



Expression quantitative trait locus fine mapping of the 17q12–21 asthma locus in African American children: a genetic association and gene expression study

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Summary

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Background African ancestry is associated with a higher prevalence and greater severity of asthma than European ancestries, yet genetic studies of the most common locus associated with childhood-onset asthma, 17q12–21, in African Americans have been inconclusive. The aim of this study was to leverage both the phenotyping of the Children's Respiratory and Environmental Workgroup (CREW) birth cohort consortium, and the reduced linkage disequilibrium in African Americans, to fine map the 17q12–21 locus.

Methods We first did a genetic association study and meta-analysis using 17q12–21 tag single-nucleotide polymorphisms (SNPs) for childhood-onset asthma in 1613 European American and 870 African American children from the CREW consortium. Nine tag SNPs were selected based on linkage disequilibrium patterns at 17q12–21 and their association with asthma, considering the effect allele under an additive model (0, 1, or 2 effect alleles). Results were meta-analysed with publicly available summary data from the EVE consortium (on 4303 European American and 3034 African American individuals) for seven of the nine SNPs of interest. Subsequently, we tested for expression quantitative trait loci (eQTLs) among the SNPs associated with childhood-onset asthma and the expression of 17q12–21 genes in resting peripheral blood mononuclear cells (PBMCs) from 85 African American CREW children and in upper airway epithelial cells from 246 African American CREW children; and in lower airway epithelial cells from 44 European American and 72 African American adults from a case-control study of asthma genetic risk in Chicago (IL, USA).

Findings 17q12–21 SNPs were broadly associated with asthma in European Americans. Only two SNPs (rs2305480 in *gasdermin-B* [*GSDMB*] and rs8076131 in *ORMDL3* sphingolipid biosynthesis regulator 3 [*ORMDL3*]) were associated with asthma in African Americans, at a Bonferroni-corrected threshold of $p < 0.0055$ (for rs2305480_G, odds ratio [OR] 1.36 [95% CI 1.12–1.65], $p = 0.0014$; and for rs8076131_A, OR 1.37 [1.13–1.67], $p = 0.0010$). In upper airway epithelial cells from African American children, genotype at rs2305480 was the most significant eQTL for *GSDMB* (eQTL effect size $[\beta]$ 1.35 [95% CI 1.25–1.46], $p < 0.0001$), and to a lesser extent showed an eQTL effect for post-GPI attachment to proteins phospholipase 3 (β 1.15 [1.08–1.22], $p < 0.0001$). No SNPs were eQTLs for *ORMDL3*. By contrast, in PBMCs, the five core SNPs were associated only with expression of *GSDMB* and *ORMDL3*. Genotype at rs12936231 (in zona pellucida binding protein 2) showed the strongest associations across both genes (for *GSDMB*, eQTL β 1.24 [1.15–1.32], $p < 0.0001$; and for *ORMDL3* (β 1.19 [1.12–1.24], $p < 0.0001$). The eQTL effects of rs2305480 on *GSDMB* expression were replicated in lower airway cells from African American adults (β 1.29 [1.15–1.44], $p < 0.0001$).

Interpretation Our study suggests that SNPs regulating *GSDMB* expression in airway epithelial cells have a major role in childhood-onset asthma, whereas SNPs regulating the expression levels of 17q12–21 genes in resting blood cells are not central to asthma risk. Our genetic and gene expression data in African Americans and European Americans indicated *GSDMB* to be the leading candidate gene at this important asthma locus.

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Introduction

Global disparities in the prevalence, severity, and natural history of asthma have been well described. African

ancestry is associated with an increased prevalence of asthma^{1,2} and, in affected individuals, with poorer responses to asthma therapies and suboptimal lung

Research in context

Evidence before this study

We searched PubMed for all genetic studies and expression quantitative trait loci (eQTL) studies of the 17q12–21 locus since it was first reported as an asthma locus on July 26, 2007, up to July 1, 2019, using the terms “*GSDMB*”, “*ORMDL3*”, “17q”, and “asthma”, with no language restrictions. The studies we identified supported the conclusion that 17q12–21 is the most highly replicated locus to be associated with childhood-onset asthma, and is nearly always the most significant locus in genome-wide association studies of asthma in populations of European ancestry. This locus has also been replicated in Latino populations, but much less so in populations of African ancestry in whom associations of the locus with asthma are considerably weaker. Single-nucleotide polymorphisms (SNPs) at this locus have been reported as eQTLs for two genes, gasdermin-B (*GSDMB*) and *ORMDL3* sphingolipid biosynthesis regulator 3 (*ORMDL3*), primarily in blood-derived cells and primarily in individuals of European ancestry. Because of the extensive linkage disequilibrium at this locus in European populations, the individual effects of SNPs (spanning approximately 150 kb) on asthma risk and the expression of

genes at this locus have been difficult to distinguish. No previous studies in African Americans have focused on asthma onset in early childhood or on eQTLs in airway cells.

Added value of this study

By leveraging the reduced linkage disequilibrium in African Americans, we were able to narrow the association with childhood-onset asthma to two SNPs. Additionally, we showed that one of the asthma-associated SNPs is also a significant eQTL for *GSDMB*, but not for *ORMDL3*, in upper airway epithelial cells from African American children and in lower airway epithelial cells from African American and 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 candidates within 17q12–21 to be associated with asthma onset in children, and identify the gasdermin-B protein as a potential therapeutic target for childhood-onset asthma.

function compared with populations of European ancestry.^{3,4} The relative contributions of genes, the environment, and gene–environment interactions to these disparities are unknown. In particular, evidence for an association of asthma with single-nucleotide polymorphisms (SNPs) at the chromosome 17q12–21 locus, the most highly replicated locus to be associated with childhood-onset asthma in populations of European ancestry,^{5–9} has been ambiguous in African ancestry populations, with weak associations at best.^{7,8,10–14} However, none of these studies in African ancestry populations focused specifically on asthma onset in early childhood and sample sizes have been small relative to studies of populations of European ancestry.

