



Published in final edited form as:

Allergy. 2015 October ; 70(10): 1309–1318. doi:10.1111/all.12683.

eQTL of bronchial epithelial cells and bronchial alveolar lavage deciphers GWAS-identified asthma genes

Xingnan Li¹, Annette T. Hastie¹, Gregory A. Hawkins¹, Wendy C. Moore¹, Elizabeth J. Ampleford¹, Jadranka Milosevic², Huashi Li¹, William W. Busse³, Serpil C. Erzurum⁴, Naftali Kaminski⁵, Sally E. Wenzel², Deborah A. Meyers¹, and Eugene R. Bleeker¹

¹Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

²Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

³Department of Medicine, University of Wisconsin, Madison, Wisconsin, USA

⁴Department of Pathobiology, The Lerner Research Institute, Cleveland, Ohio, USA

⁵Pulmonary, Critical Care and Sleep Medicine, Yale School of Medicine, New Haven, Connecticut, USA

Abstract

Background—Genome-wide association studies (GWASs) have identified various genes associated with asthma, yet, causal genes or single nucleotide polymorphisms (SNPs) remain elusive. We sought to dissect functional genes/SNPs for asthma by combining expression quantitative trait loci (eQTLs) and GWASs.

Methods—*Cis*-eQTL analyses of 34 asthma genes were performed in cells from human bronchial epithelial biopsy (BEC, n = 107) and from bronchial alveolar lavage (BAL, n = 94).

Results—For *TSLP-WDR36* region, rs3806932 (G allele protective against eosinophilic esophagitis) and rs2416257 (A allele associated with lower eosinophil counts and protective against asthma) were correlated with decreased expression of *TSLP* in BAL ($P = 7.9 \times 10^{-11}$ and 5.4×10^{-4} , respectively) and BEC, but not *WDR36*. Surprisingly, rs1837253 (consistently associated with asthma) showed no correlation with *TSLP* expression levels. For *ORMDL3-GSDMB* region, rs8067378 (G allele protective against asthma) was correlated with decreased

Correspondence: Xingnan Li, PhD, Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA, Telephone: 336-713-7514, FAX: 336-713-7566, xinli@wakehealth.edu.

Author contribution: Drs Li, Ampleford, Meyers and Bleeker and Ms Li designed the genetic study and participated in the interpretation of results. Dr. Hawkins was responsible for isolating DNA, establishing the DNA database and genotyping all samples. Dr. Milosevic isolated RNA and with Dr. Kaminski was responsible for performing and analyzing gene expression microarrays on BEC and BAL. Drs Li, Ampleford, Kaminski, Wenzel, Meyers and Bleeker and Ms Li were responsible for the analytical plan including the data base, quality control and data analysis. Drs Moore, Hastie, Busse, Erzurum, Kaminski, Wenzel, Meyers, and Bleeker were responsible for the design of the SARP clinical study, recruitment and characterization of subjects with asthma and interpretation of SARP results. All authors contributed to the writing of the manuscript.

Conflict of interest: W.W. Busse has received consultancy fees from Novartis, GlaxoSmithKline, Genentech, Pfizer and Roche; has received consultancy fees from Circassia for the Data Monitoring Board; has received consultancy fees from Boston Scientific for the Data Monitoring Board and consultancy fees from ICON for the Study Oversight Committee; has received research support from the NIH/NIAID and NIH/NHLBI; and receives royalties from Elsevier.

expression of *GSDMB* in BEC and BAL ($P = 1.3 \times 10^{-4}$ and 0.04) but not *ORMDL3*. rs992969 in the promoter region of *IL33* (A allele associated with higher eosinophil counts and risk for asthma) was correlated with increased expression of *IL33* in BEC ($P = 1.3 \times 10^{-6}$) but not in BAL.

Conclusions—Our study illustrates cell-type-specific regulation of the expression of asthma-related genes documenting SNPs in *TSLP*, *GSDMB*, *IL33*, *HLA-DQB1*, *C11orf30*, *DEXI*, *CDHR3*, and *ZBTB10* affect asthma risk through *cis*-regulation of its gene expression. Whenever possible, disease-relevant tissues should be used for transcription analysis. SNPs in *TSLP* may affect asthma risk through up-regulating *TSLP* mRNA expression or protein secretion. Further functional studies are warranted.

Keywords

asthma susceptibility genes; bronchial alveolar lavage; bronchial epithelial cells; eQTL; GWAS

Introduction

34 genes associated with asthma susceptibility (MIM 610906) have been identified by GWAS (1). Ten of these genes in six major regions have been consistently replicated: ORM1-like 3 and gasdermin B (*ORMDL3-GSDMB*) region (2–4), interleukin 33 (*IL33*) (3–5), interleukin 1 receptor-like 1 and interleukin 18 receptor 1 (*IL1RL1-IL18R1*) region (3–5), RAD50 homolog and interleukin 13 (*RAD50-IL13*) region (3, 6), thymic stromal lymphopoietin and WD repeat domain 36 region (*TSLP-WDR36*) region (3–5, 7), and major histocompatibility complex class II DR/DQ region (*HLA-DR/DQ*) (3, 6, 7). The linkage disequilibrium (LD) structure of these regions makes the task of identifying the actual disease causing genes/SNPs quite difficult.

Most of the trait-associated SNPs identified by GWASs are not coding-change variants (1). Instead, SNPs associated with complex traits are more likely to be associated with expression quantitative trait locus (eQTL) (8). eQTL analysis is an efficient approach to identify functional SNPs regulating expression of disease-associated genes. For example, the first GWAS asthma gene, *ORMDL3*, was identified through the combined approaches of GWAS and eQTL in lymphoblastoid cell lines (LCLs) (2, 9, 10). The purpose of this study was to determine which SNPs, known to confer asthma susceptibility, do so through alteration in mRNA expression levels.

