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Expression Quantitative Trait Locus (eQTL) Fine Mapping of the 17q12–21 Asthma Locus in African American Children: a Cross-Sectional Study

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C.O. wrote the initial draft of the manuscript; C.O, C.M., D.O., D.L.N., A.M.L., J.E.G. read and edited early drafts of the manuscript. All authors reviewed and/or critically revised the manuscript for important intellectual content and provided final approval.

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Abstract

Background—African ancestry is associated with higher prevalence and greater severity of asthma. Yet genetic studies of the most significant childhood onset asthma locus on 17q12–21 in African Americans have been equivocal. The goal of this study was to leverage both the careful phenotyping in the CREW birth cohort consortium and the breakdown of linkage disequilibrium in African Americans to fine map this important asthma locus.

Methods—Using nine 17q12–21 tag SNPs, we performed association studies for childhood onset asthma in 1,613 European American and 870 African American CREW children, and meta-analyzed the results with publicly available summary data from the EVE consortium (n=4,343 European American, 3,035 African ancestry). We then performed eQTL studies of the SNPs associated with childhood asthma and the expression of 17q12–21 genes in resting blood-derived and upper airway epithelial cells from 85 and 246, respectively, African American children; and in

lower airway epithelial cells from 44 European American and 72 African American adults from Chicago.

Findings—17q12–21 SNPs were broadly associated with asthma in European Americans, but only two SNPs (rs2305480 in *GSDMB* and rs8076131 in *ORMDL3*) were associated with asthma in African Americans, at a Bonferroni-corrected $P < 0.0055$. Genotype at rs2305480 was the most significant eQTL for *GSDMB* ($P < 0.0001$), and to a lesser extent for *PGAP3* ($P < 0.0001$), in upper airway epithelial cells from African American children. No SNPs were eQTLs for *ORMDL3*. These patterns were replicated in lower airway cells from European and African American adults. The SNP that was the most significant eQTL (rs12936231) for *GSDMB* and *ORMDL3* in blood cells ($P < 10^{-8}$) was not associated with asthma in African Americans.

Interpretations—Our study suggests that SNPs regulating *GSDMB* expression in airway epithelial cells play a major role in childhood onset asthma, while SNPs regulating the expression levels of 17q12–21 genes in resting blood cells are not central to asthma risk. Using both genetic and gene expression data in African Americans and European Americans, *GSDMB*, which induces cell death in response to intracellular pathogens, emerged as the leading candidate gene at this important asthma locus.

Disparities in the prevalence, severity and natural history of asthma have been well described, with African ancestry associated with increased asthma prevalence^{1,2} and poorer response to asthma therapies and suboptimal lung function.^{3,4} The relative contributions of genes, environment, or gene-environment interactions to these disparities remain unknown. In particular, the evidence for association with SNPs at the chromosome 17q12–21 locus, the most highly replicated childhood onset asthma locus in European ancestry populations,^{5–9} has been equivocal in African ancestry populations, with weak associations at best.^{7,8,10–14} Notably though, none of the studies in African Americans focused specifically on early childhood onset asthma and samples sizes have been small relative to studies of European ancestry populations.

A remarkable feature of the 17q12–21 asthma locus is extensive linkage disequilibrium (LD) that spans over 150 kb on European-derived chromosomes with lesser LD extending into the flanking regions, together encoding at least 10 genes. The asthma-associated alleles in European ancestry populations are on an extended “risk haplotype” that have been associated with the expression of two genes, *ORMDL3* and *GSDMB*, primarily in blood cells. The risk and protective haplotypes at this locus occur at approximately equal frequencies in most European ancestry populations. The extensive LD makes it difficult to differentiate the effects of individual variants on asthma risk or on gene expression, or to identify causal variants, in European ancestry individuals. In contrast, there is significant breakdown of LD at this locus on African-derived chromosomes,¹⁵ yet this feature has so far not been used to fine map the independent effects of 17q12–21 SNPs on risk of childhood-onset asthma or on the expression of genes at this locus.

We hypothesized that the breakdown of LD in African Americans would facilitate fine mapping studies at this important locus, allowing us to narrow the associations with asthma and with the expression of genes at this locus to fewer SNPs. To this end, we leveraged the Children’s Respiratory and Environmental Workgroup (CREW) Consortium,¹⁶ a component

study of the larger NIH-funded Environmental Influences on Child Health Outcomes Program (ECHO).¹⁷ CREW includes ethnically diverse subjects from 12 U.S. longitudinal birth cohorts with asthma outcome phenotypes. To first show that SNPs at this locus are associated with childhood onset asthma in the CREW children, we genotyped nine 17q12–21 tag SNPs in 1,613 European American and 870 African American CREW children. To increase power, we also performed a meta-analysis using summary statistics for 4,343 European American and 3,035 African ancestry individuals from the EVE consortium.⁷ We then sought to determine which SNPs are both associated with both childhood onset asthma and are eQTLs for 17q12–21 genes in 268 African American children from a CREW cohort, using unstimulated peripheral blood mononuclear cells (PBMCs; n=85) and upper airway epithelium (n=246), two compartments that are central to the pathobiology of asthma. Finally, we validated the eQTL results in lower airway epithelial cells from 44 European American and 72 African American adults, which allowed us to generalize our findings to the lower airway and to European Americans. Using these orthogonal approaches, we show that patterns of association among asthma-associated SNPs and gene expression differ between airway epithelial cells and PBMCs, and that *GSDMB*, and possibly *PGAP3*, in airway epithelial cells likely underlie the risk for childhood onset asthma at the 17q12–21 locus.

Methods

Sample composition

Our study included 2,483 self-identified European American or African American unrelated children from nine CREW birth cohorts who were followed to at least age 6 years and provided DNA for genotyping (Table 1). Because the 17q12–21 locus is associated with early life wheezing illnesses and early diagnosis of asthma,^{9,18–20} we focused on asthma diagnoses by a health care provider by age 6 to maximize sample size and reduce heterogeneity (see appendix p. 5 for additional details). This work was approved by the institutional review boards at the participating institutions.

