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A decade of research on the 17q12-21 asthma locus: Piecing together the puzzle



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Chromosome 17q12-21 remains the most highly replicated and significant asthma locus. Genotypes in the core region defined by the first genome-wide association study correlate with expression of 2 genes, ORM1-like 3 (*ORMDL3*) and gasdermin B (*GSDMB*), making these prime candidate asthma genes, although recent studies have implicated gasdermin A (*GSDMA*) distal to and post-GPI attachment to proteins 3 (*PGAP3*) proximal to the core region as independent loci. We review 10 years of studies on the 17q12-21 locus and suggest that genotype-specific risks for asthma at the proximal and distal loci are not specific to early-onset asthma and mediated by *PGAP3*, *ORMDL3*, and/or *GSDMA* expression. We propose that the weak and inconsistent associations of 17q single nucleotide polymorphisms with asthma in African Americans is due to the high frequency of some 17q alleles, the breakdown of linkage disequilibrium on African-derived chromosomes, and possibly different early-life asthma endotypes in these children. Finally, the inconsistent association between asthma and gene expression levels in blood or lung cells from older children and adults suggests that genotype effects may mediate asthma risk or protection during critical developmental windows and/or in response to relevant exposures in early life. Thus studies of young children and ethnically diverse populations are required to fully understand the relationship between genotype and asthma phenotype and the gene regulatory architecture at this locus. (J Allergy Clin Immunol 2018;142:749-64.)

Key words: Wheezing, genome-wide association study, immune cells, lung cells, *ORMDL3*, *GSDMB*, *PGAP3*, *GSDMA*, gene expression

The first genome-wide association study (GWAS) of asthma was reported 10 years ago by Moffatt et al,¹ who made the seminal discovery of a novel asthma locus on chromosome 17q21, which was defined by a large 206.5-kb region. This locus has since been extended to include single nucleotide polymorphisms (SNPs) both proximal and distal* to the core locus initially defined by Moffatt et al and is more accurately referred to as the 17q12-21 asthma locus, although in this review we will refer to it simply as 17q.

Subsequent GWASs and meta-analyses of GWASs have highlighted 17q as the most replicated and most significant asthma locus.²⁻¹⁰ Genotypes at SNPs in the core region defined by Moffatt et al¹ have been shown to correlate with the expression of 2 genes, ORM1-like 3 (*ORMDL3*) and gasdermin B (*GSDMB*),^{1,11-23} making these the prime candidate asthma genes at this locus. More recent studies have implicated gasdermin A (*GSDMA*)^{3,24} distal to and Post-GPI attachment to proteins 3 (*PGAP3*) (previously named *PERLDI*)^{2,20,25} proximal to the core region as potentially independent asthma loci (Fig 1, A and B).² Follow-up studies of 17q comprised a significant proportion of asthma-related genetic research over the past decade; however, many questions remain. Here we overview our current understanding of this important locus, focusing on clinical correlations and the genomic, transcriptomic, and epigenomic studies of this region. Throughout, we highlight 17 SNPs that capture the signature genetic findings (Fig 2).^{1-3,5,6,10,14,20,21,23-35} We refer readers to Das et al³⁶ for an excellent review of functional studies of genes at the 17q locus.

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PHENOTYPES AND ENDOTYPES ASSOCIATED WITH THE 17Q12-21 LOCUS

Despite the fact that the 17q locus shows the strongest associations in large GWASs and meta-analyses, the odds ratios (ORs) of these associations are modest. For example, in the Transnational Genetics of Asthma Consortium (TAGC) meta-analysis,² which included 75 independent GWASs comprising ethnically diverse populations totaling 23,948 cases and 118,538 control subjects, the lead SNP at the 17q locus had a *P* value of 2.2×10^{-30} (Fig 1, A) but an OR of only 1.16 (95% CI, 1.13-1.19). This likely reflects the extensive clinical heterogeneity

*The terms proximal and distal are used in this review to refer to locations relative to the centromere.

Abbreviations used

ASMC:	Airway smooth muscle cell
BEC:	Bronchial epithelial cell
CEU:	Utah residents with Northern and Western European ancestry from the CEPH collection
COPSAC:	Copenhagen Prospective Studies on Asthma in Childhood birth cohort
CTCF:	CCCTC-binding factor
eQTL:	Expression quantitative trait locus
ERBB2:	Erb-b2 receptor tyrosine kinase 2
ETS:	Environmental tobacco smoke
GPI:	Glycosylphosphatidylinositol
GSDMA:	Gasdermin A
GSDMB:	Gasdermin B
GWAS:	Genome-wide association study
IKZF3:	IKAROS family zinc finger 3
LCL:	Lymphoblastoid cell line
LD:	Linkage disequilibrium
OR:	Odds ratio
ORMDL3:	ORM1-like 3
PBL:	Peripheral blood leukocyte
PCA:	Principal components analysis
PGAP3:	Post-GPI attachment to proteins 3
SNP:	Single nucleotide polymorphism
TRM:	Tissue-resident memory
YRI:	Yoruba in Ibadan, Nigeria
ZPBP2:	Zona pellucida binding protein 2

of asthma, with variation in age of onset, co-occurrence with allergic sensitization, and manifestation of wheezing illnesses in early life. Moreover, asthma risk is affected significantly by environmental exposures in the first few years of life, including environmental tobacco smoke (ETS), older siblings, furred pets, and large farm animals, which also vary among GWAS subjects. In fact, studies of more clinically homogeneous cases or accounting for relevant exposures have yielded increased ORs for asthma-associated loci, including 17q.³⁷

Age of onset and early-life exposures

It is clear that the core 17q locus is associated with early-onset asthma,^{2,6,14,27,28,38,39} which was first reported by Bouzigon et al,²⁸ who showed that associations with 17q genotypes were restricted to children with onset of asthma symptoms before the age of 4 years (Fig 3, A).²⁸ Furthermore, the association with early-onset asthma was present only in children exposed to ETS in early life. Associations with early age of onset were replicated in subsequent studies that further extended this observation to Asian subjects,¹⁴ more severe symptoms in early childhood,^{14,38} bronchial hyperresponsiveness at 1 month of age,³⁸ exacerbations and hospitalization before age 6 years,⁶ older siblings,³³ rhinovirus-associated wheezing illnesses in the first 3 years of life,¹² and wheezing in the first year of life (Fig 3, B).^{12,33,40} The observation that 17q-associated asthma risk is modified by early-life exposure to ETS²⁸ was replicated in 2 Dutch birth cohorts,⁴¹ Croatian children,²⁹ and United Kingdom adults²⁴ but not in the Danish National Birth Cohort⁴² or the European GABRIEL consortium.⁴³

Surprisingly, the same alleles that are associated with asthma risk in the above studies were associated with protection from wheezing among children exposed to animal barns in the first year

of life (Fig 3, C).³³ This protection was dose dependent: exposure of more than 2 hours a week to animal barns was associated with a greater than 80% reduction in wheezing among children with “high-risk” genotypes (rs8076131 AG or GG).

In this same study there was no interaction between genotype and dog ownership on wheezing,³³ although significant interactions were reported between the 17q genotype and furred pets on wheezing in the first 18 months of life in the Danish National Birth Cohort study⁴² and on asthma at school age in Croatian children²⁹ and Danish children in the Copenhagen Prospective Studies on Asthma in Childhood birth cohort (COPSAC)₂₀₀₀ study.⁴⁴ In these studies genotypes at 17q SNPs were associated with *protection* among children with a cat or dog. In the COPSAC₂₀₀₀ study cat (but not dog) allergen levels were associated with asthma among children with the rs7216389 TT genotype.

Associations of the 17q12-21 locus with other phenotypes and diseases

Genotypes at the 17q locus show inconsistent associations with asthma-associated phenotypes. SNPs at this locus were not associated with allergic sensitization^{11,12,14,38,40,45} or atopic dermatitis^{38,46,47} in GWASs or in 17q-focused studies. Although GWASs of total serum IgE levels did not report associations with 17q SNPs,^{3,48-53} a 17q-focused study reported an association with IgE in ethnic Chinese subjects from Singapore.¹¹ Two 17q-focused studies reported associations with allergic rhinitis,^{54,55} but a GWAS⁵⁶ and other 17q-focused studies^{11,33,38} did not. Neither GWASs⁵⁷⁻⁶¹ nor 3 17q-focused studies^{24,38,45} reported associations with lung function, whereas one 17q-focused study did.²⁹ One GWAS of fraction of exhaled nitric oxide reported associations between 17q SNPs,⁶² whereas another did not show evidence of association,⁶³ and one 17q-focused study reported associations with blood eosinophilia,¹¹ although one GWAS did not.⁶⁴

Interestingly, 17q SNPs have been associated with other diseases in GWASs, all of which are autoimmune in cause: Crohn disease,⁶⁵ ulcerative colitis,⁶⁶ inflammatory bowel disease,⁶⁷ type I diabetes,^{68,69} rheumatoid arthritis,⁷⁰ and primary biliary cirrhosis.^{71,72} Although the same SNPs are associated with both asthma and autoimmune disease, the risk alleles associated with autoimmune disease are opposite of those associated with asthma risk, as previously noted.^{23,73,74} These findings suggest that genes at the core locus affect immune development broadly.²³ Moreover, the overlapping associations of asthma and autoimmune disease with SNPs at the core region, but not at the proximal or distal regions, suggest that the latter 2 regions may confer lung specificity to asthma, whereas SNPs at the core region might be more central to early-life immune responses to asthma-promoting or asthma-protective exposures.

FINE MAPPING AND ETHNIC-SPECIFIC ASSOCIATIONS AT THE 17Q LOCUS

Fine mapping at the 17q locus has been challenging because of the extensive linkage disequilibrium (LD) in populations of European, Latino, and Asian ancestry (Fig 1, D) and the weak and inconsistent evidence for association between asthma and 17q SNPs in populations of African ancestry.^{5,35,75-77} Coregulation of genes at this locus and different patterns of gene regulation in blood and lung cells impose additional layers of complexity.

Patterns of LD at the 17q locus

LD is very strong between SNPs at the 17q locus, with an extended haplotype spanning the entire core region in populations of European, Latino, and East Asian ancestry (Fig 1, D). This feature makes it virtually impossible to distinguish the effects of specific SNPs with any confidence. In fact, the evidence for association between SNPs across the core region and asthma is strong and consistent in Europeans,^{2,3,5} Latinos,^{10,35} and ethnic Chinese from Singapore¹¹ and Hong Kong.⁷⁸⁻⁸⁰ Associations are less consistent in smaller studies of Han Chinese from China⁸¹⁻⁸³ and of Korean^{14,84,85} and Japanese^{53,86} subjects.

In contrast, LD extends over much shorter distances in African Americans (Fig 1, D) and Africans (see Fig E1 and Table E1 in this article's Online Repository at www.jacionline.org), except for a region in *GSDMB* defined by 3 SNPs that shows near-perfect LD in all populations; these were the most significant SNPs in GWASs of asthma in Europeans,^{3,6} ethnically diverse US populations⁵ and in a 17q-focused study in Han Chinese.⁸² The 3 SNPs consist of a common missense variant that is predicted to be "possibly damaging" by PolyPhen (rs2305480; c.892C>T, p.P298S),^{26,87} an intronic variant that affects alternatively spliced transcript abundances (rs11078928; c.662T>C),³¹ and an intronic variant of unknown function (rs11078927). The rs11078928-T allele is associated with higher expression of *GSDMB* caused by aberrant splicing, and the very high frequency of this allele in African Americans (approximately 0.82) could contribute to the high prevalence of asthma among these subjects.

In the EVE meta-analysis the most significant association in African Americans was with rs11078927, making this the most significant association in the combined sample. Although of modest significance in African Americans ($P = .0019$), the estimated effect size for rs11078927 was similar to those reported for the other EVE populations. This is likely due to the high frequency of the 3 *GSDMB* SNPs in African Americans (0.82), which reduces power to detect associations (Fig 1, C).

The breakdown of LD in the core region in African Americans should facilitate fine mapping of the 17q locus if the true causal variants are genotyped or tagged by the SNPs typed in those studies. The inconsistency between studies raises the possibility that the 17q-associated risk might involve multiple variants across the extended haplotype that are not in LD and possibly not tagged by the GWAS SNPs on African-derived chromosomes. Ethnicity-specific rare variants at the 17q locus could also influence asthma risk, although a study of rare coding variants in 2308 African American cases and control subjects found no significant associations at 17q genes.²⁶ However, the array used included only 57% (8/14) of the missense, nonsense, or splicing variants discovered in 17q genes in whole-genome sequences from 93 of the African American asthmatic patients in this study, raising the possibility that rare variants may have been missed. These important questions will be addressed by the Consortium on Asthma among African Ancestry Populations in the Americas (CAAPA)⁸⁸ in an asthma GWAS in nearly 18,000 subjects of African ancestry by using whole-genome sequences from a subset of samples as the imputation reference panel.

Finally, it is also possible that the natural history of early-life asthma among African American children differs from that of children of European ancestry with respect to age of onset,

virus-associated wheezing illness, or environmental exposures that modify or interact with the 17q-associated risk. In fact, US inner-city African American children had lower rates of virus detection overall, fewer rhinovirus infections in particular, and higher rates of adenovirus infection and adenovirus-associated illness in the first year of life compared with children of European ancestry living in Madison, Wisconsin.⁸⁹ Thus the specific type of asthma in African American children might be less influenced by variation at the 17q locus or by different variants than are associated with asthma in other children. It is likely that 1 or more of these factors could be masking associations with SNPs at the 17q locus in populations of African ancestry.