Most eQTLs related to asthma have been performed in LCLs (2, 11), rarely in lung tissues (12). There may be extensive overlap in *cis*-eQTL signals across multiple tissues particularly with regard to housekeeping genes (13, 14). However, a study indicated that the mean levels of genetic correlation for gene expression in LCLs and whole blood were near zero (14). In addition, the expression of non-housekeeping genes were more likely to be tissue-specific than housekeeping genes (14). Thus, we hypothesize that eQTLs in target tissues which physiologically change during disease progression should display differential regulation. Selection of asthma-relevant tissues, such as bronchial epithelial cells (BEC) and bronchial alveolar lavage (BAL), may be required to discover functional variants underlying asthma risk. Therefore, we performed eQTL in asthma-relevant tissues (BEC and BAL) for the first time.

Methods

Study subjects

Subjects with mild to severe asthma and healthy controls recruited at four NHLBI funded Severe Asthma Research Program (SARP) centers were carefully characterized, including baseline spirometry with a medication withhold before testing (15). All studies were approved by the appropriate Institutional Review Boards at the participating sites including appropriate informed consent.

Primary human bronchial epithelial cells and bronchoalveolar lavage cells were obtained as part of the SARP projects from a range of asthmatics and healthy controls by bronchoscopy with endobronchial epithelial brushings and lavage. Sample preparation and array procedures were performed as previously described by us (16–18). Briefly, total RNA was extracted from BEC and BAL suspended in Qiazol solution using the QIACube system (QIAGEN Inc, Valencia, CA, USA). RNA quality was determined using the Agilent Bioanalyzer 2100 (Agilent Technologies Inc, Santa Clara, CA, USA). Complementary RNA (cRNA) labeled with the Cy5 fluorescent dye was hybridized to 4X44K v2 Whole Human Genome Microarrays. The microarrays were scanned using the Agilent Microarray Scanner and the data was extracted using the Agilent Feature Extraction software v9.5.

Genomic DNA was isolated from whole blood using DNA purification kits (QIAGEN Inc, Valencia, CA, USA). SNP genotyping was performed using the Illumina HumanHap1M BeadChip or the Illumina HumanOmniExpress700k BeadChip. Genotyping for all studies was performed using BeadStudio or GenomeStudio (Illumina, Inc., San Diego, CA, USA) (19, 20).

Statistical Analyses

The following 34 candidate genes in 23 regions were selected for analysis based on SNPs achieving genome-wide significance ($P < 5 \times 10^{-8}$) or P -value $< 10^{-6}$ with replication which were reported by NIH GWAS database (<http://www.genome.gov/gwastudies/>) (1) and the published literatures: *ORMDL3-GSDMB*, *IL33*, *IL1RL1-IL18R1*, *RAD50-IL13*, *TSLP-WDR36*, *HLA-DQB1*, *HLA-DPA1*, *TNIP1*, *PDE4D*, *DENND1B*, *SMAD3*, *IL2RB*, *RORA*, *PYHIN1*, *NOTCH4-AGER-C6orf10-PBX2*, *USP38-GAB1*, *GATA3*, *IKZF4-CDK2*, *IL6R*, *C11orf30-LRRC32*, *CDHR3*, *ZBTB10*, and *CLEC16A-DEXT*.

Quality control processes of genotypes were described previously (19, 20). In brief, subjects were removed if they 1) had genotyping call rates $< 95\%$, 2) were discrepant or ambiguous for genetic sex, 3) failed the check for family relatedness ($PI_HAT > 0.25$), or 4) were detected as an outlier (> 6 standard deviations for the first or second principal component generated from whole-genome genotyping data). After subjects meeting these criteria were excluded, SNPs were removed if 1) call rates $< 95\%$, 2) inconsistent with Hardy-Weinberg Equilibrium (HWE) ($P < 10^{-5}$), or 3) minor allele frequency (MAF) < 0.05 .

Gene expression data was normalized using a cyclic loess algorithm authored in the R programming environment using the Bioconductor suite of tools as described (18, 21). The data discussed in this manuscript have been deposited in NCBI's Gene Expression Omnibus

database (GEO), and are accessible through GEO series accession number GSE67940 (<http://www.ncbi.nlm.nih.gov/geo/>) (18). The expression values were cyclic LOESS normalized and log2 transformed. An inverse normalization transformation was applied to the residuals of expression data (adjusted for age, gender, asthma status, and the first and second components from the multidimensional scaling analysis) to remove outliers and normalize the data. A linear additive model was chosen to test association between expression levels and GWAS-identified candidate SNPs and also *cis*-SNPs (within 100kb upstream or downstream of candidate genes or regions) of 34 asthma genes using PLINK software (<http://pngu.mgh.harvard.edu/purcell/plink/>) (22). BEC was used as primary dataset because it is composed primarily of epithelial cells (> 90%). BAL is a mix of cell types, and thus was used as secondary dataset. Multiple tests adjustment was done by 10,000 permutations within candidate genes/regions using label-swapping and max(T) procedures incorporated in PLINK (22). *P*-values < 0.05 after permutations were considered significant for *cis*-SNPs. eQTLs in LCLs of 34 asthma genes were extracted from public available databases of the GABRIEL study (11, 23) or GENEVAR study (24) and compared with our eQTLs in BEC and BAL.

Results

SARP is a cohort enriched for subjects with severe asthma, but also well balanced with mild to moderate asthma and healthy controls (Table 1). After quality control was performed as described, data from 107 and 94 subjects with GWAS and expression data from BEC and BAL, respectively, was analyzed. The population was European Americans (> 60%), African Americans (> 25%), and Hispanics (< 15%) with over 75% asthmatics and greater than 40% severe asthmatics. BAL consists of macrophages, lymphocytes, neutrophils, and eosinophils (Table 2). The percentages of macrophages and neutrophils were lower and higher, respectively, in severe asthma than controls or mild/moderate asthma. The percentage of eosinophils was higher in asthma or severe asthma than controls (Table 2).

