HHS Public Access

Author manuscript

J Asthma. Author manuscript; available in PMC 2017 October 01.

Published in final edited form as:

J Asthma. 2016 October ; 53(8): 775–782. doi:10.3109/02770903.2016.1158268.

Expression of asthma susceptibility genes in bronchial epithelial cells and bronchial alveolar lavage in the Severe Asthma Research Program (SARP) cohort

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Abstract

Objective—Genome-wide association studies (GWASs) have identified genes associated with asthma, however expression of these genes in asthma-relevant tissues has not been studied. This study tested expression and correlation between GWAS-identified asthma genes and asthma or asthma severity.

Methods—Correlation analyses of expression levels of GWAS-identified asthma genes and asthma-related biomarkers were performed in cells from human bronchial epithelial biopsy (BEC, n=107) and bronchial alveolar lavage (BAL, n=94).

Results—Expression levels of asthma genes between BEC and BAL and with asthma or asthma severity were weakly correlated. The expression levels of *IL18R1* were consistently higher in asthma than controls or in severe asthma than mild/moderate asthma in BEC and BAL ($p<0.05$). In *RAD50-IL13* region, the expression levels of *RAD50*, not *IL4*, *IL5*, or *IL13*, were positively correlated between BEC and BAL (rho=0.53, $P=4.5\times10^{-6}$). The expression levels of *IL13* were positively correlated with *IL5* in BEC (rho=0.35, $P=1.9\times10^{-4}$) and *IL4* in BAL (rho=0.42, $P=2.5\times10^{-5}$), respectively. rs3798134 in *RAD50*, a GWAS-identified SNP, was correlated with *IL13* expression and the expression levels of *IL13* were correlated with asthma (P=0.03).

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

rs17772583 in *RAD50* was significantly correlated with *RAD50* expression in BAL and BEC ($P=7.4\times10^{-7}$ and 0.04) but was not associated with asthma.

Conclusions—This is the first report studying the expression of GWAS-identified asthma genes in BEC and BAL. *IL13*, rather than *RAD50*, *IL4*, or *IL5*, is more likely to be the asthma susceptibility gene. Our study illustrates tissue-specific expression of asthma-related genes. Therefore, whenever possible, disease-relevant tissues should be used for transcription analysis.

Keywords

asthma susceptibility genes; bronchial alveolar lavage; bronchial epithelial cells; genome-wide association; mRNA expression

Introduction

Genome-wide association studies (GWASs) have revealed association of a variety of genes with susceptibility to asthma [1]. Consistent replication has been demonstrated for 6 genomic regions: *ORMDL3-GSDMB* region [2–4], *IL33* [3–5], *IL1RL1-IL18R1* region [3–5], *RAD50-IL13* region [3,6], *TSLP-WDR36* region [3–5,7], and *HLA-DR/DQ* region [3,6,7]. Biomarkers involved in Th2 (*IL13*), Th1 (*IFNG* and *IL12A*), Th17 (*IL17A*), regulatory T cell (*IL10*), and inflammatory pathways (*IL6*, *CHI3L1*, and *TNF*) are critical for diagnosing and monitoring asthma development [8]. Genetic markers identified through GWAS and biomarkers identified through expression analyses and functional studies are not completely overlapped [8,9]. Correlation between expression of asthma susceptibility genes and asthma or asthma severity has not been systemically studied in asthma-relevant tissues from subjects with asthma.

Trait-associated single nucleotide polymorphisms (SNPs) are rarely coding-change variants [1]. Expression quantitative trait locus (eQTL) SNPs are more likely to associate with complex trait [10]. For example, comparison of lymphoblastoid cell lines (LCLs) and whole blood demonstrates near zero genetic correlation of gene expression; while tissue-specific expression of non-housekeeping genes is more likely [11]. Recently, we have identified SNPs in *GSDMB*, *TSLP*, *IL33*, and *HLA-DQB1* affect asthma via tissue-specific cis-regulation of gene expression [12]. Thus, we hypothesize differential regulation of asthma gene and biomarker expression in target tissues, such as bronchial epithelial cells (BEC) and bronchial alveolar lavage (BAL), which change physiologically during asthma progression.

Methods

Study subjects

Pulmonary function testing in healthy control and asthmatics (with medication withheld) was performed at the Severe Asthma Research Program (SARP) centers [13]. Studies were approved by each center's Institutional Review Board including written informed consent.

