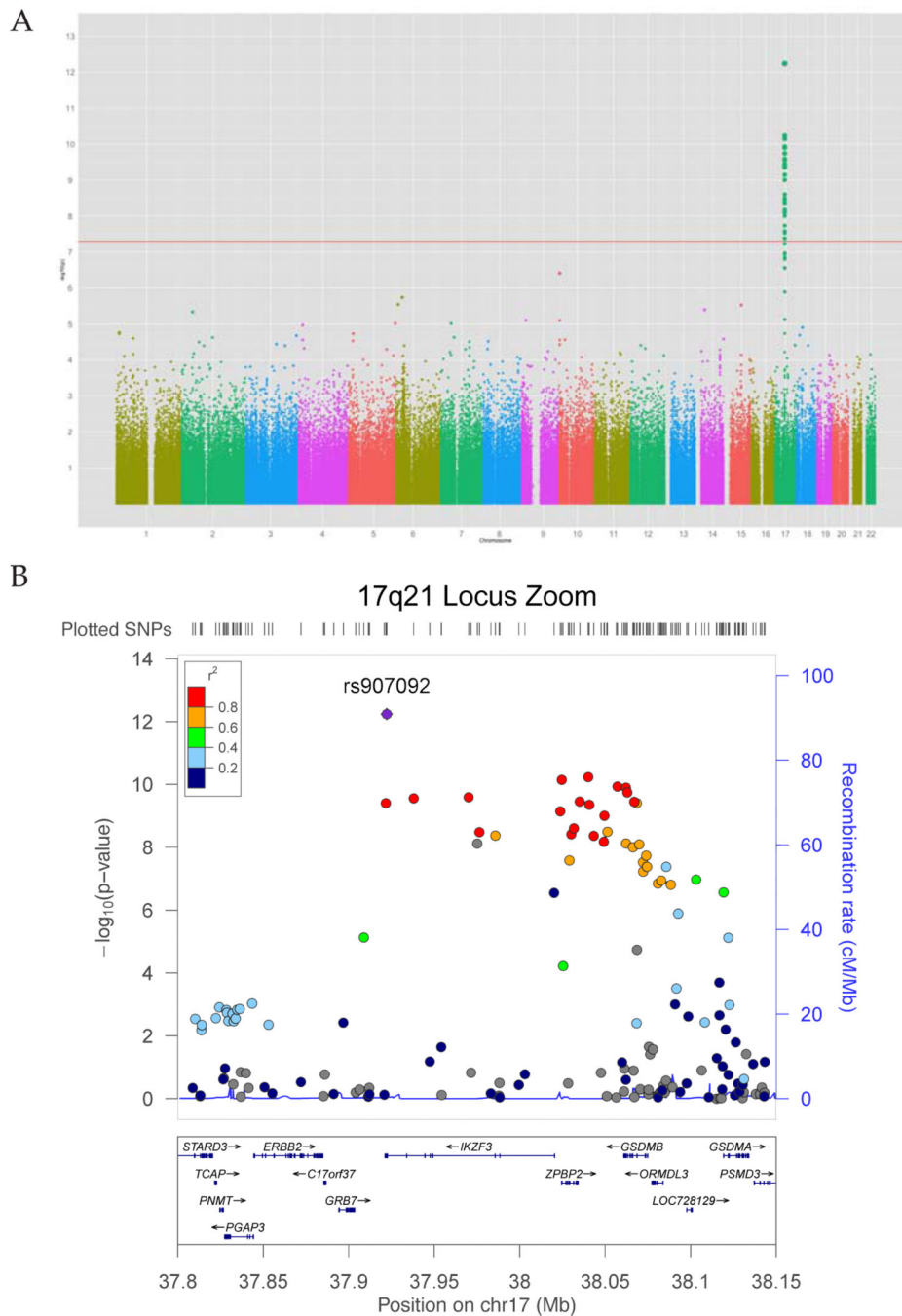