Evidence for 3 independent asthma associations at the 17q locus

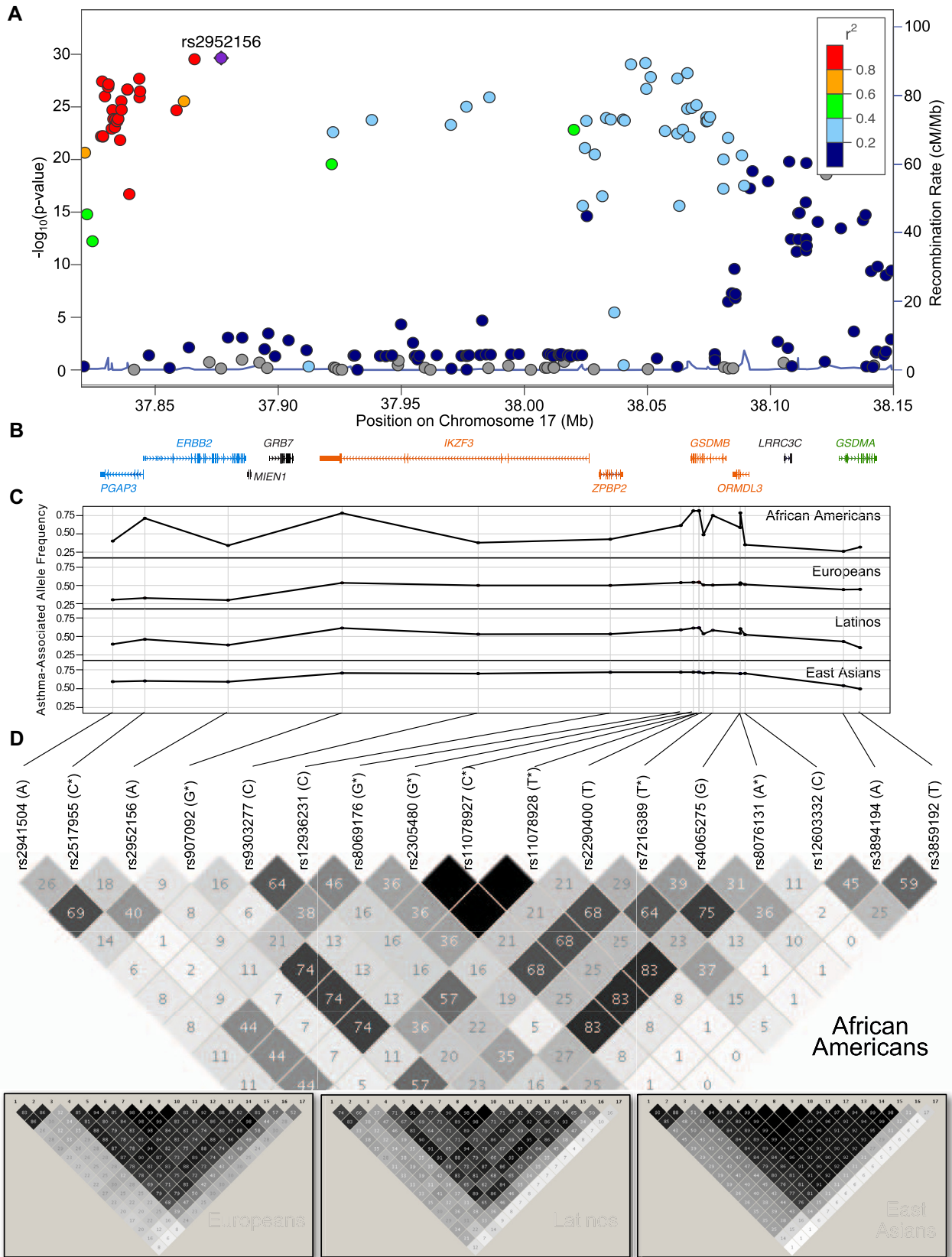
Several lines of evidence suggest that SNPs in *GSDMA* and *PGAP3* can have effects that are independent of SNPs at the core locus. In all populations the LD that characterizes the core region decays in the distal region encoding *GSDMA* and the proximal region encoding *PGAP3* and Erb-b2 receptor tyrosine kinase 2 (*ERBB2*; LD $r^2 \leq 0.5$; Fig 1, D), and residual associations with asthma remained at rs3894194 in *GSDMA*³ and rs2941504 in *PGAP3*¹⁴ after conditioning on SNPs at the core locus. Moreover, because the association with asthma at both regions is reduced in a pediatric sample (see Fig E8 in Demenais et al²), the effects of variation at the proximal and distal loci might not be specific to childhood-onset asthma. SNP rs2941504 in *PGAP3* was also associated with allergic asthma, but not with atopy among nonasthmatic subjects, in ethnic Chinese adults from Singapore.²⁵ SNP rs3894194 in *GSDMA* was also associated with current asthma, FEV₁, and airway hyperresponsiveness, as well as showing interactive effects with cigarette smoking and current asthma in a 17q-focused study of 1018 United Kingdom adults.²⁴ Another SNP in *GSDMA*, rs3859192, is a strong expression quantitative trait locus (eQTL) for *GSDMA* in whole lung tissue,^{15,30} although it shows little LD in all populations with the asthma-associated *GSDMA* SNP rs3894194.

CELL-SPECIFIC EXPRESSION AND eQTL STUDIES OF 17Q GENES

Patterns of gene expression and eQTLs in asthma-relevant tissues (ie, immune cells and lung cells) can provide clues as to which genes contribute to asthma pathogenesis and potentially which SNPs are either themselves causal or tag causal variation. Although nearly all the 17q genes are expressed ubiquitously in asthma-relevant cell types, relative expression patterns and associations with SNPs (ie, eQTLs) differ between these cells.

Patterns of gene expression

Six of the 7 genes at the 17q locus potentially implicated in asthma risk (*PGAP3*, *ERBB2*, IKAROS family zinc finger 3 [*IKZF3*], *GSDMB*, *ORMDL3*, and *GSDMA*; Fig 1, B, shown in red) are expressed at detectable levels in RNA from whole blood,³⁰ lymphoblastoid cell lines (LCLs),²³ PBMCs,⁹⁰ peripheral blood leukocytes (PBLs; Stein, unpublished), lung CD4⁺ tissue-resident memory (TRM) cells (Schoettler, unpublished), whole-lung tissue,³⁰ freshly isolated bronchial epithelial cells (BECs),²⁰ and cultured primary airway smooth



Location	SNP	bp position	SNP Type	Associations in published studies
<i>PGAP3</i>	rs2941504	17:37,830,900	Synonymous (c.465G>A, p.Val104)	Associated with allergic asthma in ethnic Chinese from Singapore ²⁵ and asthma in Icelanders plus four European populations ¹⁴ ; eQTL for <i>PGAP3</i> in white blood cells ¹⁴
<i>PGAP2</i>	rs2517955	17:37,843,681	Intron (C>T)	eQTL for <i>ORMDL3</i> and meQTL for a nearby CpG site in airway epithelial cells ²⁰
<i>ERBB2</i>	rs2952156	17:37,876,835	Intron (G>A)	Lead SNP in the TAGC GWAS ²
<i>IKZF3</i>	rs907092	17:37,922,259	Synonymous (c.1314G>A; p.Ser438)	Lead SNP in asthma GWAS in Puerto Ricans ¹⁰ and in the EVE study of exome SNPs in Latinos ²⁶
<i>IKZF3</i>	rs9303277	17: 37,976,469	Intron (T>C)	Lead SNP with childhood onset asthma ²⁷
<i>ZPBP2</i>	rs12936231	17: 38,029,120	Intron (T>C)	Lead functional candidate for gene expression and chromatin effects ²³ ; C disrupts a CTCF-binding motif, which insulates upstream <i>cis</i> -regulatory elements from interacting with the <i>ORMDL3</i> promoter in immune cells ²¹
Intergenic	rs8069176	17: 38,057,197	(G>A)	Lead SNP for early onset asthma among children exposed to ETS in early life ^{28,29} and asthma in children in the TAGC GWAS ²
<i>GSDMB</i>	rs2305480	17:38,062,196	Missense (c.892G>A, p.Pro298Ser)	Lead SNP in the GABRIEL GWAS ³ and the Exacerbation GWAS ⁶ ; lung eQTL for <i>ORMDL3</i> , <i>GSDMB</i> , <i>GSDMA</i> ³⁰
<i>GSDMB</i>	rs11078927	17:38,064,405	Intron (C>T)	Lead SNP in EVE GWAS ⁵
<i>GSDMB</i>	rs11078928	17:38,064,469	Splice variant (c.662T>C)	C allele results in skipping of exon 6 and reduced levels of <i>GSDMB</i> transcript ³¹
<i>GSDMB</i>	rs2290400	17:38,066,240	Intron (T>C)	Interactive with smoking on asthma risk ²⁴
<i>GSDMB</i>	rs7216389	17:38,069,949	Intron (T>C)	Lead SNP in first GWAS and eQTL for <i>ORMDL3</i> and <i>GSDMB</i> in LCLs ¹ ; associated with early onset asthma ¹² ; associated with RV-wheezing interaction in early life and eQTL for <i>ORMDL3</i> and <i>GSDMB</i> in whole blood ³² ; lung eQTL for <i>ORMDL3</i> , <i>GSDMB</i> and <i>GSDMA</i> ³⁰
<i>ORMDL3</i>	rs4065275	17: 38,080,865	Intron (A>G)	G allele creates a CTCF-binding motif associated with increased expression of <i>ORMDL3</i> ²¹
<i>ORMDL3</i>	rs8076131	17:38,080,912	Promoter (A>G)	Strongest association with protective farming effect ³³
<i>ORMDL3</i>	rs12603332	17:38,082,807	5'UTR (T>C)	meQTL and eQTL for <i>ORMDL3</i> and <i>GSDMB</i> in blood leukocytes ³⁴ ; lead SNP for asthma in Mexican Americans and African Americans among 7 SNPs tested ³⁵
<i>GSDMA</i>	rs3894194	17: 38,121,993	Missense (c.536G>A, p.Ala18Gln)	Independent GWAS signal for asthma ^{3, 24}
<i>GSDMA</i>	rs3859192	17: 38,128,648	Intron (C>T)	eQTL for <i>GSDMA</i> in lung tissue ³⁰

FIG 2. Seventeen SNPs in the extended 17q12-21 region that are reported to be associated with asthma, gene expression, or epigenetic modification. Base pair position from build hg19 is shown. SNP type is shown as ancestral > derived alleles. TAGC, Transnational Genetics of Asthma Consortium.

muscle cells (ASMCs; Thompson, unpublished). By using quantitative PCR to measure transcript levels, zona pellucida binding protein 2 (*ZPBP2*) expression was also detected in LCLs²³ and cord blood mononuclear cells.³² Among immune cell types, transcript levels of *ORMDL3* and *GSDMB* were most highly expressed in T and B cells,^{21,34} and in one study *ORMDL3* was most highly expressed in eosinophils.¹⁷

ORMDL3 and *GSDMB* transcription increases in response to viral infection. Expression levels of both *ORMDL3* and *GSDMB*, but not *IKZF3*, increased in PBMCs from 96 adults after exposure to rhinovirus,⁹⁰ and *ORMDL3* expression levels were higher in PBMCs from 10 children with respiratory syncytial virus-induced bronchiolitis compared with 15 uninfected children.⁹¹ *ORMDL3* expression was increased in primary human lung fibroblasts, but not in primary human BECs or primary human ASMCs, after exposure to polyinosinic:polycytidylic

acid, a Toll-like receptor 3 ligand but not in response to LPS in any of the cells.⁵³ The response of these genes to important respiratory tract viruses further supports an important role for this locus in early-life wheezing illness and childhood-onset asthma.

As expected, relative expression of the 17q genes varies by cell type, but the patterns of variation are revealing. In all cells examined here (Fig 4 and see the Methods section in this article's Online Repository at www.jacionline.org),²⁰ *GSDMA* transcripts were expressed at levels just greater than the lower limits of detection in most subjects, and therefore conclusions about this gene should be tempered by this observation. Moreover, this broader view of gene expression across the 17q locus shows that within subjects, transcript levels of *ORMDL3* were most correlated with those of *PGAP3* and not those of *GSDMB* in PBLs, BECs, and ASMCs (Figs 4 and 5).²⁰ In lung CD4⁺ TRM cells, *ORMDL3* expression was not correlated with expression

FIG 1. The 17q12-21 asthma locus. **A**, Regional association plot of 17q SNPs with asthma in TAGC (children plus adults). The lead SNP, rs2952156 (purple diamond), is in *ERBB2* in the proximal 17q region. All other SNPs are colored based on their LD with the lead SNP (see inset). Modified with permission from Demenais et al.² **B**, Location of genes at the 17q locus. The 6 genes highlighted in this review are shown in blue, orange, and green to correspond to the proximal, core, and distal regions, respectively. **C**, Frequencies of asthma-associated alleles at 17 SNPs in 1000 Genomes reference panels: African Americans (African American Ancestry in SW USA [ASW] and African Caribbeans in Barbados [ACB]), Europeans (CEU, British in England and Scotland [GBR], and Tuscans in Italy [TSI]), Latinos (Mexican Ancestry from Los Angeles, USA [MXL] and Puerto Ricans from Puerto Rico [PUR]), East Asians (Han Chinese in Beijing [CHB], Japanese in Tokyo [JPT], Southern Han Chinese [CHS], Chinese Dai in Xishuangbanna [CDX], and Kinh in HoChi Minh City [KHV]). **D**, LD (r^2) among the 17 SNPs described in Fig 2 in African Americans (upper panel), Europeans (lower left panel), Latinos (lower middle panel), and East Asians (lower right panel) determined by using the same 1000 Genomes reference panels as in Fig 1, C. Asthma-associated alleles are shown for each SNP. *Associated allele is ancestral. Data for Fig 1, C and D, are from [ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/](http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/).

