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Cosmopolitan and Ethnic-specific Replication of Genetic Risk Factors for Asthma in Two Latino Populations

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Abstract

Background—Although Mexicans and Puerto Ricans are jointly classified as “Hispanic/Latino”, there are significant differences in asthma prevalence, severity, and mortality between the two groups. We sought to examine the possibility that population-specific genetic risks contribute to this disparity.

Objectives—Over 100 candidate genes have been associated with asthma and replicated in an independent population, and seven genomewide association studies in asthma have been performed. We compared the pattern of replication of these associations in Puerto Ricans and Mexicans.

Methods—We genotyped Mexican and Puerto Rican trios using an Affymetrix 6.0 Genechip, and used a family based analysis to test for genetic associations in 124 genes previously associated with asthma.

Results—We identified 32 SNPs in 17 genes associated with asthma in at least one of the two populations. Twenty-two of these SNPs in eleven genes were significantly associated with asthma in the combined population and showed no significant heterogeneity of association, while five SNPs were associated in only one population and showed statistically significant effect heterogeneity. In a gene-based approach, two additional genes were associated with asthma in the

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combined population and three additional genes displayed ethnic-specific associations with heterogeneity.

Conclusions—Our results show that only a minority of genetic association studies replicate in our population of Mexican and Puerto Rican asthmatics. Among SNPs that were successfully replicated, most showed no significant heterogeneity across populations. However, we identified several population-specific genetic associations.

Keywords

asthma; genetics; Hispanics; Latinos; Mexicans; Puerto Ricans; replication; genomewide association; candidate genes; effect heterogeneity

Introduction

Although both Puerto Ricans and Mexicans are classified as “Hispanic/Latino”, the two populations have widely divergent asthma risk.¹ Puerto Ricans have the highest, and Mexicans the lowest prevalence of asthma in the United States. Asthma exacerbations, hospitalizations and mortality follow a similar pattern.² The reasons for these discrepancies are unknown and are likely related to genetic,³ social,³ and environmental factors, as well as their interaction.^{4, 5} Although several studies have explored the epidemiology,⁶ lung function,⁷ and response to albuterol⁸ in Latino populations, we are unaware of any study that has systematically explored differences in asthma susceptibility genes between Puerto Ricans and Mexicans.

There have been over 200 published candidate gene associations with asthma, as well as multiple reviews,^{4, 11, 12} candidate gene meta-analyses,^{13–15} and seven genomewide association studies,^{16–21} including one consortium based study that included over 25,000 subjects of European descent recruited in 23 studies.²² Many of the initial candidate gene association studies were never replicated in an independent population, but a 2006 review by Ober and Hoffjien found that 79 genes were associated with asthma in at least two populations.²³ The majority of these genetic association studies in asthma have been carried out in European populations, and their relevance to Latino populations is unknown.

While the number of comparisons used in GWAS limit their power to detect all but the strongest effect sizes,³⁰ genomewide genotyping data can be used to examine a more limited set of associations, replicating SNPs or genes previously associated with a disease or phenotype.³¹ Such analyses demonstrate that although a minority of genetic associations identified prior to the availability of genomewide genotyping are replicated using GWAS data, those that can be conclusively replicated can have substantial genetic effects. Two asthma studies have formally examined candidate genes using genomewide data.^{32, 33} In each study, only a minority of the evaluated genes were associated with asthma. Moreover, only an association between *DPP10* and asthma was replicated in both studies.

In this study, we performed a literature search to identify genes that had previously been associated with asthma in a GWAS study or at least two candidate gene studies. We used genomewide data to test whether asthma-associated SNPs in those genes are associated with asthma in two populations of Latino asthmatics enrolled in the Genetics of Asthma in Latino Americans (GALA) study.³⁴ To test for the possibility that different variants within those genes are associated with asthma, we also performed a gene-based study, testing all genotyped SNPs in or near the genes, adjusting for multiple comparisons using a gene-based permutation approach. The process of SNP and gene selection is summarized in Figure 1. Because the study includes individuals of both Mexican and Puerto Rican descent, we

sought to identify genes that confer risk in both populations, as well as genes that confer ethnic-specific risk.