We also included two additional samples. First, to increase power in the association studies, asthma GWAS summary data were included from the EVE consortium⁷ for 6,141 (2,088 cases) European American and 3,976 (1,612 cases) African ancestry individuals from cases-control studies, in whom asthma was determined by a doctor's diagnosis. Second, to provide validation of the eQTLs in the lower airway and in European Americans, we performed eQTL studies in bronchial epithelial cells from 44 (32 cases) European American and 72 (48 cases) African American participants in an case-control study in Chicago.²¹ Asthma was diagnosed based on a doctor's diagnosis, current symptoms and asthma medication use (appendix p 5).

These studies were approved by the local Institutional Review Boards from each participating center.

Genotyping and quality control in CREW

DNA (500 ng at concentrations of 5 ng/μl) was shipped from the coordinating center of each CREW cohort to the University of Chicago for genotyping. Nine SNPs were selected based on LD patterns at the extended 17q12–21 locus and previously reported associations with asthma or gene expression (appendix p 9). Genotyping methods and quality control are described in the appendix (p 5).

SNP association tests

To avoid selection bias, we included all CREW cohorts, regardless of sample size. However, we repeated all analyses including cohorts with at least 20 cases to assure that the small samples were not skewing results. The genotype of each SNP was considered under an additive model (0, 1, or 2 effect alleles) and tested for association with asthma using logistic regression, including sex as a covariate. Because genome-wide SNPs were not available to estimate ancestry principal components (PCs), we also included the study site as a covariate to account for differences in allele and asthma frequencies among the cohorts. We considered the allele at each SNP that has been associated with increased asthma risk in European ancestry populations as the “effect” allele in all tests¹⁵. *P*-values were adjusted for nine tests; *P*<0.0055 was considered significant.

To increase power, we also included published summary data from the EVE Consortium.⁷ The majority of EVE cases had asthma onset in childhood (median age of onset <8 years in the European Americans and <7 years in the African ancestry subjects). We extracted summary statistics for seven of the nine SNPs that were available in EVE, and meta-analyzed across the two studies within each racial/ethnic group, using a fixed effect model in which we assumed each study’s log-transformed odds ratio (OR) estimate was centered around the true log-transformed OR with known study-specific variance. Statistical significance of the meta-analysis test statistics was assessed using standard normal approximations; analyses were performed in R (v3.3.3).

Gene expression studies

RNA-seq data were available in African American children from one of the CREW cohorts (URECA) in unstimulated PBMCs for 85 children (48 cases, 37 controls)²² and in upper airway (nasal) cells for 246 children (125 cases, 121 controls) at the age 11 visit (appendix p 5). We used an additive effects linear model to test for eQTL studies of the five 17q core region SNPs with expression of each of the 10 genes at the extended locus, including as covariates sex, sample collection site, and 10 latent factors²³ in the PBMC studies and sex, study site, batch id, epithelial cell proportion and 12 latent factors in the epithelial cell studies. Latent factors were included to correct for unwanted variation.²³ *P*-values were adjusted for 40 tests (5 SNPs x 8 genes); *P*<0.0012 was considered significant. Differential expression between cases and controls were tested in using a linear model, including the same covariates as described above.

We further examined associations between the same five SNPs with *GSDMB* and *ORMDL3* expression in lower (bronchial) airway epithelial cells from 44 European American adults (32 cases, 12 controls) and African American adults (48 cases, 24 controls) who participated

in a case-control study in Chicago (appendix pp 5–6, 10). Normalized gene expression counts were adjusted for age, sex, current smoking status, sequencing pool, the first three ancestry PCs, and cell composition (appendix p 5). Linear regression considering additive genotype effects on gene expression was performed using limma in R (v3.3.3). Differential expression between cases and controls were tested in the normalized data described above using a linear model.

Role of the funding source

Funding sources had no role in the study design, in the collection, analysis or interpretation of the data, in writing the report, or in decisions regarding choice of journals.

Results

LD patterns between 17q12–21 SNPs

Correlations between the nine SNPs in CREW children (Figure 1) are similar to those reported for European and African American populations (appendix p 12),^{11,15} both reflecting significant breakdown of LD in African Americans compared to European Americans. Throughout this paper we refer to the five SNPs within the approximately 150 kb LD block that includes the *GSDMB* and *ORMDL3* genes as the core region; and the two SNPs in each of the flanking regions that show less LD with core region SNPs and less association with childhood onset asthma, as the proximal (encoding *PGAP3* and *ERBB2*) and the distal (encoding *GSDMA*) regions, respectively, following Stein et al.¹⁵

Associations between 17q12–21 SNPs and childhood onset asthma

The alleles at the nine SNPs were relatively common in CREW children (Table 2), as previously reported.¹⁵ All five SNPs in the core region were associated with asthma in the European American CREW children and in the meta-analysis with EVE (Table 2), reflecting the strong LD at this locus.

None of the SNPs were significant in the African American CREW children, possibly due to the smaller sample size and the high frequency of the risk alleles compared to European American children. However, the overlapping confidence intervals for two SNPs in the core region (SNP4 [rs2305480] and SNP5 [rs7216389] in *GSDMB* and SNP7 [rs8076131] in *ORMDL3*) in the CREW samples suggest that the ORs are not different between these groups (Table 2; Figure 1B). Analyses including only CREW cohorts with 20 cases yielded similar results (appendix p 10). In the meta-analysis with EVE African American subjects, two SNPs were significantly associated with asthma: SNP4 (rs2305480) in *GSDMB* and SNP7 (rs8076131) in *ORMDL3* (Table 2; Figure 1B).

Gene expression studies at the 17q12–21 locus

The meta-analysis in African American subjects narrowed the association with asthma to two candidate SNPs. However, whether the effects of these SNPs are independently associated with asthma, or whether the associations are due to LD cannot be distinguished by genetic association studies. Therefore, to further investigate these possibilities, we performed eQTL fine mapping for the five core SNPs in African American CREW children

and the expression of eight genes at this locus in two asthma-relevant cell compartments: PBMCs and upper airway epithelial cells.

