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

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Abstract

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.)

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Keywords

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 Moffat et al[1] 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 *PERLD1*)[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 al[36] for an excellent review of functional studies of genes at the 17q locus.

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) metaanalysis,[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 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]

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

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 ETS[28] was replicated in 2 Dutch birth cohorts,[41] Croatian children,[29] and United Kingdom adults[24] but not in the Danish National Birth Cohort[42] 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 study[42] and on asthma at school age in Croatian children[29] 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 $COPSAC_{2000}$ 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 GWAS[56] and other 17q-focused studies[11,33,38] did not. Neither GWASs[57–61] 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 Singapore[11] and Hong Kong.[78–80] Associations are less consistent in smaller studies of Han Chinese from China[81–83] 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 9 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 populations[5] 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 sizeforrs11078927wassimilartothosereportedforthe 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 the17q-associated risk might involve multiplevariants 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 al[2]), 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 adultsfromSingapore.[25] SNP rs3894194 in *GSDMA* was also associated with current asthma, FEV_1, 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 muscle cells (ASMCs; Thompson, unpublished). By using quantitative PCR to measure transcript levels, zona pellucida binding protein 2 (*ZPBP2*) expression was also detected in LCLs[23] 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,aToll-likereceptor3ligandbutnotinresponsetoLPSinany ofthecells.[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 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 inTableI.[1,11–23] Tofurtherevaluatetheeffectsofgenotypeatthe17 SNPs highlighted in this review on the expression levels of the 6 candidate 17q genes, we extracted data from the GenotypeTissue 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 *ZPBP2* ($P = 5.35 \times 10^{-10}$); 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 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 children[34] 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 subjects[20] or in 42 adults with mild-tomoderate 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 al[96] 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 al[23] 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 *ZPBP2* 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 *ZPBP2* 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 *ZPBP2* (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]

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 *ZPBP2* 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 rs4065275A), 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* promoter and enhancer activity in LCLs (CEU), Berlivet et al[99] provided further evidence for an enhancer of *ORMDL3* located in ZPBP2. Acevedo et al[34] 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 al[20] performedgenome-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.

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 expressers 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 Africanderived 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.

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 μL 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 millionuniquely mapped reads underwent trimmedmeans 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.[E5]

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-cm^2 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.

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Abbreviations

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

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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?