Airway epithelial cells and alveolar lavage cells were obtained via bronchoscopy with epithelial brushings. Sample were prepared and microarrays were performed as described [12,14]. Total RNA extracted from the samples was suspended in Qiazol solution using the

QIACube system (QIAGEN Inc., Valencia, CA, USA). Complementary RNA (cRNA) labeled with the Cy5 fluorescent dye was hybridized to 4×44K v2 Whole Human Genome Microarrays and data were extracted using the Agilent Feature Extraction software v9.5 (Agilent Technologies Inc., Santa Clara, CA, USA).

Genomic DNA from whole blood was isolated using DNA purification kits (QIAGEN Inc., Valencia, CA, USA). Illumina HumanHap1M BeadChip or the Illumina HumanOmniExpress700k BeadChip was used for genotyping (Illumina, Inc., San Diego, CA, USA) [15,16].

Statistical analyses

34 candidates for analysis were chosen from asthma genes which achieved genome-wide significance or near genome-wide significance with replication from NIH GWAS database (http://www.genome.gov/gwastudies/) and the published literatures.

Gene expression data was normalized using a cyclic loess algorithm and analyzed using BRB ArrayTools version 4.3.0 as described [12,14]. The data has been deposited in NCBI's Gene Expression Omnibus database (GEO), and is accessible through GEO series accession number GSE67940 and GSE43696 (http://www.ncbi.nlm.nih.gov/geo/) [12,14].

The residual of expression data (after adjustment for asthma status, age, gender, first and second principal components obtained through multidimensional scaling analysis of genome-wide genotyping data) were inverse normally transformed to remove outliers and normalize the data. Spearman's rank correlation was performed on the 34 asthma genes expression probes either across genes within BEC (n=107) and BAL (n=94) separately, or for the same gene between BEC and BAL (n=67 with two tissues collected from the same individuals) using the programming package R (http://www.r-project.org/). Correlation analysis was also performed between seven biomarkers (*IL6*, *CHI3L1*, *TNF*, *IFNG*, *IL12A*, *IL17A*, and *IL10*) and asthma genes expression levels within BEC and BAL. Significance of correlation was defined stringently as Bonferroni adjusted P values ($P<0.05/34/(17+7)=6.13\times10^{-5}$). The correlation between expression values of asthma genes or biomarkers and asthma or asthma severity was accomplished using a logistic model adjusted for age, gender, and the first and second principal components. Significance of correlation was defined as $p<0.05$ because these genes were confirmed asthma genes identified through GWAS or functional studies.

Association between inverse normalized expression levels and SNPs of candidate genes (*RAD50-IL13* region and *CHI3L1*) was tested using a linear additive model in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) [17]. P values<0.05 were considered significant because GWAS-identified SNPs were used. GWAS results were extracted from the published TENOR study [6] and GABRIEL study [3] (http://www.cng.fr/gabriel; accession number: EGAS00000000077).

Due to large amount of information presented, we summarized the data in Supplementary Table S1.

Results

Correlation of the expression levels of asthma genes

The SARP cohort consists of healthy controls and asthmatics ranging from mild to severe, with over-recruitment of severe subjects (Table 1). GWAS and expression data from 107 subjects with BEC and 94 subjects with BAL (including 67 subjects with both BAL and BEC) were included in the analyses. The expression probes of 34 candidate genes were listed in Supplementary Table S2.

Expression levels from the same individual in different tissues displayed significant correlation, primarily in housekeeping genes (rho>0.7 and P $<10^{-10}$) (Supplementary Table S3). These genes fell into two groups: Group 1 were enzymes: glutathione S-transferase family (*GSTM4*, *GSTM5*, and *GSTT1*), sulfotransferase family (*SULT1A1*, *SULT1A2*, and *SULT1A4*), and other enzymes (*PPIL3*, *ACSM1*, *DECR2*, *GATC*, *FN3KRP*, *METTL21A*, *ABHD12*, and *MTRR*); The second group were structural proteins related to transcription and translation including mitochondrial ribosomal protein L43 (*MRPL43*), ribosomal protein S26 (RPS26), and eukaryotic translation initiation factor 2A (*EIF2A*). Signal transducer and activator of transcription 6 (*STAT6*) and interferon regulatory factor 5 (*IRF5*) are involved in signal transduction in immunological pathways thus might be relevant to asthma (Supplementary Table S3). Among 34 asthma genes, the expression levels of *AGER*, *USP38*, *ZBTB10*, *TSLP*, and *GSDMB* were mildly positively correlated (P<0.05) (Table 2) while only *RAD50* displayed significant correlation after multiple test adjustment (rho=0.53 and P=4.5×10^{-6}) between BEC and BAL.