A notable feature of the 17q12–21 asthma locus is extensive linkage disequilibrium that spans over 150 kb on European-derived chromosomes, with lesser linkage disequilibrium extending into the flanking regions, altogether encoding at least ten genes. The asthma-associated alleles in populations of European ancestry are on an extended, so-called risk haplotype that has been associated with the expression of two genes, *ORMDL3* sphingolipid biosynthesis regulator 3 (*ORMDL3*) and gasdermin-B (*GSDMB*), primarily in blood cells. The risk haplotype and protective haplotype at this locus occur at approximately equal frequencies in most populations of European ancestry. Because of the extent of the linkage disequilibrium, studies have had difficulty in differentiating the effects of individual variants on asthma risk and gene expression, and in identifying causal variants and genes, in individuals of European ancestry. In contrast, considerably less linkage disequilibrium is observed at this locus on

African-derived chromosomes;¹⁵ however, this feature has not been used to fine map the independent effects of SNPs at 17q12–21 on the risk of childhood asthma or the expression of genes at this locus.

We hypothesised that the breakdown of linkage disequilibrium in African Americans would facilitate fine mapping studies at the 17q12–21 locus, and allow us to narrow the associations with asthma and with the expression of genes at this locus to a lessened number of SNPs. To this end, we leveraged the Children’s Respiratory and Environmental Workgroup (CREW) consortium,¹⁶ a component study of the larger Environmental Influences on Child Health Outcomes Program funded by the US National Institutes of Health. CREW includes ethnically diverse participants from 12 US longitudinal birth cohorts with asthma-related phenotypes. In the current study, to first show that SNPs at the 17q12–21 locus are associated with childhood-onset asthma in the CREW children, we genotyped nine tag SNPs of 17q12–21 in European American and African American children enrolled at birth between 1980 and 2003. To increase the power to detect associations with asthma, we also included summary statistics from a meta-analysis of European American and African ancestry individuals from the EVE consortium.⁷ We then sought to identify SNPs associated with childhood-onset asthma that are also expression quantitative trait loci (eQTLs) for 17q12–21 genes in 268 African American children from a CREW cohort, using unstimulated peripheral blood mononuclear cells (PBMCs) and upper airway epithelial cells, as two compartments that are central to the pathobiology of asthma. Finally, we validated the eQTL results in lower airway epithelial cells from

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See Online for appendix

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44 European American and 72 African American adults involved in a case-control study of genetic risk in asthma,¹⁷ to allow us to generalise our findings to the lower airway and to European Americans.

Methods

Study design and sample composition

We did a genetic association study with a meta-analysis of 2483 unrelated children from nine CREW birth cohorts, followed by a gene expression study. Included children were reported by parents to be European American or African American, and had been followed up to at least age 6 years and provided DNA for genotyping (table 1). Because the 17q12–21 locus is associated with wheezing illnesses in the first 3 years of life and asthma diagnosis by age 5 years,^{18–21} we focused on asthma diagnosed by a health-care provider by age 6 years to maximise sample size and reduce heterogeneity. Further details on our CREW sample composition are provided in the appendix (p 6). The genetic studies at all CREW sites were approved by the institutional review boards at the participating institutions (appendix p 35).

We also included two additional samples. First, to increase statistical power in the association studies, summary data from a meta-analysis of genome-wide association studies (GWASs) of the EVE consortium⁷ were included for 4303 European American individuals (2125 cases and 2178 controls) and 3034 African ancestry individuals (1567 cases and 1467 controls) from case-control studies, in which asthma had been diagnosed by a doctor (appendix p 6). Second, to validate any eQTL identified in CREW children, we studied eQTL in bronchial epithelial cells from 44 European American participants (32 cases and 12 controls) and 72 African American participants (48 cases and 24 controls) from a case-control study in Chicago (IL, USA).¹⁷ Asthma was diagnosed on the basis of a doctor's diagnosis, current symptoms, and use of asthma medication (appendix p 7). Approval for the case-control study was obtained from the University of Chicago (Chicago).

Genotyping and quality control in the CREW study

DNA (500 ng at concentrations of 5 ng/μL) was shipped from the coordinating centre of each CREW cohort to the University of Chicago (Chicago, IL, USA) for genotyping. Nine SNPs were selected based on linkage disequilibrium patterns at the extended 17q12–21 locus and previously reported associations with asthma or gene expression (appendix p 11). The genotyping and quality control methods are described in the appendix (p 6). Linkage disequilibrium plots and r^2 values were generated with LocusView 2.0 (Broad Institute, Cambridge, MA, USA).

SNP association tests

We considered the genotype of each SNP 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, 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 an increased risk of asthma in populations of European ancestry as the effect allele in all tests.¹⁵ p values were adjusted for nine tests; $p < 0.0055$ was considered to indicate statistical significance. To avoid selection bias, we included all CREW cohorts, regardless of sample size. However, we repeated all analyses on cohorts with at least 20 cases to assure that the small samples were not skewing results.

In these tests, we also included published summary data from the EVE consortium.⁷ Most EVE cases had asthma onset in childhood (median age of onset <8 years in European Americans and <7 years in individuals of African ancestry). Summary statistics were available for seven of the nine SNPs in EVE, which were extracted and meta-analysed with all association data across the CREW and EVE cohorts within each ancestry group, using a fixed-effect model in which we assumed the log-transformed odds ratio (OR) estimate from each study was centred around the true log-transformed OR with known study-specific variance. Statistical significance of test statistics from the meta-analysis was assessed with standard normal approximations; analyses were done in R (version 3.3.3).