To study asthma gene expression in disease-relevant tissues, BEC and BAL were obtained by bronchoscopy with endobronchial epithelial brushings and lavage. The SNPs and expression probes of 34 candidate genes reported by GWASs of asthma are listed in Table 3. One gene may be represented by one or more probes on the microarray. In this study, the probe with the most significant eQTL was used to represent the specific gene (Table 3) (2–7, 19, 25–30). The expression levels of 34 candidate genes were moderately correlated with asthma status (data will be presented in a separate manuscript). In brief, only the expression levels of *IL18R1* were consistently higher in asthma than controls in BEC and BAL ($p < 0.05$).

eQTL analyses of six most consistently replicated regions in asthma

10 genes in six regions were consistently replicated by GWASs at least twice, and thus, these genes were prioritized in this study (Table 4). rs8067378 in *ORMDL3-GSDMB* region was significantly correlated with the expression levels of *GSDMB* in BEC ($P = 1.3 \times 10^{-4}$) and was replicated in BAL ($P = 0.04$). No SNPs were correlated with the expression levels of *ORMDL3* in BEC or BAL. Multiple SNPs (including rs3806932, rs3806933, and

rs2289276) in *TSLP-WDR36* region were significantly correlated with the expression levels of *TSLP* but not *WDR36* in BEC and BAL. Our results indicated that *GSDMB* and *TSLP* were more likely to be functional genes in *ORMDL3-GSDMB* and *TSLP-WDR36* regions in BEC and BAL, respectively. SNPs in the promoter region of *IL33* (including rs992969 and rs3939286) were significantly correlated with the expression levels of *IL33* only in BEC. rs1063355, located at 3' UTR of *HLA-DQB1*, was significantly correlated with the expression levels of *HLA-DQB1* in BEC. rs12999517, located in the intron of *IL1RL1*, was significantly correlated with the expression levels of *IL1RL1* in BAL. No SNPs were correlated with the expression levels of *IL18R1*, *IL13*, or *RAD50*.

eQTL analyses of 24 other asthma genes

24 genes in 17 regions were identified by GWAS just once (Table 3 and Table 5). Six SNPs (rs2513525, etc.) in *C11orf30-LRRC32* region were significantly correlated with the expression levels of *C11orf30* in BAL but not in BEC (Table 5). rs2513525 was in moderate LD ($r^2=0.35$) with rs7130588 which was identified through GWAS of asthma (28). No SNPs were correlated with the expression levels of *LRRC32* in BEC or BAL. Our results indicated that *C11orf30* was more likely to be functional gene in *C11orf30-LRRC32* region. Similarly, rs12919083, in the intron of *CLEC16A*, was correlated with the expression levels of *DEXT* in BAL, but not *CLEC16A* (Table 5). rs12919083 was in moderate LD ($r^2=0.34$) with rs62026376 which was identified through GWAS of asthma with hay fever (30). Our results indicated that *DEXT* was more likely to be the functional gene in the *CLEC16A-DEXT* region, as previously reported (30). eQTL SNPs were identified for *CDHR3* (rs17152490) and *ZBTB10* (rs1543857) in BEC, and these eQTL SNPs were in LD with the GWAS SNPs (rs6967330 in *CDHR3* (29) and rs7009110 in *ZBTB10* (30), respectively). eQTL SNPs were also identified for *TNIP1* (rs871269), *NOTCH4* (rs2071279), and *SMAD3* (rs4776890); however, these eQTL SNPs were not in LD with the GWAS SNPs (rs10036748, rs404860, and rs744910 for *TNIP1* (19), *NOTCH4* (7), and *SMAD3* (3), respectively). No eQTL SNPs were identified for the other 17 genes (*HLA-DPA1*, *PDE4D*, *DENND1B*, *IL2RB*, *RORA*, *PYHIN1*, *AGER-C6orf10-PBX2*, *USP38-GAB1*, *GATA3*, *IKZF4-CDK2*, *IL6R*, *LRRC32*, and *CLEC16A*).

Cell-type-specific eQTL

eQTLs of 34 asthma genes in BEC, BAL, and LCLs (11, 23, 24) are summarized in Table 6. 15 GWAS SNPs for 17 genes (*TSLP-WDR36*, *SMAD3*, *PDE4D*, *DENND1B*, *IL2RB*, *RORA*, *PYHIN1*, *NOTCH4*, *USP38-GAB1*, *GATA3*, *IL6R*, *TNIP1*, *C11orf30-LRRC32*, and *CLEC16A*) were not eQTL SNPs in BEC, BAL, or LCLs. 15 GWAS SNPs for 17 genes (*ORMDL3-GSDMB*, *IL33*, *IL1RL1-IL18R1*, *RAD50-IL13*, *HLA-DQB1*, *HLA-DPA1*, *AGER-C6orf10-PBX2*, *IKZF4-CDK2*, *CDHR3*, *ZBTB10*, and *DEXT*) were eQTL SNPs, but not consistently observed in BEC, BAL, and LCLs. For example, rs992969 was correlated with the expression levels of *IL33* in BEC, but not in BAL and LCLs. Our study shows that eQTLs of asthma-related genes in BEC, BAL, and LCLs often do not overlap.

Discussion

In this study, we performed eQTL analysis in asthma-relevant tissues (BEC and BAL) for the first time, and compared our eQTL results with published eQTL in LCLs. eQTLs of asthma-related genes in BEC, BAL, and LCLs often differed (Table 6), indicating that the expression of asthma-associated genes are cell-type-specific. Tissue-specific transcriptional regulation is not uncommon. Previous studies have shown tissue-specific or cell-type specific eQTLs between whole blood and LCLs (14), among blood, liver, subcutaneous tissue, visceral adipose tissue, and skeletal muscle (31), and among primary fibroblasts, T cells and LCLs (32). In addition, SNPs associated with complex diseases more often affect gene expression in a tissue-dependent manner (31).