The correlations of the expression levels of 34 asthma genes in each of the BEC and BAL datasets were shown in Supplementary Table S4 and Supplementary Table S5. In general, the correlation of expression levels of 34 asthma genes within a tissue was stronger than the correlation of the same genes between tissues (Table 2). In BEC, the expression levels of most of asthma genes were positively correlated (*GATA3*, *IL13*, *HLA-DQB1*, *HLA-DPA1*, *IL2RB*, and *PYHIN1*) (Supplementary Table S4). In BAL, the expression levels of a different set of genes were strongly positively correlated to each other (*IL18R1*, *RORA*, *IL2RB*, *SMAD3*, *PDE4D*, *GSDMB*, and *PYHIN1*) (Supplementary Table S5).

Correlation of the expression levels of asthma genes with asthma and asthma severity

The expression levels of *IL18R1* were consistently higher in asthma or severe asthma than controls or mild/moderate asthma in BEC and BAL (p<0.05) (Table 3 and Table 4). The expression levels of *IL6R* were consistently higher in asthma or severe asthma than controls or mild/moderate asthma in BAL. The expression levels of *ORMDL3* were higher in severe asthma than controls in BEC; while the expression levels of *GSDMB* were higher in severe asthma than mild/moderate asthma in BAL. The expression levels of *IL13* were higher in asthma and severe asthma than controls in BAL.

Expression and genetic association of RAD50-IL13 (chr5q31) region

Chr5q31 contains a Th2 cytokine region with *RAD50* separating *IL5* from *IL4* and *IL13*. Of the 34 asthma genes, only *RAD50* displayed strong correlation in expression levels between

BEC and BAL (Table 2). The expression levels of *IL4*, *IL5*, and *IL13* were significantly correlated with each other within BEC or BAL (Supplementary Table S6) but were not significantly correlated with *RAD50*. To further dissect the genetic association and gene expression in this important asthma locus, GWAS results were extracted from TENOR [6] and GABRIEL study publications [3] and eQTL analysis was performed (Table 5). There are multiple SNPs in a linkage disequilibrium (LD) block spanning *RAD50* and *IL13*, including rs1881457 and rs3798134 associated with asthma [3,6]. These asthma associated SNPs were correlated with the expression levels of *IL13* in BAL (P=0.03). Three SNPs (rs17772583, rs2069812, and rs11739623) were identified by eQTL analysis in BAL in an adjacent LD block spanning *RAD50*/*IL5* which were significantly correlated with the expression levels of *RAD50*. These three SNPs were not significantly associated with asthma (Table 5).

Expression and genetic association of asthma-related Biomarkers

T cell differentiation is an essential for asthma development. We studied the expression of Th1 (*IFNG* and *IL12A*), Th17 (*IL17A*), regulatory T cell (*IL10*), and inflammatory biomarkers (*IL6*, *CHI3L1*, and *TNF*) in addition to Th2 pathway genes (*IL4*, *IL5*, and *IL13*) (Supplementary Table S7 and Supplementary Table S8). Expression levels of the Th1 biomarker *IL12A* were negatively correlated with *GATA3* in BEC. The expression levels of *IFNG* were positively correlated with *IL2RB* and *PYHIN1* in both BEC and BAL. The expression levels of the Th17 biomarker *IL17A* were positively correlated with *ORMDL3* in BAL. Regulatory T-cell biomarker *IL10* expression was negatively correlated with *RORA*, *C11orf30*, and *TSLP* in BEC, but positively correlated with *TNIP1*, *IL2RB*, *IL6R* and *PYHIN1* in BAL.

The expression levels of *TNF* were positively correlated with *GATA3* in BEC. The expression levels of *CHI3L1* were positively correlated with *IL6R*, but negatively correlated with *IL1RL1* in BEC. The expression levels of *IL6* and *CHI3L1* were lower in asthma or severe asthma than controls in BEC (Supplementary Table S9). rs10399931, 5′ of *CHI3L1*, was significantly correlated with the expression levels of *CHI3L1* in BEC and BAL ($P=8.1\times10^{-3}$ and 0.02, respectively), but not associated with asthma (Table 6).

