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General Article

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GENERAL ARTICLE

Genome-wide analysis revealed sex-specific gene expression in asthmatics

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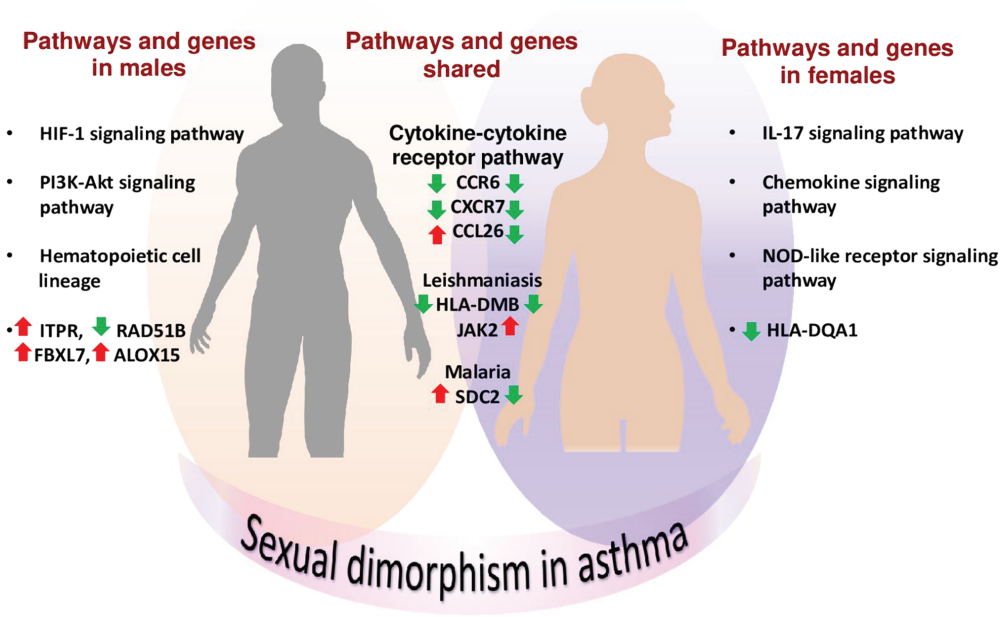
Abstract



Global gene-expression analysis has shown remarkable difference between males and females in response to exposure to many diseases. Nevertheless, gene expression studies in asthmatics have so far focused on sex-combined analysis, ignoring inherent variabilities between the sexes, which potentially drive disparities in asthma prevalence. The objectives of this

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study were to identify (1) sex-specific differentially expressed genes (DEGs), (2) genes that show sex-interaction effects and (3) sex-specific pathways and networks enriched in asthma risk. We analyzed 711 males and 689 females and more than 2.8 million transcripts covering 20 000 genes leveraged from five different tissues and cell types (i.e. epithelial, blood, induced sputum, T cells and lymphoblastoids). Using tissue-specific meta-analysis, we identified 439 male- and 297 female-specific DEGs in all cell types, with 32 genes in common. By linking DEGs to the genome-wide association study (GWAS) catalog and the lung and blood eQTL annotation data from GTEx, we identified four male-specific genes (FBXL7, ITPR3 and RAD51B from epithelial tissue and ALOX15 from blood) and one female-specific gene (HLA-DQA1 from epithelial tissue) that are disregulated during asthma. The hypoxia-inducible factor 1 signaling pathway was enriched only in males, and IL-17 and chemokine signaling pathways were enriched in females. The cytokine–cytokine signaling pathway was enriched in both sexes. The presence of sex-specific genes and pathways demonstrates that sex-combined analysis does not identify genes preferentially expressed in each sex in response to diseases. Linking DEG and molecular eQTLs to GWAS catalog represents an important avenue for identifying biologically and clinically relevant genes.

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Introduction

The prevalence and severity of asthma as well as several other complex diseases, including autoimmune disease, psychiatric disorders, heart disease and pain, are characterized by sexual dimorphism (1–3). Pre-pubertal boys are twice as likely to develop asthma compared with girls (4–7), but after puberty, women are twice as likely to develop asthma as men (http://www.cdc.gov). Similarly, boys have a higher total serum immunoglobulin E (IgE) and severe wheezing compared to girls, but it later recedes when they reach puberty (8–11). On the other hand, increased body mass index (BMI), obesity and serum leptin are associated with asthmatic girls, while higher neck circumference is associated with asthmatic boys (12–14). Animal models have also shown sexual dimorphism in the asthmatic phenotype. Female and male mice models of asthma respond differently to allergen sensitization, with female mice having a higher level of airway remodeling and allergic hyperresponsiveness than male mice (15,16). Prior candidate gene-based studies using sex-stratified analysis have unmasked strong sex-specific effect on asthma than sex-combined analysis (1).