The *ORMDL3-GSDMB* region (including rs7216398 and rs2305480 (2, 3)) is the most reproduced loci associated with asthma susceptibility. Identification of functional genes/ SNPs in *ORMDL3-GSDMB* region is difficult due to very strong and long LD in this region and co-expression of *ORMDL3* and *GSDMB* in LCLs. An allele-specific chromatin remodeling study indicated that rs12936231 and rs8067378 (in complete LD) might be functional SNPs (9). In our study, rs8067378 was identified as the most significant eQTL SNP for *GSDMB* in BEC ($P = 1.3 \times 10^{-4}$) and BAL ($P = 0.04$). However there was no correlation with *ORMDL3* in either BEC or BAL (Table 4). The G allele of rs8067378 (protective against asthma (2–4)) was correlated with decreased expression of *GSDMB*, implying that A allele is the risk allele for asthma by up-regulating the expression of *GSDMB*. A recent study indicated that *ORMDL3* or *GSDMB* might affect childhood asthma through interaction with human rhinovirus wheezing illnesses (10). BEC provides the first line of defense against viral/bacterial infection. SNPs in this region were correlated with the expression of *GSDMB* in BEC but not *ORMDL3*, indicating that *GSDMB* is more likely to be the functional gene in this process.

IL33 may sense the damage of epithelial cells, induce the expression of Th2-type cytokines, and lead to eosinophil infiltration into the airway. SNPs in *IL33* have been reported to be associated with eosinophil counts in blood (rs3939286) and asthma (rs992969) (3, 5). All asthma-associated SNPs in *IL33* region are located in the 5' or first intron, making them good candidates for eQTL SNPs; however, no published data support this speculation (33). Our study showed for the first time that SNPs in the promoter region of *IL33* (including rs3939286 and rs992969) were correlated with *IL33* expression in BEC but not in BAL, confirming its 'alarmin' role in BEC (Table 4). We further tested the correlation between rs3939286 and blood eosinophil percentage using 107 SARP subjects with BEC data. The A allele of rs3939286 was associated with increased eosinophil percentage in blood with p value of 0.0077 (GG: 2.88%; AG: 3.92%; AA: 4.94%). The A allele of rs3939286 may be associated with higher eosinophil counts and affect asthma risk by up-regulating *IL33* expression. Published eQTL analyses showed that rs996929 was not correlated with *IL33* expression in LCLs (Table 5) (23, 24), emphasizing the importance to perform eQTL in disease-relevant tissues. rs1420101 and rs3771166 in *IL1RL1-IL18* region are associated with eosinophil counts in blood and asthma (3, 5). In this study, a different SNP, rs12999517 was correlated with *IL1RL1* (the receptor of *IL33*) expression in BAL (Table 4), which was not in LD with asthma or eosinophil associated SNPs.

SNPs in *TSLP-WDR36* region are associated with eosinophil counts in blood (rs2416257) (5), eosinophilic esophagitis (rs3806932) (34), and asthma (rs1837253) (4, 7). We found multiple SNPs correlated with the expression levels of *TSLP* but not *WDR36* in both BEC and BAL (Table 4). Decreased expression of *TSLP* in BAL was correlated with the G allele of rs3806932 (protective against eosinophilic esophagitis) and the A allele of rs2416257 (associated with lower eosinophil counts and protective against asthma) at *P*-values of 7.9×10^{-11} and 5.4×10^{-4} , respectively. rs1837253, which is most consistently associated with asthma (4, 5, 7), and correlated with the secretion levels of TSLP protein from human nasal epithelial cells (35), was not correlated with *TSLP* mRNA expression levels, nor was it in LD with any other SNPs in this region. Our findings and previous evidence suggest that eosinophilic or atopic asthma may be associated with up-regulation of *TSLP* mRNA expression while asthma may also be affected via mechanisms other than *TSLP* mRNA expression, such as the regulation of TSLP protein secretion (35).

eQTL is a good approach for detecting SNPs regulating gene expression; however, other mechanisms that do not require changes in gene expression, such as protein structure changes and post-translational modification, will also be important for the manifestation and progression of disease. Thus, the aim of this study is to identify functional SNPs through *cis*-eQTL, not to exclude the potential candidate asthma genes simply based on the negative findings of eQTLs. The purpose of this study was to determine which SNPs associated with asthma susceptibility were also correlated with changes in mRNA expression in tissues which are important in asthma physiology. Since a relatively small sample size was available, exploration of *trans*-eQTLs was not performed and provided the rationale for analyzing only *cis*-eQTLs for known asthma genes. The expression data from different ethnic groups with or without asthma were included in this study to increase the sample size and power (Table 1). We have adjusted gene expression levels with asthma status and the first and second principal components generated from GWAS data to reduce the influence of ethnicity and disease status. The GWAS-driven focus of this work refines and identifies the subset of genes/SNPs which should be analyzed by reporter gene and quantitative PCR in the future.

BEC, the first line of defense against viral/bacterial infection, recognizes allergens and initiates airway inflammation. For example, we observed eQTL SNPs of *IL33* (alarmin to induce Th2 pathway) and *HLA-DQB1* (allergen recognition) in BEC but not in BAL. BAL consists of macrophages, lymphocytes, neutrophils, and eosinophils, and reflects current inflammatory process in the airway (Table 2). Since the composition of BAL is mixed cell types and may vary based on disease status, the interpretation of findings from BAL is not as straight forward. We identified eQTL SNPs of *TSLP* in both BEC and BAL and observed a much stronger effect size in BAL, suggesting that TSLP is involved in the early induction of Th2 pathway in BEC and highly induced in inflammatory cells in BAL. Unfortunately, we can not find additional expression datasets from BEC and BAL with available genotyping information to replicate our findings. Future replication studies are necessary to confirm our findings. Furthermore, other tissues such as bronchial smooth muscle are also asthma-relevant and may have different expression patterns as what we have observed in BEC and BAL.