Methods

Subjects

Mexican and Puerto Rican probands age 8–40 with asthma and their biological parents were recruited for the Genetics of Asthma in Latino Americans (GALA) study.³⁴ Puerto Rican probands were recruited from Puerto Rico and New York City, and Mexican probands were recruited in Mexico City and the San Francisco Bay Area. Probands were invited to participate if they had a physician diagnosis of asthma and were either taking a medication for asthma or had two or more asthma-related symptoms (wheezing, coughing, and/or dyspnea) in the preceding year. Participants were included if their self-identified ethnicity was Mexican or Puerto Rican and if all four of their biological grandparents were of the same Latino ethnicity. Detailed recruitment criteria and participant characteristics are described elsewhere and in the online repository.^{8, 34} Local institutional review boards approved all the studies. All subjects and, in the case of minors, their parents provided written consent and age-appropriate assent.

Genotyping

Genotyping was performed on the Affymetrix 6.0 GeneChip Array, which contains > 900,000 SNPs prior to quality control measures. Markers were included based on 95% call rates, Hardy-Weinberg equilibrium p -values $>10^{-5}$, and < 5 heterozygous genotype calls in males for X-linked markers. We filtered out SNPs with ambiguous mappings in the hg18 annotation track and those with high inconsistency in allele calling between plates. Subjects were excluded if they had less than a 95% call rate, unexpected cryptic relatedness, or Mendelian inconsistencies.

SNP-based replication

Figure 1 summarizes the procedure used to select SNPs. Genes were included if they were associated with asthma in a genomewide association study or at least two candidate gene studies. A further description of the criteria and search strategy used to identify genes for replication is available in the online repository.

We used the program BEAGLE v3.2³⁵ to phase and impute genotypes of 3.5 million SNPs. We used the combined consensus set of phased haplotypes in Release 21 of HapMap Phase II, using Yorubans, CEPH Europeans and the combined Chinese and Japanese populations as reference populations.³⁶ We tested all SNPs previously implicated in asthma within the identified genes using the transmission disequilibrium test (TDT test) as implemented in the program PLINK v1.07³⁷, in the combined population, and separately in Puerto Ricans and Mexicans. We tested whether the effect magnitude (odds ratio) between Mexicans and Puerto Ricans was homogenous or heterogeneous by comparing the ratio of transmitted and untransmitted alleles, using a chi-squared test with three degrees of freedom, where, the null hypothesis states that there is no difference between the odds ratio in Mexicans and Puerto Ricans (*i.e.*, the effect is homogenous). Homogeneity in the minor allele frequency of Mexican and Puerto Rican parents was compared using a chi-squared test.

Gene-based replication

Given the possibility of a failure to replicate an association due to differing LD patterns between the GALA populations and those in which the association was first reported, we supplemented our analysis by performing a “gene-based” replication. For every gene identified above, we tested all genotyped SNPs within 1 kb of the transcription start and stop

sites of RefSeq genes (release 43).³⁸ Because the gene was the unit of replication, we corrected the results for the number of comparisons made within each gene using a permutation procedure. We performed 10,000 gene-dropping permutations, whereby the transmitted allele was permuted with a 50:50 probability while maintaining LD between SNPs. Each observed test statistic was compared against the maximum of all permuted statistics (i.e. over all SNPs in the gene) to control for the gene-wide error rate.³⁷

Results

Subjects

Demographic, clinical, and spirometric characteristics of all asthmatic probands who enrolled in this study are shown in Table 1. There were a total of 509 subjects with complete spirometric data (Mexican, $n = 273$; Puerto Rican, $n = 336$). The median age of the Mexican and Puerto Rican subjects with asthma was 13 and 12 years, respectively.

A boxplot of ancestry estimates for the Puerto Rican and Mexican probands is given in Figure 2. A multidimensional scaling plot of the two populations can be found in the online repository (Figure E1). Overall, Mexicans had 39% (95% CI 37% to 41%, $p < 0.0001$) higher Native American ancestry than Puerto Ricans, while Puerto Ricans had a 25% (95% CI 23% – 27%, $p < 0.0001$) higher European ancestry than Mexicans.