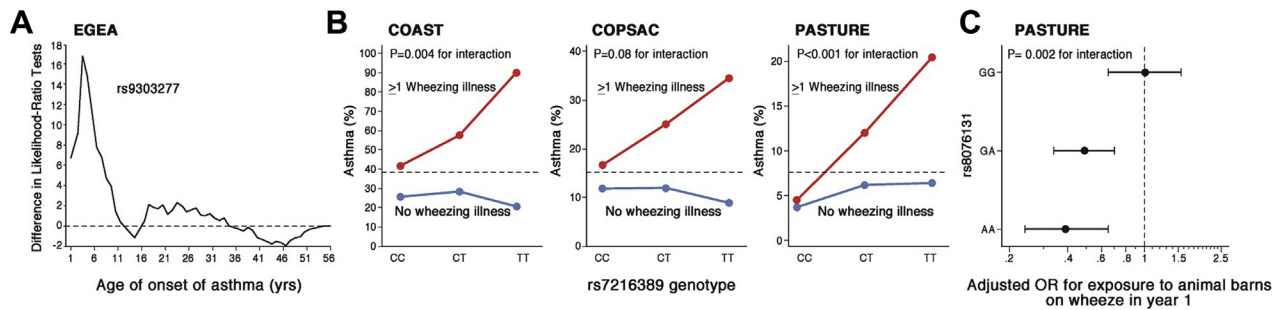


FIG 3. SNPs in the 17q core region are associated with early-life onset and risk of or protection from wheezing illness. **A**, Ordered-subset regression analysis of asthma was performed for 11 SNPs associated with asthma in the Epidemiological Study on Genetics and Environment of Asthma sample (EGEA; $n = 1511$). The maximal difference in likelihood ratio test statistics for association between the ordered age-specific subsets and the total sample was for an onset of asthma of 4 years or younger. SNP rs9303277 showed the largest difference in the likelihood ratio test statistics. The EGEA included children from 388 nuclear families with at least 1 asthmatic member living in one of 5 French cities. Modified with permission from Bouzigon et al.²⁸ **B**, Risk for asthma based on the rs7216389 genotype is confined to children who wheezed in the first 3 years of life (Childhood Origins of Asthma [COAST], $n = 200$; COPSAC, $n = 297$)¹² or in the first year of life (Protection against Allergy Study in Rural Environments [PASTURE] birth cohort, $n = 696$).³³ Dashed horizontal lines show the overall prevalence of asthma at age 6 years in each sample. COAST, COPSAC, and PASTURE are birth cohort studies in Madison, Wisconsin; Copenhagen, Denmark; and rural areas of Europe, respectively. The children in COAST had at least 1 parent with asthma or allergies, the children in COPSAC had a mother with asthma, and the children in PASTURE were unselected with respect to asthma or allergy. Modified from Bønnelykke et al.⁴⁰ **C**, In the PASTURE study children with the asthma-associated allele rs8076131-A and exposed to animal barns have significantly fewer wheezing illnesses in the first year of life. Used with permission from Loss et al.³³

of any other 17q genes, although the sample size was smallest for these cells.

Cell type-specific eQTLs

Published eQTL studies of 17q SNPs and genes are summarized in Table 1.^{1,11-23} To further evaluate the effects of genotype at the 17 SNPs highlighted in this review on the expression levels of the 6 candidate 17q genes, we extracted data from the Genotype-Tissue Expression (GTEx) project for whole blood and lung tissue (Fig 6).³⁰ In whole blood all 17 SNPs were significant eQTLs for *ORMDL3* and *GSDMB* but not for any of the other 17q genes. At each SNP, the asthma-associated allele was correlated with increased expression of *ORMDL3* and *GSDMB*. Thus, as in previous studies (Table I), SNPs at the 17q core locus are strong eQTLs for these 2 genes in peripheral blood cells from populations of primarily non-African ancestry.

A different picture emerges in lung tissue cells. SNPs across the region are significant eQTLs for *ORMDL3*, which is similar to studies in blood but with overall smaller effects (Fig 6). However, only the 6 most significant eQTLs for *ORMDL3* are also more modest eQTLs for *GSDMB*. Notably, the splice variant in *GSDMB* is not an eQTL for *GSDMB* in lung tissue. In contrast to eQTLs in blood, SNPs in the core and distal regions are also eQTLs for *GSDMA*. Additionally, SNPs in the proximal and core regions are strong eQTLs for *PGAP3*. The asthma-associated alleles at each of these SNPs are correlated with increased expression of both *PGAP3* and *ORMDL3*, whereas in the distal region the asthma risk alleles at rs3894194 and rs3859192 are correlated with lower expression of *GSDMA*, as previously reported.^{15,92} These data further suggest that expression of *ORMDL3* and *GSDMB* are not coregulated in lung cells, as they are in blood cells, as suggested previously in studies of BECs.²⁰

To examine eQTL patterns in a population with less LD across this region, we extracted eQTL data for 17q SNPs from a study

of LCLs from the Nigerian HapMap population (Yoruba in Ibadan, Nigeria [YRI]; Fig 7).^{5,93} In contrast to studies in whole blood and in populations of primarily non-African ancestry, the 17q SNPs were only significant eQTLs for *ORMDL3* in African-derived LCLs, showing considerably less evidence as eQTLs for *GSDMB*. Moreover, among the SNPs in the core region, the strongest eQTL for *ORMDL3* was rs12936231 in *ZBP2* ($P = 5.35 \times 10^{-10}$); the strength of the associations between other SNPs and *ORMDL3* expression were correlated with the degree of LD with rs12936231, a strong functional candidate for the observed associations with 17q SNPs (discussed below).^{21,23} Additionally, 2 SNPs in *PGAP3* are associated with *ORMDL3* expression, despite little LD between these SNPs in these regions (Fig 1, D). This possibly reflects long-range interactions between a putative enhancer in *PGAP3* and the *ORMDL3* promoter in LCLs,²⁰ further suggesting independent effects of SNPs in the proximal region on *ORMDL3* expression. Although we cannot exclude the 6 less informative SNPs in the core region from being eQTLs because of the very low power to detect associations in this sample, these data indicate that the effect of rs12936231 on *ORMDL3* expression levels is independent of the other 17q SNPs. Curiously, rs12936231 was not associated with asthma in African Americans in the EVE Consortium,⁵ despite having an allele frequency near 0.50 and high statistical power to detect association. These findings suggest that increased expression of *GSDMB* or *ORMDL3* in circulating immune cells might not be causally related to asthma, as has been previously suggested.¹⁴

Gene expression differences between asthmatic and nonasthmatic subjects

The observation that 17q SNPs are associated both with asthma risk and expression levels of 17q genes does not consistently

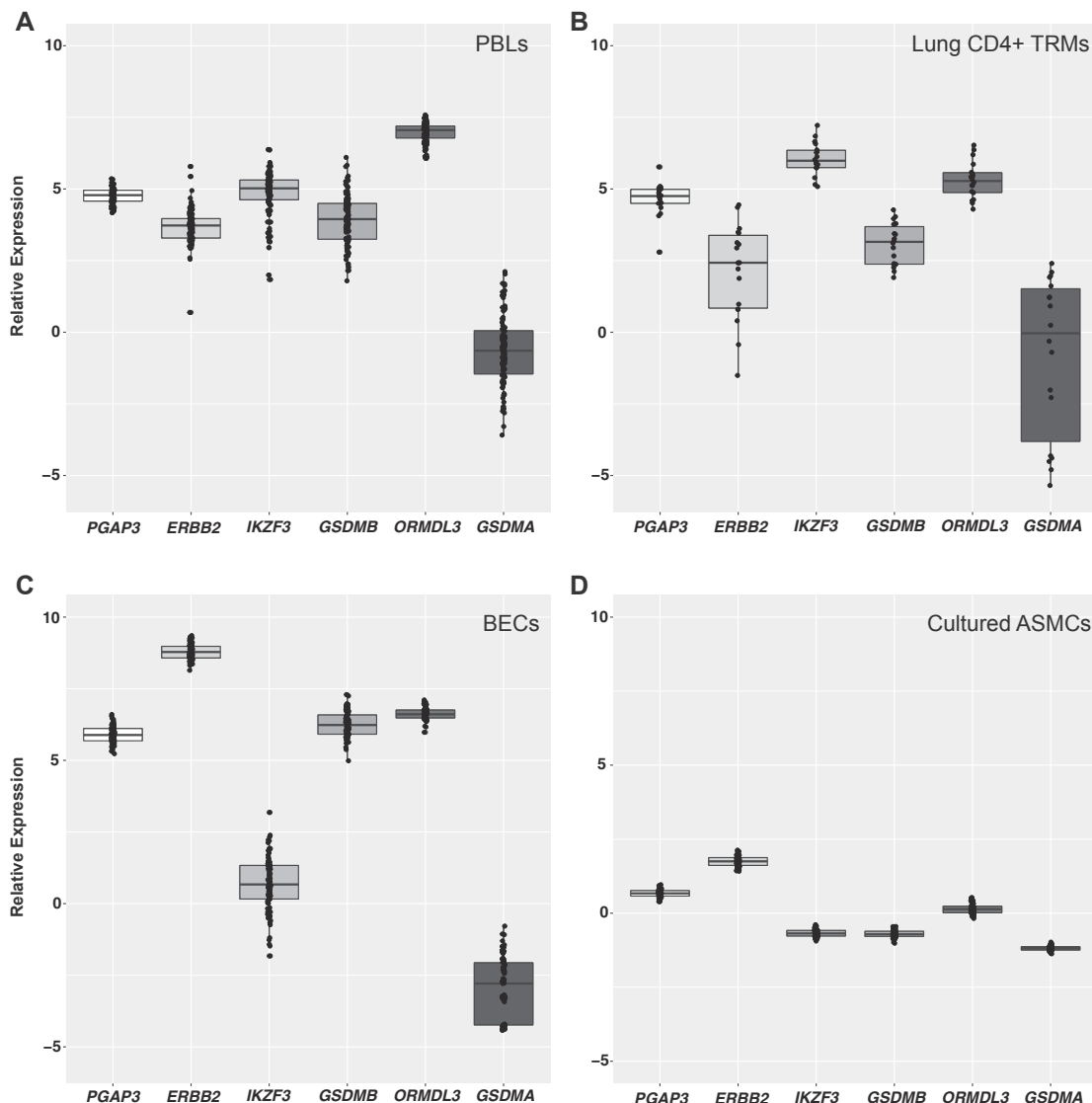


FIG 4. Relative expression of 17q genes in different tissues and cells. **A**, PBLs (n = 112). **B**, Lung CD4⁺ TRMs (n = 18). **C**, BECs (n = 85).²⁰ **D**, ASMCs (n = 67). Expression levels of *ORMDL3* and *PGAP3* were most correlated in PBLs (*ORMDL3* vs *PGAP3* $r = 0.410$, $P = 7.3 \times 10^{-6}$ cf. *ORMDL3* vs *GSDMB* $r = 0.369$, $P = 6.2 \times 10^{-5}$), BECs (*ORMDL3* vs *PGAP3* $r = 0.693$, $P = 1.9 \times 10^{-13}$ cf. *ORMDL3* vs *GSDMB* $r = 0.320$, $P = 2.8 \times 10^{-3}$), and ASMCs (*ORMDL3* vs *PGAP3* $r = 0.450$, $P = 1.3 \times 10^{-4}$ cf. *ORMDL3* vs *GSDMB* $r = 0.221$, $P = .07$). *ORMDL3* expression was not correlated with expression of any 17q genes in lung CD4⁺ TRMs (*ORMDL3* vs *PGAP3* $r = 0.077$, $P = .76$ cf. *ORMDL3* vs *GSDMB* $r = 0.400$, $P = .10$). Gene expression levels in Fig 4, A-C, are from RNA-seq; counts are shown as relative expression normalized to all genes detected as expressed within each cell type. Gene expression in Fig 4, D, is based on microarrays; expression levels were normalized to have a mean of zero and scaled between -5 and 10. Methods for PBLs, CD4⁺ TRMs, and ASMCs are described in the [Methods](#) section in this article's Online Repository; methods for BECs are reported in Nicodemus-Johnson et al.²⁰

extend to differences in 17q gene expression levels between asthmatic and nonasthmatic subjects. For example, one small study of Swedish asthmatic and nonasthmatic school-aged children³⁴ reported differences in 17q gene expression levels in PBMCs: *ORMDL3* transcript abundance was greater in 16 children with controlled asthma compared with that in 15 healthy control subjects and 16 children with severe asthma ($P = .002$, ANOVA); however, there were no differences between children with severe asthma and control subjects. In the original GWAS by Moffatt et al,¹ transcript levels of *ORMDL3* in LCLs were

not significantly different between 112 asthmatic and 266 nonasthmatic children. None of the 17q genes were differentially expressed in BECs from 55 adults with mild-to-severe asthma and 26 healthy control subjects²⁰ or in 42 adults with mild-to-moderate asthma and healthy control subjects.⁹⁴ In contrast, *ORMDL3* expression was increased in BECs from 19 adults with stable mild asthma and 16 healthy control subjects (false discovery rate, 8.5×10^{-4}).⁹⁵ Although *GSDMB* transcripts were not increased in BECs from asthmatic patients in any of these studies, Das et al⁹⁶ reported increased *GSDMB* protein

using immunohistochemistry in BECs from 14 asthmatic patients compared with 7 healthy control subjects ($P < .001$), with significantly more GSDMB⁺ cells among 7 patients with severe asthma compared with control subjects ($P < .01$).