In 1993, the US Food and Drug Administration issued guidance with regard to evaluating sex differences in drug development that emphasized the importance of understanding pharmacokinetics and pharmacodynamics of drugs by sex (https://www.fda.gov/media/75648/download. The landmark Institute of Medicine report entitled *Exploring the Biological Contributions to Human Health: Does Sex Really Matters?* concluded that sex matters in all aspects of cellular processes and physiology from 'womb to tomb' (17). The 2014 National Academies of Sciences report entitled 'Sex-Specific Medical Research: Why Women's Health Can't Wait' acknowledged that although notable advances have been made in maternal health, sex differences are frequently excluded from the early stage of the research process (18). The report recommends that US federal agencies, including the National Institutes of Health (NIH), ensure sex-based research at all levels, from basic science to clinical trials (18). The Office of Research on Women's Health and the NIH have developed policies for increased attention to sex differences to achieve equitable health outcomes and to improve the quality of care (19).

Despite ample evidence about the importance of sexual dimorphism in asthma and the recent efforts by federal and private funding agencies to include sex as a biological variable (20,21), most genetic studies have not considered the inherent differences between males and females. Instead, most studies continue to combine sexes in their analysis to increase sample size and gain power. Although such strategy seems logical, this is not universally true. In fact, sex-combined analysis may reduce power (instead of resulting in a perceived increase in power) due to 'canceling effects' of the true outcome when sex-specific effects exist. Hence, failure to account for sex differences in asthma research can lead to either missing relevant biological phenomena entirely or overgeneralization of the findings. Consideration of sex as a biological variable could identify sex-specific differences in asthma and offer an opportunity for delivering sex-specific interventions for asthmatic patients. The purpose of this study is to determine sex-specific gene expression between asthmatic males and females by performing genome-wide gene expression analysis and comparing asthmatic versus non-asthmatic control from a separate male and female analysis.

Results

Figure 1 illustrates an overview of our experimental workflow. First, we curated 12 different public data sets (steps 1–2) and extensively evaluated the differences in gene expression profiles between asthmatic males and females (step 3a). Second, we performed tissue-based sex-specific gene expression (meta-) analysis (step 3b) across five different tissues and cellular types (epithelial, blood, induced sputum, T cells and lymphoblastoid). We used two complementary approaches, sex stratified (Fig. 1, step 4a) and pooled control (Fig. 1, step 4b), to determine sex-specific gene expression. Both approaches are useful in detecting sex interaction effects; however, pooled controls have one particular advantage. They can identify genes with marginal interaction effects, which are otherwise not detected due to low power from reduced sample size in stratified analyses. A similar pooling approach was previously applied on a genome-wide genotype-by-sex interaction study (22). In addition, we identified the differentially expressed genes (DEGs) linked with eQTL in lung and blood from GTEx databases (Fig. 1, step 5) and further evaluated for sex-specific gene expression in the genome-wide association study (GWAS) catalog (23). Finally, we performed sex-specific functional enrichment analysis to explore the biological insights underpinning the sex-disparity of asthma.

Data filtering and selection

We used 12 Gene Expression Omnibus (GEO) datasets that met our selection and quality control criteria as summarized in steps 1 and 2 in Figure 1. Two datasets (GSE89809 and GSE64913) consisted of gene expression data from multiple tissues and