Discussion

It was not surprising to find that the most significantly correlated genes (rho>0.7) between BEC and BAL were housekeeping rather than asthma genes (Table 2 and Supplementary Table S3). However the correlations of the expression levels of the same asthma genes between BEC and BAL were weaker than the correlations of the genes within BEC or BAL (Table 2, Supplementary Table S4 and Supplementary S5). This differential expression implies cell-type-specific regulation of asthma gene expression in these tissues. The correlations of the expression levels of 34 genes with asthma or asthma severity were also not consistent between BEC and BAL (Table 3 and Table 4), further indicating cell-type-specific expression of asthma-associated genes. Tissue-specific regulation of transcription is not uncommon. Previous work has revealed tissue-specific or cell-type-specific expression regulation between various tissues, including brain and blood [18], whole blood and LCLs [11], blood, liver, subcutaneous tissue, visceral adipose tissue, and skeletal muscle [19], and

primary fibroblasts, T cells and LCLs [20]. In a recent study, we have identified tissue-specific eQTL of asthma candidate genes among BEC, BAL, and LCLs [12].

Correlations of the expression levels of 34 asthma genes with asthma or asthma severity were lower than expected. The expression levels of *IL18R1* were consistently higher in asthma or severe asthma than controls or mild/moderate asthma in both BEC and BAL (Table 3 and Table 4), indicating that *IL18R1* may be a potential biomarker for asthma susceptibility and severity. IL18R1 is a cytokine receptor of IL18 and essential for IL18 mediated signal transduction. IL18 may induce both Th1 and Th2 responses dependent on cytokine environment [21]. IL18 together with IL12 will induce the expression of IFRG and Th1 pathway; however IL18 itself can induce serum IgE expression and Th2 pathway [21]. Thus, IL18-IL18R1 is functional important for asthma development but with complex effects. The expression levels of the inflammatory biomarker *CHI3L1* were negatively correlated with Th2 pathway gene (*IL1RL1*) in BEC (Supplementary Table S7), implying that *CHI3L1* is more likely downstream in the immune responses in asthma.

rs2244012 and rs20541 (in *RAD50* and *IL13*, respectively) are associated with asthma (Table 5) [3,6]. It is difficult to dissect disease causal SNPs on the basis of genetic studies alone due to the degree of LD present in the *RAD50-IL13* region. Expression levels of *RAD50* were correlated between BEC and BAL (Supplementary Table S6), but not correlated with asthma within BEC or BAL (Table 3 and Table 4). rs17772583 (in *RAD50*) was significantly correlated with the expression levels of *RAD50*, but not associated with asthma (Table 5). *RAD50* codes for a protein which functions in housekeeping (DNA double-strand break repair) with an expression level consistent between BEC and BAL but not correlated with asthma. Thus its function may not relate directly to asthma. Genes nearby (*IL4*, *IL5*, and *IL13*) in the Th2 cytokines locus are better asthma causal candidates based on function. A 25 kb fragment at the 3′ end of *Rad50* was identified as a Th2 locus control region (LCR) using transgenic mice [22]. LCR is defined as regulating the expression of linked genes in a tissue-specific manner. The Th2 LCR alters chromatin configuration to re-organize promoters of *IL4*, *IL5*, and *IL13* [23]. In this study, the expression levels of *IL4*, *IL5*, and *IL13* were correlated in BEC and in BAL indicating co-expression, but not correlated between BEC and BAL indicating tissue-specific expression (Supplementary Table S6). In the same LD block as rs2244012 and rs20541, rs1881457 (in *IL13*) and rs3798134 (in *RAD50*) were correlated with the expression levels of *IL13* (Table 5) and the expression levels of *IL13* were correlated with asthma in BAL (Table 4), and thus *IL13* is more likely to be the functional asthma gene in the region.

Genetic markers identified through GWAS (Supplementary Table S2) generally differ from biomarkers identified through expression (Supplementary Table S7). For example, YKL-40 protein (encoded by *CHI3L1*) is a good biomarker for inflammation and its level is highly correlated with asthma and asthma severity [24]. rs4950928 in *CHI3L1* has been identified to be correlated with serum YKL-40 levels [25]. In this study, the expression levels of *CHI3L1* were correlated with asthma in BEC (Supplementary Table S9). rs10399931 (in LD with rs4950928: r^2=0.79) were significantly correlated with the expression levels of *CHI3L1* in BEC and BAL, but not associated with asthma (Table 6). GWAS-identified genes/SNPs are causal factors for asthma development, however, we may not be able to see significant

expression level difference between asthma and controls even if SNPs regulate gene expression. The probable explanation is that GWAS identifies the SNPs with allele frequency difference between asthmatics and controls, but that difference may not be large enough to translate into expression level difference between asthmatics and controls. Biomarkers identified through expression analyses have the largest fold changes between asthmatics and controls, but may be downstream genes of signal transduction pathways instead of causal genes. In summary, genetic markers identified through GWAS and biomarkers identified through expression analyses may not overlap. However both types of marker may complement each other in the quest for personalized medicine.