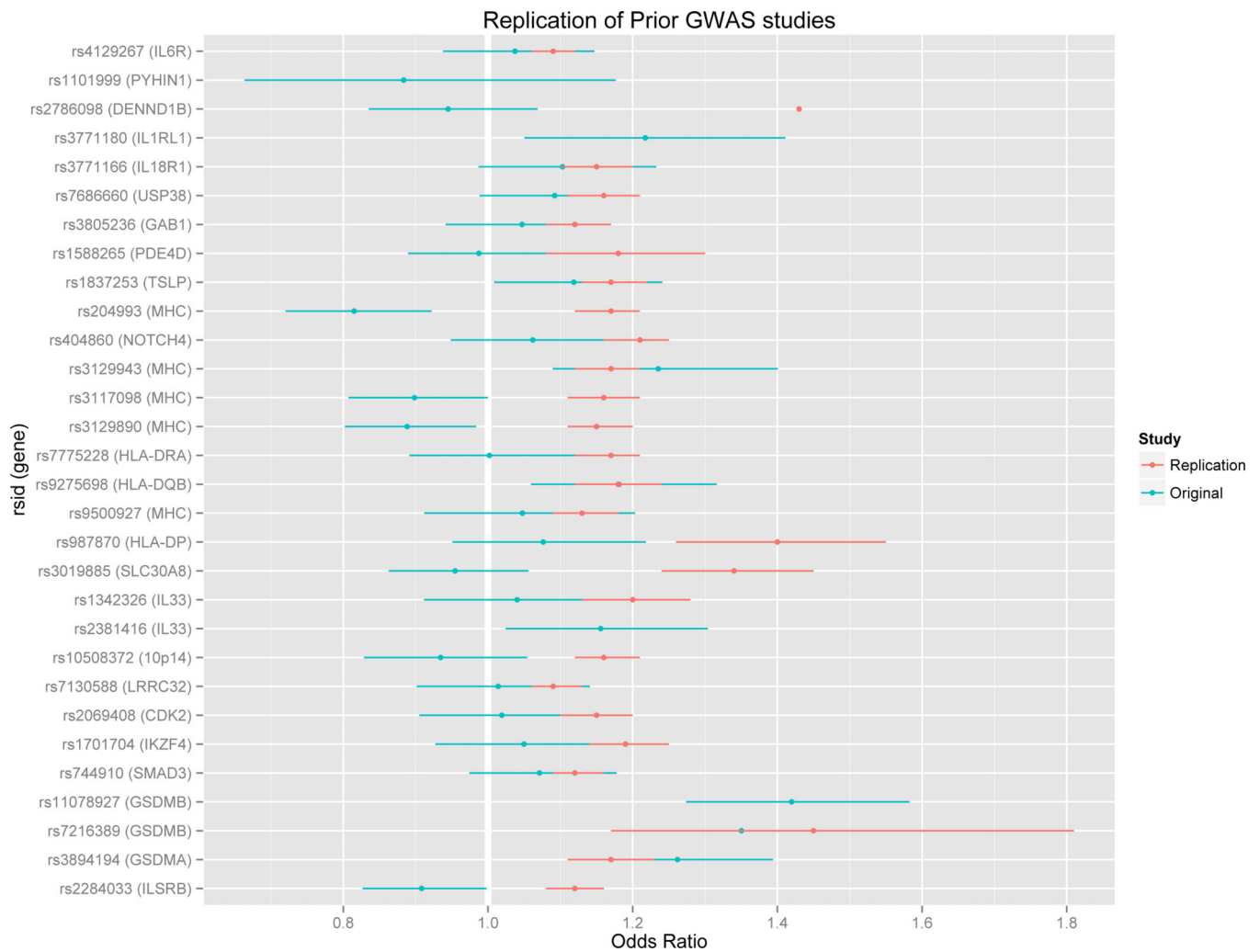