The inconsistent results and paucity of studies demonstrating increased expression of 17q genes in asthmatic patients is surprising. This could reflect insufficient power to detect gene expression differences,^{1,20} a focus on cell types or asthma endotypes that are unrelated to the 17q-associated risk, and/or collection of samples at developmental windows or in environments that are irrelevant to the 17q-associated asthma risk.

CHROMATIN AND DNA METHYLATION STUDIES AT THE 17Q LOCUS

Studies of chromatin architecture and DNA methylation patterns in blood and lung cells have provided insight into the specific SNPs that regulate gene expression at this locus.

Allele-specific chromatin states and DNA methylation in immune cells

Verlaan et al²³ were the first to study *cis* regulatory elements associated with gene expression patterns and genotypes at SNPs at the core 17q locus. Using LCLs from 53 European (Utah residents with Northern and Western European ancestry from the CEPH collection [CEU]) HapMap samples, they first showed that the asthma-associated alleles were on an extended haplotype that was associated with increased expression of *ORMDL3* and *GSDMB*. They further showed that the nonrisk G allele at rs12936231 in *ZBPB2* was depleted of nucleosomes (a signature of regions with regulatory function) and enriched for inactive chromatin marks and binding of CCCTC-binding factor (CTCF), an important protein that can act as both an insulator between regulatory domains and a mediator of enhancer-promoter interactions.⁹⁷ Therefore this study discovered an asthma-associated SNP, rs12936231, that alters the chromatin state of a regulatory domain and is correlated with expression of *ORMDL3* and *GSDMB*.

This observation was extended by Schmiedel et al,²¹ who assessed the open chromatin state in 62 primary cell types at the core 17q locus (from *IKZF3* to *GSDMA*), as well as allele-specific associations with enhancer activity, CTCF binding, and gene expression in 10 immune cell types and BECs. Overall, 17q genes were more transcriptionally active in immune cells than in nonimmune cells, as measured by the number of open chromatin sites assessed based on DNase hypersensitivity (DNase-hypersensitive sites). Many of the DNase-hypersensitive sites in T cells, B cells, and natural killer cells overlapped asthma-associated SNPs, including the rs12936231 SNP in *ZBPB2* identified by Verlaan et al,²³ as well as SNPs in the first intron of *ORMDL3*, including rs4065275. These SNPs, among others, were enriched for H3K27ac chromatin marks of active enhancer activity in T cells; asthma risk alleles were associated with increased enrichment of H3K27ac. Additionally, these 2 SNPs were predicted to disrupt CTCF-binding motifs, which were experimentally confirmed by using chromatin immunoprecipitation sequencing: the asthma-associated allele in *ZBPB2* (rs12936231-C) disrupted a CTCF-binding site, and the asthma-associated allele in *ORMDL3* (rs4065275-G) introduced a CTCF-binding site (Fig 8, A).²¹

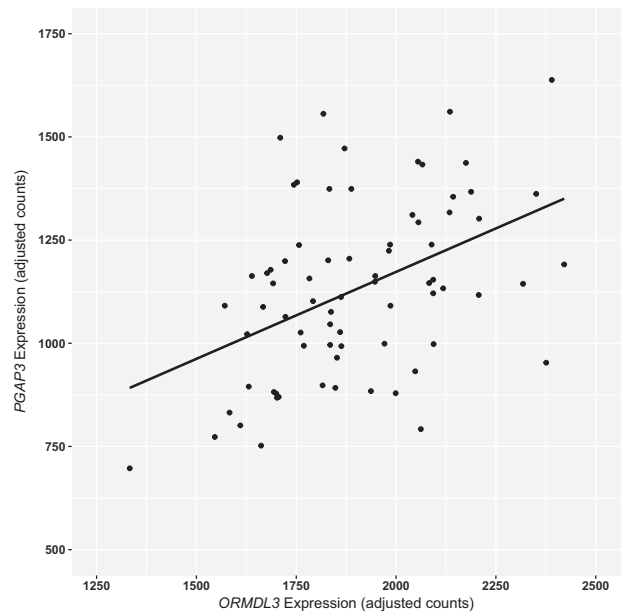


FIG 5. Expression of *ORMDL3* and *PGAP3* transcripts in BECs from 85 adults. Correlation between *ORMDL3* (*x*-axis) and *PGAP3* (*y*-axis) in 85 subjects ($r = 0.693$, $P = 1.9 \times 10^{-13}$, as Fig 4). Expression levels are shown as read counts adjusted for unwanted variation, as described in Nicodemus-Johnson et al.²⁰

Finally, chromatin conformation assays (4C-seq) showed that the *ORMDL3* promoter interacts with a long-range enhancer in *IKZF3* that promotes transcription of *ORMDL3* in cells that also express *IKZF3*. The binding of CTCF on chromosomes with the rs12936231-G allele in *ZBPB2* blocks this interaction, resulting in reduced transcription of *ORMDL3* on haplotypes with the rs12936231-G allele, presumably independent of rs4065275 (Fig 8, B).⁹⁸ Because *IKZF3* is a transcription factor in B cells, T cells, and selected other immune cells, the chromatin looping and physical interaction between the *IKZF3* enhancer and the *ORMDL3* promoter occurs only in immune cells. This likely accounts for the very high expression of *ORMDL3* in immune cells compared with airway cells, where this interaction does not occur.²¹

Because of the LD structure at the 17q locus, nearly all non-African-ancestry haplotypes will carry either both asthma-associated alleles (rs12936231-C and rs4065275-G) or neither asthma-associated allele (rs12936231-G and rs4065275-A), which correspond to high and low expressers of *ORMDL3*, respectively (Fig 8, A). However, a recombinant haplotype with rs12936231-G and rs4065275-G is relatively common on African-derived chromosomes and is associated with low expression of *ORMDL3* in YRI LCLs, despite carrying the high expressing allele rs4065275-G (Fig 8, B). As a result, approximately 30% of African-derived chromosomes carrying the asthma-associated rs4065275-G allele will be low expressers of *ORMDL3*, whereas nearly all European- or Asian-derived chromosomes with rs4065275-G will be high expressers of *ORMDL3*. This could explain why rs4065275 is a very weak eQTL for *ORMDL3* in YRI LCLs (Fig 7).

Two studies examined allele-specific differences in DNA methylation patterns at the core locus. Combining DNA methylation patterns with nucleosome occupancy and *in vitro*

TABLE I. Published eQTL studies of 17q12-21 SNPs and genes in blood and lung cells

Study	SNP	17q12-21 genes						
		<i>PGAP3</i> , P value	<i>ERBB2</i> , P value	<i>IKZF3</i> , P value	<i>ZBP2</i> , P value	<i>GSDMB</i> , P value	<i>ORMDL3</i> , P value	<i>GSDMA</i> , P value
Blood cells								
Schmiedel et al ²¹ (n = 34), sorted cells*	rs7216389					Naive CD4 ⁺ T cells: <.05 Treg cells: <.05 T _H 1 cells: <.05 B cells: <.01 NK cells: <.01	Naive CD4 ⁺ T cells: <.01 Treg cells: <.01 T _H 1 cells: <.001 T _H 2 cells: <.001 T _H 17 cells: <.001 B cells: <.001 NK cells: <.01 PBMCs: ≤.03	
Liu et al ¹⁷ (n = 19), sorted cells*	rs7216389						PBMCs, RV inf: ≤.03 CD19 ⁺ B cells: .004 CD19 ⁺ B cells, RV inf: <.001 CD8 ⁺ T cells, RV inf: ≤.03 CD19 ⁻ CD8 ⁻ cells, RV inf: ≤.03	
Moffat et al ¹ (n = 378), LCLs†	rs7216389 rs93003277 rs2290400 rs3894194 rs3859192						3.9 × 10 ⁻²³ 1.2 × 10 ⁻²² 3.9 × 10 ⁻²³ 1.0 × 10 ⁻¹¹ 3.1 × 10 ⁻⁴	
Verlaan et al ²³ (n = 53), LCLs*	rs907091/rs12603332 haplotype				.0037	2.7 × 10 ⁻⁵	3.8 × 10 ⁻³	
Dixon et al ¹³ (n = 308), LCLs†	rs2941504 rs2517955 rs907092 rs9303277 rs2305480 rs2290440 rs2716389 rs3894194 rs3859192					3 × 10 ⁻⁴	2.6 × 10 ⁻⁸ 7.5 × 10 ⁻⁸ 2.0 × 10 ⁻²² 1.2 × 10 ⁻²² 4.9 × 10 ⁻²¹ 3.7 × 10 ⁻²³ 4.2 × 10 ⁻²³ 1.1 × 10 ⁻¹¹ 3.0 × 10 ⁻⁴	
Andiappan et al ¹¹ (n = 71), whole blood†	rs8076131			NS	.023	.026	.0051	
Çalışkan et al ¹² (n = 100), PBMCs*	rs7216389			Untreated: .11 RV inf: .16		Untreated: <.001 RV inf: <.001	Untreated: .07 RV inf: .03	
Lluis et al ¹⁸ (n = 200), CBMCs*	rs7216389				NS	NS	Der p 1 stim: .01	PHA stim: .0001 Der p 1 stim: .05
Halapi et al ¹⁴ (n = 473), WBCs†	rs2941504 rs907092 rs9303277 rs2305480 rs2290400 rs7216389					1.2 × 10 ⁻¹¹ 5.1 × 10 ⁻³⁶ 1.1 × 10 ⁻⁴¹ 5.5 × 10 ⁻³⁷ 1.3 × 10 ⁻⁴¹ 2.3 × 10 ⁻³⁸	3.6 × 10 ⁻²¹ 5.1 × 10 ⁻⁶³ 8.3 × 10 ⁻⁶⁸ 9.1 × 10 ⁻⁶⁵ 1.2 × 10 ⁻⁶⁰ 8.8 × 10 ⁻⁵⁸	
Sharma et al ²² (n = 200), CD4 ⁺ T cells†	rs4795405						3.0 × 10 ⁻¹⁰	
Murphy et al ¹⁹ (n = 200), CD4 ⁺ T cells†	rs2290400 rs7216389	NS NS	NS NS	NS NS			3.1 × 10 ⁻⁹ 1.6 × 10 ⁻⁸	NS NS

(Continued)

TABLE I. (Continued)

Study	SNP	17q12-21 genes						
		<i>PGAP3</i> , P value	<i>ERBB2</i> , P value	<i>IKZF3</i> , P value	<i>ZBP2</i> , P value	<i>GSDMB</i> , P value	<i>ORMDL3</i> , P value	<i>GSDMA</i> , P value
Lung cells								
Nicodemus-	rs2517944	2.1×10^{-8}	NS	NS		NS	2.6×10^{-5}	NS
Johnson et al ²⁰	rs8076131	8.0×10^{-5}	NS	NS		NS	3.2×10^{-5}	NS
(n = 81), BECs†	rs7216389	7.1×10^{-5}	NS	NS		NS	8.0×10^{-5}	NS
	rs2305480	NS	NS	NS		NS	1.1×10^{-4}	NS
	rs11078928	NS	NS	NS		NS	1.2×10^{-4}	NS
	rs2941504	NS	NS	NS		NS	1.3×10^{-4}	NS
	rs2941504	4.7×10^{-7}	NS	NS		NS	4.0×10^{-5}	NS
	rs4065275	2.1×10^{-5}	NS	NS		NS	5.5×10^{-5}	NS
Hao et al ¹⁵	rs2290400	NS	NS	NS		4.0×10^{-15}	NS	NS
(n = 1111), lung	rs7216389	NS	NS	NS		4.2×10^{-14}	1.3×10^{-6}	8.8×10^{-25}
tissue†	rs2305480	NS	NS	NS		2.5×10^{-12}	2.1×10^{-5}	7.4×10^{-32}
	rs3859192	NS	NS	NS		3.9×10^{-5}	NS	3.6×10^{151}
	rs3894194	NS	NS	NS		1.6×10^{-7}	NS	2.8×10^{-73}
Li et al ¹⁶ , n = 107	rs8067378					BECS: 1.9×10^{-3} ;	BECS: NS; BAL fluid:	
for BECs† and	rs2305480					BAL fluid: .04	NS	
n = 94 for BAL	rs7216389					BECS: 4.7×10^{-3} ;	BECS: NS; BAL fluid:	
fluid†						BAL fluid: NS	NS	
						BECS: 1.1×10^{-3} ;	BECS: NS; BAL fluid:	
						BAL fluid: NS	NS	

BAL, Bronchoalveolar lavage; CBMC, cord blood mononuclear cell; NK, natural killer; NS, not significant; RV inf, rhinovirus infection; Treg, regulatory T. Symbols after references designate whether gene expression studies were performed by using quantitative PCR (*), microarray (†), or RNA-seq (‡). P values are shown as reported in each article for the genes included in each study.