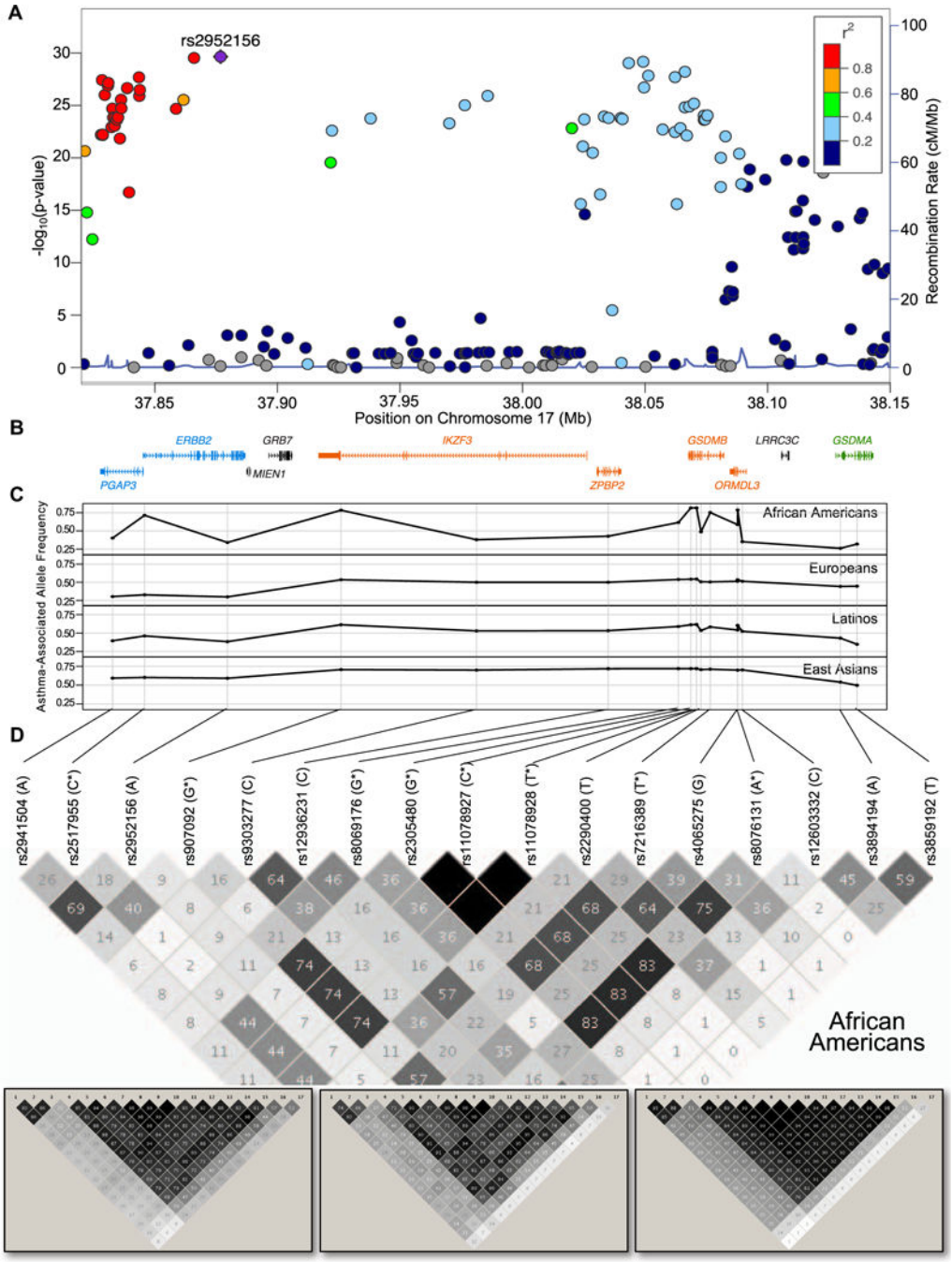

FIG 1.

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

(American of African Ancestry in SW USA [ASW] and African Caribbeans in Barbados [ACB]), Europeans (CEU, British in England and Scotland [GBR], and Toscani 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/.

Location	SNP	bp position	SNP Type	Associations in published studies
PGAP3	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 populatons[14]; eQTL for *PGAP3* in white blood cells[14]
PGAP2	rs2517955	17:37,843,681	Intron (C>T)	eQTL for *ORMDL3* and meQTL for a nearby CpG site in airway epithelial cells[20]
ERBB2	rs2952156	17:37,876,835	Intron (G>A)	Lead SNP in the TAGC GWAS[2]
IKZF3	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]
IKZF3	rs9303277	17: 37,976,469	Intron (T>C)	Lead SNP with childhood onset asthma[27]
ZPBP2	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 *cis*-regulatory elements from interacting with the *ORMDL3* 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]
GSDMB	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 *ORMDL3*, *GSDMB*, *GSDMA*[30]
GSDMB	rs11078927	17:38,064,405	Intron (C>T)	Lead SNP in EVE GWAS[5]
GSDMB	rs11078928	17:38,064,469	Splice variant (c.662T>C)	C allele results in skipping of exon 6 and reduced levels of *GSDMB* transcript[31]
GSDMB	rs2290400	17:38,066,240	Intron (T>C)	Interactive with smoking on asthma risk[24]
GSDMB	rs7216389	17:38,069,949	Intron (T>C)	Lead SNP in first GWAS and eQTL for *ORMDL3* and *GSDMB* in LCLs[1]; associated with early onset asthma[12]; associated with RV-wheezing interaction in early life and eQTL for *ORMDL3* and *GSDMB* in whole blood[32]; lung eQTL for *ORMDL3*, *GSDMB* and *GSDMA*[30]
ORMDL3	rs4065275	17: 38,080,865	Intron (A>G)	G allele creates a CTCF-binding motif associated with increased expression of *ORMDL3*[21]
ORMDL3	rs8076131	17:38,080,912	Promoter (A>G)	Strongest association with protective farming effect[33]
ORMDL3	rs12603332	17:38,082,807	5'UTR (T>C)	meQTL and eQTL for *ORMDL3* and *GSDMB* in blood leukocytes[34]; lead SNP for asthma in Mexican Americans and African Americans among 7 SNPs tested[35]
GSDMA	rs3894194	17: 38,121,993	Missense (c.536G>A, p.Ala18Gln)	Independent GWAS signal for asthma[3, 24]
GSDMA	rs3859192	17: 38,128,648	Intron (C>T)	eQTL for *GSDMA* 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.