Indeed, the breakdown of LD in the African American children allowed us to differentiate the eQTL effects at this locus (Figure 2, Table 3), which had not been possible in previous studies performed primarily European ancestry individuals (e.g. ^{24,25}). In PBMCs, genotypes at the five core SNPs were associated only with expression of *GSDMB* and *ORMDL3*. The strongest associations were with SNP3 (rs12936231), an intronic SNP in the *ZPBP2* gene (*GSDMB*: eQTL $\beta=1.23$ [95% CI 1.15, 1.32]; $P<0.0001$; *ORMDL3*: eQTL $\beta = 1.18$ [95% CI 1.12, 1.24], $P<0.0001$), with weaker associations at the other core region SNPs (Figure 2 upper panel, Table 3), consistent with earlier studies.^{26,27} The fact that SNP3 (rs12936231) genotype was most associated with *GSDMB* and *ORMDL3* expression in PBMCs, and not associated with risk for childhood onset asthma in the African American CREW children or in the meta-analysis, suggests that expression of *GSDMB* and *ORMDL3* in PBMCs may not underlie risk for asthma.

Very different patterns of association with gene expression were revealed in upper airway epithelial cells (Figure 2 lower panel, Table 3). The asthma-associated SNP in *GSDMB* (SNP4; rs2305480) was most strongly associated with expression of *GSDMB* (eQTL $\beta=1.35$ [95% CI 1.25, 1.46]; $P<0.0001$), and with *PGAP3* to a lesser extent (eQTL $\beta=1.22$ [95% CI 1.14, 1.31]; $P<0.0001$), but not for any other genes at this locus, including *ORMDL3* (eQTL $P=0.66$). The eQTL effects for SNP4 (rs2305480) were similar in children with and without asthma (appendix pp 10 and 14). The association between SNP4 (rs2305480) and *PGAP3* expression may be due to LD between SNP4 and SNP2 (rs2517955) in *PGAP3* ($r^2=0.40$ in African American CREW children; Figure 1A). In fact, SNP2 (rs2517955) is a very strong eQTL for *PGAP3* in upper airway epithelial cells (eQTL $\beta=1.15$ [95% CI 1.10, 1.20]; $P<0.0001$) (appendix p 15) in these children. Other SNPs (SNP5 [rs7216389] in *GSDMB* and SNP7 [rs8076131] in *ORMDL3*) showed orders of magnitude less significant association with *GSDMB* expression, likely reflecting LD with SNP4 (rs2305480). None of the SNPs were associated with the expression of any other genes. These combined data suggest that the effects of SNP4 (rs2305480), and/or other untyped SNPs that are in strong LD with SNP4, on airway epithelial cell expression of *GSDMB* may be responsible for modifying the risk for childhood onset asthma at this locus in African American children.

To assess the robustness of this finding, we performed parallel studies in lower airway epithelial cells from European American and African American adults. Similar to our observations in African American CREW children, SNPs in the core region were associated with the expression of *GSDMB*, but not with *ORMDL3*, in the European Americans (Figure 3), although no eQTLs reached significance after Bonferroni correction, possibly due to the small sample size ($n=44$). In the African American adults, SNP4 (rs2305480) was the most significant eQTL for *GSDMB*, but three other core region SNPs were also significant eQTLs for *GSDMB*. Four of the SNPs showed modest associations with *ORMDL3*, but none were significant. These results replicate our findings in CREW children indicating that asthma-associated variation at the 17q12–21 locus is most strongly associated with *GSDMB* expression levels in airway epithelial cells, and suggest that *GSDMB* expression in these cells underlies risk for childhood onset asthma in both European Americans and African

Americans. The studies also raise the possibility that *ORMDL3* expression in the lower airway may additionally contribute to risk in African American adults.

Finally, we tested for differential expression of *GSDMB* and *ORMDL3* between asthma cases and controls in resting blood-derived cells and upper airway epithelial cells from the African American children and in lower airway epithelial cells from the African American and European American adults. None of the analyses revealed differences in the overall abundance of transcripts from either gene between cases and controls, with and without including genotype for SNP4 (rs2305480) as a covariate (appendix pp 11–12).

Discussion

Asthma is the most common chronic disease of childhood, affecting about 8% of children in the U.S. this past decade.² During this time, the prevalence of asthma was 15.7% among non-Hispanic black children, who also showed greater severity and poorer response to treatment compared to U.S. white populations.^{1–4} Among the more than 120 loci associated with childhood onset asthma in populations of European ancestry, a broad locus on chromosome 17q12–21 remains the most replicated to date.^{5–9} Yet, the effects of SNPs and genes at this locus on childhood onset asthma risk in African ancestry populations have been equivocal^{7,8,10–14}. Moreover, the strong LD across more than 150 kb in European ancestry populations makes it particularly challenging to identify the SNPs and genes at this locus that underlie the strong associations with childhood onset asthma risk.

In this study, we used several approaches to identify the source of the asthma risk attributed to the 17q12–21 locus. First, the breakdown of LD in African ancestry populations allowed us to narrow the association signal to two tag SNPs. Second, eQTL studies in unstimulated PBMCs and airway epithelial cells from African American children revealed that the strongest eQTL in epithelial cells (SNP4; rs2305480), but not the strongest eQTL in PMBCs (SNP3; rs12936231), was also associated with asthma in African Americans. SNP3 (rs12936231) in the *ZBP2* gene is a known eQTL for *ORMDL3* and *GSDMB* in immune cells,^{26,27} consistent with our findings. Yet, despite its profound effect on expression of *ORMDL3* in immune cells from both European and African ancestry individuals,^{26,27} it has never been reported as the lead SNP at this locus in asthma GWASs.^{5–9} These combined observations suggest that expression of *GSDMB* in airway epithelial cells, but not *ORMDL3* or *GSDMB* in PMBCs, modulates asthma risk at this locus. Moreover, we attribute the most of the association between SNP4 (rs2305480) with airway epithelial cell expression of *PGAP3*, which is located at the proximal end of the locus, to LD with a SNP in *PGAP3* (SNP2, rs2517955; $r^2=0.40$), which is a strong eQTL for *PGAP3* in lung tissue¹⁵ and in the airway epithelial cells ($P<0.0001$) (appendix p 15). Third, our studies in lower airway epithelial cells from European American and African American adults replicated the finding that asthma-associated SNPs at this locus are strong eQTLs for *GSDMB* in airway cells and extended these observations to the lower airway, to adults, and to European Americans. Modest associations with *ORMDL3* levels in lower airway cells raise the possibility that this gene may also contribute to asthma risk in African American adults. These combined data provide strong convergent lines of evidence that asthma risk at the 17q12–21 locus is modulated by genetic regulation of *GSDMB* expression in airway epithelial cells in African