SNP rsID	Asthma Risk Allele	Whole Blood (n=338)				Lung Tissue (n=278)							
		<i>GSDMB</i>		<i>ORMDL3</i>		<i>PGAP3</i>		<i>GSDMB</i>		<i>ORMDL3</i>		<i>GSDMA</i>	
		Effect Size	P-value	Effect Size	P-value	Effect Size	P-value	Effect Size	P-value	Effect Size	P-value	Effect Size	P-value
rs2941504	A	0.19	1.00E-07	0.15	5.60E-05	0.22	1.70E-09	n.s.	n.s.	0.2	1.30E-06	n.s.	n.s.
rs2517955	C	0.17	8.00E-07	0.16	1.50E-05	0.23	1.00E-09	n.s.	n.s.	0.18	9.10E-06	n.s.	n.s.
rs2952156	A	0.21	1.80E-09	0.17	6.60E-06	0.23	1.50E-09	n.s.	n.s.	0.22	2.70E-07	n.s.	n.s.
rs907092	G	0.29	6.90E-20	0.32	3.10E-21	n.s.	n.s.	n.s.	n.s.	0.19	3.50E-06	-0.36	6.30E-06
rs9303277	C	0.34	6.70E-28	0.34	5.80E-26	0.15	1.40E-05	0.17	9.20E-07	0.27	4.60E-13	-0.36	1.90E-06
rs12936231	C	0.33	1.30E-29	0.35	5.70E-29	n.s.	n.s.	0.16	9.80E-07	0.29	6.40E-15	-0.37	4.70E-07
rs8069176	G	0.29	7.70E-22	0.33	1.30E-26	n.s.	n.s.	n.s.	n.s.	0.23	4.80E-09	-0.42	1.50E-08
rs2305480	G	0.31	8.00E-22	0.35	8.00E-26	n.s.	n.s.	n.s.	n.s.	0.23	2.60E-08	-0.43	3.10E-08
rs11078927	C	0.31	1.00E-21	0.35	8.80E-26	n.s.	n.s.	n.s.	n.s.	0.23	2.50E-08	-0.44	2.80E-08
rs11078928	T	0.31	1.50E-21	0.36	3.90E-27	n.s.	n.s.	n.s.	n.s.	0.24	1.10E-08	-0.45	2.00E-08
rs2290400	T	0.31	2.10E-25	0.34	2.10E-27	0.14	6.30E-05	0.16	2.10E-06	0.29	1.20E-14	-0.38	2.50E-07
rs7216389	T	0.31	1.70E-22	0.36	9.00E-29	n.s.	n.s.	0.19	1.50E-07	0.28	2.50E-12	-0.41	1.60E-07
rs4065275	G	0.34	2.20E-27	0.34	1.60E-25	n.s.	n.s.	0.16	3.30E-06	0.27	2.50E-12	-0.47	4.70E-10
rs8076131	A	0.32	3.40E-23	0.37	1.10E-29	n.s.	n.s.	n.s.	n.s.	0.24	4.50E-09	-0.5	4.40E-10
rs12603332	C	0.32	3.20E-27	0.33	1.10E-25	0.14	4.30E-05	0.17	8.70E-07	0.28	1.70E-13	-0.47	1.10E-10
rs3894194	A	0.23	1.00E-13	0.23	5.90E-12	n.s.	n.s.	n.s.	n.s.	0.22	7.00E-09	-0.60	4.80E-17
rs3859192	T	0.19	1.70E-09	0.19	1.60E-08	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.77	1.50E-30

FIG 6. GTEx project eQTLs for 17q genes in whole blood and lung tissue at a false discovery rate of 5%. 17q SNPs were eQTLs for only 2 of the 6 genes in whole blood and 4 of the 6 genes in lung tissue; the remaining genes (without significant eQTLs) are not shown. SNPs in the proximal region are shown on a blue background, SNPs in the core region are shown on an orange background, and SNPs in the distal region are shown on a green background (see Fig 1). The GTEx project sample composition is approximately 84% white, 14% African American, 1% other, and 1% unknown (<https://gtexportal.org/home/tissueSummaryPage>). n.s., Not significant.

SNP rsID	Asthma Risk Allele	LD r^2 with rs12936231 in YRI	Asthma Risk Allele Frequency in YRI	eQTLs in YRI LCLs (n=56)		EVE Meta-Analysis of Asthma GWAS in African Americans	
				<i>GSDMB</i> P-value	<i>ORMDL3</i> P-value	Meta P-value	OR
rs2941504	A	0.09	0.49	0.59	1.3E-03	0.196	1.05
rs2517955	C	0.00	0.89	0.40	0.77	0.144	1.06
rs2952156	A	0.09	0.52	0.57	0.01	0.607	1.02
rs907092	G	0.02	0.97	0.10	0.69	0.022	1.14
rs9303277	C	0.73	0.56	0.01	5.48E-07	0.115	1.06
rs12936231	C	1.0	0.54	0.03	5.35E-10	0.348	1.06
rs8069176	G	0.39	0.67	0.11	2.43E-04	0.064	1.11
rs2305480	G	0.07	0.96	0.29	0.31	0.009	1.17
rs11078927	C	0.07	0.96	0.34	0.21	0.001	1.23
rs11078928	T	0.07	0.96	0.26	0.32	n.i.	n.i.
rs2290400	T	0.50	0.61	0.04	1.96E-04	0.174	1.03
rs7216389	T	0.16	0.89	0.29	0.06	0.097	1.06
rs4065275	G	0.28	0.75	0.16	0.01	n.i.	n.i.
rs8076131	A	0.10	0.09	0.47	0.53	0.003	1.17
rs12603332	C	0.43	0.56	0.24	2.52E-03	0.089	1.05
rs3894194	A	0.17	0.30	0.50	0.09	0.241	1.05
rs3859192	T	0.10	0.32	0.77	0.03	0.433	1.03

FIG 7. eQTLs for *ORMDL3* and *GSDMB* in Nigerian (YRI) LCLs⁹³ and associations with asthma in African Americans.⁵ LD between each SNP and the lead eQTL (rs12936231) are shown in the *third column*. eQTLs and GWAS *P* values of .01 or less are shown in boldface. SNPs in the proximal region are shown on a blue background, SNPs in the core region are shown on an orange background, and SNPs in the distal region are shown on a green background (see Fig 1). *n.i.*, No information available (SNPs not imputed).

promoter and enhancer activity in LCLs (CEU), Berlivet et al⁹⁹ provided further evidence for an enhancer of *ORMDL3* located in *ZBP2*. Acevedo et al³⁴ focused on 3 SNPs within CpG dinucleotides: the risk allele at rs7216389 in *GSDMB* removes a CpG site, and the risk alleles at rs4065275 and rs12603332 in *ORMDL3* create CpG sites. These SNPs affected methylation levels both at the position at which they are located and at other CpG sites in the 5' untranslated region of *ORMDL3*. The asthma-associated SNPs and allele-specific methylation patterns were associated independently with expression of *ORDML3* and *GSDMB* in PBLs, but only methylation patterns at, but not expression of, *ORMDL3* differed between children with and without asthma.

DNA methylation and gene regulation in BECs

Nicodemus-Johnson et al²⁰ performed genome-wide eQTL and meQTL mapping in freshly isolated BECs from 74 asthmatic and 41 nonasthmatic adults. The most significant eQTL for *ORMDL3* was rs2517955, which is located 240 bp from *ORMDL3* in an intron of *PGAP3* (eQTL $P = 1.56 \times 10^{-5}$) and shows little LD with genotypes at the core locus (Fig 1, D). This SNP was also a significant eQTL for *PGAP3* ($P = 2.07 \times 10^{-8}$) but not for *GSDMB* or any other genes at the core locus. SNP rs2517955

was also a meQTL for a nearby CpG, cg05616858 (meQTL $P = 8.95 \times 10^{-13}$), which was itself correlated with expression of *ORMDL3* ($P = 3.87 \times 10^{-4}$).

A conditional analysis that accounted for genotype at the core 17q locus confirmed that the association between rs2517955 and *ORMDL3* expression is independent of SNPs at the core locus. To elucidate the causal relationship between rs2517955 genotype, cg05616858 methylation, and *ORMDL3* gene expression, the investigators used Mendelian randomization and showed that methylation at this CpG site directly influences *ORMDL3* expression level independent of rs2517955. This SNP was also associated with asthma in the TAGC Consortium ($P = 7.6 \times 10^{-29}$).

Interestingly, rs2517955 resides within a peak of H3K27ac histone marks in ENCODE data (all cell types pooled), which is suggestive of an enhancer in this region. Chromatin capture (Hi-C) studies in LCLs demonstrated looping and physical interaction between the putative enhancer at rs2517955 and the promoter of *ORMDL3*, approximately 240 kb away,²⁰ which is consistent with the correlated expression levels of these 2 genes in PBLs, BECs, and ASMCs (Fig 4), providing a potential mechanism for these observations and supporting an independent asthma risk locus in the proximal 17q region.

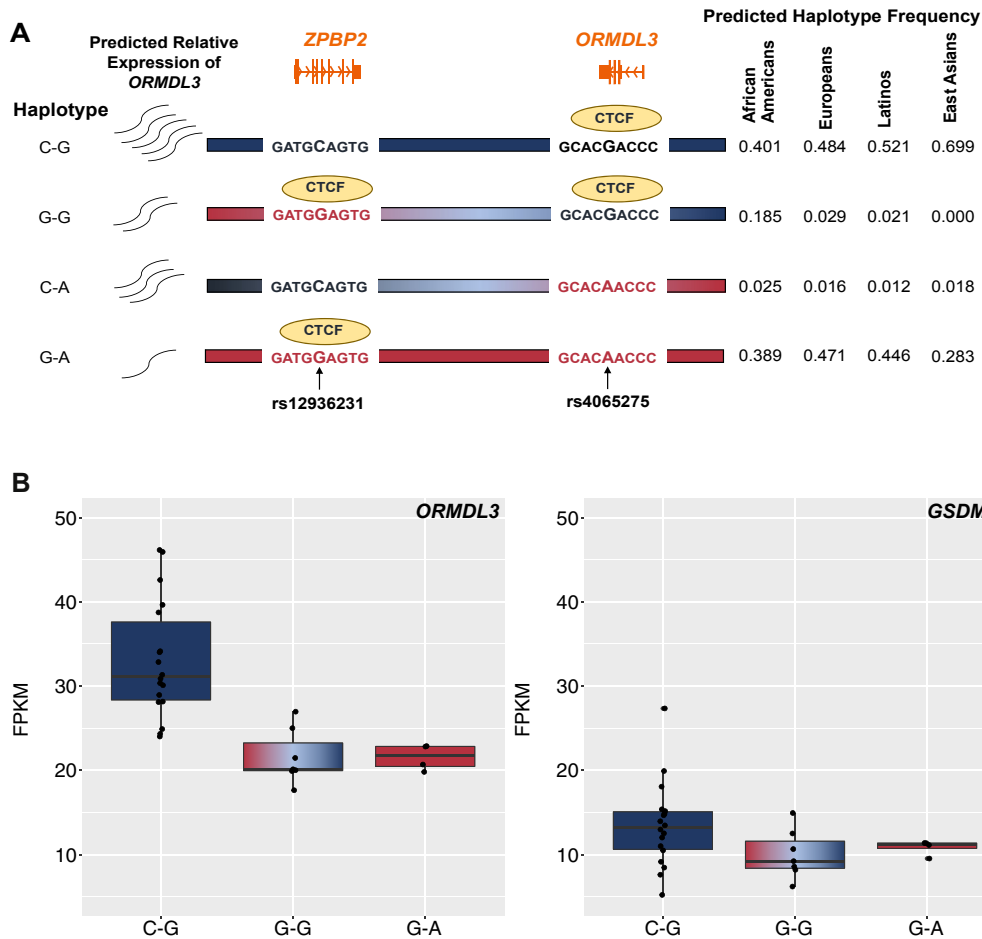


FIG 8. Allele-specific chromatin modification and gene expression at the 17q locus. **A**, Schematic representation of the CTCF-binding motifs that overlap with the asthma-associated SNPs rs12936231 in *ZPBP2* and rs4065275 in *ORMDL3* (modified from Schmiedel et al²¹). Relative expression of *ORMDL3* from the C-A haplotype was inferred from 1 YRI subject who was heterozygous C-A/G-G-A (*ORMDL3* fragments per kb million [FPKM] = 26; see Fig 8, B). Predicted haplotype frequencies are based on allele frequencies and LD estimates (see Fig 1). **B**, *ORMDL3* and *GSDMB* gene expression in LCLs and phased genotype data for 29 YRI subjects⁹⁸ who are homozygous for 3 of the rs12936231-rs4065274 haplotypes; no subjects were homozygous for the C-A haplotype. The *y*-axis shows FPKM, a measure of gene expression after normalizing for sequence depth and gene length.

SYNTHESIS AND FUTURE DIRECTIONS

Although our understanding of the 17q asthma locus has deepened significantly since its discovery, research over the past few years has revealed appreciable levels of complexity for this locus. Importantly, several lines of evidence support the presence of 3 independent asthma-associated loci at the extended 17q12-21 region.