Some asthma genes show cell-type-specific regulation of expression, and thus it is essential to study gene expression in disease-relevant tissues whenever possible. SNPs in *IL33*, *GSDMB*, *TSLP*, *HLA-DQB1*, *C11orf30*, *DEXT*, *CDHR3*, and *ZBTB10* identified through GWASs affect asthma risk through *cis*-regulation of gene expression. For *TSLP*, two distinct mechanisms may affect asthma risk: one involving regulation of *TSLP* mRNA expression while the other may regulate TSLP protein secretion. The identification of functional genes/SNPs may help to gain understanding of molecular mechanisms underlying disease and target new therapeutic approaches, and thus, facilitate ‘personalized medicine’ for complex diseases. Such findings stimulate further functional studies of asthma genes in post-GWAS era.

Acknowledgments

Funding: Genetic studies for SARP were funded by NIH HL87665 and Go Grant RC2HL101487.

References

1. Hindorf LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci*. 2009; 106:9362–9367. [PubMed: 19474294]
2. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature*. 2007; 448:470–473. [PubMed: 17611496]
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; 363:1211–1221. [PubMed: 20860503]
4. 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; 43:887–892. [PubMed: 21804549]
5. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadóttir A, Sulem P, Jonsdóttir GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet*. 2009; 41:342–347. [PubMed: 19198610]
6. Li X, Howard TD, Zheng SL, Haselkorn T, Peters SP, Meyers DA, et al. Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. *J Allergy Clin Immunol*. 2010; 125:328–335. [PubMed: 20159242]
7. Hirota T, Takahashi A, Kubo M, Tsunoda T, Tomita K, Doi S, et al. Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. *Nat Genet*. 2011; 43:893–896. [PubMed: 21804548]
8. Nicolae DL, Gamazon E, Zhang W, Duan S, Dolan ME, Cox NJ. Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. *PLoS Genet*. 2011; 6:e1000888. [PubMed: 20369019]
9. Verlaan DJ, Berlivet S, Hunninghake GM, Madore AM, Larivière M, Moussette S, et al. Allele-specific chromatin remodeling in the ZPBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet*. 2009; 85:377–393. [PubMed: 19732864]
10. Cali kan 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; 368:1398–1407. [PubMed: 23534543]
11. 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; 39:1202–1207. [PubMed: 17873877]
12. Hao K, Bosse Y, Nickle DC, Paré PD, Postma DS, Laviolette M, et al. Lung eQTLs to help reveal the molecular underpinnings of asthma. *PLoS Genet*. 2012; 8:e1003029. [PubMed: 23209423]

13. Ding J, Gudjonsson JE, Liang L, Stuart PE, Li Y, Chen W, et al. Gene expression in skin and lymphoblastoid cells: refined statistical method reveals extensive overlap in cis-eQTL signals. *Am J Hum Genet.* 2010; 87:779–789. [PubMed: 21129726]
14. Powell JE, Henders AK, McRae AF, Wright MJ, Martin NG, Dermitzakis ET, et al. Genetic control of gene expression in whole blood and lymphoblastoid cell lines is largely independent. *Genome Res.* 2012; 22:456–466. [PubMed: 22183966]
15. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the severe asthma research program. *Am J Respir Crit Care Med.* 2010; 181:315–323. [PubMed: 19892860]
16. Cabrera S, Selman M, Lonzano-Bolaños A, Konishi K, Richards TJ, Kaminski N, et al. Gene expression profiles reveal molecular mechanisms involved in the progression and resolution of bleomycin-induced lung fibrosis. *Am J Physiol Lung Cell Mol Physiol.* 2013; 304:L593–601. [PubMed: 23457188]
17. Herazo-Maya JD, Noth I, Duncan SR, Kim S, Ma SF, Tseng GC, et al. Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. *Sci Transl Med.* 2013; 5:205ra136.
18. Voraphani N, Gladwin MT, Contreras AU, Kaminski N, Tedrow JR, Milosevic J, et al. An airway epithelial iNOS-DUOX2-thyroid peroxidase metabolome drives Th1/Th2 nitrate stress in human severe asthma. *Mucosal Immunol.* 2014; 7:1175–1185. [PubMed: 24518246]
19. 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; 130:861–868. [PubMed: 22694930]
20. Li X, Hawkins GA, Ampleford EJ, Moore WC, Li H, Hastie AT, et al. Genome-wide association study identifies TH1 pathway genes associated with lung function in asthmatic patients. *J Allergy Clin Immunol.* 2013; 132:313–320. [PubMed: 23541324]
21. Wu W, Dave N, Tseng GC, Richards T, Xing EP, Kaminski N. Comparison of normalization methods for CodeLink Bioarray data. *BMC Bioinformatics.* 2005; 6:309. [PubMed: 16381608]
22. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. Plink: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81:559–575. [PubMed: 17701901]
23. Li L, Kabesch M, Bouzigon E, Demenais F, Farrall M, Moffatt MF, et al. Using eQTL weights to improve power for genome-wide association studies: a genetic study of childhood asthma. *Front Genet.* 2013; 4:103. [PubMed: 23755072]
24. Stranger BE, Montgomery SB, Dimas AS, Parts L, Stegle O, Ingle CE, et al. Patterns of cis regulatory variation in diverse human populations. *PLoS Genet.* 2012; 8:e1002639. [PubMed: 22532805]
25. Himes BE, Hunninghake GM, Baurley JW, Rafaels NM, Sleiman P, Strachan DP, et al. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. *Am J Hum Genet.* 2009; 84:581–593. [PubMed: 19426955]
26. Sleiman PM, Flory J, Imielinski M, Bradfield JP, Annaiah K, Willis-Owen SA, et al. Variants of DENND1B associated with asthma in children. *N Engl J Med.* 2010; 362:36–44. [PubMed: 20032318]
27. 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. [PubMed: 21814517]
28. Ferreira MA, Matheson MC, Duffy DL, Marks GB, Hui J, Le Souëf P, et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet.* 2011; 378:1006–1014. [PubMed: 21907864]
29. 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; 46:51–55. [PubMed: 24241537]
30. Ferreira MA, Matheson MC, Tang CS, Granell R, Ang W, Hui J, et al. Genome-wide association analysis identifies 11 risk variants associated with the asthma with hay fever phenotype. *J Allergy Clin Immunol.* 2014; 133:1564–1571. [PubMed: 24388013]