and European ancestry populations. The strength and specificity of association between 17q12–21 asthma-associated alleles with expression of *GSDMB* in airway epithelial cells further highlights the important role of epithelial function in the etiology of childhood onset asthma⁹.

GSDMB is an outstanding candidate asthma gene due to its critical role in pyroptosis, a form of necrotic and inflammatory cell death mediated by inflammatory caspases.²⁸ Acting through a non-canonical pathway, the N-terminus of *GSDMB* binds to the CARD domain of caspase-4 in response to intracellular microbes, directly leading to activation of other caspases and cleavage of the N-terminus of another gasdermin, *GSDMD*, which then orchestrates pyroptosis by forming pores in the plasma membrane.²⁹ SNP4 (rs2305480) is a missense variant (c. 892G>A; p.Pro298Ser) in *GSDMB* at a highly conserved amino acid. This SNP is in near perfect LD ($r^2 \sim 1$) in all populations with two *GSDMB* intronic SNPs,¹⁵ which were not genotyped in our study (appendix p 13). One intronic SNP, rs11078928 (c.662T>C), affects splicing efficiency of the *GSDMB* transcript by skipping exon 6,³⁰ resulting in reduced expression of both the *GSDMB* transcript and *GSDMB* protein on the non-risk (C) allele. The second untyped SNP, rs11078927, is of unknown function. Thus, the lead candidate SNP in our studies represents the effects of three SNPs in *GSDMB*, including one with possible effects on protein function (SNP4; rs2305480) and one with known effects on transcript and protein levels (rs11078928).

The disparate frequencies of the asthma-associated alleles in *GSDMB*, ~ 0.80 in African American and ~ 0.50 in European American CREW children are notable. For example, only $\sim 4\%$ of African American children are expected to be homozygous for the protective allele compared to nearly 25% of European American children. Among children with one or two copies of the risk allele, 19% of the European American and 36% of the African American children had a diagnosis of asthma by 6 years of age. On the one hand, the overall high penetrance of the risk genotypes in both groups may be due to inclusion of four CREW cohorts (accounting for 45% of European American children and 51% of African American children) in which participants were selected based on a family history of asthma or allergies (Table 1). On the other hand, the less than 100% penetrance of 17q12–21 genotypes in both groups, and the central role *GSDMB* in innate immune response to intracellular microbes, is consistent with the many interactions observed between 17q12–21 genotypes and early life exposures, including tobacco smoke,^{18,31} rhinovirus and wheezing illnesses,^{19,20} farm animals,²⁰ and pets³² on childhood onset asthma risk.

There are several strengths and some caveats to this study. First, the CREW sample is relatively small for genetic association studies, particularly in the African American children. However, these children who were enrolled at birth and comprehensively monitored longitudinally improved the accuracy of the asthma phenotyping to an extent not achievable in the large samples needed for GWAS. In fact, prospectively collected “doctor-diagnosed asthma” definitions closely agree with other definitions that include lung function and other symptom-based criteria.³³ Combining the CREW association results with published GWAS results increased power and revealed largely overlapping effect sizes at the associated loci in the CREW and EVE African Americans. This, together with the breakdown of LD in the African American children, allowed us to make several important

observations that could not have been made in European ancestry samples. Second, because genome-wide genotypes for estimating ancestry were not available for the CREW children, we could not adjust for different levels of admixture. This potential limitation was minimized by focusing on a single locus and correcting for differences in asthma prevalence and allele frequencies by including study site as a covariate.

Third, this study included cells from different cellular compartments for gene expression studies, and the contrasting findings in airway and blood cells were informative. However, we cannot exclude that other genes at this locus, including *ORMDL3*, may be important in other tissues, in response to exposures, or at different stages of development. Lastly, we could not relate differential expression of *GSDMB* or *ORMDL3* in these samples to asthma, similar to earlier studies (reviewed in Stein et al.¹⁵). This observation further suggests that expression differences of these genes between asthma cases and controls may only be evident during critical windows of development or in response to certain exposures, such as respiratory infections in early life.^{18,19}

In conclusion, this study demonstrates the power of using populations of different ethnicities for association studies and eQTL studies in asthma-relevant cells to fine map the effects of SNPs on both disease risk and gene expression, and of using birth cohorts in which children have been carefully phenotyped during early life when disease risk trajectories are established. Our study highlights *GSDMB* as an important asthma gene at the 17q12–21 locus and airway epithelial cells as the most likely target for modulating the effects of variation at this locus on the development of childhood onset asthma. Combined with previous studies of *GSDMB* function, these results suggest a potential target for therapeutic intervention in children with the high-risk genotype at this locus.