Gene expression studies

RNA sequencing data were available in African American children from one of the CREW cohorts (Urban Environment and Childhood Asthma study [URECA]); in unstimulated PBMCs for 85 children (48 cases and 37 controls)²² and in upper airway (nasal) cells for 246 children (125 cases and 121 controls) at the follow-up visit, all at age 11 years (appendix p 6). We used an additive effects linear model to test for eQTL effect sizes (β) between the five 17q core-region SNPs and each of the expressed genes at the extended locus, including sex, sample collection site, and ten latent factors²³ as covariates in the PBMC studies, and sex, study site, sequencing batch, epithelial cell proportion, and 12 latent factors²³ as covariates in the epithelial cell studies. Latent factors were included to correct for unwanted variation.²³ p values were adjusted for 40 tests (five SNPs multiplied by eight expressed genes); $p < 0.0012$ was considered to indicate statistical significance. We also tested differential expression between cases and controls using a standard linear regression model with gene expression (log counts per million) as the dependent variable and covariates as the dependent variables, including the same covariates as in the previous models. Additionally, based on results of our eQTL studies of core region SNPs, we did a post-hoc eQTL analysis of rs2517955 in the proximal region and

of post-GPI attachment to proteins phospholipase 3 (*PGAP3*) expression.

We further examined associations between the same five SNPs with *GSDMB* and *ORMDL3* expression in lower (bronchial) airway epithelial cells from the 44 European American adults and 72 African American adults involved in the case-control study in Chicago (appendix pp 7–8). Gene expression counts were normalised by the trimmed mean of M-values method were adjusted for age, sex, current smoking status (appendix p 7). Linear regression considering additive genotype effects on gene expression was done with the *limma* package in R (v3.3.3), including as covariates age, sex, smoking at the time of the study, sequencing batch, the first three ancestry principle components (to correct for admixture), and cell composition (appendix pp 7–8). We also tested the normalised data for differential expression between cases and controls using a linear model, including as covariates age, sex, smoking at the time of the study, sequencing batch, the first three ancestry PCs, and cell composition; and as a post-hoc covariate, rs2305480 genotype (appendix pp 7–8).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The correlations we identified between the nine 17q12–21 SNPs in children of the CREW consortium (figure 1A)

were similar to those previously reported in European and African American populations (appendix p 18),^{11,15} confirming substantial breakdown of linkage disequilibrium in African Americans compared with European Americans. Throughout this paper, following the approach of Stein and colleagues,¹⁵ we refer to the five SNPs within the approximately 150-kb linkage disequilibrium block, including the *GSDMB* and *ORMDL3* genes, as the core region; and the two SNPs in each of the flanking regions that show less linkage disequilibrium with core region SNPs, as the proximal region (encoding *PGAP3* and erb-b2 receptor tyrosine kinase 2 [*ERBB2*, also known as *HER2*]) and the distal region (encoding *GSDMA*).

The effect alleles at the nine SNPs were common in children from the CREW cohorts (table 2), as previously reported.¹⁵ All five SNPs in the core region were associated with asthma in European American children from the CREW cohorts. These associations were validated in our meta-analysis for the four core SNPs included in EVE (table 2 and figure 1B), reflecting the high linkage disequilibrium between core region SNPs.

None of the SNPs showed significant associations with asthma in African American children of the CREW cohorts, possibly because of the smaller sample size and increased frequency of risk alleles compared with European American children. However, the confidence intervals were overlapping for three SNPs in the core region (rs2305480 and rs7216389 in *GSDMB* and rs8076131 in *ORMDL3*) between African American children and European American children of the CREW cohorts, suggesting that the ORs are not different between these groups (table 2 and figure 1B). Analysis including

	Childhood Asthma Study	Cincinnati Childhood Allergy and Air Pollution Study	Columbia Center for Children's Environmental Health study	Childhood Origins of Asthma study	Epidemiology of Home Allergens and Asthma Study	Infant Immune Study	Tucson Children's Respiratory Study	Urban Environment and Childhood Asthma study	Wayne County Health, Environment, Allergy, and Asthma Longitudinal Study	Total
Recruitment sites	Detroit, MI, USA	Cincinnati, OH, USA	New York, NY, USA	Madison, WI, USA	Boston, MA, USA	Tucson, AZ, USA	Tucson, AZ, USA	Boston, MA; Baltimore, MD; New York, NY; St Louis, MO, USA	Detroit, MI, USA	..
Recruitment years	1987–89	2001–03	1998–2006	1998–2000	1994–96	1997–2003	1980–84	2004–06	2003–07	..
High risk*	No	Yes	No	Yes	Yes	No	No	Yes	No	..
European American individuals	294	324	0	169	238	198	236	3	151	1613
Cases (% female)	33 (42%)	40 (35%)	..	56 (34%)	81 (26%)	34 (44%)	23 (43%)	1 (0%)	28 (46%)	296 (36%)
Controls (% female)	261 (51%)	284 (47%)	..	113 (50%)	157 (47%)	164 (57%)	213 (49%)	2 (50%)	123 (47%)	1317 (50%)
African American individuals	3	89	71	8	21	3	11	328	336	870
Cases (% female)	0	27 (37%)	3 (100%)	5 (40%)	11 (55%)	2 (50%)	1 (0%)	159 (47%)	111 (45%)	319 (45%)
Controls (% female)	3 (33%)	62 (42%)	68 (65%)	3 (67%)	10 (50%)	1 (0%)	10 (70%)	169 (53%)	225 (54%)	551 (54%)

Further details provided in Gern et al.¹⁶ *Children had at least one parent with asthma or allergy (or both).