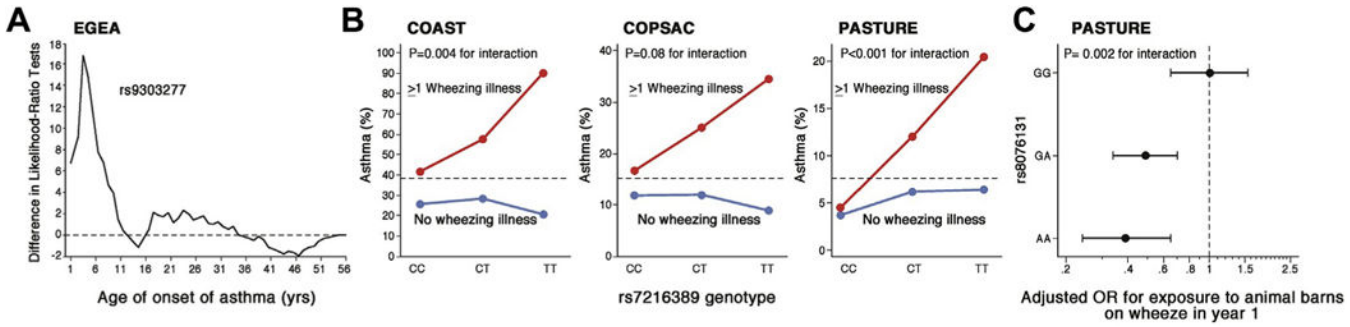

FIG 3.

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 5 297)[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]