Research in Context

Evidence before this study

We searched PubMed for all genetic and eQTL studies of the 17q12–21 locus since it was first reported as an asthma locus in 2007 through July 1, 2019, using the terms *GSDMB*, *ORMDL3*, 17q, and asthma. These studies supported the conclusion that this is the most highly replicated childhood onset asthma locus and is nearly always the most significant locus in GWASs of asthma in European ancestry populations. This locus has also been replicated in Latino populations, but much less so in African ancestry populations in whom associations with asthma are considerably weaker. SNPs at this locus have been reported as eQTLs for two genes, *GSDMB* and *ORMDL3*, primarily in blood-derived cells and primarily in individuals of European ancestry. Moreover, due to the extensive linkage disequilibrium (LD) at this locus in European ancestry populations, it has not been possible to distinguish the individual effects of SNPs spanning approximately 150 kb on asthma risk or on the expression of genes at this locus. No previous studies in African Americans focused on early childhood onset asthma or on eQTL studies in airway cells.

Added value of this study

Leveraging the breakdown of LD in African Americans, we were able to narrow the association with childhood onset asthma to two SNPs, we showed that one of the asthma-associated SNPs is also a strong eQTL for *GSDMB*, but not *ORMDL3*, in upper airway epithelial cells from African American children and in lower airway epithelial cells from African American adults. We further showed that 17q12–21 SNPs are eQTLs for *GSDMB*, but not *ORMDL3*, in lower airway epithelial cells from European American adults. The SNP that was the most significant eQTL for *GSDMB* and *ORMDL3* in blood-derived cells from African American children was not associated with asthma.

Implications of all the available evidence

Our results indicate that *GSDMB* expression in airway epithelial cells, and the SNPs that regulate its expression, are the leading 17q12–21 asthma candidates, and identify *GSDMB* as a potential therapeutic target for childhood onset asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of Interest

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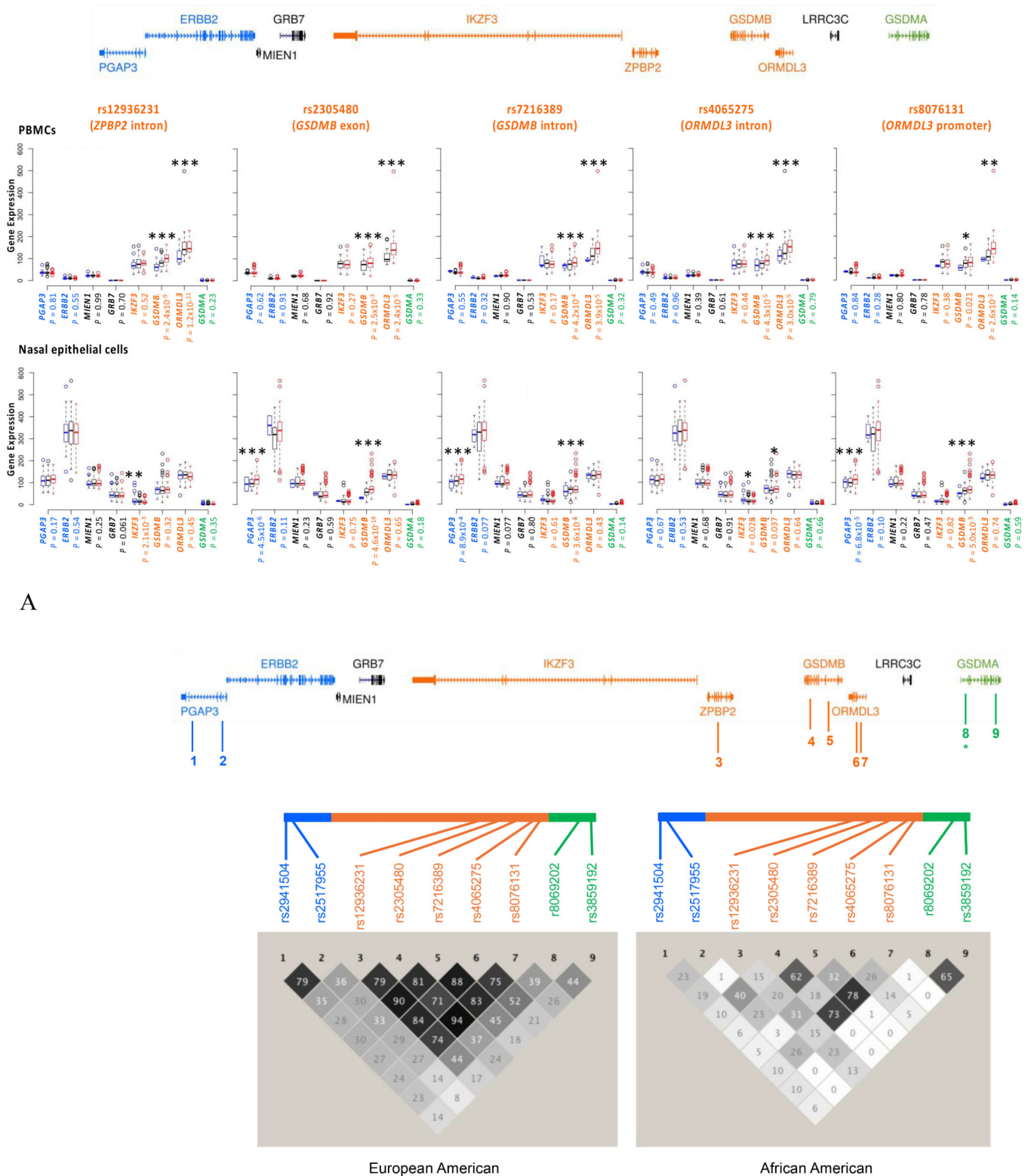


Figure 1. Asthma association studies and LD nine SNPs at the 17q12–21 locus in CREW European American and African American children.