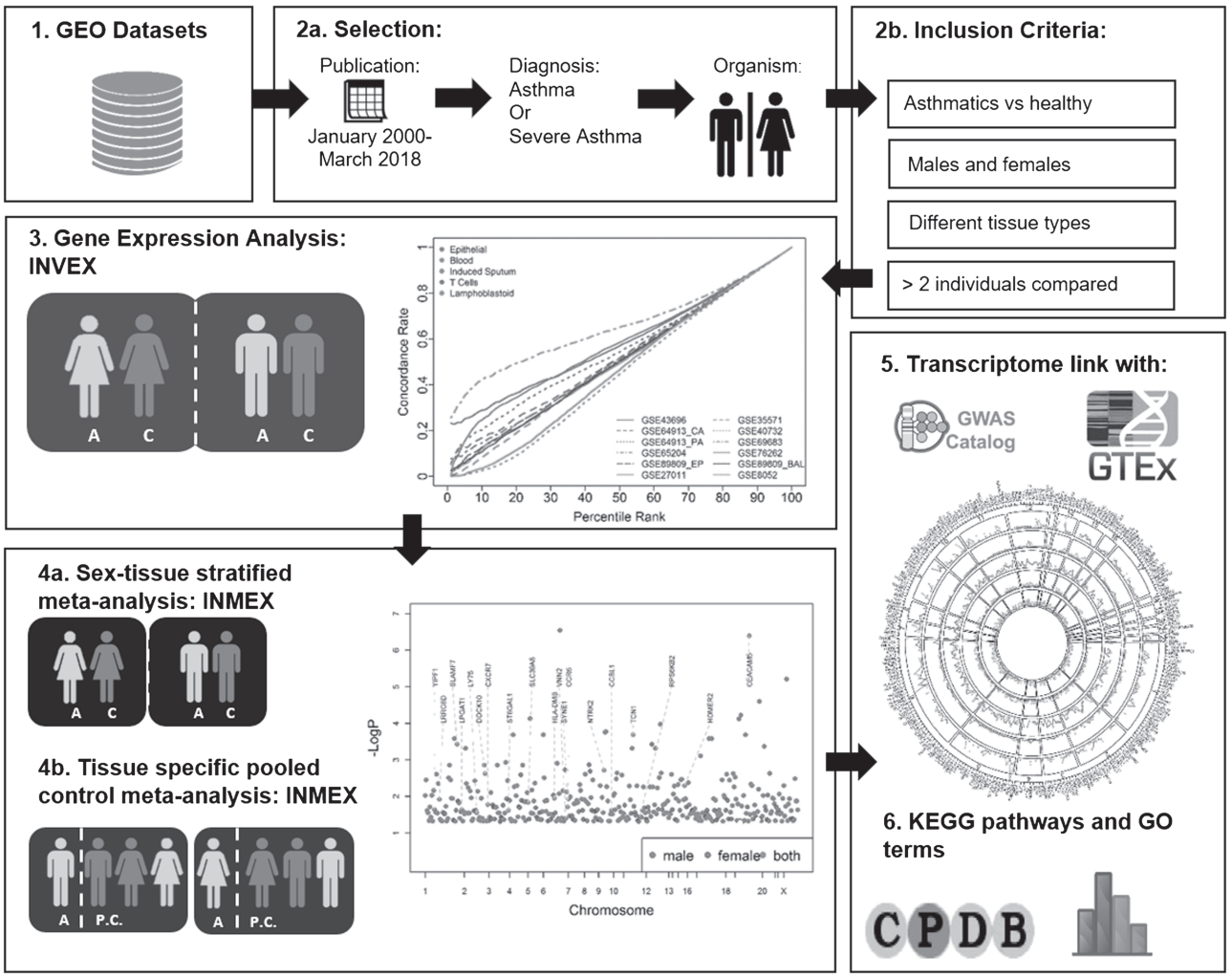

Figure 1. Flow chart. Schematic representation of the analysis procedures in the study. Curated gene expression data from 12 different sources were extensively evaluated for differences in gene expression profiles between asthmatic males and females followed by tissue-based sex-specific gene expression analysis across five different tissue and cellular types (epithelial, blood, induced sputum, T-cells and lymphoblastoid). Sex stratified and pooled control were used to determine the sex-specific expression of genes. The identified DEGs were linked to eQTL in the lung and blood from GTEx databases and further evaluated for sex-specific gene expression in the GWAS catalog. Finally, we performed sex-specific functional enrichment analysis to explore the biological insights underpinning the sex-disparity of asthma.

were further split into independent datasets. GSE89809 consisted of gene expression data measured from three different cell types—epithelial, CD3+ T cells derived from Broncho alveolar lavage and CD3+ T cells derived from induced sputum—and was divided into three datasets—GSE89809_EP, GSE89809_BAL and GSE89809_IS. However, the sex stratification of the GSE89809_IS dataset resulted in a cohort with only one sample and was dropped from the analysis. Similarly, GSE64913 dataset consisted of gene expression data from epithelial cells from central airways and from peripheral airways and was divided into two datasets (GSE64913_CA and GSE64913_PA) and was further stratified by sex. This resulted in 12 different datasets grouped into 5 major tissue types—blood, epithelial cells, T cells, lymphoblastoid cells and induced sputum. Table 1 presents the summary of each dataset including the GEO accession number, tissue type, sample sizes by sex and reference to the original publication. In total, we had gene expression data on 711 male (421 asthmatic and 290 controls) and 689 female (432 asthmatic and 257 controls) samples.