At least 2 SNPs at the core locus directly regulate the expression of *ORMDL3* or *GSDMB* in immune cells. The asthma-associated allele in *GSDMB*, rs11078928-T, is associated with higher expression of this gene because of aberrant splicing associated with the alternate C allele, and the rs12936231-C allele in *ZPBP2* that destroys a CTCF motif is associated with increased expression of *ORMDL3*. Because of LD, 2 haplotypes corresponding to high and low expressors of *ORMDL3* and *GSDMB* account for 95% of non-African haplotypes. However, these 2 SNPs have very different allele frequencies and are in

low LD on African-derived chromosomes (Fig 1, C and D), potentially breaking up the coregulation of these 2 genes in African ancestry populations. Moreover, 18.5% of African-derived chromosomes are recombinants carrying the rs12603332-G allele, which is associated with low expression of *ORMDL3*, and the rs4065275-G allele, which is associated with high expression of *ORMDL3*, in subjects of non-African ancestry (Fig 8, A). However, in African subjects this haplotype is associated with low expression of *ORMDL3* (Fig 8, B), indicating that rs12936231 in *ZPBP2* has a “dominant” effect on expression of *ORMDL3*. Therefore it is not surprising that rs12936231 is the most significant eQTL for *ORMDL3* in whole blood and lung tissue cells (Fig 5) and in African-derived LCLs (Fig 6). Thus if high expression of *ORMDL3* by itself was the underlying cause of asthma, this SNP in *ZPBP2* should be associated with asthma in GWASs. Yet rs12936231 has never been reported as a lead SNP in GWASs.

The long-range looping and correlated expression patterns between *PGAP3* in the proximal region and *ORMDL3* in the core region and the correlated expression of their transcripts are intriguing and raise additional questions. Are the combinations of genotypes at the proximal and core locus SNPs more associated with asthma than each individually? Are the chromatin interactions and looping between *PGAP3* and *ORMDL3* inhibited by the binding of CTCF at rs12936231 as for looping within the core locus? Do *PGAP3* and *ORMDL3* proteins functionally interact to promote asthma? At the present time, little is known about the function of *PGAP3*, which is a glycosylphosphatidylinositol (GPI)-specific phospholipase that is expressed ubiquitously and localizes primarily to the Golgi apparatus. It is predicted to encode a 7-transmembrane protein that removes fatty acids from GPI, which might be important for proper association between GPI-anchored proteins and lipid rafts.¹⁰⁰ A role for *PGAP3* in asthma has not yet been explored. In contrast, many potential functions have been attributed to *ORMDL3*, including sphingolipid metabolism,¹⁰¹⁻¹⁰⁶ ER stress,^{17,107-110} eosinophil trafficking,¹¹¹ and responses to *Alternaria* species,¹¹² respiratory syncytial virus,⁹¹ polyinosinic: polycytidylic acid,⁵³ and IL-17 secretion (reviewed in Das et al).^{18,36}

Although less is known about *GSDMA*, a missense variant (rs3894194) has shown independent associations for asthma and specific lung phenotypes, and an SNP (rs3859192) in this gene is an eQTL for *GSDMA* in lung tissue cells. These 2 SNPs show very little LD with each other in all populations. As a result, a relationship between the eQTL or between *GSDMA* expression and asthma *per se* has not been established. Additionally, the eQTL should be interpreted cautiously because of the very low expression of *GSDMA* in lung cells.

Finally, the combined observations of consistently strong associations between 17q SNPs with both early-onset asthma and expression of 17q genes but conflicting evidence for increased expression of 17q genes in immune cells or airway cells in older children and adults with asthma is enigmatic and might suggest that the critical window for regulating the expression of *ORMDL3*, *GSDMB*, or other 17q genes occurs in the first few years of life. In that case studying children at school age or later will miss this relationship. Additionally, specific exposures, such as viruses or other microbes, can enhance genotype-specific differences in transcript abundance between asthmatic and nonasthmatic children, as suggested by a study in cord blood mononuclear cells.¹⁸ Prospective birth cohorts will be required to examine early-life gene expression and epigenetic patterning and the subsequent development of asthma. Ideally, these studies would be performed in both immune and airway cells during periods of wellness and during respiratory tract infections in the first few years of life and include ethnically diverse children and children exposed to different environments in infancy. More comprehensive and integrative studies of 17q genotype with gene expression and epigenetic variation using systems biology approaches in carefully phenotyped children will yield further insights into the genetic risk architecture of this important locus. Given the current pace of research, we expect the next decade to yield answers to these questions and translate findings into asthma prevention strategies for children with the 17q high-risk genotype.

What do we know?

- The asthma locus on 17q12-21 is the most replicated and most significant finding in GWASs of asthma.
- Variation at this locus is specifically associated with very early-onset asthma, possibly before age 3 years, when it manifests as wheezing illness.
- The 17q genotype's effects on asthma risk and protection are modified by early-life exposures.
- The extensive LD across this region in populations of European and Asian ancestry is greatly reduced in populations of African ancestry.
- The asthma-associated 17q SNPs are eQTLs for the nearby genes *GSDMA*, *ORMDL3*, *GSDMB*, and *PGAP3* in immune cells, lung cells, or both.
- The regulatory architecture of the 17q genes differs in immune and lung cells.
- Variation at this locus is also associated with autoimmune diseases; alleles associated with asthma risk are often associated with protection from autoimmune disease and *vice versa*.

What is still unknown?

- Is genetic variation at the extended 17q12-21 locus associated with early-life wheezing illness and childhood-onset asthma in African American children?
- Which 17q gene or genes (*ORMDL3*, *GSDMB*, *PGAP3*, and/or *GSDMA*) are involved in early-life wheezing and asthma pathogenesis?
- What are the functions of and through what mechanisms do the 17q genes affect asthma onset and severity?
- In which cell types (blood cells, airway cells, or both) are the expression of these genes most relevant to the 17q asthma-associated risks or specific endotypes?
- Do the same 17q gene or genes modulate both protection against asthma in children exposed to animals and risk for asthma in children not exposed to animals?
- What is the natural history of asthma in later childhood and after puberty in subjects with the high-risk 17q genotype?
- Which genes and what mechanisms account for the opposite associations of 17q alleles with asthma and autoimmune diseases?

REFERENCES

1. Moffatt MF, Kabisch 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;448:470-3.
2. Demenais F, Margaritte-Jeannin P, Barnes KC, Cookson WO, Altmueller J, Ang W, et al. Multi-ancestry genome-wide association study identifies new asthma susceptibility loci that co-localize with immune cell enhancer histone marks. *Nat Genet* 2018;50:42-53.
3. 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;363:1211-21.
4. Pickrell JK. Joint analysis of functional genomic data and genome-wide association studies of 18 human traits. *Am J Hum Genet* 2014;94:559-73.

5. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011;43:887-92.
6. Bønnelykke K, Sleiman P, Nielsen K, Kreiner-Møller E, Mercader JM, Belgrave D, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. *Nat Genet* 2014;46:51-5.
7. Ferreira MA, McRae AF, Medland SE, Nyholt DR, Gordon SD, Wright MJ, et al. Association between ORMDL3, IL1RL1 and a deletion on chromosome 17q21 with asthma risk in Australia. *Eur J Hum Genet* 2011;19:458-64.
8. Galanter JM, Gignoux CR, Torgerson DG, Roth LA, Eng C, Oh SS, et al. Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: the Genes-environments & Admixture in Latino Americans study. *J Allergy Clin Immunol* 2014;134:295-305.
9. Wan YI, Shrine NR, Soler Artigas M, Wain LV, Blakey JD, Moffatt MF, et al. Genome-wide association study to identify genetic determinants of severe asthma. *Thorax* 2012;67:762-8.
10. Yan Q, Brehm J, Pino-Yanes M, Forno E, Lin J, Oh SS, et al. A meta-analysis of genome-wide association studies of asthma in Puerto Ricans. *Eur Respir J* 2017;49.
11. Andiappan AK, Sio YY, Lee B, Suri BK, Matta SA, Lum J, et al. Functional variants of 17q12-21 are associated with allergic asthma but not allergic rhinitis. *J Allergy Clin Immunol* 2016;137:758-66.e3.
12. Çalıřkan M, Bochkov YA, Kreiner-Møller E, Bønnelykke K, Stein MM, Du G, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med* 2013;368:1398-407.
13. Dixon AL, Liang L, Moffatt MF, Chen W, Heath S, Wong KC, et al. A genome-wide association study of global gene expression. *Nat Genet* 2007;39:1202-7.
14. Halapi E, Gudbjartsson DF, Jonsdottir GM, Bjornsdottir US, Thorleifsson G, Helgadóttir H, et al. A sequence variant on 17q21 is associated with age at onset and severity of asthma. *Eur J Hum Genet* 2010;18:902-8.
15. Hao K, Bosse Y, Nickle DC, Pare PD, Postma DS, Laviolette M, et al. Lung eQTLs to help reveal the molecular underpinnings of asthma. *PLoS Genet* 2012;8:e1003029.
16. Li X, Hastie AT, Hawkins GA, Moore WC, Ampleford EJ, Milosevic J, et al. eQTL of bronchial epithelial cells and bronchial alveolar lavage deciphers GWAS-identified asthma genes. *Allergy* 2015;70:1309-18.
17. Liu YP, Rajamanikham V, Baron M, Patel S, Mathur SK, Schwantes EA, et al. Association of ORMDL3 with rhinovirus-induced endoplasmic reticulum stress and type I Interferon responses in human leucocytes. *Clin Exp Allergy* 2017;47:371-82.
18. Lluís A, Schedel M, Liu J, Illi S, Depner M, von Mutius E, et al. Asthma-associated polymorphisms in 17q21 influence cord blood ORMDL3 and GSDMA gene expression and IL-17 secretion. *J Allergy Clin Immunol* 2011;127:1587-94.e6.
19. Murphy A, Chu JH, Xu M, Carey VJ, Lazarus R, Liu A, et al. Mapping of numerous disease-associated expression polymorphisms in primary peripheral blood CD4+ lymphocytes. *Hum Mol Genet* 2010;19:4745-57.
20. Nicodemus-Johnson J, Myers RA, Sakabe NJ, Sobreira DR, Hogarth DK, Nareckas ET, et al. DNA methylation in lung cells is associated with asthma endotypes and genetic risk. *JCI Insight* 2016;1:e90151.
21. Schmiedel BJ, Seumois G, Samaniego-Castruita D, Cayford J, Schulten V, Chavez L, et al. 17q21 asthma-risk variants switch CTCF binding and regulate IL-2 production by T cells. *Nat Commun* 2016;7:13426.
22. Sharma S, Zhou X, Thibault DM, Himes BE, Liu A, Szeffler SJ, et al. A genome-wide survey of CD4(+) lymphocyte regulatory genetic variants identifies novel asthma genes. *J Allergy Clin Immunol* 2014;134:1153-62.
23. Verlaan DJ, Berlivet S, Hunninghake GM, Madore AM, Larivière 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;85:377-93.
24. Marinho S, Custovic A, Marsden P, Smith JA, Simpson A. 17q12-21 variants are associated with asthma and interact with active smoking in an adult population from the United Kingdom. *Ann Allergy Asthma Immunol* 2012;108:402-11.e9.
25. Anantharaman R, Andiappan AK, Nilkanth PP, Suri BK, Wang de Y, Chew FT. Genome-wide association study identifies PERLD1 as asthma candidate gene. *BMC Med Genet* 2011;12:170.
26. Igartua C, Myers RA, Mathias RA, Pino-Yanes M, Eng C, Graves PE, et al. Ethnic-specific associations of rare and low-frequency DNA sequence variants with asthma. *Nat Commun* 2015;6:5965.
27. Kreiner-Møller E, Strachan DP, Linneberg A, Husemoen LL, Bisgaard H, Bønnelykke K. 17q21 gene variation is not associated with asthma in adulthood. *Allergy* 2015;70:107-14.
28. Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. *N Engl J Med* 2008;359:1985-94.
29. Blekic M, Kijaic Bukvic B, Aberle N, Marinho S, Hankinson J, Custovic A, et al. 17q12-21 and asthma: interactions with early-life environmental exposures. *Ann Allergy Asthma Immunol* 2013;110:347-53.e2.
30. GTEx Consortium. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 2015;348:648-60.
31. Morrison FS, Locke JM, Wood AR, Tuke M, Pasko D, Murray A, et al. The splice site variant rs11078928 may be associated with a genotype-dependent alteration in expression of GSDMB transcripts. *BMC Genomics* 2013;14:627.
32. Schedel M, Michel S, Gaertner VD, Toncheva AA, Depner M, Binia A, et al. Polymorphisms related to ORMDL3 are associated with asthma susceptibility, alterations in transcriptional regulation of ORMDL3, and changes in TH2 cytokine levels. *J Allergy Clin Immunol* 2015;136:893-903.e14.
33. Loss GJ, Depner M, Hose AJ, Genuneit J, Karvonen AM, Hyvarinen A, et al. The early development of wheeze. Environmental determinants and genetic susceptibility at 17q21. *Am J Respir Crit Care Med* 2016;193:889-97.
34. Acevedo N, Reinius LE, Greco D, Gref A, Orsmark-Pietras C, Persson H, et al. Risk of childhood asthma is associated with CpG-site polymorphisms, regional DNA methylation and mRNA levels at the GSDMB/ORMDL3 locus. *Hum Mol Genet* 2015;24:875-90.
35. 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;177:1194-200.
36. Das S, Miller M, Broide DH. Chromosome 17q21 Genes ORMDL3 and GSDMB in asthma and immune diseases. *Adv Immunol* 2017;135:1-52.
37. Bønnelykke K, Ober C. Leveraging gene-environment interactions and endotypes for asthma gene discovery. *J Allergy Clin Immunol* 2016;137:667-79.
38. Bisgaard H, Bønnelykke K, Sleiman PM, Brasholt M, Chawes B, Kreiner-Møller E, et al. Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. *Am J Respir Crit Care Med* 2009;179:179-85.
39. Smit LA, Bouzigon E, Pin I, Siroux V, Monier F, Aschard H, et al. 17q21 variants modify the association between early respiratory infections and asthma. *Eur Respir J* 2010;36:57-64.
40. Bønnelykke K, Matheson MC, Pers TH, Granell R, Strachan DP, Alves AC, et al. Meta-analysis of genome-wide association studies identifies ten loci influencing allergic sensitization. *Nat Genet* 2013;45:902-6.
41. van der Valk RJ, Duijts L, Kerkhof M, Willemsen SP, Hofman A, Moll HA, et al. Interaction of a 17q12 variant with both fetal and infant smoke exposure in the development of childhood asthma-like symptoms. *Allergy* 2012;67:767-74.
42. Brauner EV, Loft S, Raaschou-Nielsen O, Vogel U, Andersen PS, Sorensen M. Effects of a 17q21 chromosome gene variant, tobacco smoke and furred pets on infant wheeze. *Genes Immun* 2012;13:94-7.
43. Scholtens S, Postma DS, Moffatt MF, Panasevich S, Granell R, Henderson AJ, et al. Novel childhood asthma genes interact with in utero and early-life tobacco smoke exposure. *J Allergy Clin Immunol* 2014;133:885-8.
44. Stokholm J, Chawes BL, Vissing N, Bønnelykke K, Bisgaard H. Cat exposure in early life decreases asthma risk from the 17q21 high-risk variant. *J Allergy Clin Immunol* 2017 [Epub ahead of print].
45. Granell R, Henderson AJ, Timpson N, St Pourcain B, Kemp JP, Ring SM, et al. Examination of the relationship between variation at 17q21 and childhood wheeze phenotypes. *J Allergy Clin Immunol* 2013;131:685-94.
46. Paternoster L, Standl M, Chen CM, Ramasamy A, Bønnelykke K, Duijts L, et al. Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. *Nat Genet* 2012;44:187-92.
47. Kim KW, Myers RA, Lee JH, Igartua C, Lee KE, Kim YH, et al. Genome-wide association study of recalcitrant atopic dermatitis in Korean children. *J Allergy Clin Immunol* 2015;136:678-84.e4.
48. Levin AM, Mathias RA, Huang L, Roth LA, Daley D, Myers RA, et al. A meta-analysis of genome-wide association studies for serum total IgE in diverse study populations. *J Allergy Clin Immunol* 2013;131:1176-84.
49. Granada M, Wilk JB, Tuzova M, Strachan DP, Weidinger S, Albrecht E, et al. A genome-wide association study of plasma total IgE concentrations in the Framingham Heart Study. *J Allergy Clin Immunol* 2012;129:840-5.e21.
50. Pino-Yanes M, Gignoux CR, Galanter JM, Levin AM, Campbell CD, Eng C, et al. Genome-wide association study and admixture mapping reveal new loci associated with total IgE levels in Latinos. *J Allergy Clin Immunol* 2015;135:1502-10.