Gene selection

We identified 124 candidate genes for analysis based on a search of asthma and related phenotypes conducted in September, 2010. Of these, ten genes were initially identified in a genomewide association study, and 114 were found associated with asthma in at least two independent candidate gene studies (Figure 1). Within these genes, 418 SNPs were identified as having been associated with asthma in at least one study. 273 SNPs were genotyped ($n = 78$) or imputed ($n = 195$) in our sample population; the remaining SNPs were not among the 3.5 million SNPs in the HapMap II reference populations. Seventeen genes had no asthma-associated SNPs genotyped or imputed for analysis. Three SNPs were monomorphic in our population, leaving 270 for analysis. In the gene-based replication study, we identified 1537 genotyped SNPs that were within 1 kb of 117 genes; seven of the 124 genes selected for analysis had no genotyped SNPs that met criteria for analysis.

SNP-based replication

Figure 3 shows the significant results of the SNP-based replication for asthma. The significant and full results of the SNP-based replication can be found in the online repository (Tables E1 and E2). A QQ-plot comparing observed versus expected p-values in the Puerto Rican, Mexican, and combined samples can be found in the online repository (Figure E2) and shows an excess of low p-values. P-values were lower in the combined sample than in each of the individual samples, consistent with the combined sample's larger sample size and greater power.

Out of the 270 genotyped or imputed SNPs in the 107 genes analyzed, 32 SNPs in 17 genes were associated with asthma in at least one of the two populations ($p < 0.05$). In the combined population, 22 SNPs in 11 genes were associated with asthma in the combined population and did not display significant heterogeneity between Mexicans and Puerto Ricans ($p_{\text{het}} > 0.1$). The most significant association was rs1342326 in the *IL33* gene, whose C allele (minor allele) was found to confer a risk for asthma with an odds ratio of 1.65 (95% CI 1.29 – 2.11, $p = 6 \times 10^{-5}$) in the combined population. In all but one SNP, rs2069762 in *IL2* (see table E1A), there was a statistically significant difference in the minor allele

frequency between Mexicans and Puerto Ricans, ranging from 4.4% to 18.8%, consistent with the differences in ancestry between the two groups (Figure 2).

Five genes had SNPs that displayed significant effect heterogeneity between the two groups ($P_{\text{het}} < 0.1$) and were associated with asthma in only one of the two populations ($p < 0.05$), including three associated with Mexicans but not Puerto Ricans (Figure 3B and Table E1B in the online repository). These include a SNP in *ORMDL3*, rs4795408, which showed an odds ratio of 1.55 (95% CI: 1.18 – 2.03, $p = 0.001$) in Mexicans but an odds ratio of 0.98 (95% CI: 0.76 – 1.25, $p = 0.8$) in Puerto Ricans and a p -value for heterogeneity of 0.02. This SNP was in partial linkage disequilibrium with SNP rs8076131, the second most strongly associated SNP in the combined population (Figure 3A and Table E1A in the online repository) and in both ethnic groups. However, linkage between rs4795408 and rs8076131 was substantially stronger in Mexicans ($r^2 = 0.46$) than Puerto Ricans ($r^2 = 0.26$), as shown in Figure E3 in the online repository. Two genes had SNPs associated with asthma in Puerto Ricans, but not Mexicans and had significant effect heterogeneity at $p < 0.1$, *ADRA1B* and *NOD1*. A Venn diagram summarizing the replication pattern of the genes analyzed is shown in Figure 4.

Gene-based replication

Among the 124 genes evaluated for replication, we found at least one SNP to test for association in 117 genes. Within these, 148 SNPs in 45 genes were nominally associated with asthma in at least one of the two populations, and 78 SNPs were nominally associated with asthma in the combined population. After adjusting for multiple comparisons with permutation testing, 16 SNPs in 11 genes were associated with asthma in at least one population. Table E3 in the online repository shows the six genes associated with asthma in the combined population without effect heterogeneity, and the five genes associated with asthma in one of the two populations with significant heterogeneity.