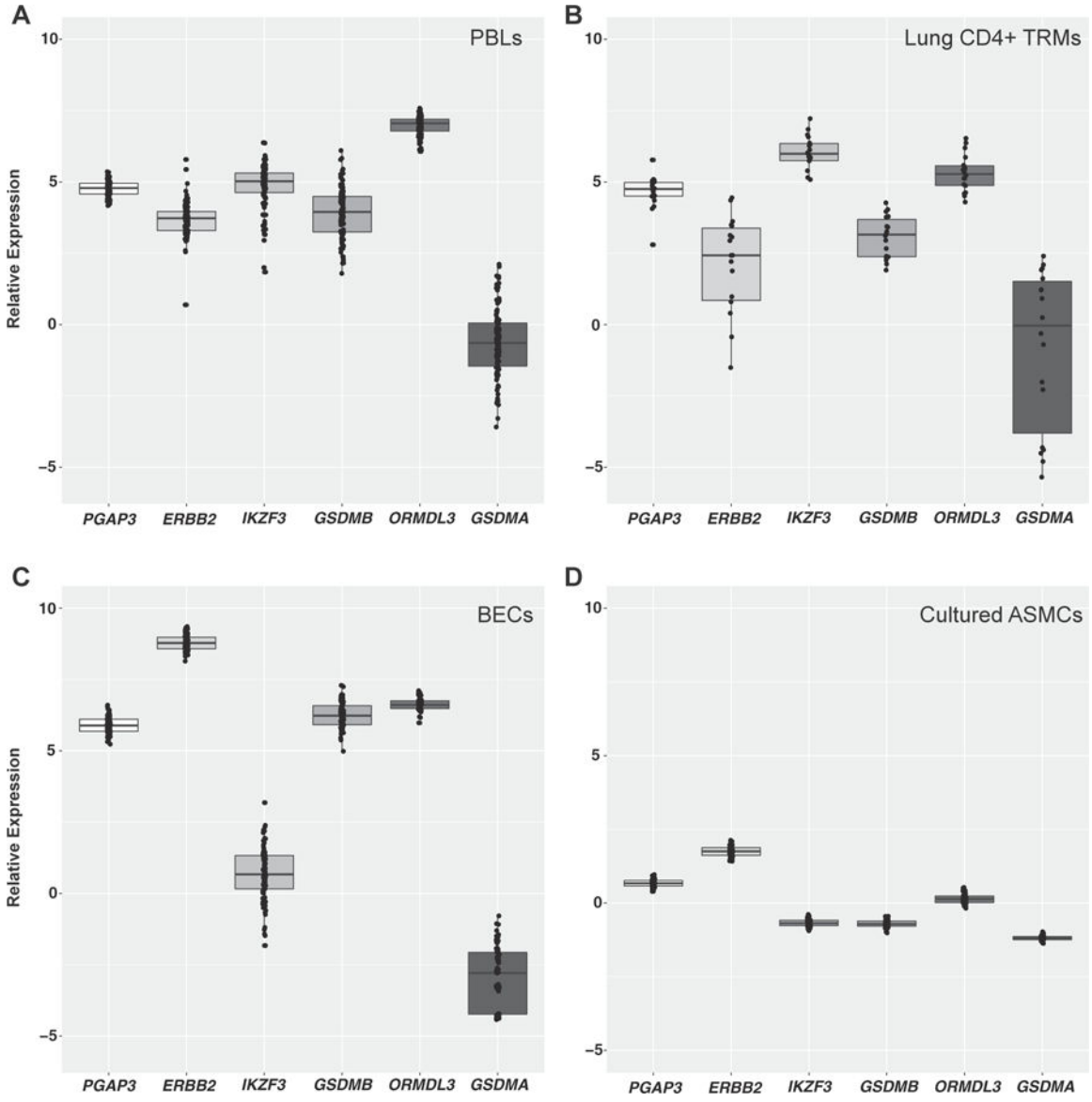

FIG 4.

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* $r = 0.369$, $P = 6.2\ 3\ 10^{-5}$), 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]