51. Weidinger S, Gieger C, Rodriguez E, Baurecht H, Mempel M, Klopp N, et al. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. *PLoS Genet* 2008;4:e1000166.
52. Yatagai Y, Sakamoto T, Masuko H, Kaneko Y, Yamada H, Iijima H, et al. Genome-wide association study for levels of total serum IgE identifies HLA-C in a Japanese population. *PLoS One* 2013;8:e80941.
53. Hirota T, Harada M, Sakashita M, Doi S, Miyatake A, Fujita K, et al. Genetic polymorphism regulating ORM1-like 3 (*Saccharomyces cerevisiae*) expression is associated with childhood atopic asthma in a Japanese population. *J Allergy Clin Immunol* 2008;121:769-70.
54. Fuertes E, Soderhall C, Acevedo N, Becker A, Brauer M, Chan-Yeung M, et al. Associations between the 17q21 region and allergic rhinitis in 5 birth cohorts. *J Allergy Clin Immunol* 2015;135:573-6.
55. Tomita K, Sakashita M, Hirota T, Tanaka S, Masuyama K, Yamada T, et al. Variants in the 17q21 asthma susceptibility locus are associated with allergic rhinitis in the Japanese population. *Allergy* 2013;68:92-100.
56. Ramasamy A, Curjiric I, Coin LJ, Kumar A, McArdle WL, Imboden M, et al. A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. *J Allergy Clin Immunol* 2011;128:996-1005.
57. Joost O, Wilk JB, Cupples LA, Harmon M, Shearman AM, Baldwin CT, et al. Genetic loci influencing lung function: a genome-wide scan in the Framingham Study. *Am J Respir Crit Care Med* 2002;165:795-9.
58. Repapi E, Sayers I, Wain LV, Burton PR, Johnson T, Obeidat M, et al. Genome-wide association study identifies five loci associated with lung function. *Nat Genet* 2010;42:36-44.
59. Soler Artigas M, Loth DW, Wain LV, Gharib SA, Obeidat M, Tang W, et al. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nat Genet* 2011;43:1082-90.
60. Tang W, Kowgier M, Loth DW, Soler Artigas M, Joubert BR, Hodge E, et al. Large-scale genome-wide association studies and meta-analyses of longitudinal change in adult lung function. *PLoS One* 2014;9:e100776.
61. Yao TC, Du G, Han L, Sun Y, Hu D, Yang JJ, et al. Genome-wide association study of lung function phenotypes in a founder population. *J Allergy Clin Immunol* 2014;133:248-55, e1-10.
62. van der Valk RJ, Duijts L, Timpson NJ, Salam MT, Standl M, Curtin JA, et al. Fraction of exhaled nitric oxide values in childhood are associated with 17q11.2-q12 and 17q12-q21 variants. *J Allergy Clin Immunol* 2014;134:46-55.
63. Bouzigon E, Nadif R, Thompson EE, Concas MP, Kuldaneck S, Du G, et al. A common variant in RAB27A gene is associated with fractional exhaled nitric oxide levels in adults. *Clin Exp Allergy* 2015;45:797-806.
64. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadóttir A, Sulem P, Jonsson GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009;41:342-7.
65. Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008;40:955-62.
66. Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011;43:246-52.
67. Huang C, Haritunians T, Okou DT, Cutler DJ, Zwick ME, Taylor KD, et al. Characterization of genetic loci that affect susceptibility to inflammatory bowel diseases in African Americans. *Gastroenterology* 2015;149:1575-86.
68. Saleh NM, Raj SM, Smyth DJ, Wallace C, Howson JM, Bell L, et al. Genetic association analyses of atopic illness and proinflammatory cytokine genes with type 1 diabetes. *Diabetes Metab Res Rev* 2011;27:838-43.
69. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 2009;41:703-7.
70. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat Genet* 2010;42:508-14.
71. Mells GF, Floyd JA, Morley KI, Cordell HJ, Franklin CS, Shin SY, et al. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet* 2011;43:329-32.
72. Hirschfeld GM, Liu X, Xu C, Lu Y, Xie G, Lu Y, et al. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 2009;360:2544-55.
73. Kreiner E, Waage J, Standl M, Brix S, Pers TH, Couto Alves A, et al. Shared genetic variants suggest common pathways in allergy and autoimmune diseases. *J Allergy Clin Immunol* 2017;140:771-81.
74. Li X, Ampleford EJ, Howard TD, Moore WC, Torgerson DG, Li H, et al. Genome-wide association studies of asthma indicate opposite immunopathogenesis direction from autoimmune diseases. *J Allergy Clin Immunol* 2012;130:861-8.e7.
75. 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;122:1225-7.
76. White MJ, Risse-Adams O, Goddard P, Contreras MG, Adams J, Hu D, et al. Novel genetic risk factors for asthma in African American children: precision medicine and the SAGE II study. *Immunogenetics* 2016;68:391-400.
77. Almoguera B, Vazquez L, Mentch F, Connolly J, Pacheco JA, Sundaresan AS, et al. Identification of four novel loci in asthma in European American and African American populations. *Am J Respir Crit Care Med* 2017;195:456-63.
78. Tang MF, Sy HY, Kong AP, Ko FW, Wang SS, Liu TC, et al. Genetic effects of multiple asthma loci identified by genomewide association studies on asthma and spirometric indices. *Pediatr Allergy Immunol* 2016;27:185-94.
79. Fang Q, Zhao H, Wang A, Gong Y, Liu Q. Association of genetic variants in chromosome 17q21 and adult-onset asthma in a Chinese Han population. *BMC Med Genet* 2011;12:133.
80. Yu X, Yu C, Ren Z, Deng Y, Song J, Zhang H, et al. Genetic variants of 17q21 are associated with childhood-onset asthma and related phenotypes in a northeastern Han Chinese population: a case-control study. *Tissue Antigens* 2014;83:330-6.
81. Chen J, Zhang J, Hu H, Jin Y, Xue M. Polymorphisms of RAD50, IL33 and IL1RL1 are associated with atopic asthma in Chinese population. *Tissue Antigens* 2015;86:443-7.
82. Li FX, Tan JY, Yang XX, Wu YS, Wu D, Li M. Genetic variants on 17q21 are associated with asthma in a Han Chinese population. *Genet Mol Res* 2012;11:340-7.
83. Yang FF, Huang Y, Li QB, Dai JH, Fu Z. Single nucleotide polymorphisms in the ORM1-like 3 gene associated with childhood asthma in a Chinese population. *Genet Mol Res* 2012;11:4646-53.
84. Kang MJ, Yu HS, Seo JH, Kim HY, Jung YH, Kim YJ, et al. GSDMB/ORMDL3 variants contribute to asthma susceptibility and eosinophil-mediated bronchial hyperresponsiveness. *Hum Immunol* 2012;73:954-9.
85. Yu J, Kang MJ, Kim BJ, Kwon JW, Song YH, Choi WA, et al. Polymorphisms in GSDMA and GSDMB are associated with asthma susceptibility, atopy and BHR. *Pediatr Pulmonol* 2011;46:701-8.
86. Noguchi E, Sakamoto H, Hirota T, Ochiai K, Imoto Y, Sakashita M, et al. Genome-wide association study identifies HLA-DP as a susceptibility gene for pediatric asthma in Asian populations. *PLoS Genet* 2011;7:e1002170.
87. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248-9.
88. Mathias RA, Taub MA, Gignoux CR, Fu W, Musharoff S, O'Connor TD, et al. A continuum of admixture in the Western Hemisphere revealed by the African Diaspora genome. *Nat Commun* 2016;7:12522.
89. Gern JE, Pappas T, Visness CM, Jaffee KF, Lemanske RF, Togias A, et al. Comparison of the etiology of viral respiratory illnesses in inner-city and suburban infants. *J Infect Dis* 2012;206:1342-9.
90. Çalıřkan M, Baker SW, Gilad Y, Ober C. Host genetic variation influences gene expression response to rhinovirus infection. *PLoS Genet* 2015;11:e1005111.
91. Wang XH, Shu J, Jiang CM, Zhuang LL, Yang WX, Zhang HW, et al. Mechanisms and roles by which IRF-3 mediates the regulation of ORMDL3 transcription in respiratory syncytial virus infection. *Int J Biochem Cell Biol* 2017;87:8-17.
92. Nieuwenhuis MA, Siedlinski M, van den Berge M, Granell R, Li X, Niens M, et al. Combining genomewide association study and lung eQTL analysis provides evidence for novel genes associated with asthma. *Allergy* 2016;71:1712-20.
93. Lappalainen T, Sammeth M, Friedlander MR, t Hoen PA, Monlong J, Rivas MA, et al. Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* 2013;501:506-11.
94. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;180:388-95.
95. Bhakta NR, Christenson SA, Nerella S, Solberg OD, Nguyen CP, Choy DF, et al. Interferon-stimulated gene expression, type-2 inflammation and endoplasmic reticulum stress in asthma. *Am J Respir Crit Care Med* 2017 [Epub ahead of print].
96. Das S, Miller M, Beppu AK, Mueller J, McGeough MD, Vuong C, et al. GSDMB induces an asthma phenotype characterized by increased airway responsiveness and remodeling without lung inflammation. *Proc Natl Acad Sci U S A* 2016;113:13132-7.