Three genes with effect heterogeneity were associated with asthma in Mexicans but not Puerto Ricans, including *NPSR1* (OR in Mexicans 2.00, 95% CI 1.29 – 3.1, $p_{\text{permuted}} = 0.045$, $P_{\text{heterogeneity}} = 0.02$), *MS4A2* (OR in Mexicans 0.65, 95% CI 0.47 – 0.90, $p_{\text{permuted}} = 0.008$, $P_{\text{heterogeneity}} = 0.01$), and *ADRA1B* (OR in Mexicans 2.50, 95% CI 1.28 – 4.88, $p_{\text{permuted}} = 0.048$, $P_{\text{heterogeneity}} = 0.02$) (Table E2B in the online repository). In this analysis, the SNP in *ADRA1B* associated with asthma (rs6884129) was different than the one associated with asthma in Puerto Ricans in the SNP-based association (rs10515802). These two SNPs in *ADRA1B* are not in linkage disequilibrium in either of the two populations ($r^2 = 0.01$ in Puerto Ricans, $r^2 = 0$ in Mexicans). Two genes, *HNMT* (OR in Puerto Ricans 0.66, 95% CI 0.48 – 0.90, $p_{\text{permuted}} = 0.03$, $P_{\text{heterogeneity}} = 0.06$) and *NOS3* (OR in Puerto Ricans 0.48, 95% CI 0.33 – 0.71, $p_{\text{permuted}} = 0.0007$, $P_{\text{heterogeneity}} = 0.053$) were associated with asthma in Puerto Ricans, but not Mexicans.

In the combined analysis, eleven SNPs in six genes were associated with asthma and had no significant effect heterogeneity (Table E3A). Only two of the six genes that showed gene-based replication, *HLA-DQB1* (combined OR 0.75, 95% CI 0.60 – 0.94, $p = 0.01$) and *IL16* (combined OR 1.34, 95% CI 1.11 – 1.61, $p = 0.002$), did not have SNPs associated with asthma in the SNP-based replication.

Discussion

In this study, we performed a literature search and identified 124 genes that were previously associated with asthma in a genomewide association study or two independent candidate gene studies and tested their association in two Latino populations. Our SNP-level replication found that 38 SNPs in 17 genes were associated with asthma in at least one

population, while an additional 4 genes were associated with at least one population in the gene-based study. The pattern of replication of these genes falls into several categories, as shown in Figure 4.

SNPs in three of the genes, *ORMDL3*, *GSDMB*, and *IL33* replicate in both populations, and generally had no significant effect heterogeneity between the two Latino populations. These three genes represent the most confident replications. Interestingly, all three of these genes were identified in genomewide association studies, and all three are associated with asthma in the large GABRIEL study.²² It is not surprising that the two genes in the 17q21 locus (*ORMDL3* and *GSDMB*) replicate in our population, as we had previously been able to replicate the association in these two Latino populations in GALA as well as in an African American population.²⁹

Several genes were associated with asthma in only one of the two populations, and failed to show substantial heterogeneity of effect, or association in the combined population. It is difficult to interpret the meaning of these findings, as they could represent spurious associations. However, they could also represent cases of ethnic-specific replication for which the test of heterogeneity was underpowered to detect differences between the two ethnic groups. For example, SNP rs17576 showed an association in Puerto Ricans (OR of 1.34) but not Mexicans (OR of 0.96). However, the test for heterogeneity failed to reject the null hypothesis ($p_{\text{het}} = 0.2$). It is possible that in a larger sample, the test for heterogeneity would have been significant. However, because the 95% confidence interval for the odds ratio for Mexicans was broad (0.71 – 1.31), an effect magnitude comparable to that in Puerto Ricans is also possible.

A third category contains genes associated with asthma in the combined population, without significant heterogeneity between the populations. In some cases, though the effect magnitude was similar and in the same direction in the two populations, the association failed to reach statistical significance in at least one of the two groups. This may be the result of loss of power from dividing the sample into subgroups by ethnicity.

Finally, there are several genes that show an ethnic specific replication pattern. Specifically, SNPs on genes *NOD1*, *HNMT*, and *NOS3* in Puerto Ricans and *NPSR1* and *MS4A2* in Mexicans were associated with asthma in one population but not the other, and there was substantial effect heterogeneity between the two populations. SNPs in two other genes, *ORMDL3* and *ADRA1B*, also showed this pattern. In the case of *ORMDL3*, other SNPs in this gene in partial linkage disequilibrium showed a robust association in both populations, while in the case of *ADRA1B*, different, unlinked SNPs in the gene were associated with asthma separately in each population.