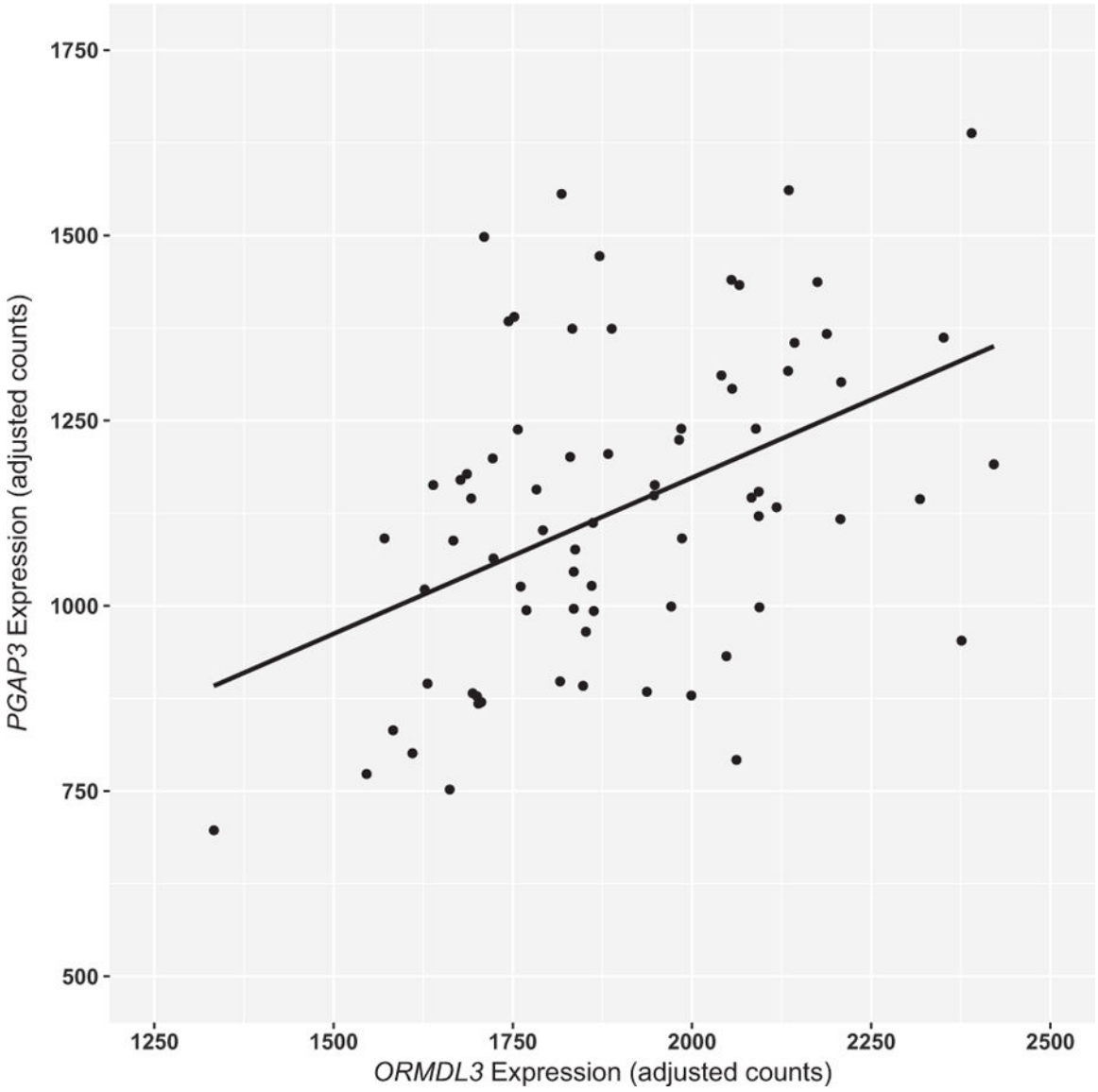

FIG 5.

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]

SNP rsID	Asthma Risk Allele	Whole Blood (n=338)				Lung Tissue (n=278)							
		GSDMB		*ORMDL3*		*PGAP3*		*GSDMB*		*ORMDL3*		*GSDMA*	
		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	
				GSDMB P-value	*ORMDL3* 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) LCLs[93] 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).