Table 1: Cohorts from the Children's Respiratory and Environmental Workgroup consortium included in this study

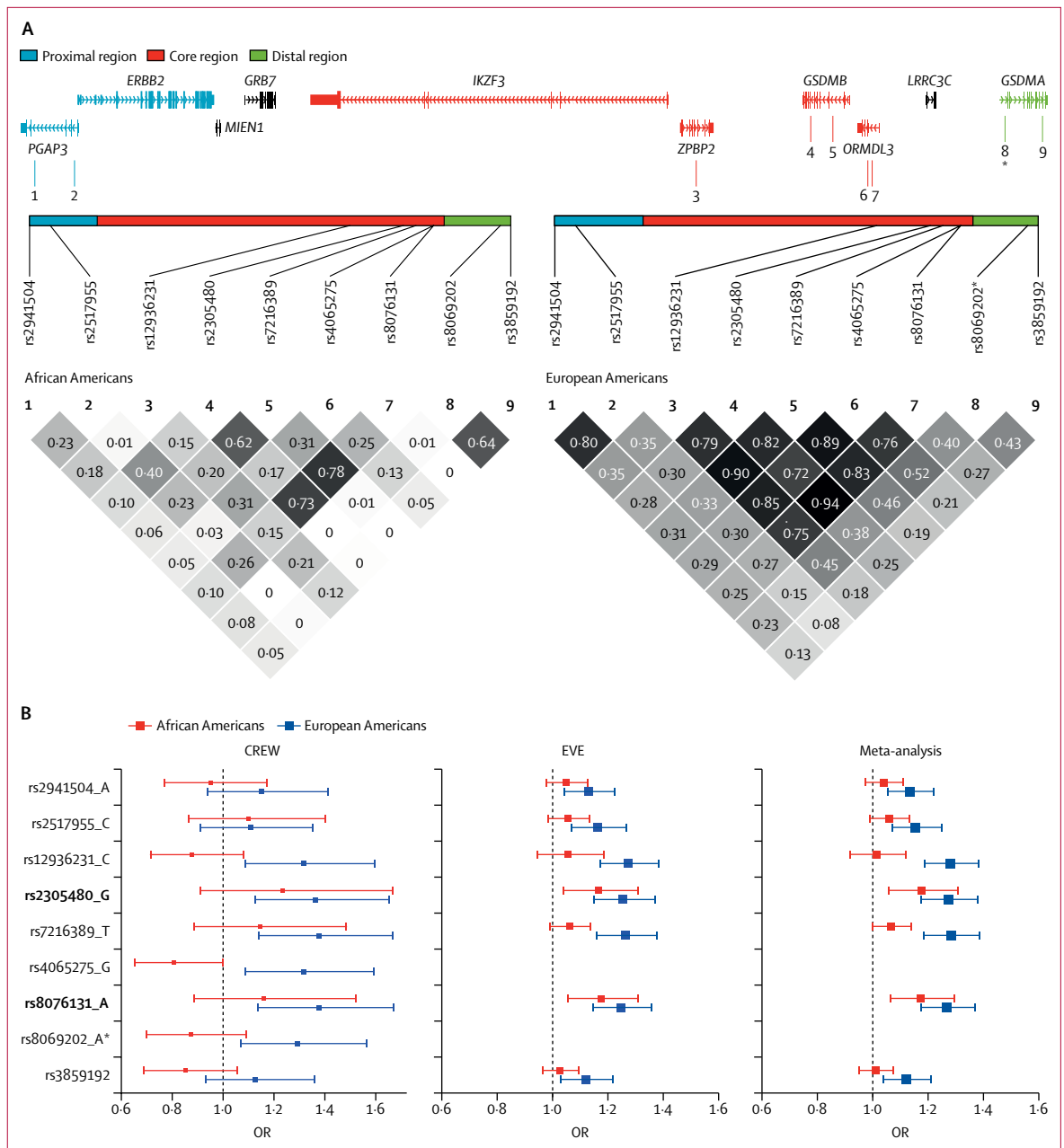


Figure 1: Linkage disequilibrium and asthma association of nine SNPs at the 17q12-21 locus in European American and African American children
 (A) Pairwise linkage disequilibrium between SNPs. The top panel shows the location of genes at the locus, with the relative positions of the nine SNPs genotyped in the CREW children indicated by vertical lines (numbered 1-9). The lower panel shows the linkage disequilibrium plots in each population. The r^2 values for pairs of SNPs are shown within the plots. Designation of proximal, core, and distal regions was as previously described.¹⁵ (B) Forest plots of SNP associations with asthma in European Americans and African Americans. Error bars represent 95% CIs. The size of each square is proportional to the square root of the sample size. The SNPs in bold were associated with asthma in the meta-analysis of African Americans (Bonferroni-corrected threshold $p < 0.0055$; p value for each SNP shown in table 2). SNP=single-nucleotide polymorphism. PGAP3=post-GPI attachment to proteins phospholipase 3. ERBB2=erb-b2 receptor tyrosine kinase 2. MIEN1=migration and invasion enhancer 1. GRB7=growth factor receptor bound protein 7. IKZF3=IKAROS family zinc finger 3. ZPBP2=zona pellucida binding protein 2. GSDMB=gasdermin-B. ORMDL3=ORMDL sphingolipid biosynthesis regulator 3. LRR3C=leucine rich repeat containing protein 3C. GSDMA=gasdermin-A. CREW=Children's Respiratory and Environmental Workgroup. *A TaqMan genotyping assay was not available for rs3894194 and thus we used the surrogate SNP rs8069202, which the 1000 Genomes Project has shown to be in high linkage disequilibrium with rs3894194 in both Europeans ($r^2=0.984$) and African Americans ($r^2=0.953$).

only CREW cohorts with at least 20 cases yielded similar results (appendix p 12). In the meta-analysis with data on African American cases from the EVE consortium,

two SNPs were significantly associated with asthma: rs2305480 in *GSDMB* and rs8076131 in *ORMDL3* (table 2 and figure 1B).