Several factors could explain this ethnic-specific heterogeneity of effect. The first, and most likely, is that SNPs of interest may be indirectly tagging underlying causal variants. Differing patterns of linkage disequilibrium between the underlying causal variant(s) and the genotyped SNPs in Puerto Ricans and Mexicans contribute significantly to observed effect heterogeneity. Bryc and colleagues found that Mexicans have a larger degree of linkage disequilibrium (r^2) as a function of physical distance on the chromosome than Puerto Ricans.¹⁰ This is due to the greater proportion of Native American ancestry in Mexicans and African ancestry in Puerto Ricans; Native Americans have higher linkage disequilibrium as a function of physical distance than Africans. In the case of *ORMDL3*, where several SNPs were strongly associated with asthma in both populations, greater linkage disequilibrium in Mexicans than Puerto Ricans likely explains the association of SNP rs4795408 with asthma in the former group but not the latter (Figure E2).

Secondly, while the “common disease, common variant” hypothesis suggests that multiple common, older variants that predated population divergence confer relatively small risks individually but jointly contribute to asthma susceptibility, recent work has suggested that rare variants that arose more recently with stronger effects may be responsible for common diseases.³⁹ Since rare variants may have arisen after populations diverged, they are more likely to be population-specific. These rare variants may be overrepresented in specific ethnic groups.⁴⁰ Thus, the presence of undetected rare causal variants in only one of the populations may explain the difference in observed effect and success in replication.

Third, in at least one gene, *ADRA1B*, different polymorphisms in the same gene had different effects in each population. These polymorphisms were in complete linkage equilibrium, suggesting that while a particular gene may be a common risk factor across populations, different polymorphisms may confer that risk.

Finally, it is possible that the heterogeneity in effect arises because of gene-gene or gene-environment interactions.⁴¹ As seen in Figure E1 of the online repository, the two populations separate almost completely over the first principal coordinate, so interactions between tested genetic variants and unknown variants elsewhere in the genome may explain the heterogeneity. If genetic effects are manifest only in the presence of environmental conditions to which only one ethnic group has significant exposure, then only that group would show a genetic effect. Though our two populations were recruited using identical recruitment criteria to minimize heterogeneity in asthma ascertainment and demographic factors, the population of Mexicans was recruited in the San Francisco Bay Area and Mexico City, while the population of Puerto Ricans was recruited in Puerto Rico and New York. Thus, they may have been exposed to systemically different environments. We used a mixed effects model to determine the proportion of heterogeneity between sites that was accounted for by differences in ethnicity and found that with the exception of SNP rs4795408 in *ORMDL3*, ethnicity accounts for almost all of the heterogeneity observed between sites (see online repository for details of methods and Table E4 for results).

Population-specific differences in minor allele frequency are well-established between continental populations.³⁶ In this study, we have shown that heterogeneity in minor allele frequency can be substantial across two Latino populations, as expected given the differences in ancestral proportions of our admixed population.⁹ This has profound implications for case/control studies seeking to recruit Latino participants, as it presents a high risk for confounding due to population stratification. Because we analyzed our population using a transmission disequilibrium test, this study was robust to confounding due to population substructure.⁴³

Only a minority of candidate asthma genes were replicated in this study, which is consistent with previous findings.^{31–33} In the majority of cases, genes that replicated showed similar association across the two populations. However, there were several genes that showed substantial heterogeneity in effect magnitude ($p_{\text{het}} < 0.1$). Our findings are similar to those by Ioannidis *et al*, evaluating the effects of “race” on genetic associations.⁴² We found that there were substantial differences in minor allele frequency between our populations while differences in the magnitude of genetic associations were rare but occasionally substantial. While these differences may be explained by differing patterns of linkage disequilibrium between our two populations, the existence of these differences were statistically significant and should not be entirely dismissed.

In conclusion, we have attempted to replicate over 100 asthma candidate genes and the results of seven genomewide association studies. Given that a minority of the genes replicated in our two populations of Latinos, caution should be used when attempting to

generalize the results of genetic association studies to Latinos. Moreover, while our results indicate there are shared genetic risk factors among our two Latino subgroups, we found differences between the two groups in the genetic factors associated with asthma. These may partially account for disparities in asthma prevalence between the two groups. These differences also suggest that studies attempting to analyze different Latino populations jointly should do so judiciously, and only after establishing that there is no significant between-group heterogeneity. Finally, caution should be exercised in applying the results from genetic studies in one Latino population to others.