In conclusion, expression levels of GWAS-identified asthma genes were weakly correlated between BEC and BAL and with asthma or asthma severity. Our study illustrates tissue-specific expression of asthma-related genes. eQTL analysis indicates *IL13* is the functional asthma gene in *RAD50-IL13* region.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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

References

1. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA. 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, Depner M, 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, von Mutius E, 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, Himes BE, 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, Helgadottir A, Sulem P, Jonsdottir GM, Thorleifsson G, 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, Bleecker ER. 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, Fujita K, 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. Zissler UM, Esser-von Bieren J, Jakwerth CA, Chaker AM, Schmidt-Weber CB. Current and future biomarkers in allergic asthma. Allergy. 2015; [Epub ahead of print]. doi: 10.1111/all.12828
9. Ortiz RA, Barnes KC. Genetics of allergic diseases. Immunol Allergy Clin North Am. 2015; 35(1): 19–44. [PubMed: 25459575]

10. 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.
11. Powell JE, Henders AK, McRae AF, Wright MJ, Martin NG, Dermitzakis ET, Montgomery GW, 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]
12. Li X, Hastie AT, Hawkins GA, Moore WC, Ampleford EJ, Milosevic J, Li H, et al. eQTL of bronchial epithelial cells and bronchial alveolar lavage deciphers GWAS-identified asthma genes. Allergy. 2015; 70:1309–1318. [PubMed: 26119467]
13. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R Jr, 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]
14. Voraphani N, Gladwin MT, Contreras AU, Kaminski N, Tedrow JR, Milosevic J, Bleecker ER, et al. An airway epithelial iNOS-DUOX2-thyroid peroxidase metabolome drives Th1/Th2 nitrative stress in human severe asthma. Mucosal Immunol. 2014; 7:1175–1185. [PubMed: 24518246]
15. Li X, Ampleford EJ, Howard TD, Moore WC, Torgerson DG, Li H, Busse WW, 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]
16. Li X, Hawkins GA, Ampleford EJ, Moore WC, Li H, Hastie AT, Howard TD, 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]
17. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, 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]
18. Hernandez DG, Nalls MA, Moore M, Chong S, Dillman A, Trabzuni D, Gibbs JR, et al. Integration of GWAS SNPs and tissue specific expression profiling reveal discrete eQTLs for human traits in blood and brain. Neurobiol Dis. 2012; 47:20–28. [PubMed: 22433082]
19. Fu J, Wolfs MG, Deelen P, Westra HJ, Fehrmann RS, Te Meerman GJ, Buurman WA, et al. Unraveling the regulatory mechanisms underlying tissue-dependent genetic variation of gene expression. PLoS Genet. 2012; 8:e1002431. [PubMed: 22275870]
20. Dimas AS, Deutsch S, Stranger BE, Montgomery SB, Borel C, Attar-Cohen H, Ingle C, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. Science. 2009; 325:1246–1250. [PubMed: 19644074]
21. Nakanishi K1, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. Cytokine Growth Factor Rev. 2001; 12:53–72. [PubMed: 11312119]
22. Lee GR, Fields PE, Griffin TJ, Flavell RA. Regulation of the Th2 cytokine locus by a locus control region. Immunity. 2003; 19:145–153. [PubMed: 12871646]
23. Spilianakis CG, Flavell RA. Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. Nat Immunol. 2004; 5:1017–1027. [PubMed: 15378057]
24. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. N Engl J Med. 2007; 357:2016–2027. [PubMed: 18003958]
25. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, Radford S, et al. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med. 2008; 358:1682–1691. [PubMed: 18403759]