97. Shukla S, Kavak E, Gregory M, Imashimizu M, Shutinoski B, Kashlev M, et al. CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature* 2011;479:74-9.
98. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al. A global reference for human genetic variation. *Nature* 2015;526:68-74.
99. Berlivet S, Moussette S, Ouimet M, Verlaan DJ, Koka V, Al Tuwaijri A, et al. Interaction between genetic and epigenetic variation defines gene expression patterns at the asthma-associated locus 17q12-q21 in lymphoblastoid cell lines. *Hum Genet* 2012;131:1161-71.
100. NCBI Gene Report on PGAP3. Available at: <https://www.ncbi.nlm.nih.gov/gene/93210>. Accessed September 10, 2017.
101. Breslow DK, Collins SR, Bodenmiller B, Aebersold R, Simons K, Shevchenko A, et al. Orm family proteins mediate sphingolipid homeostasis. *Nature* 2010;463:1048-53.
102. Miller M, Rosenthal P, Beppu A, Gordillo R, Broide DH. Oroscomucoid like protein 3 (ORMDL3) transgenic mice have reduced levels of sphingolipids including sphingosine-1-phosphate and ceramide. *J Allergy Clin Immunol* 2017;139:1373-6.e4.
103. Miller M, Tam AB, Mueller JL, Rosenthal P, Beppu A, Gordillo R, et al. Cutting edge: targeting epithelial ORMDL3 increases, rather than reduces, airway responsiveness and is associated with increased sphingosine-1-phosphate. *J Immunol* 2017;198:3017-22.
104. Oyeniran C, Sturgill JL, Hait NC, Huang WC, Avni D, Maceyka M, et al. Aberrant ORM (yeast)-like protein isoform 3 (ORMDL3) expression dysregulates ceramide homeostasis in cells and ceramide exacerbates allergic asthma in mice. *J Allergy Clin Immunol* 2015;136:1035-46.e6.
105. Yu F, Sun Y, Yu J, Ding Z, Wang J, Zhang L, et al. ORMDL3 is associated with airway remodeling in asthma via the ERK/MMP-9 pathway. *Mol Med Rep* 2017;15:2969-76.
106. Zhakupova A, Debeuf N, Krols M, Toussaint W, Vanhoutte L, Alecu I, et al. ORMDL3 expression levels have no influence on the activity of serine palmitoyltransferase. *FASEB J* 2016;30:4289-300.
107. 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;19:111-21.
108. Li M, Zhang S, Qiu Y, He Y, Chen B, Mao R, et al. Upregulation of miR-665 promotes apoptosis and colitis in inflammatory bowel disease by repressing the endoplasmic reticulum stress components XBP1 and ORMDL3. *Cell Death Dis* 2017;8:e2699.
109. Miller M, Rosenthal P, Beppu A, Mueller JL, Hoffman HM, Tam AB, et al. ORMDL3 transgenic mice have increased airway remodeling and airway responsiveness characteristic of asthma. *J Immunol* 2014;192:3475-87.
110. Miller M, Tam AB, Cho JY, Doherty TA, Pham A, Khorram N, et al. ORMDL3 is an inducible lung epithelial gene regulating metalloproteases, chemokines, OAS, and ATF6. *Proc Natl Acad Sci U S A* 2012;109:16648-53.
111. Ha SG, Ge XN, Bahaie NS, Kang BN, Rao A, Rao SP, et al. ORMDL3 promotes eosinophil trafficking and activation via regulation of integrins and CD48. *Nat Commun* 2013;4:2479.
112. Loser S, Gregory LG, Zhang Y, Schaefer K, Walker SA, Buckley J, et al. Pulmonary ORMDL3 is critical for induction of *Alternaria*-induced allergic airways disease. *J Allergy Clin Immunol* 2017;139:1496-507.e3.

METHODS

PBLs were obtained from 112 Hutterite subjects (age range, 7-76 years), a founder population of European ancestry in a study approved by the Institutional Review Board at the University of Chicago. Written consent for these studies was obtained from the adult participants and parents of children less than 18 years of age; written assent was obtained from all children. One milliliter of whole blood was drawn into a TruCulture (Myriad RBM, Austin, Tex) tube containing proprietary TruCulture media and incubated upright in a dry heat block at 37°C for 30 hours. Samples were washed twice with Buffer EL (Qiagen, Hilden, Germany), and cell pellets were resuspended in 350 μ L of RLT Buffer (Qiagen) and frozen on dry ice.

RNA was extracted from thawed cell pellets by using AllPrep DNA/RNA Mini Kits (Qiagen). RNA-seq libraries were made with the TruSeq Library kit (Illumina, San Diego, Calif); quality and concentration of libraries were assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, Calif) and quantitative PCR by using the Kapa library quantification kit (Kapa Biosystems, Wilmington, Mass). Samples were sequenced in pools of 16 to 18 samples across 3 flow cells of an Illumina HiSeq 2500; 119 samples with low read counts were resequenced on 2 flow cells on the same machine. Reads were mapped to hg19, and genes were counted with STAR.^{E1} Samples with more than 7 million uniquely mapped reads underwent trimmed means of M-value normalization and a voom transformation, which was used to correct for differences in library sizes.^{E2} Confounding technical effects were assessed in the normalized expression data by using principal components analysis (PCA), and the sequencing pool was adjusted by using the function RemoveBatchEffect() from the R package Limma.^{E3}

Lung CD4 TRM cells were sorted from 20 human organ donors whose lungs were not used for transplantation and provided by the Gift of Hope Organ and Tissue Donor Network. Fifty million lung leukocytes were thawed and centrifuged over Histopaque 1077 (Sigma, St Louis, Mo) gradient, as previously described.^{E4} Cells were then stained with antibodies for fluorescence-activated cell sorting of CD4 TRMs with the CD45⁺CD206⁻CD14⁻CD3⁺CD11b⁻CD4⁺CD8⁻CDRO⁺CD11a⁺ phenotype. RNA was extracted from cells by using AllPrep DNA/RNA Mini Kits (Qiagen). RNA-seq libraries were made by using the SMART seq v4 Ultra Low Input RNA kit (Takara, Shiga, Japan); quality and concentration of libraries were assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies), and quantitative PCR was done with the Kapa library quantification kit (Kapa Biosystems). Samples were sequenced in pools with 84 samples (including other samples) across 3 flow cells of an Illumina HiSeq 2500; 4 samples with low read counts were resequenced on 2 flow cells on the same machine. Reads were mapped to hg19, and genes were counted by using TopHat2.^{E5} Samples with greater than 8 million uniquely mapped reads underwent trimmed means of M-value normalization and a voom transformation, which was used to correct for differences in library sizes.^{E2} Confounding technical effects were assessed in the normalized expression data using PCA, and no confounding effects were seen with extraction batch, library batch, pool, or lane. Effects for age, sex, race, and date of cell sorting were removed by using linear regression in the R package Limma.^{E3}

Primary ASMCS were isolated from 75 human donor lungs that were not suitable for transplantation and provided by the Gift of Hope Organ and Tissue Donor Network. Cells were isolated from trachea and main bronchi by using established techniques.^{E6} Cells were cultured in 75-cm² flasks in DMEM/F-12 media (Invitrogen, Carlsbad, Calif) supplemented with 10% FBS, 5% nonessential amino acids (Invitrogen), and 5% antibiotic/antimycotic (Invitrogen). RNA was isolated by using the QIAgen AllPrep Kit (Qiagen) and hybridized to Illumina Human HT-12 v4 arrays at the University of Chicago Genomics Facility. Probe-level raw intensity values across arrays were normalized by using quantile normalization, and background-corrected normalized expression values were obtained for each probe by using the R package lumi.^{E7} A total of 67 samples had adequate array intensities and were further processed. Probes that were indistinguishable from background intensity ($P < .01$), contained more than 1 HapMap SNP, or mapped to multiple locations in the genome^{E8} were removed. Median probe intensity was used to represent the transcriptional abundance of each gene. Extraction batch, chip, RNA concentration, cell line age, and plate were identified as potential confounders by using PCA^{E9} of gene expression data. The effects of culture and extraction batch, chip, and plate were removed by using ComBat,^{E10} whereas the effects of the quantitative variables (RNA concentration and cell line age) were removed by using linear regression.

REFERENCES

- E1. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013;29:15-21.
- E2. Law CW, Chen Y, Shi W, Smyth GK. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol* 2014;15:R29.
- E3. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015;43:e47.
- E4. Adegunsoye A, Hrusch CL, Bonham CA, Jaffery MR, Blaine KM, Sullivan M, et al. Skewed lung CCR4 to CCR6 CD4+ T cell ratio in idiopathic pulmonary fibrosis is associated with pulmonary function. *Front Immunol* 2016;7.
- E5. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 2013;14:R36.
- E6. Panettieri RA Jr. Isolation and culture of human airway smooth muscle cells. *Methods Mol Med* 2001;56:155-60.
- E7. Du P, Kibbe WA, Lin SM. lumi: a pipeline for processing Illumina microarray. *Bioinformatics* 2008;24:1547-8.
- E8. Nicodemus-Johnson J, Naughton KA, Sudi J, Hogarth K, Naurekas ET, Nicolae DL, et al. Genome-wide methylation study identifies an IL-13-induced epigenetic signature in asthmatic airways. *Am J Respir Crit Care Med* 2016; 193:376-85.
- E9. Leek JT, Scharpf RB, Bravo HC, Simcha D, Langmead B, Johnson WE, et al. Tackling the widespread and critical impact of batch effects in high-throughput data. *Nat Rev Genet* 2010;11:733-9.
- E10. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 2007;8:118-27.

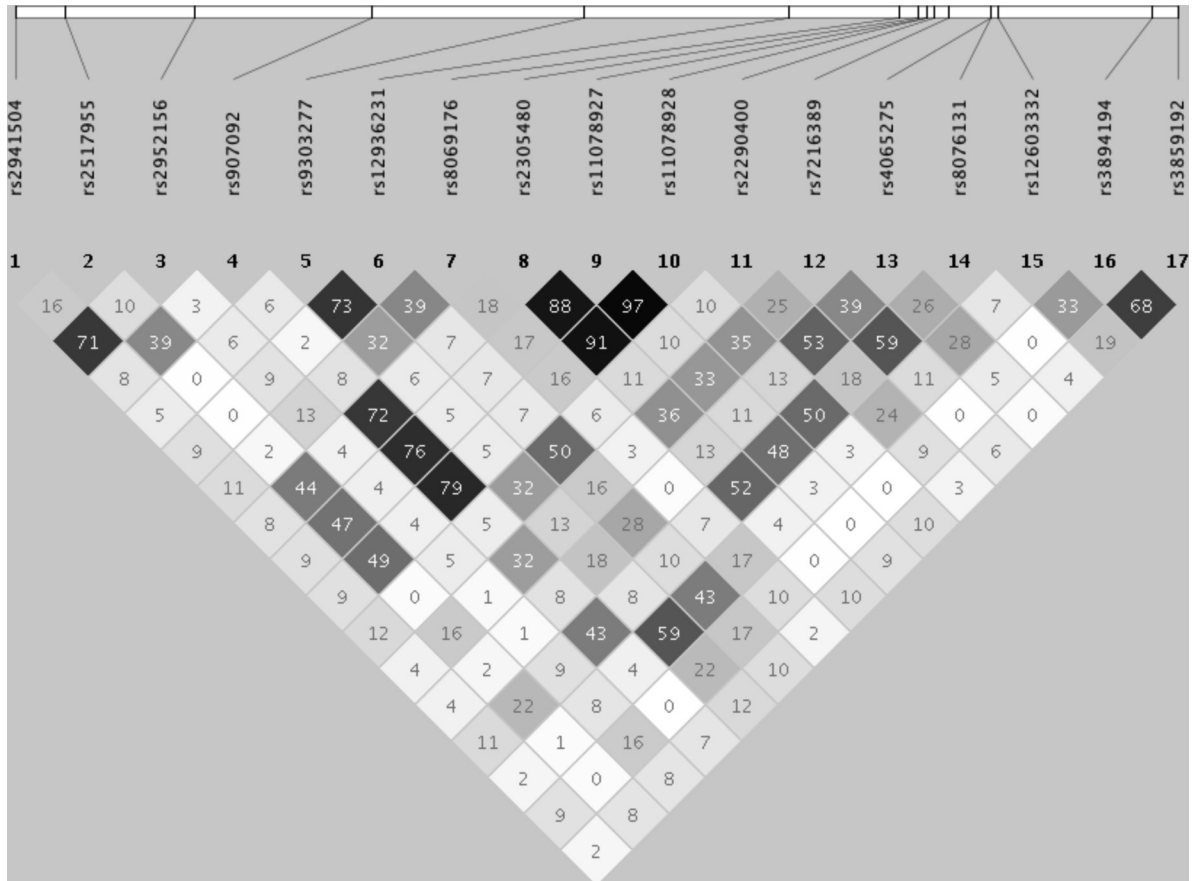


FIG E1. LD (r^2) among the 17 SNPs described in Fig 2 in Nigerians (YRI) by using the 1000 Genomes reference panel.

TABLE E1. Asthma-associated allele frequencies in Nigerians (YRI) from the 1000 Genomes reference panel

SNP	Asthma-associated allele	Frequency	No.
rs2941504	A	0.5	207
rs2517955	C	0.8575	207
rs2952156	A	0.4469	207
rs907092	G	0.8986	207
rs9303277	C	0.372	207
rs12936231	C	0.4227	207
rs8069176	G	0.6377	207
rs2305480	G	0.9058	207
rs11078927	C	0.91063	207
rs11078928	T	0.91304	207
rs2290400	T	0.5266	207
rs7216389	T	0.814	207
rs4065275	G	0.6667	207
rs8076131	A	0.8599	207
rs12603332	C	0.3623	207
rs3894194	A	0.2681	207
rs3859192	T	0.3213	207