Key Messages

- A minority of genetic association studies replicate in a population of Latino asthmatics.
- Among SNPs that were successfully replicated, most showed consistent association in Mexicans and Puerto Ricans.
- We identified several associations that appear to be population-specific and show heterogeneity between the two populations.

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Abbreviations

GWAS	Genomewide association study
GALA	Genetics of Asthma in Latino Americans
CEPH	Centre d'Etude du Polymorphisme Humain
TDT	Transmission disequilibrium test
LD	Linkage disequilibrium
SNP	Single nucleotide polymorphism
OR	Odds ratio

References

1. Lara M, Akinbami L, Flores G, Morgenstern H. Heterogeneity of childhood asthma among Hispanic children: Puerto Rican children bear a disproportionate burden. *Pediatrics*. 2006; 117:43–53. [PubMed: 16396859]
2. Homa DM, Mannino DM, Lara M. Asthma mortality in U.S. Hispanics of Mexican, Puerto Rican, and Cuban heritage, 1990–1995. *Am J Respir Crit Care Med*. 2000; 161:504–509. [PubMed: 10673193]
3. Chen E, Chim LS, Strunk RC, Miller GE. The role of the social environment in children and adolescents with asthma. *Am J Respir Crit Care Med*. 2007; 176:644–649. [PubMed: 17556714]
4. Ober C, Thompson EE. Rethinking genetic models of asthma: the role of environmental modifiers. *Curr Opin Immunol*. 2005; 17:670–678. [PubMed: 16214315]
5. Kleeberger SR, Peden D. Gene-environment interactions in asthma and other respiratory diseases. *Annu Rev Med*. 2005; 56:383–400. [PubMed: 15660518]

6. Freeman NC, Schneider D, McGarvey P. Household exposure factors, asthma, and school absenteeism in a predominantly Hispanic community. *J Expo Anal Environ Epidemiol.* 2003; 13:169–176. [PubMed: 12743611]
7. Naqvi M, Thyne S, Choudhry S, Tsai HJ, Navarro D, Castro RA, et al. Ethnic-specific differences in bronchodilator responsiveness among African Americans, Puerto Ricans, and Mexicans with asthma. *J Asthma.* 2007; 44:639–648. [PubMed: 17943575]
8. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J, et al. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. *Am J Respir Crit Care Med.* 2005; 171:563–570. [PubMed: 15557128]
9. Wang S, Ray N, Rojas W, Parra MV, Bedoya G, Gallo C, et al. Geographic patterns of genome admixture in Latin American Mestizos. *PLoS Genet.* 2008; 4:e1000037. [PubMed: 18369456]
10. Bryc K, Velez C, Karafet T, Moreno-Estrada A, Reynolds A, Auton A, et al. Colloquium paper: genome-wide patterns of population structure and admixture among Hispanic/Latino populations. *Proc Natl Acad Sci U S A.* 2010; 107 Suppl 2:8954–8961. [PubMed: 20445096]
11. Ober C. Perspectives on the past decade of asthma genetics. *J Allergy Clin Immunol.* 2005; 116:274–278. [PubMed: 16083779]
12. Weiss ST, Raby BA, Rogers A. Asthma genetics and genomics 2009. *Curr Opin Genet Dev.* 2009; 19:279–282. [PubMed: 19481925]
13. Aoki T, Hirota T, Tamari M, Ichikawa K, Takeda K, Arinami T, et al. An association between asthma and TNF-308G/A polymorphism: meta-analysis. *J Hum Genet.* 2006; 51:677–685. [PubMed: 16865291]
14. Nishimura F, Shibasaki M, Ichikawa K, Arinami T, Noguchi E. Failure to find an association between CD14-159C/T polymorphism and asthma: a family-based association test and meta-analysis. *Allergol Int.* 2006; 55:55–58. [PubMed: 17075287]
15. Finkelstein Y, Bournissen FG, Hutson JR, Shannon M. Polymorphism of the ADRB2 gene and response to inhaled beta- agonists in children with asthma: a meta-analysis. *J Asthma.* 2009; 46:900–905. [PubMed: 19905915]
16. Hancock DB, Romieu I, Shi M, Sienra-Monge JJ, Wu H, Chiu GY, et al. Genome-wide association study implicates chromosome 9q21.31 as a susceptibility locus for asthma in mexican children. *PLoS Genet.* 2009; 5:e1000623. [PubMed: 19714205]
17. Himes BE, Hunninghake GM, Baurley JW, Rafaels NM, Sleiman P, Strachan DP, et al. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. *Am J Hum Genet.* 2009; 84:581–593. [PubMed: 19426955]
18. Li X, Howard TD, Zheng SL, Haselkorn T, Peters SP, Meyers DA, et al. Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. *J Allergy Clin Immunol.* 2010; 125:328–335. e11. [PubMed: 20159242]
19. Mathias RA, Grant AV, Rafaels N, Hand T, Gao L, Vergara C, et al. A genome-wide association study on African-ancestry populations for asthma. *J Allergy Clin Immunol.* 2010; 125:336–346. e4. [PubMed: 19910028]
20. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature.* 2007; 448:470–473. [PubMed: 17611496]
21. Sleiman PM, Flory J, Imielinski M, Bradfield JP, Annaiah K, Willis-Owen SA, et al. Variants of DENND1B associated with asthma in children. *N Engl J Med.* 2010; 362:36–44. [PubMed: 20032318]
22. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med.* 2010; 363:1211–1221. [PubMed: 20860503]
23. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun.* 2006; 7:95–100. [PubMed: 16395390]
24. Cantero-Recasens G, Fandos C, Rubio-Moscardo F, Valverde MA, Vicente R. The asthma-associated ORMDL3 gene product regulates endoplasmic reticulum-mediated calcium signaling and cellular stress. *Hum Mol Genet.* 2010; 19:111–121. [PubMed: 19819884]