Table 1

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

	Bronchial epithelial cells (BEC)			Bronchial alveolar lavage (BAL)			Overlapped tissues (BEC & BAL)		
	Cases	**Controls**	**All**	**Cases**	**Controls**	**All**	**Cases**	**Controls**	**All**
N	88	19	107	66	28	94	54	13	67
Age (y)	37.6 ± 12.2	33.3 ± 13.5	37.1 ± 12.6	35.7 ± 12.3	28.8 ± 11.5	33.6 ± 12.3	35.9 ± 12.0	32.4 ± 13.5	35.2 ± 12.3
Sex (% female)	72	53	68	69	54	61	72	46	67
Race (Caucasian/African American/others)	49/27/12	14/2/3	63/29/15	38/22/6	21/1/6	59/23/12	31/19/4	10/1/2	41/20/6
ATS classification (Mild/Moderate/Severe)	31/18/39	NA	31/18/39	28/10/28	NA	28/10/28	21/10/23	NA	21/10/23
FEV_1 (%)	73.2 ± 22.3	96.1 ± 7.49	76.8 ± 22.4	76.9 ± 21.2	101 ± 10.4	84.4 ± 21.6	75.3 ± 21.8	96.8 ± 5.56	79.5 ± 21.4
FVC (%)	84.3 ± 18.4	98.6 ± 10.6	86.7 ± 18.0	87.3 ± 17.6	102 ± 11.1	92.0 ± 17.2	86.2 ± 18.4	98.9 ± 7.34	88.7 ± 17.5
FEV_1/FVC	0.70 ± 0.13	0.80 ± 0.04	0.72 ± 0.12	0.72 ± 0.12	0.83 ± 0.07	0.75 ± 0.12	0.71 ± 0.12	0.81 ± 0.05	0.73 ± 0.12
Log total IgE (geometric mean)	2.08 ± 0.69	1.31 ± 0.64	1.93 ± 0.75 (85.5)	2.05 ± 0.67	1.03 ± 1.02	1.71 ± 0.93 (51.1)	2.05 ± 0.70	1.27 ± 0.68	1.88 ± 0.76 (76.2)

Table 2

Correlations of the expression levels of 34 asthma genes between BEC and BAL (n=67)

Gene	Gene Full Name	rho*	P value
RAD50	RAD50 homolog (S. cerevisiae)	0.53	4.5×10^{-6}
AGER	advanced glycosylation end product-specific receptor	0.32	9.5×10^{-3}
USP38	ubiquitin specific peptidase 38	0.30	0.01
ZBTB10	zinc finger and BTB domain containing 10	0.29	0.02
TSLP	thymic stromal lymphopoietin	0.25	0.04
GSDMB	gasdermin B	0.25	0.04
IL13	interleukin 13	0.23	0.06
IL33	interleukin 33	0.23	0.06
IL1RL1	interleukin 1 receptor-like 1	0.20	0.11
C6orf10	chromosome 6 open reading frame 10	−0.18	0.14
RORA	RAR-related orphan receptor A	−0.17	0.16
CDHR3	cadherin-related family member 3	0.15	0.21
PYHIN1	pyrin and HIN domain family, member 1	0.15	0.23
IL18R1	interleukin 18 receptor 1	0.14	0.24
PBX2	pre-B-cell leukemia homeobox 2	−0.12	0.34
GAB1	GRB2-associated binding protein 1	−0.11	0.36
SMAD3	SMAD family member 3	−0.11	0.37
NOTCH4	notch 4	0.11	0.39
IKZF4	IKAROS family zinc finger 4 (Eos)	0.10	0.40
HLA-DPA1	major histocompatibility complex, class II, DP alpha 1	0.09	0.45
LRRC32	leucine rich repeat containing 32	0.09	0.49
DEXI	Dexi homolog (mouse)	0.09	0.49
HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	0.08	0.51
IL6R	interleukin 6 receptor	0.08	0.53
DENND1B	DENN/MADD domain containing 1B	−0.07	0.57
CDK2	cyclin-dependent kinase 2	0.07	0.59
IL2RB	interleukin 2 receptor, beta	0.06	0.62
ORMDL3	ORM1-like 3 (S. cerevisiae)	−0.06	0.65
C11orf30	chromosome 11 open reading frame 30	−0.04	0.75
WDR36	WD repeat domain 36	−0.02	0.86
TNIP1	TNFAIP3 interacting protein 1	0.01	0.90
PDE4D	phosphodiesterase 4D, cAMP-specific	0.01	0.92
CLEC16A	C-type lectin domain family 16, member A	0.01	0.92
GATA3	GATA binding protein 3	0.01	0.95

*rho: correlation coefficient from Spearman's rank sum test. Bonferroni adjusted P value is $0.05/34=1.5\times10^{-3}$.