31. Fu J, Wolfs MG, Deelen P, Westra HJ, Fehrmann RS, Te Meerman GJ, et al. Unraveling the regulatory mechanisms underlying tissue-dependent genetic variation of gene expression. *PLoS Genet.* 2012; 8:e1002431. [PubMed: 22275870]
32. Dimas AS, Deutsch S, Stranger BE, Montgomery SB, Borel C, Attar-Cohen H, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. *Science.* 2009; 325:1246–1250. [PubMed: 19644074]
33. Grotenboer NS, Ketelaar ME, Koppelman GH, Nawijn MC. Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology. *J Allergy Clin Immunol.* 2013; 131:856–865. [PubMed: 23380221]
34. Rothenberg ME, Spergel JM, Sherrill JD, Annaiah K, Martin LJ, Cianferoni A, et al. Common variants at 5q22 associated with pediatric eosinophilic esophagitis. *Nat Genet.* 2010; 42:289–291. [PubMed: 20208534]
35. Hui CC, Yu A, Heroux D, Akhabir L, Sandford AJ, Neighbour H, et al. Thymic stromal lymphopoietin (TSLP) secretion from human nasal epithelium is a function of TSLP genotype. *Mucosal Immunol.* 2014;10.1038/mi.2014.126

Table 1

Demographics of subjects with both GWAS data and expression data in BEC or BAL cells

	Bronchial epithelial cells	Bronchial alveolar lavage
N	107	94
Age (y)	37.1 ± 12.6	33.6 ± 12.3
Sex (% female)	68	61
Race (non-Hispanic white/African American/others)	63/29/15	59/23/12
Asthma (Yes/No)	88/19	66/28
ATS classification (Mild/Moderate/Severe)	31/18/39	28/10/28
FEV ₁ (%)	76.8 ± 22.4	84.4 ± 21.6
FVC (%)	86.7 ± 18.0	92.0 ± 17.2
FEV ₁ /FVC	0.72 ± 0.12	0.75 ± 0.12
Log total IgE (geometric mean)	1.93 ± 0.75 (85.5)	1.71 ± 0.93 (51.1)

Table 2

The composition of cell types in BAL

	Controls* (n=28)	Mild/moderate* (n=37)	Severe* (n=28)	Cases* (n=65)	P value	
					Controls vs. Cases	Controls vs. Severe Mild/moderate vs. Severe
Macrophages (%)	86.5 (8.69)	88.2 (7.69)	80.7 (12.1)	84.9 (10.4)	0.48	0.043 0.0034
Lymphocytes (%)	9.36 (6.10)	8.56 (6.82)	10.7 (8.95)	9.48 (7.81)	0.94	0.51 0.28
Neutrophils (%)	3.61 (4.19)	2.00 (1.62)	6.42 (6.61)	3.91 (4.98)	0.79	0.063 0.0002
Eosinophils (%)	0.40 (0.66)	1.07 (2.06)	2.17 (3.55)	1.55 (2.83)	0.038	0.012 0.12

* Mean (standard deviation) of percentage of different cell types in BAL was reported.

Table 3

List of SNPs and probes of 34 genes identified through GWAS of asthma

Gene	Chr	GWAS (reported, ref.*)	Population (reported)	SNP (reported)	SNP (tested)	Probe (tested)
<i>ORMDL3</i>	17	2	European	rs7216389	rs7216389	A_23_P38190
<i>IL1RL1</i>	2	5	European	rs1420101	rs1420101	A_23_P51126
<i>WDR36</i>	5	5	European	rs2416257	rs2416257	A_23_P110598
<i>PDE4D</i>	5	25	European American	rs1588265	rs1588265	A_23_P124456
<i>DENND1B</i>	1	26	European American, African American	rs2786098	rs2786098	A_23_P201605
<i>RAD50</i>	5	6	European American	rs2244012	rs2244012	A_24_P226198
<i>IL13</i>	5	6	European American	rs20541	rs20541	A_23_P251031
<i>HLA-DQB1</i>	6	6	European American	rs1063355	rs1063355	A_23_P8108
<i>GSDMB</i>	17	3	European	rs2305480	rs2305480	A_23_P66454
<i>IL33</i>	9	3	European	rs1342326	rs992969 (r ² =0.58, CEU)	A_23_P31945
<i>IL18R1</i>	2	3	European	rs3771166	rs3771166	A_23_P39735
<i>SMAD3</i>	15	3	European	rs744910	rs744910	A_23_P48936
<i>IL2RB</i>	22	3	European	rs2284033	rs2284033	A_24_P203000
<i>RORA</i>	15	3	European	rs11071559	rs9920560 (r ² =0.94, CEU)	A_23_P26124
<i>HLA-DPA1</i>	6	27	Japanese	rs987870	rs987870	A_23_P30913
<i>TSLP</i>	5	4	European American, African American, Hispanic	rs1837253	rs1837253	A_23_P121987
<i>PYHIN1</i>	2	4	African American	rs1101999	rs856090 (r ² =0.64, YRI)	A_23_P365834
<i>NOTCH4</i>	6	7	Japanese	rs404860	rs404860	A_23_P365614
<i>AGER</i>	6	7	Japanese	rs204993	rs204993	A_23_P93360
<i>C6orf10</i>	6	7	Japanese	rs3129943	rs3129943	A_23_P136734
<i>PBX2</i>	6	7	Japanese	rs204993	rs204993	A_23_P214658
<i>IKZF4</i>	12	7	Japanese	rs1701704	rs1701704	A_23_P358904
<i>CDK2</i>	12	7	Japanese	rs2069408	rs2069408	A_23_P98898
<i>USP38</i>	4	7	Japanese	rs7686660	rs7686660	A_23_P44734
<i>GAB1</i>	4	7	Japanese	rs3805236	rs3805236	A_23_P18505
<i>GATA3</i>	10	7	Japanese	rs10508372	rs10508372	A_23_P75056
<i>IL6R</i>	1	28	European	rs4129267	rs4129267	A_24_P379413
<i>LRRC32</i>	11	28	European	rs7130588	rs7130588	A_24_P389916