Gene	European Americans					African Americans					
	CREW (n=1613)			Meta-analysis (CREW and EVE; n=5916)		CREW (n=870)			Meta-analysis (CREW and EVE; n=3904)		
	EAf	OR (95% CI)	p value	OR (95% CI)	p value	EAf	OR (95% CI)	p value	OR (95% CI)	p value	
Proximal											
rs2941504_A	PGAP3	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
rs2517955_C	PGAP3 or ERBB2	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
Core											
rs12936231_C	ZBP2	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
rs2305480_G*	GSDMB	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
rs7216389_T	GSDMB	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
rs4065275_G	ORMDL3	0.52	1.31 (1.09–1.59)	0.0043	NA	NA	0.62	0.81 (0.66–1.005)	0.055	NA	NA
rs8076131_A*	ORMDL3	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
Distal											
rs8069202_A	GSDMA	0.45	1.29 (1.07–1.56)	0.0077	NA	NA	0.32	0.87 (0.70–1.09)	0.25	NA	NA
rs3859192_T	GSDMA	0.47	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

Proximal, core, and distal denotes location in the 17q12–21 as in figure 1A. Effect alleles are the asthma-associated alleles in Europeans as previously described.¹⁵ p values on meta-analysis were weighted by the number of cases. SNP=single-nucleotide polymorphism. CREW=Children's Respiratory and Environmental Workgroup. EAf=effect allele frequency. OR=odds ratio. PGAP3=post-GPI attachment to proteins phospholipase 3. ERBB2=erb-b2 receptor tyrosine kinase 2. ZBP2=zona pellucida binding protein 2. GSDMB=gasdermin-B. ORMDL3=ORMDL sphingolipid biosynthesis regulator 3. NA=not available for EVE.⁷ GSDMA=gasdermin-A. *SNPs associated with asthma in the meta-analysis of African American samples at the Bonferroni-corrected threshold (nine SNPs; p<0.0055).

Table 2: Associations of 17q12–21 SNPs with asthma

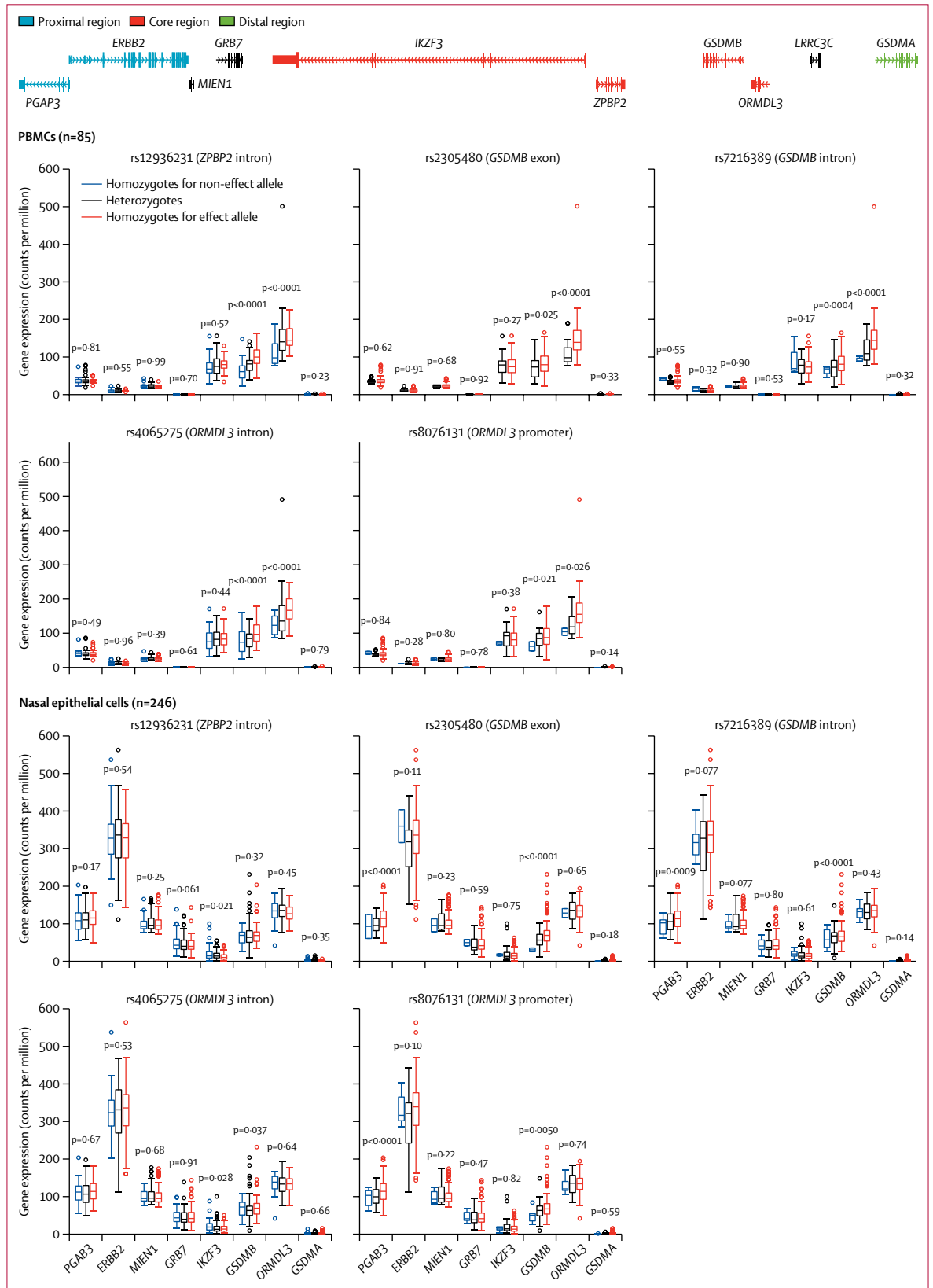
The meta-analysis in African American participants narrowed the association with asthma to two candidate SNPs. However, whether the effects of these SNPs are independently associated with asthma, or whether the associations are due to linkage disequilibrium, could not be distinguished by genetic association studies. Therefore, to investigate these possibilities, we performed eQTL fine mapping for the five core SNPs in African American children of the URECA cohort in the CREW study, and of the eight genes at this locus that were expressed in two cell compartments relevant to asthma: PBMCs and upper airway epithelial cells.