A) Pairwise LD between (see also appendix p 12). The top panel shows the location of genes at the locus, with the relative positions of the nine SNPs genotyped in the CREW children shown by vertical lines. The lower panel shows the LD plots in each population. The r^2 value for pairs of SNPs are shown within the diamond shapes for all values <1.0 . SNPs. Genes in the proximal region shown in blue, in the core region shown in orange, and in the distal region in green, as previously described.¹⁵ *rs3894194 was replaced in this study with rs8069202, which is in high LD with rs3894194 in both Europeans ($r^2=0.984$) and African Americans ($r^2=0.953$) (1000 Genomes). *PGAP3*, post-GPI attachment to proteins 3; *ERBB2*, erb-b2 receptor tyrosine kinase 2; *MEN1*, multiple endocrine neoplasia type 1; *GRB7*, growth factor receptor-bound protein 7; *IKZF3*, IKAROS family zinc finger 3; *ZBP2*, zona pellucida binding protein 2; *GSDMB*, gasdermin B; *ORMDL3*, ORM1-like 3; *LRRC3C*, leucine-rich repeat-containing protein 3C; *GSDMA*, gasdermin A. **B)** Forest plots of association results in European Americans (EuAm) and African Americans (AfAm). Asthma odds ratio estimates (filled squares) and 95% confidence intervals (horizontal bars) are shown for the AfAm and EuAm samples in the CREW, EVE and CREW+EVE meta analyses. The size of the squares is proportional to the square root of the sample size. The bolded SNPs are the two that are associated with asthma in the AfAm meta-analysis (Bonferroni $P<0.0055$).

B

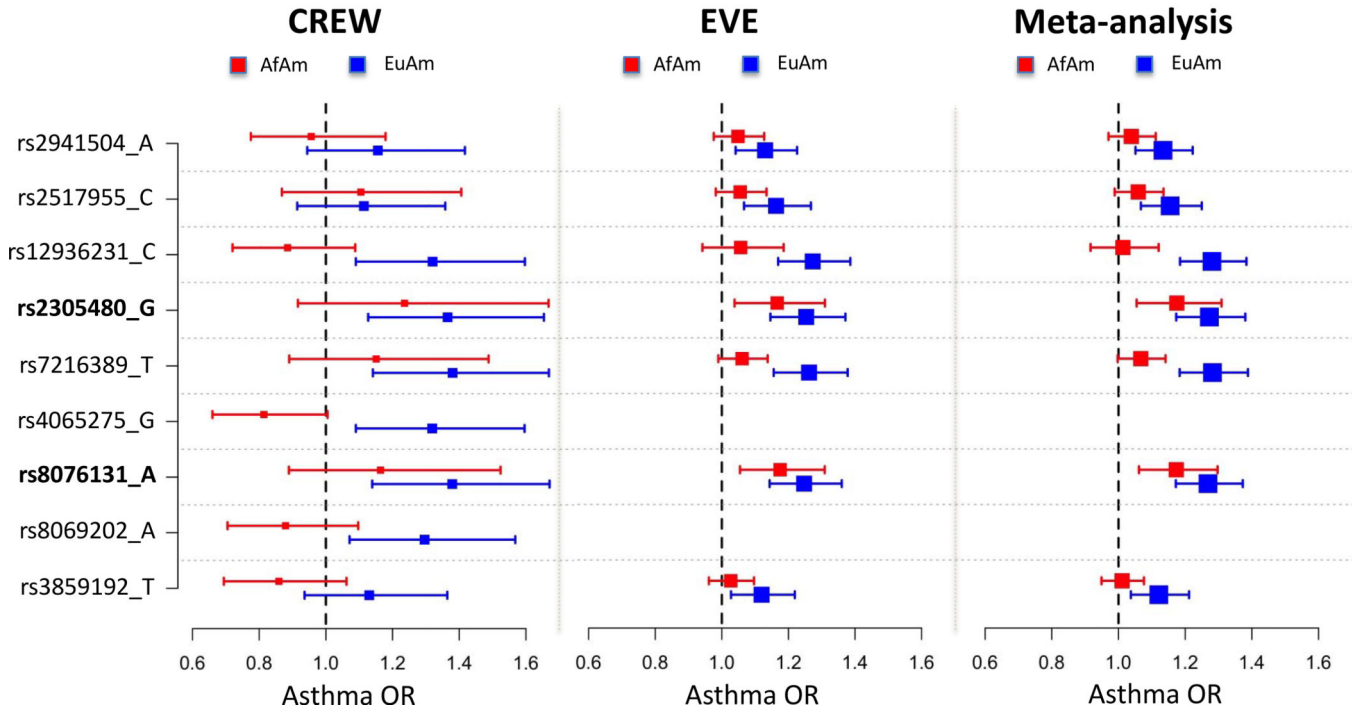


Figure 2. Associations between five core region SNPs and expression levels of genes at the extended 17q12–21 locus in African American children in A) PBMCs (n=85) and B) upper airway epithelial cells (n=246).

The relative location of genes at this locus are shown in the upper panel. *ZPBP2* and *LRRC3C* were not detected as expressed in these cells. SNPs and the genes in which they reside are shown above each panel. Gene names are shown on the x-axes; normalized gene expression in counts per million are shown on the y-axis (see Methods). At each SNP, blue bars correspond to homozygotes for the non-effect (non-risk) alleles, black bars to heterozygotes, and red bars to homozygotes for the effect (risk) alleles. For SNP rs2305480, the AA and AG genotypes were combined in PBMCs because there was only one homozygote for the A allele. See Table 3 for beta values (95% CIs) and exact *P*-values. **P* < 0.05, ***P* < 0.01, ****P* < 0.0012