Table 3

Correlation of the expression levels of asthma genes with asthma and asthma severity in BEC

Gene	Controls* (n=19)	Mild/moderate* (n=49)	Severe* (n=39)	Cases* (n=88)	P value#		
					Controls vs. Cases	Controls vs. Severe	Mild/moderate vs. Severe
ORMDL3	6.62 (0.2)	6.74 (0.3)	6.85 (0.27)	6.79 (0.29)	0.09	0.03	0.17
IL18R1	2.52 (0.75)	3.02 (0.81)	3.24 (0.99)	3.12 (0.9)	0.05	0.04	0.42
RORA	9.59 (0.2)	9.41 (0.3)	9.38 (0.29)	9.39 (0.29)	0.0067	0.02	0.81
DENND1B	7.47 (0.29)	7.46 (0.34)	7.26 (0.26)	7.37 (0.32)	0.31	0.06	0.03
IKZF4	3.34 (0.46)	3.75 (0.62)	3.56 (0.53)	3.67 (0.59)	0.02	0.10	0.16
USP38	5.02 (0.46)	5.19 (0.44)	4.92 (0.33)	5.07 (0.41)	0.49	0.71	0.02
GAB1	3.88 (0.51)	3.53 (0.52)	3.36 (0.60)	3.45 (0.56)	0.0008	0.002	0.31

*Log2 transformed expression values (standard deviation) were reported.

#Only entries with P values<0.05 were included.

Table 4

Correlation of the expression levels of asthma genes with asthma and asthma severity in BAL

Gene	Controls* (n=28)	Mild/moderate* (n=38)	Severe* (n=28)	Cases* (n=66)	P value		
					Controls vs. Cases	Controls vs. Severe	Mild/moderate vs. Severe
GSDMB	7.64 (0.54)	7.24 (0.48)	7.7 (0.68)	7.44 (0.61)	0.75	0.14	0.0034
IL18R1	2.76 (1)	3.03 (0.87)	3.81 (1.33)	3.36 (1.15)	0.02	0.0038	0.02
IL13	2.18 (0.9)	2.44 (1.23)	2.31 (0.99)	2.38 (1.13)	0.03	0.04	0.68
SMAD3	5.34 (0.82)	4.99 (0.7)	5.51 (1.14)	5.21 (0.94)	0.52	0.08	0.02
IL6R	7.87 (0.4)	7.99 (0.37)	8.23 (0.37)	8.09 (0.38)	0.04	0.0088	0.05
USP38	5.97 (0.36)	6.19 (0.33)	6.02 (0.24)	6.12 (0.31)	0.58	0.28	0.0078
AGER	8.73 (0.48)	8.35 (0.34)	8.51 (0.46)	8.42 (0.4)	0.03	0.29	0.11
DEXI	9.57 (0.42)	9.58 (0.32)	9.82 (0.43)	9.68 (0.39)	0.16	0.05	0.03

*Log2 transformed expression values (standard deviation) were reported.

#Only entries with P values<0.05 were included.

J Asthma. Author manuscript; available in PMC 2017 October 01.