25. Verlaan DJ, Berlivet S, Hunninghake GM, Madore AM, Lariviere M, Moussette S, et al. Allele-specific chromatin remodeling in the ZBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet.* 2009; 85:377–393. [PubMed: 19732864]
26. Wu H, Romieu I, Sienra-Monge JJ, Li H, del Rio-Navarro BE, London SJ. Genetic variation in ORM1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. *Allergy.* 2009; 64:629–635. [PubMed: 19133921]
27. Sleiman PM, Annaiah K, Imielinski M, Bradfield JP, Kim CE, Frackelton EC, et al. ORMDL3 variants associated with asthma susceptibility in North Americans of European ancestry. *J Allergy Clin Immunol.* 2008; 122:1225–1227. [PubMed: 18760456]
28. Tavendale R, Macgregor DF, Mukhopadhyay S, Palmer CN. A polymorphism controlling ORMDL3 expression is associated with asthma that is poorly controlled by current medications. *J Allergy Clin Immunol.* 2008; 121:860–863. [PubMed: 18395550]
29. Galanter J, Choudhry S, Eng C, Nazario S, Rodriguez-Santana JR, Casal J, et al. ORMDL3 gene is associated with asthma in three ethnically diverse populations. *Am J Respir Crit Care Med.* 2008; 177:1194–1200. [PubMed: 18310477]
30. Spencer CC, Su Z, Donnelly P, Marchini J. Designing genome-wide association studies: sample size, power, imputation, and the choice of genotyping chip. *PLoS Genet.* 2009; 5:e1000477. [PubMed: 19492015]
31. Siontis KC, Patsopoulos NA, Ioannidis JP. Replication of past candidate loci for common diseases and phenotypes in 100 genome-wide association studies. *Eur J Hum Genet.* 2010; 18:832–837. [PubMed: 20234392]
32. Wu H, Romieu I, Shi M, Hancock DB, Li H, Sienra-Monge JJ, et al. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. *J Allergy Clin Immunol.* 2010; 125:321–327. e13. [PubMed: 19910030]
33. Rogers AJ, Raby BA, Lasky-Su JA, Murphy A, Lazarus R, Klanderman BJ, et al. Assessing the reproducibility of asthma candidate gene associations, using genome-wide data. *Am J Respir Crit Care Med.* 2009; 179:1084–1090. [PubMed: 19264973]
34. Burchard EG, Avila PC, Nazario S, Casal J, Torres A, Rodriguez-Santana JR, et al. Lower bronchodilator responsiveness in Puerto Rican than in Mexican subjects with asthma. *Am J Respir Crit Care Med.* 2004; 169:386–392. [PubMed: 14617512]
35. Browning BL, Browning SR. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am J Hum Genet.* 2009; 84:210–223. [PubMed: 19200528]
36. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. *Nature.* 2007; 449:851–861. [PubMed: 17943122]
37. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81:559–575. [PubMed: 17701901]
38. Pruitt KD, Tatusova T, Maglott DR. NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res.* 2005; 33:D501–D504. [PubMed: 15608248]
39. Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic variation and its contribution to complex traits. *Nat Rev Genet.* 2009; 10:241–251. [PubMed: 19293820]
40. Keen-Kim D, Mathews CA, Reus VI, Lowe TL, Herrera LD, Budman CL, et al. Overrepresentation of rare variants in a specific ethnic group may confuse interpretation of association analyses. *Hum Mol Genet.* 2006; 15:3324–3328. [PubMed: 17035247]
41. Tcheurekdjian H, Via M, De Giacomo A, Corvol H, Eng C, Thyne S, et al. ALOX5AP and LTA4H polymorphisms modify augmentation of bronchodilator responsiveness by leukotriene modifiers in Latinos. *J Allergy Clin Immunol.* 2010
42. Ioannidis JP, Ntzani EE, Trikalinos TA. 'Racial' differences in genetic effects for complex diseases. *Nat Genet.* 2004; 36:1312–1318. [PubMed: 15543147]
43. Lewis CM. Genetic association studies: design, analysis and interpretation. *Brief Bioinform.* 2002; 3:146–153. [PubMed: 12139434]