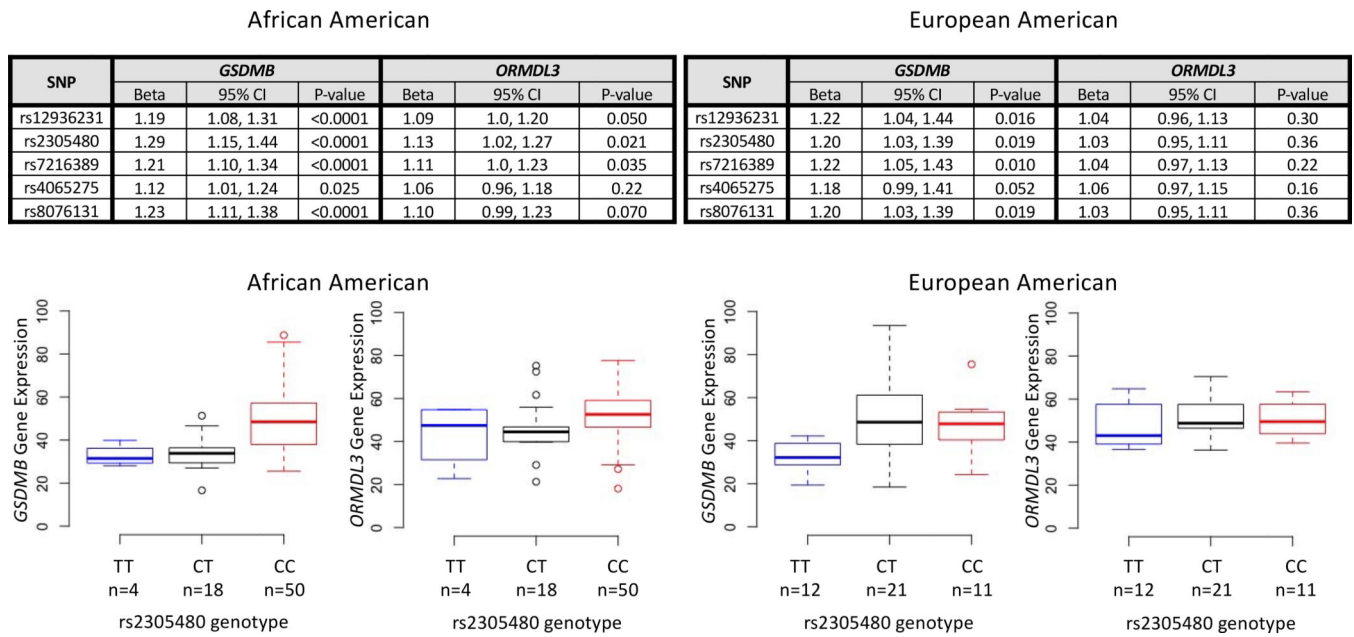


Figure 3. eQTL studies of five core region SNPs and expression levels of *GSDMB* and *ORMDL3* in lower airway (bronchial) epithelial cells from European American (n=44) and African American (n=72) adults.

Upper panel: eQTL results with five core SNPs. The effect sizes (beta) reflect the expected increase in expression associated with each copy of the asthma risk allele. Bonferroni corrected *P*-value threshold (5 SNPs × 2 genes) = 0.0055. Lower panel: Boxplots showing associations between rs2305480 genotype and expression levels of *GSDMB* and *ORMDL3*.

Table 1.

Description of the nine CREW cohorts included in this study. See Gern et al.¹⁶ for more details.

Cohort	Recruitment Sites	Recruitment Years	High Risk*	European American (case/control) [%Female]	African American (case/control) [%Female]
CAS	Detroit, MI	1987 – 1989	No	294 (33/261) [42%/51%]	3 (0/3) [-/33%]
CCAAPS	Cincinnati, OH	2001 – 2003	Yes	324 (40/284) [35%/47%]	89 (27/62) [37%/42%]
CCCEH	New York, NY	1998 – 2006	No	0	71 (3/68) [100%/65%]
COAST	Madison, WI	1998 – 2000	Yes	169 (56/113) [34%/50%]	8 (5/3) [40%/67%]
EHAAS	Boston, MA	1994 – 1996	Yes	238 (81/157) [26%/47%]	21 (11/10) [55%/50%]
IIS	Tucson, AZ	1997 – 2003	No	198 (34/164) [44%/57%]	3 (2/1) [50%/0%]
TCRS	Tucson, AZ	1980 – 1984	No	236 (23/213) [43%/49%]	11 (1/10) [10%/70%]
URECA	Boston, MA/Baltimore, MD/New York, NY/St. Louis, MO	2004 – 2006	Yes	3 (1/2) [10%/50%]	328 (159/169) [47%/53%]
WHEALS	Detroit, MI	2003 – 2007	No	151 (28/123) [46%/47%]	336 (111/225) [45%/54%]
Total (case/control)				1,613 (296/1,317) [36%/50%]	870 (319/551) [45%/54%]

* High risk refers to families ascertained based on having at least one parent with asthma and/or allergy.

Table 2.

Results of single SNP analysis of the 17q12–21 SNPs and asthma.

Different colors demarcate SNPs in the proximal, core, and distal regions of this locus (Figure 1A). Effect alleles are the asthma-associated alleles in Europeans, following ¹⁵. SNP numbers are from Figure 1A. The two rows in bolded font show the two SNPs associated with asthma in the meta-analysis of African American samples at the Bonferroni corrected *P*-value threshold (9 SNPs) = 0.005. The meta-analysis *P*-values were weighted by the number of cases. CI, confidence interval; EAF, effect allele frequency; n/a, SNP not available for EVE⁷.