Table 5

eQTL and GWAS of *RAD50-IL13* region

SNP	Gene	Location	Distance to Gene	Minor Allele	Major Allele	MAF	IL13 expression				RAD50 expression				GWAS			
							BEC		BAL		BEC		BAL		TENOR		GABRIEL	
							Beta	P value	Beta	P value	Beta	P value	Beta	P value	OR	P value	OR	P value
rs4143832	*IL5*	3′	−14159	A	C	0.23	−0.22	0.18	0.20	0.25	0.18	0.27	0.26	0.13	1.23	0.05	1.07	9.9×10^{-3}
rs11739623	*IL5*	3′	−12984	T	C	0.18	0.01	0.94	−0.04	0.81	−0.28	0.10	−0.65	7.1×10^{-5}	0.91	0.33	0.94	4.4×10^{-3}
rs2079103	*IL5*	3′	−12630	T	G	0.33	−0.14	0.32	−0.01	0.96	0.25	0.07	0.27	0.08	1.06	0.52	1.06	0.02
rs17690122	*IL5*	3′	−9301	G	A	0.17	−0.06	0.74	0.22	0.26	0.19	0.31	0.25	0.19	1.22	0.06	NA	NA
rs743562	*IL5*	3′	−4753	T	C	0.34	0.06	0.70	0.10	0.50	−0.07	0.61	−0.32	0.02	1.06	0.52	0.98	0.31
rs739719	*IL5*	3′	−4271	T	G	0.22	−0.22	0.14	−0.20	0.23	0.11	0.44	0.09	0.59	0.79	0.17	1.01	0.78
rs2069812	*IL5*	5′	−702	T	C	0.43	−0.10	0.45	−0.10	0.50	−0.13	0.33	−0.58	5.8×10^{-5}	0.85	0.07	0.93	3.5×10^{-3}
rs2244012	*RAD50*	intron	−6166	C	T	0.38	−0.21	0.11	0.19	0.18	0.10	0.47	0.05	0.71	1.64	3.0×10^{-7}	NA	NA
rs2301713	*RAD50*	intron	−174	C	T	0.29	−0.15	0.34	0.29	0.07	0.13	0.42	0.23	0.16	1.59	1.5×10^{-6}	NA	NA
rs17772583	*RAD50*	intron	−252	G	A	0.17	0.03	0.87	−0.01	0.96	−0.37	0.04	−0.85	7.4×10^{-7}	0.89	0.23	0.92	3.4×10^{-4}
rs3798134	*RAD50*	intron	−7628	T	C	0.30	−0.18	0.21	0.33	0.03	0.12	0.44	0.19	0.23	1.59	1.5×10^{-6}	NA	NA
rs2237060	*RAD50*	intron	−1922	C	A	0.29	0.16	0.29	−0.26	0.11	0.09	0.55	0.38	0.02	0.88	0.12	0.95	0.02
rs1881457	*IL13*	5′	−1456	C	A	0.23	0.02	0.89	0.38	0.02	0.14	0.36	0.26	0.10	NA	NA	NA	NA
rs1295686	*IL13*	intron	−24	A	G	0.42	−0.08	0.55	0.20	0.18	0.05	0.73	0.06	0.67	1.45	2.2×10^{-4}	1.15	1.4×10^{-8}
rs20541	*IL13*	coding	97_10	T	C	0.25	0.05	0.76	0.32	0.07	0.17	0.27	0.12	0.48	1.44	2.5×10^{-4}	1.15	2.0×10^{-8}
rs1295685	*IL13*	3UTR	470_356	T	C	0.21	−0.04	0.80	0.31	0.08	0.19	0.25	0.20	0.26	1.49	7.4×10^{-5}	NA	NA
rs848	*IL13*	3UTR	525_301	T	G	0.38	0.00	0.99	0.21	0.18	0.10	0.51	0.06	0.71	1.49	7.6×10^{-5}	NA	NA
rs2243204	*IL13*	3′	−2693	T	C	0.26	−0.04	0.81	0.00	0.98	−0.07	0.64	−0.11	0.50	1.69	1.3×10^{-4}	1.19	5.3×10^{-7}
rs2243248	*IL4*	5′	−729	G	T	0.11	0.43	0.05	−0.04	0.89	0.06	0.79	0.05	0.83	0.91	0.52	1.00	0.91
rs2070874	*IL4*	5UTR	337_32	T	C	0.28	−0.07	0.61	0.12	0.41	0.16	0.24	0.08	0.60	1.12	0.34	NA	NA
rs2243268	*IL4*	intron	−1443	C	A	0.23	−0.04	0.80	0.11	0.50	0.19	0.19	0.19	0.23	1.11	0.38	NA	NA
rs2243290	*IL4*	intron	−9	A	C	0.25	−0.02	0.88	0.10	0.52	0.14	0.34	0.14	0.36	1.11	0.38	1.08	5.5×10^{-3}

*SNPs were in two separate linkage disequilibrium blocks (r^2>0.5). Block 1 includes rs11739623, rs2069812, and rs17772583. Block 2 includes rs2244012, rs2301713, rs3798134, rs1881457, rs1295686, rs20541, rs1295685, rs848, and rs2243204.

Table 6

eQTL and GWAS of *CHI3L1*

SNP	Location	Distance to Gene	Minor Allele	Major Allele	MAF	CHI3L1 expression				GWAS			
						BEC		BAL		TENOR		GABRIEL	
						Beta	P value	Beta	P value	OR	P value	OR	P value
rs903358	3′	−791	G	T	0.12	0.00	1.00	−0.19	0.43	NA	NA	NA	NA
rs7542294	intron	−683	A	G	0.27	0.04	0.76	0.16	0.37	0.96	0.72	0.98	0.46
rs880633	coding	32_118	C	T	0.47	0.07	0.56	−0.12	0.39	0.96	0.62	1.04	0.04
rs1538372	intron	−19	A	G	0.26	−0.09	0.54	0.05	0.78	0.99	0.86	NA	NA
rs10399931	5′	−203	T	C	0.22	−0.41	8.1×10^{-3}	−0.40	0.02	0.9	0.28	NA	NA
rs10920579	5′	−3095	A	G	0.13	−0.62	2.6×10^{-3}	−0.30	0.13	0.9	0.34	0.98	0.32