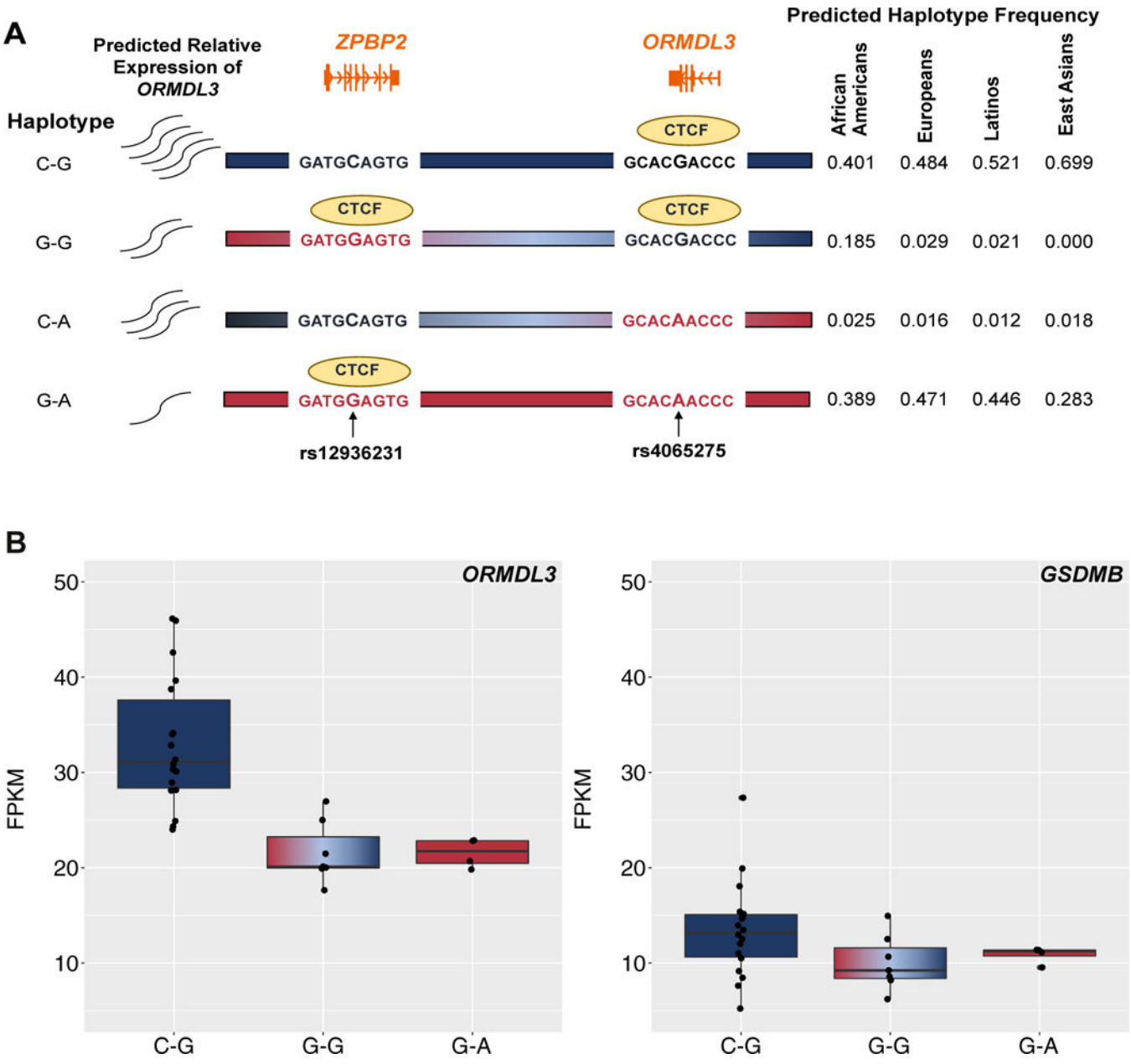

FIG 8.

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 al[21]). Relative expression of *ORMDL3* from the C-A haplotype was inferred from 1 YRI subject who was heterozygous C-A/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 subjects[98] 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.