Gene	Chr	GWAS (reported, ref. *)	Population (reported)	SNP (reported)	SNP (tested)	Probe (tested)
<i>C11orf30</i>	11	28	European	rs7130588	rs7130588	A_23_P380834
<i>TNIP1</i>	5	19	European American	rs10036748	rs10036748	A_23_P19036
<i>CDHR3</i>	7	29	European	rs6967330	rs17152490 ($r^2=0.58$, CEU)	A_32_P204239
<i>ZBTB10</i>	8	30	European	rs7009110	rs1543857 ($r^2=0.97$, CEU)	A_24_P64071
<i>CLEC16A</i>	16	30	European	rs62026376	rs12919083 ($r^2=0.34$, CEU)	A_32_P194246
<i>DEXI</i>	16	30	European	rs62026376	rs12919083 ($r^2=0.34$, CEU)	A_24_P144377

* The first GWAS studies reported the novel genes/SNPs were cited and GWAS studies were in chronological order.

Table 4

eQTL of six most consistently replicated regions for asthma susceptibility

SNP	Gene	Chr	Location	Distance to Gene	Minor Allele	MAF	GSDMB			ORMDL3		
							BEC	BAL	BEC	BAL	BEC	BAL
							Beta* P-value (adjusted)	Beta* P-value (adjusted)	Beta* P-value (adjusted)	Beta* P-value (adjusted)	Beta* P-value (adjusted)	Beta* P-value (adjusted)
rs8067378	GSDMB	17	3'	-9500	G	0.50	-0.52 1.3x10 ⁻⁴ (1.9x10 ⁻³)	-0.35 0.04	-0.05 0.74	0.11 0.52		
rs2305480	GSDMB	17	coding	96_42	T	0.35	-0.45 4.4x10 ⁻⁴ (4.7x10 ⁻³)	-0.23 0.12	0.15 0.27	-0.05 0.75		
rs7216389	GSDMB	17	intron	-1199	C	0.39	-0.43 1.1x10 ⁻³ (0.01)	-0.24 0.10	0.07 0.62	0.00 0.99		
WDR36												
rs1837253	TSLP	5	5'	-5518	T	0.26	-0.24 0.15	-0.03 0.86	-0.07 0.69	0.05 0.80		
rs3806932	TSLP	5	5'	-1715	G	0.50	-0.39 2.3x10 ⁻³ (0.05)	-0.85 7.9x10 ⁻¹¹ (1.0x10 ⁻⁴)	-0.07 0.61	-0.04 0.76		
rs3806933	TSLP	5	5'	-648	T	0.36	-0.43 7.8x10 ⁻⁴ (0.02)	-0.82 1.5x10 ⁻⁹ (1.0x10 ⁻⁴)	-0.06 0.66	-0.01 0.94		
rs2289276	TSLP	5	5UTR	117_81	T	0.24	-0.51 5.2x10 ⁻⁴ (0.01)	-0.66 2.7x10 ⁻⁵ (8.0x10 ⁻⁴)	-0.21 0.15	-0.09 0.57		
rs2416257	WDR36	5	intron	-798	A	0.12	-0.17 0.44	-0.81 5.5x10 ⁻⁴ (0.02)	0.29 0.20	0.20 0.41		
RAD50												
rs22444012	RAD50	5	intron	-6166	C	0.38	-0.21 0.11	0.19 0.18	0.10 0.47	0.05 0.71		
rs20541	IL13	5	coding	97_10	T	0.25	0.05 0.76	0.32 0.07	0.17 0.27	0.12 0.48		
IL18R1												
rs1420101	IL1RL1	2	coding	68_101	A	0.38	-0.16 0.30	-0.22 0.21	0.02 0.88	-0.10 0.57		
rs12999517	IL1RL1	2	intron	-236	C	0.14	-0.02 0.90	-0.68 4.4x10 ⁻⁴ (9.5x10 ⁻³)	0.33 0.08	-0.12 0.56		
rs3771166	IL18R1	2	intron	-1694	T	0.41	0.15 0.27	-0.14 0.31	-0.16 0.23	-0.06 0.68		
IL33												
rs992969	IL33	9	5'	-31981	A	0.27	0.69 1.3x10 ⁻⁶ (1.0x10 ⁻⁴)	0.11 0.53				
rs3939286	IL33	9	5'	-31579	A	0.33	0.50 2.0x10 ⁻⁴ (5.9x10 ⁻³)	0.21 0.18				
HLA-DQB1												

SNP	Gene	Chr	Location	Distance to Gene	Minor Allele	MAF	GSDMB				ORMDL3			
							Beta *	P-value (adjusted)	Beta *	P-value (adjusted)	Beta *	P-value	Beta *	P-value
rs1063355	HLA-DQB1	6	3UTR	50_298	A	0.46	0.63	8.0x10 ⁻⁷ (1.0x10 ⁻⁴)	0.05					0.71

* Beta is the correlation coefficient of SNP vs. gene expression value on the basis of minor allele from linear additive model.