Figure 1.
Process of SNP and gene selection.

Figure 2. Boxplot of individual ancestry estimates of GALA Mexicans and Puerto Ricans. On average Puerto Ricans have higher African and European ancestry than Mexicans while Mexicans have higher Native American ancestry.

Significant associations in SNP-based replications

Significant ethnic-specific associations in SNP-based replications

Figure 3.

SNP based replication showing odds ratio for Mexican, Puerto Rican, and combined samples. The top graph shows the genes that replicated in the combined population with low heterogeneity; the bottom graph shows genes that replicated in one population and had significant heterogeneity.

Figure 4.

Venn diagram showing the relationships between the replicated genes. An asterisk (*) indicates that there was heterogeneity between ethnicities. Genes within boxes are additional genes identified only in the gene-based replication; the rest of the genes were identified in the SNP-based replication. In three genes, *ADRA1B*, *ORMDL3*, and *RAD50*, different SNPs in the genes fit into the diagram differently so the gene is shown twice.

Table 1

Demographic and baseline characteristics for the GALA probands. For continuous variables, the median and interquartile range is displayed, and differences between the two populations were tested using a t-test. A chi-squared test was used to test for differences in proportions.

	Puerto Ricans	Mexicans	p-value for difference
N	373 (241 from PR, 132 from NY)	300 (99 from MX, 201 from SF)	
Age, yrs	11.9 (10 – 15)	13.2 (11 – 20)	<0.001
Sex, % male	55.5%	55.2%	0.8
BMI, kg/m ²	21.3 (17 – 26)	23.8 (20 – 28)	<0.001
Serum total IgE, IU/mL	262 (96 – 618)	249 (94 – 599)	0.7
Severity, % severe	66.9%	67.0%	0.3
Spirometry (n with full data)	363	273	
Pre-FEV1, % predicted	84 (74 – 93)	89 (77 – 100)	<0.001
FVC, % predicted	93.8 (83 – 105)	97.8 (87 – 109)	0.2
FEV1/FVC, %	79.0 (72 – 85)	80.7 (75 – 86)	0.007
ΔFEV1, relative %	4.9 (0.6 – 10)	7.4 (4 – 14)	<0.001

PR = Puerto Rico, NY = New York, MX = Mexico City, SF = San Francisco, BMI = Body Mass Index, FEV1 = Forced Expiratory Volume in 1 second, ΔFEV1 = Change in FEV1 after the administration of albuterol.