The breakdown of linkage disequilibrium in the African American children allowed us to differentiate the eQTL effects at this locus (figure 2 and appendix pp 13–14). In PBMCs, genotypes at the five core SNPs were associated only with expression of *GSDMB* and *ORMDL3*. The strongest associations were with rs12936231, an intronic SNP in the zona pellucida binding protein 2 (*ZBP2*) gene (*GSDMB*: eQTLβ 1.24 [95% CI 1.15–1.32], p<0.0001; *ORMDL3*: eQTLβ 1.19 [95% CI 1.12–1.24], p<0.0001), with weaker associations at the other core region SNPs (figure 2 and appendix p 13–14), consistent with earlier studies in HapMap lymphoblastoid cell lines²⁴ and primary T cells and B cells.²⁵

Different patterns of association with gene expression were revealed in upper airway epithelial cells (figure 2 and appendix pp 13–14). The SNP associated with asthma in *GSDMB* (rs2305480) was most strongly associated with expression of *GSDMB* (eQTLβ 1.35 [95% CI 1.25–1.46], p<0.0001) and with *PGAP3* to a lesser extent

(eQTLβ 1.15 [1.08–1.22], p<0.0001), but not for any other genes at this locus, including *ORMDL3* (eQTLβ 0.99 [0.95–1.04], p=0.66). The eQTL effects for rs2305480 were similar in children with and without asthma (appendix pp 15, 19). The association between rs2305480 and *PGAP3* expression might be due to linkage disequilibrium between rs2305480 and the proximal SNP, rs2517955, in *PGAP3* (r²=0.40 in African American CREW children; figure 1A). In addition, on post-hoc analysis, rs2517955 was shown to be a strong eQTL for *PGAP3* in upper airway epithelial cells (eQTLβ 1.15 [1.10–1.20], p<0.0001; appendix p 20) in these children. Other SNPs showing significant associations with *GSDMB* expression were rs7216389 in *GSDMB* and rs8076131 in *ORMDL3* (figure 2), possibly reflecting linkage disequilibrium with rs2305480 (figure 1A). None of the SNPs were associated with the expression of any other genes.

To assess the robustness of these findings, we studied in parallel the lower airway epithelial cells of European American and African American adults. Similar to our observations in African American children of the CREW consortium, SNPs in the core region were associated with the expression of *GSDMB*, but not with *ORMDL3*, in European American adults; however, no eQTLs reached significance after Bonferroni correction (figure 3A), possibly because of the small sample size (n=44). In the African American adults, rs2305480 had the greatest eQTL effect for *GSDMB* (eQTLβ 1.29 [1.15–1.44], p<0.0001); three other core-region SNPs were also significant eQTLs for *GSDMB*. Four of the SNPs showed modest associations with *ORMDL3*, but



none were significant. Figure 3B illustrates the SNP (rs2305480) with the largest effect size on expression levels of *GSDMB* and *ORMDL3* in African Americans. These results were in accordance with our findings in CREW children, in whom asthma prevalence was highest in children with the rs2305480 CC genotype (appendix p 16).

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 African American children, and in lower airway epithelial cells from African American and European American adults. None of our analyses revealed differences in the overall abundance of transcripts from either gene between cases and controls, even in post-hoc analyses including genotype for rs2305480 as a covariate (appendix pp 16–17).

Discussion

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} However, the effects of SNPs and genes at this locus on the risk of childhood-onset asthma in populations of African ancestry have been ambiguous.^{7,8,10–14} Furthermore, the strong linkage disequilibrium spanning more than 150 kb at the 17q12–21 locus in European populations creates a particular challenge in identifying the SNPs and genes at this locus that underlie the associations with childhood-onset asthma.