Table 5

eQTL of 7 of 24 other asthma genes (with significant eQTL)

SNP	Gene	Location	Distance to Gene	Chr	Position	Major Allele	Minor Allele	MAF	TNIP1		
									BEC	BAL	
									Beta*	P-value (adjusted)	Beta* P-value (adjusted)
rs10036748	<i>TNIP1</i>	intron	-2295	5	150458146	C	T	0.43	0.02	0.87	0.04
rs871269	<i>TNIP1</i>	intron	-568	5	150432388	C	T	0.38	0.10	0.49	1.5x10 ⁻³ (0.03)
NOTCH4											
rs404860	<i>NOTCH4</i>	intron	-377	6	32184345	T	C	0.2103	0.21	0.22	0.11
rs2071279	<i>NOTCH4</i>	intron	-25	6	32164874	G	T	0.28	-0.13	0.37	3.3x10 ⁻⁴ (0.05)
C11orf30											
rs4300410	<i>C11orf30</i>	intron	-150	11	76163151	C	T	0.43	-0.08	0.52	-0.48
rs10793169	<i>C11orf30</i>	intron	-346	11	76164012	G	A	0.43	-0.08	0.52	-0.48
rs4245443	<i>C11orf30</i>	intron	-40	11	76183924	G	A	0.42	-0.09	0.51	-0.46
rs2508740	<i>C11orf30</i>	intron	-4	11	76227182	A	G	0.39	-0.04	0.75	-0.45
rs2513513	<i>C11orf30</i>	3'UTR	342_1056	11	76261533	G	A	0.42	-0.08	0.52	-0.44
rs2513525	<i>C11orf30</i>	3'	-4119	11	76266708	C	A	0.42	-0.06	0.62	-0.43
rs7130588	<i>C11orf30</i>	3'	-8094	11	76270683	A	G	0.32	-0.23	0.11	-0.26
SMAD3											
rs4776890	<i>SMAD3</i>	intron	-34347	15	67393045	T	G	0.36	0.48	1.0x10 ⁻⁴ (5.3x10 ⁻³)	-0.06
rs744910	<i>SMAD3</i>	intron	-10448	15	67446785	G	A	0.40	0.09	0.57	0.11
CDHR3											
rs17152490	<i>CDHR3</i>	3'	-3361	7	105677579	G	A	0.14	0.52	0.01	-0.08
ZBTB10											
rs1543857	<i>ZBTB10</i>	5'	-103224	8	81295224	A	G	0.48	-0.32	0.02	-0.12
											0.41

* Beta is the correlation coefficient of SNP vs. gene expression value on the basis of minor allele from linear additive model.

Table 6

eQTL comparison of BEC, BAL, and LCLs

SNP	Chr	Location	Expressed Gene	Bronchial epithelial cells (BEC)	Bronchial alveolar lavage (BAL)	Lymphoblastoid cell lines (LCLs) GABRIEL/GENEVAR (11, 23, 24)	Consistency
rs2305480	17	coding	<i>GSDMB</i>	Yes	No	Yes/No	No
rs7216389	17	intron	<i>ORMDL3</i>	No	No	Yes/Yes	No
rs1837253	5	5'	<i>TSLP/WD36</i>	No	No	No/No	Yes
rs2244012	5	intron	<i>RAD50/IL13</i>	No	No	Yes/No	No
rs3771166	2	intron	<i>IL18R1</i>	No	No	Yes/No	No
rs12999517	2	intron	<i>IL1RL1</i>	No	Yes	NA/No	No
rs992969	9	5'	<i>IL33</i>	Yes	No	No (rs2381416; $r^2=1$ with rs992969)/No	No
rs1063355	6	3UTR	<i>HLA-DQB1</i>	Yes	No	No (rs9273349; $r^2=0.46$ with rs1063355)/NA	No
rs10036748	5	intron	<i>TNIP1</i>	No	No	NA/No	Yes
rs7130588	11	3'	<i>C11orf30/LRRC32</i>	No	No	No/No	Yes
rs404860	6	intron	<i>NOTCH4</i>	No	No	No/No	Yes
rs744910	15	intron	<i>SMAD3</i>	No	No	No/No	Yes
rs1588265	5	5'	<i>PDE4D</i>	No	No	No/No	Yes
rs9920560	15	intron	<i>RORA</i>	No	No	No (rs11071559; $r^2=0.94$ with rs9920560)/No	Yes
rs2284033	22	intron	<i>IL2RB</i>	No	No	No/No	Yes
rs856090	1	intron	<i>PYHIN1</i>	No	No	No (rs1101999; $r^2=0.64$ with rs856090)/No	Yes
rs4129267	1	intron	<i>IL6R</i>	No	No	No/No	Yes
rs2786098	1	intron	<i>DENND1B</i>	No	No	No/No	Yes
rs1701704	12	5'	<i>IKZF4</i>	No	No	Yes/Yes	No
rs2069408	12	intron	<i>CDK2</i>	No	No	Yes/No	No
rs7686660	4	5'	<i>USP38</i>	No	No	No/No	Yes
rs3805236	4	intron	<i>GAB1</i>	No	No	No/No	Yes
rs10508372	10	5'	<i>GATA3</i>	No	No	No/No	Yes
rs204993	6	intron	<i>PBX2/AGER</i>	No	No	Yes/NA	No
rs3129943	6	intron	<i>C6orf10</i>	No	No	Yes/NA	No
rs987870	6	5'	<i>HLA-DPA1/DPB1</i>	No	No	Yes/NA	No
rs17152490	7	3'	<i>CDHR3</i>	Yes	No	No/No	No
rs1543857	8	5'	<i>ZBTB10</i>	Yes	No	No/No	No

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

SNP	Chr	Location	Expressed Gene	Bronchial epithelial cells (BEC)	Bronchial alveolar lavage (BAL)	Lymphoblastoid cell lines (LCLs) Gabriel/ GENEVAR (11, 23, 24)	Yes/No	No
rs12919083	16	intron	CLEC16A-DEXI	No	Yes			