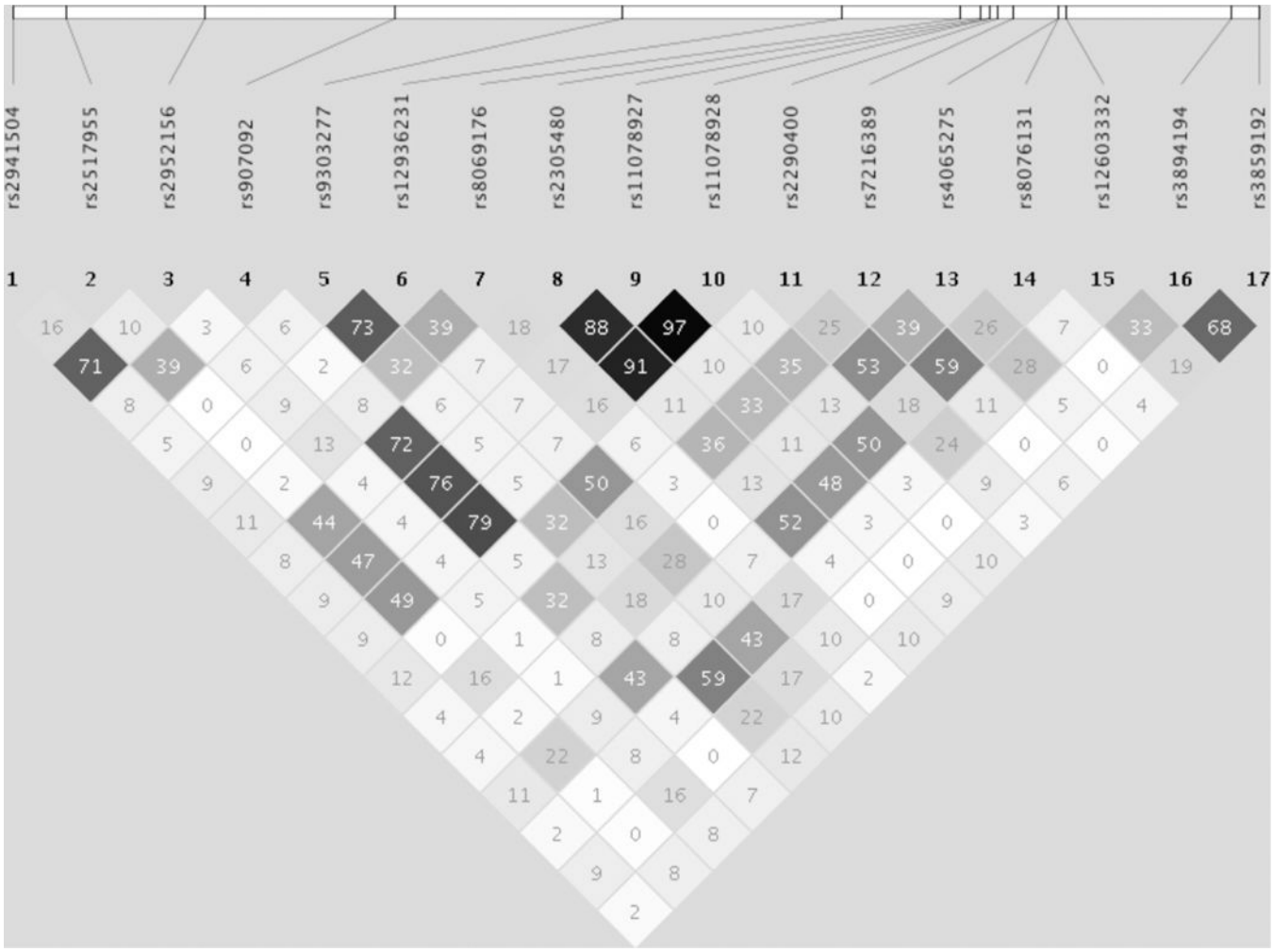

FIG E1.

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

TABLE I.

Published eQTL studies of 17q12–21 SNPs and genes in blood and lung cells

		17q12–21 genes						
Study	**SNP**	***PGAP3*, *P* value**	***ERBB2*, *P* value**	***IKZF3*, *P* value**	***ZPBP2*, *P* value**	***GSDMB*, *P* value**	***ORMDL3*, *P* value**	***GSDMA*, *P* value**
Blood cells								
schmiedel et al [21] (n=34), sorted cells*	rs 7216389					Naive $CD4^+$ T cells: <.05 Treg cells: <.05 T_H1 cells: <.05 B cells: <.01 NK cells : <.01	Naive $CD4^+$ T cells: <.01 Treg cells: <.01 T_H1 cells: <.001 T_H2 cells: <.001 T_H17 cells: <.001 B cells: <.001 NK cells: <.01	
Liu et al [17] (n=19), sorted cells*	rs 7216389						PBMCs: ≤.03 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^{-1} 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	
Çalişkan 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

Study	SNP	17q12–21 genes						
		PGAP3, *P* value	*ERBB2*, *P* value	*IKZF3*, *P* value	*ZPBP2*, *P* value	*GSDMB*, *P* value	*ORMDL3*, *P* value	*GSDMA*, *P* value
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 X 10^{-8}	NS NS
Lung cells								
Nicodemus-Johnson et al[20] (n = 81), BECs ‡	rs2517944 rs8076131 rs7216389 rs2305480 rs11078928 rs2941504 rs2941504 rs4065275	2.1×10^{-8} 8.0×10^{-5} 7.1×10^{-5} NS NS NS 4.7×10^{-7} 2.1×10^{-5}	NS NS NS NS NS NS NS NS	NS NS NS NS NS NS NS NS		NS NS NS NS NS NS NS NS	2.6×10^{-5} 3.2×10^{-5} 8.0×10^{-5} 1.1×10^{-4} 1.2×10^{-4} 1.3×10^{-4} 4.0×10^{-5} 5.5×10^{-5}	NS NS NS NS NS NS NS NS
Hao et[15] (n= 1111), lung tissue †	rs2290400 rs7216389 rs2305480 rs3859192 rs3894194	NS NS NS NS NS	NS NS NS NS NS	NS NS NS NS NS		4.0×10^{-15} 4.2×10^{-14} 2.5×10^{-12} 3.9×10^{-5} 1.6×10^{-7}	NS 1.3 3 10^{-6} 2.1 3 10^{-5} NS NS	NS 8.8×10^{-25} 7.4×10^{-32} 3.6×10^{151} 2.8×10^{-73}
Li et al[16], n =107 for BECs† and n = 94 for BAL fluid †	rs8067378 rs2305480 rs7216389					BECs: 1.9×10^{-3}; BAL fluid: .04 BECs: 4.7×10^{-3}; BAL fluid: NS BECs: 1.1×10^{-3}; BAL fluid: NS	BECs: NS; BAL fluid: NS BECs: NS; BAL fluid: NS BECs: NS; BAL fluid: 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.

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