Figure 4. Genome-wide association study results. A. Manhattan plot of the GWAS, showing a genome-wide significant peak at chromosome 17q21. B. Locus-zoom plot of the 17q21 peak, which shows a broad area of linkage disequilibrium, with the most significant finding centered on the *IKZF3* gene.

**Figure 5.**

Replication of previous genome-wide findings in the GALA II study. When provided, the original study's effect estimate and confidence interval are given in red, while the effect estimate and confidence interval in the present study are shown in teal. One study, including three SNPs reported only a summary p-value.⁽¹⁰⁾ A second study, including one SNP, reported an odds ratio, but not confidence interval.⁽⁵⁶⁾ + Replication was in the same direction as the original study.³ Replication was in the opposite direction as the original study.[?] The direction of replication could not be assessed. MHC major histocompatibility complex region.

Table I

Characteristics of GALA II participants

n	1893	1881	
Age (yrs)	12 [10: 15]	13 [11: 16]	< 0.001
Sex (male)	1046 (55%)	822 (44%)	< 0.001
Ethnicity			
Mexican	596 (31%)	661 (35%)	
Puerto Rican	894 (47%)	894 (48%)	
Mixed	62 (3%)	44 (2%)	
Other	341 (18%)	282 (15%)	
Ancestry			
African	15.3% [5%: 22%]	13.8% [4%: 20%]	< 0.001
Native American	30% [10%: 50%]	33% [10%: 55%]	< 0.001
European	55% [42%: 70%]	53% [39%: 70%]	0.007
Total IgE (IU/mL)	232 [68: 650]	80 [26: 248]	< 0.001
History of atopy	1515 (80.0%)	606(32.2%)	< 0.001
Lung Function			
	n = 422		
FEV ₁ (% predicted)	81.3:100.6]	98.8 [89.8:107.1]	< 0.001
FEF ₂₅₋₇₅ (% predicted)	82.4 [65.5:97.9]	100.6 [86.1:113.3]	< 0.001
FVC (% predicted)	95.1 [85.2:105.2]	98.1 [88.6:106.4]	0.001
FEV ₁ /FVC (%)	84.4 [80:90]	89 [85:93]	< 0.001
FEV1 (% change)	10.3 [4.8:13.7]	-	-

For continuous variables, the median and interquartile range are displayed, and the differences between cases and controls were tested with a t-test.

For categorical variables, the number and proportion of subjects in each category are displayed, and a χ^2 test was used. History of atopy was defined as self-report allergic rhinitis or eczema.

Table II

Significant and suggestive allelic associations with asthma (top: genotyped SNPs; bottom: imputed SNPs)

rs12328045	2	intergenic	0.221	1.33	1.18 - 1.50	4.6×10^{-6}
rs4566922	6	intergenic	intergenic 0.440	0.79	0.72 - 0.87	2.9×10^{-6}
rs3804327	6	<i>DCDC2</i>	0.160	1.37	1.20 - 1.56	1.8×10^{-6}
rs7086533	10	intergenic	0.117	1.51	1.29 - 1.77	3.8×10^{-7}
KG14-33639557	14	<i>NPAS3</i>	0.014	0.36	0.23 - 0.56	4.0×10^{-6}
rs12594238	15	<i>RAB11A</i>	0.169	0.73	0.64 - 0.83	3.0×10^{-6}
rs907092	17	<i>IKZF3</i>	0.304	0.67	0.61 - 0.75	5.7×10^{-13}
rs148728975	1	<i>VPS13D</i>	0.890	0.032	0.35 - 0.63	7.0×10^{-7}
rs10211025	2	Intergenic	0.992	0.214	0.65 - 0.83	9.7×10^{-7}
rs3936215	9	<i>BNC2</i>	0.996	0.263	1.21 - 1.56	8.6×10^{-7}
rs11252191	10	Intergenic	0.962	0.121	1.31 - 1.80	1.3×10^{-7}
rs182722816	12	<i>BTBD11</i>	0.708	0.011	2.86 - 11.34	7.0×10^{-7}