SNP Number	SNP_Ref_Allele	Gene	European Americans						African Americans							
			CREW (n = 1,613)			Meta-Analysis (CREW+EVE, n=5,916)			CREW (n = 870)			Meta-Analysis (CREW+EVE, n=3,904)				
			RAF	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	RAF	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>
1	rs2941504_A	<i>PGAP3</i>	0.31	1.15	0.94, 1.41	0.16	1.13	1.05, 1.22	0.0011	0.45	0.95	0.77, 1.17	0.67	1.038	0.97, 1.11	0.27
2	rs2517955_C	<i>PGAP3/ERBB2</i>	0.35	1.11	0.91, 1.35	0.12	1.15	1.06, 1.25	0.0003	0.76	1.10	0.86, 1.40	0.41	1.059	0.98, 1.13	0.10
3	rs12936231_C	<i>ZFPBP2</i>	0.50	1.32	1.09, 1.59	0.0043	1.28	1.18, 1.38	<0.0001	0.47	0.88	0.72, 1.08	0.24	1.013	0.91, 1.12	0.79
4	rs2305480_G	<i>GSDMB</i>	0.55	1.36	1.12, 1.65	0.0014	1.27	1.17, 1.38	<0.0001	0.84	1.23	0.91, 1.66	0.16	1.17	1.05, 1.30	0.0035
5	rs7216389_T	<i>GSDMB</i>	0.51	1.38	1.11, 1.66	0.0008	1.28	1.18, 1.38	<0.0001	0.79	1.15	0.89, 1.48	0.28	1.067	0.99, 1.14	0.059
6	rs4065275_G	<i>ORMDL3</i>	0.52	1.31	1.09, 1.59	0.0043	n/a	n/a	n/a	0.62	0.81	0.66, 1.005	0.055	n/a	n/a	n/a
7	rs8076131_A	<i>ORMDL3</i>	0.55	1.37	1.13, 1.67	0.0010	1.26	1.17, 1.34	<0.0001	0.81	1.16	0.88, 1.52	0.26	1.17	1.06, 1.29	0.0018
8	rs8069202_A	<i>GSDMA</i>	0.45	1.29	1.07, 1.56	0.0077	n/a	n/a	n/a	0.32	0.87	0.70, 1.09	0.25	n/a	n/a	n/a
9	rs3859192_T	<i>GSDMA</i>	0.465	1.13	0.93, 1.36	0.12	1.12	1.03, 1.21	0.0040	0.35	0.85	0.69, 1.06	0.16	1.011	0.94, 1.07	0.73

Table 3.

Effect sizes (beta), 95% confidence intervals (CI), and P-values for five core SNPs and expression of the eight 17q12–21 genes that are expressed in PBMCs (A) and upper airway epithelial cells (B).

The effect sizes (beta) reflect the expected increase in expression associated with each copy of the asthma risk allele. P-values were corrected for 40 tests (5 SNPs x 8 genes); $P < 0.0012$ are considered significant (shown in bolded font) (also see Figure 2).

A. PBMCs (n=85)												
SNP	PGAP3			ERBB2			MIEN1			GRB7		
	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	
rs12936231	1.00	0.96, 1.041	0.81	0.976	0.91, 1.037	0.55	0.994	0.96, 1.024	0.99	1.02	0.82, 1.25	0.70
rs2305480	1.01	0.95, 1.084	0.62	1.0	0.89, 1.11	0.91	0.987	0.93, 1.040	0.68	0.973	0.67, 1.40	0.92
rs7216389	0.98	0.93, 1.042	0.55	0.945	0.86, 1.036	0.32	0.993	0.95, 1.038	0.90	0.888	0.65, 1.21	0.53
rs4065275	0.98	0.95, 1.026	0.49	0.992	0.93, 1.057	0.96	1.008	0.97, 1.039	0.39	1.032	0.83, 1.27	0.61
rs8076131	1.00	0.95, 1.064	0.84	0.942	0.85, 1.033	0.28	0.99	0.94, 1.035	0.8	0.943	0.69, 1.29	0.78
SNP	IKZF3			GSDMB			ORMDL3			GSDMA		
	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value
rs12936231	0.967	0.91, 1.027	0.52	1.238	1.15, 1.32	<0.0001	1.186	1.12, 1.24	<0.0001	1.202	0.91, 1.58	0.23
rs2305480	0.938	0.84, 1.045	0.27	1.218	1.067, 1.39	0.0025	1.232	1.11, 1.35	<0.0001	1.279	0.78, 2.094	0.33
rs7216389	0.915	0.83, 1.001	0.17	1.239	1.11, 1.38	0.0004	1.203	1.10, 1.30	<0.0001	1.279	0.84, 1.93	0.32
rs4065275	1.003	0.94, 1.068	0.44	1.185	1.10, 1.27	<0.0001	1.133	1.071, 1.19	<0.0001	1.067	0.80, 1.42	0.79
rs8076131	0.94	0.85, 1.029	0.38	1.158	1.034, 1.29	0.021	1.151	1.058, 1.25	0.0026	1.408	0.92, 2.13	0.14
B. Upper Airway Epithelial Cells (n=246)												
SNP	PGAP3			ERBB2			MIEN1			GRB7		
	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value
rs12936231	1.026	0.989, 1.064	0.17	0.993	0.970, 1.016	0.54	1.009	0.993, 1.025	0.25	0.949	0.899, 1.002	0.061
rs2305480	1.147	1.081, 1.216	<0.0001	1.031	0.992, 1.071	0.12	1.016	0.991, 1.042	0.22	0.980	0.895, 1.074	0.67
rs7216389	1.081	1.032, 1.133	0.0010	1.028	0.998, 1.060	0.070	1.018	0.998, 1.039	0.070	0.990	0.921, 1.064	0.78
rs4065275	1.034	0.997, 1.072	0.74	1.008	0.985, 1.032	0.49	1.003	0.988, 1.019	0.67	1.003	0.949, 1.060	0.93
rs8076131	1.107	1.052, 1.164	<0.0001	1.028	0.995, 1.063	0.093	1.014	0.993, 1.036	0.20	0.971	0.898, 1.050	0.46
SNP	IKZF3			GSDMB			ORMDL3			GSDMA		
	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value

	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value
rs12936231	0.872	0.799, 0.951	0.0021	1.026	0.975, 1.080	0.32	0.989	0.962, 1.017	0.45	0.908	0.741, 1.112	0.35
rs2305480	0.976	0.850, 1.119	0.73	1.353	1.251, 1.464	<0.0001	0.990	0.945, 1.036	0.66	1.275	0.911, 1.785	0.16
rs7216389	0.971	0.874, 1.078	0.58	1.144	1.073, 1.220	<0.0001	1.015	0.979, 1.051	0.43	1.398	1.078, 1.813	0.11
rs4065275	0.913	0.843, 0.989	0.026	1.054	1.003, 1.108	0.038	1.006	0.979, 1.035	0.65	0.962	0.786, 1.178	0.71
rs8076131	0.985	0.877, 1.105	0.79	1.225	1.144, 1.312	<0.0001	0.994	0.956, 1.034	0.78	1.328	0.998, 1.767	0.051