In this study, we used orthogonal approaches to identify the source of the asthma risk attributed to the 17q12–21 locus. First, the breakdown of linkage disequilibrium in populations of African ancestry allowed us to narrow the association signal to two tag SNPs, which had not been possible in previous studies primarily in individuals of European ancestry.^{26,27} Second, eQTL studies in unstimulated PBMCs and airway epithelial cells from

African American children revealed that the strongest eQTL in epithelial cells (rs2305480), but not the strongest eQTL in PBMCs (rs12936231), was also associated with asthma in African Americans. rs12936231 in the *ZBPB2* gene is a known eQTL for *ORMDL3* and *GSDMB* in immune cells,^{24,25} consistent with our findings. However, despite its profound effect on expression of *ORMDL3* in immune cells in individuals of either European or African ancestry,^{24,25} it has never been reported as the lead SNP at this locus in GWASs of asthma.^{5–9} The fact that the rs12936231 genotype was the SNP most associated with *GSDMB* and *ORMDL3* expression in PBMCs, but not associated with risk of childhood-onset asthma in African American children in our association studies, suggests that expression of *GSDMB* and *ORMDL3* in PBMCs might not underlie the risk of asthma. Collectively our observations suggest that expression of *GSDMB* in airway epithelial cells modulates asthma risk at the 17q12–21 locus. Furthermore, we can attribute most of the association between rs2305480 and the airway epithelial cell expression of *PGAP3*, which is located at the proximal end of the locus, to linkage disequilibrium with rs2517955 in *PGAP3* ($r^2=0.40$), which is a strong eQTL for *PGAP3* in lung tissue,¹⁵ as we observed in airway epithelial cells (appendix p 15). Third, our studies in lower airway epithelial cells from European American and African American adults replicated our finding, that asthma-associated SNPs at 17q12–21 are strong eQTLs for *GSDMB* in airway cells, and extended these observations to the lower airway and to European Americans. Modest associations with *ORMDL3* levels in lower airway cells raise the possibility that this gene might also contribute to the risk of asthma in African American adults. These combined data provide convergent evidence that the association between asthma risk and the 17q12–21 locus is modulated by genetic regulation of *GSDMB* expression in airway epithelial cells in populations of African and European ancestry. The strength and specificity of association between asthma-associated alleles at 17q12–21 and expression of *GSDMB* in airway epithelial cells further highlights the important role of epithelial function in the origins of childhood-onset asthma.⁹

GSDMB is a noteworthy candidate asthma gene because of the crucial role of its protein product, gasdermin-B, in pyroptosis, a form of necrotic and inflammatory cell death mediated by inflammatory caspases.²⁸ Acting via a non-canonical pathway, the N-terminus of gasdermin-B binds to the caspase activation and recruitment domain of caspase-4 in response to intracellular microbes. This binding directly leads to the activation of other caspases and cleavage of the N-terminus of another gasdermin, gasdermin-D, which then orchestrates pyroptosis by forming pores in the plasma membrane.²⁹ rs2305480 is a missense variant (c.892G→A; p.Pro298Ser) in *GSDMB* at a highly conserved amino acid. This SNP is in near perfect linkage disequilibrium ($r^2=1$) in all populations with

Figure 2: Associations between five core region SNPs and expression levels of genes at the extended 17q12–21 locus in African American children in PBMCs and upper airway epithelial cells

ZBPB2 and *LRR3C* expression was not detected in these cells. Normalised gene expression counts are shown. Boxplots extend to the first and third quartiles and show the medians (horizontal bars); the whiskers extend to 1.5-times the IQR, and open circles show individual values outside of that range. p values represent the increase in expression associated with each copy of the effect (risk) allele. For SNP rs2305480, the AA (non-effect allele) and AG genotypes were combined in PBMCs because only one homozygote was recorded for the A allele. See appendix (pp 13–14) for β values and exact p values beyond $p<0.0001$. SNP=single-nucleotide polymorphism. PBMC=peripheral blood mononuclear cells. *PGAP3*=post-GPI attachment to proteins phospholipase 3. *ERBB2*=erb-b2 receptor tyrosine kinase 2. *MIEN1*=migration and invasion enhancer 1. *GRB7*=growth factor receptor bound protein 7. *IKZF3*=IKAROS family zinc finger 3. *ZBPB2*=zona pellucida binding protein 2. *GSDMB*=gasdermin-B. *ORMDL3*=ORMDL sphingolipid biosynthesis regulator 3. *LRR3C*=leucine rich repeat containing protein 3C. *GSDMA*=gasdermin-A.

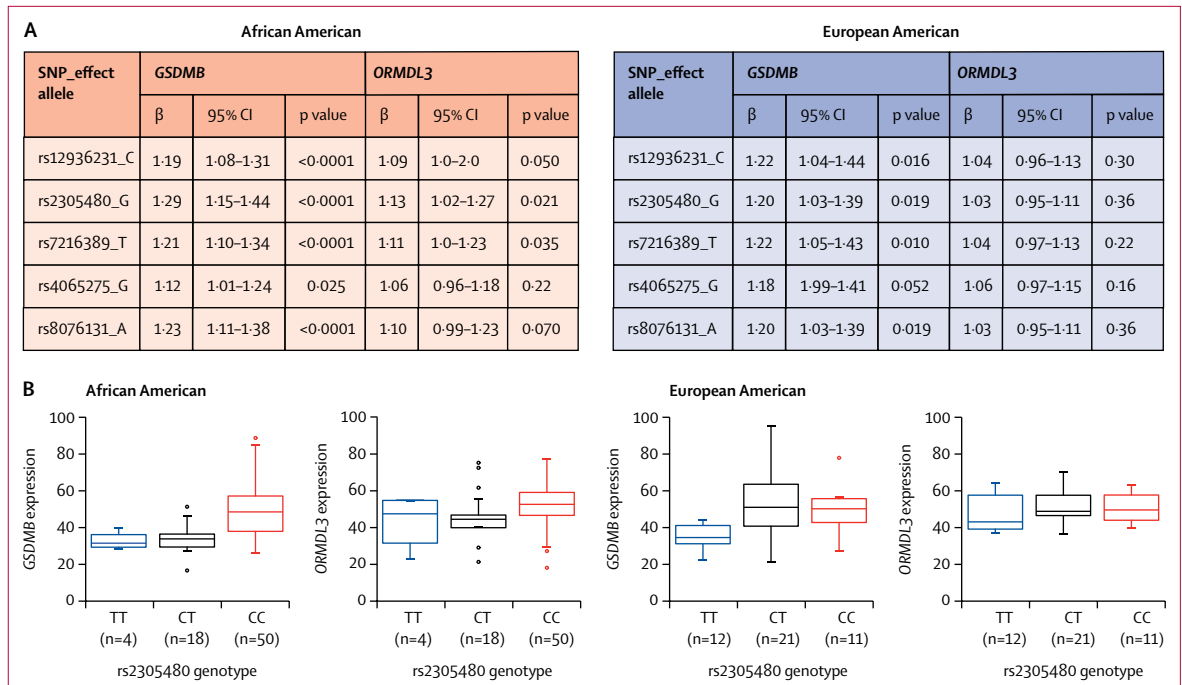


Figure 3: eQTL studies of five core-region SNPs and expression levels of GSDMB and ORMDL3 in bronchial epithelial cells from European American and African American adults

(A) eQTL results for the five core SNPs. The effect sizes (β) reflect the expected increase in expression associated with each copy of the asthma risk allele. The Bonferroni-corrected threshold was $p < 0.0055$ (five SNPs \times two genes). (B) Boxplots showing associations between rs2305480 genotype (SNP with the largest effect size in African Americans in part A) and expression levels of GSDMB and ORMDL3. Boxplots extend to the first and third quartiles and show the medians (horizontal bars); the whiskers extend to 1.5-times the IQR, and open circles show individual values outside of that range. eQTL=expression quantitative trait loci. SNP=single-nucleotide polymorphism. GSDMB=gasdermin-B. ORMDL3=ORMDL sphingolipid biosynthesis regulator 3.

two GSDMB intronic SNPs (rs11078928 and rs11078927),¹⁵ which were not genotyped in our study (appendix p 18). 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 gasdermin-B 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 (rs2305480) and one with known effects on transcript and protein levels (rs11078928). Such effects of rs2305480 in airway epithelial cells might be responsible for modifying the risk of childhood-onset asthma in African American children.

The disparate frequencies of the asthma-associated rs2305480_G allele in GSDMB, being 0.84 in African American children and 0.55 in European American children of the CREW cohorts, are noteworthy. Based on allele frequencies observed in the CREW children, and assuming Hardy-Weinberg equilibrium, only around 4% of African American children can be expected to be homozygous for the protective allele, compared with nearly 25% of European American children. Furthermore, in our study, among homozygotes for the risk allele (rs2305480_C), 112 (23%) of 494 European

American and 244 (39%) of 621 African American children manifested asthma (appendix p 16). The overall high penetrance of the risk genotypes in both groups might be a result of inclusion of four high-risk CREW cohorts (accounting for 734 [46%] of 1613 European American children and 446 [51%] of 870 African American children of our CREW consortium; table 1), in which participants were selected on the basis of a family history of asthma or allergies (table 1). Furthermore, the less than 100% penetrance of asthma among both European American and African American children with the high-risk genotype (appendix p 16), and the central role of GSDMB in the innate immune response to intracellular microbes, is consistent with the many interaction effects on childhood-onset asthma risk between 17q12–21 genotypes and early life exposures, including tobacco smoke,^{18,31} rhinovirus and wheezing illnesses,^{19,20} farm animals,²⁰ and pets.³²

The present study had several strengths and limitations. First, the CREW sample is relatively small for genetic association studies, particularly in African American children. However, children in the CREW cohorts, 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 GWASs. In fact, the

prospectively collected definitions of doctor-diagnosed asthma agree with definitions that include lung function and other symptom-based criteria.³³ Combining the CREW association results with published GWAS data increased statistical power and revealed largely overlapping effect sizes at the associated loci in African Americans across the CREW and EVE populations. This similarity, together with the breakdown of linkage disequilibrium in the African American children, allowed us to make several important observations that could not have been made in individuals of European ancestry alone. 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 minimised by focusing on a single locus and correcting for differences in asthma prevalence and allele frequencies by including study site as a covariate. Third, we 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 the 17q12–21 locus, including *ORMDL3*, might be important in other tissues, in response to exposures, or at different stages of development. Finally, we could not relate the differential expression of *GSDMB* or *ORMDL3* in the samples to asthma, similar to earlier studies (reviewed in Stein et al¹⁵). This observation further suggests that expression differences in these genes between individuals with and without asthma might only be evident during crucial windows of development or in response to particular exposures, such as respiratory infections in early life.^{18,19}

In conclusion, this study shows the potential of including populations of different ethnicities in association studies and eQTL studies in cells relevant to asthma, to fine map the effects of SNPs on disease risk and gene expression. Additionally, we showed the benefits 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-related gene at the 17q12–21 locus, and indicates airway epithelial cells to be the most probable target for modulating the effects of variation at this locus on the development of childhood-onset asthma. Combined with previous studies of gasdermin-B protein function, our results suggest a potential target for therapeutic intervention in children with the high-risk genotype at this locus.

Contributors

CO, AML, FDM, DLN, CW, DB, GW, SH, BH, SVL, ALW, and JEG contributed to the conception or design of the study. MCA, MGR, LBB, LG, DRG, TH, GKKH, DKH, DJJ, CCJ, MK, RFL, EAM, RLM, ETN, GTO'C, DO, CMS, SRW, RAW, ALW, EMZ, FDM, and JEG contributed patient samples and data. CGMcK, KMM, CS, DLN, and AML oversaw or did the statistical analyses. KAN did the genotyping. CO wrote the initial draft of the manuscript. CO, CGMcK, DO, DLN, AML, and JEG read and edited early drafts of the manuscript. All authors reviewed or critically revised the manuscript for important intellectual content and gave final approval.

Declaration of interests

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