DEGs showed low concordance between sexes

We performed gene expression analysis of asthmatic versus control on each of the 12 data sets by sex, using the limma algorithm implemented in INVEX web tool (33). We ranked the genes based on *P*-value and computed the concordance rate for each dataset by finding the proportion of overlapped genes between male and female within the top t ($t = 1, 2, \ldots, 100$) percentile. Among the genes in the top percentiles, as shown in Figure 2A, concordance rate was consistently low across all datasets (see Materials and Methods section for detail). In particular, within the top 5 percentiles (potential for finding significant differential expressed genes) in males and females, the concordance rate varied from 0.006 to 0.325. The high concordance rate between males and females was observed in the blood.

Epithelial and T cells exhibited a wider range of average fold change than the average fold changes in blood and lymphoblastoid in both sexes (Table 2). Among the epithelial tissues, the widest range of fold change among males (−4.55, 2.99) was

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Table 1. Description of GEO datasets used in the study

Accession number	Tissue	Sex	Asthma	Control	Reference
Epithelial cells					
GSE64913_CA	Central airway epithelium	Males	8	14	(24)
		Females	5	9	
GSE64913_PA	Peripheral airway epithelium	Males	8	11	(24)
		Females	7	8	
GSE43696	Fresh bronchial epithelial cells	Males	10	9	(25)
		Females	28	11	
GSE65204	Nasal epithelial cells	Males	19	15	(26)
		Females	17	18	
GSE89809_EP	Endobronchial epithelial brushings	Males	2	11	(27)
		Females	4	4	
T cells					
GSE89809_BAL	CD3+ T-cells (broncho alveolar lavage)	Males	3	11	(27)
		Females	5	3	
Blood					
GSE69683	Blood	Males	85	53	(28)
		Females	161	34	
GSE27011	White blood cells	Males	10	15	(29)
		Females	7	3	
GSE35571	Peripheral blood sample	Males	39	37	(30)
		Females	21	27	
GSE40732	Peripheral blood mononuclear cells	Males	56	41	(26)
		Females	41	56	
Induced sputum					
GSE76262	Induced sputum	Males	18	15	(31)
		Females	31	6	
Lymphoblastoid					
GSE8052	Peripheral blood lymphocytes	Males	163	58	(32)
		Females	105	78	

in the GSE89809 dataset. In females, the widest range of fold change (−2.54, 3.70) was in the GSE43696 dataset. Within the blood group, the log fold change ranged from −1.66 to 1.61 in the GSE27011 dataset among males and from −0.97 to 1.74 in the GSE69683 in females.

We compared the overall average gene expression in males and females by cohort across different data sets. In both cases and controls, the genes were highly correlated between males and females. The lowest correlation in average gene expression between asthmatic males and asthmatic females was observed in the dataset GSE89809_BAL ($r = 0.943$), and the highest value was observed in the dataset GSE69683 ($r = 0.999$ for both cases and controls) (Table 2). We further compared gene expression differences between males and females using 1.5 or higher log fold change and correlation analysis. Fold change among epithelial tissues consistently correlated between males and females except for GSE89809 ($r = 0.0631$). In the blood, we observed inconsistent correlation between males and females varying from strong negative ($r = -0.7622$) to no correlation ($r = 0.0631$) to strong positive ($r = 0.9518$) correlation (Table 2). The fold change in induced sputum was highly correlated ($r = 0.8037$), whereas the fold change in CD3+ T cells had weak negative correlation ($r = -0.1752$) (Table 2).

We further refined our male versus female comparison to the top 100 DEGs, selected based on *P*-values (Supplementary Material, Table S1). Genes were ranked from most to least significant based on the *P*-value. Among the top 100 genes, there was considerable inconsistency in the fold change values between males and females across different datasets with several genes showing fold changes in opposite direction including T cells and epithelial cells (Fig. 2B). Furthermore, there was a very little or no overlap among the top 100 DEGs in males and females. Across the different tissues, males and females shared the most similar gene expression in the epithelial cells, followed by in the blood samples. No DEGs were shared between males and females in lymphoblastoid cells.

Meta-analysis of DEGs based on sex and tissue type

To identify sex-specific DEGs across the five different tissue groups (i.e. epithelial, whole blood, induced sputum, T cells and lymphoblastoid cells), we used a meta-analysis approach. Meta-analyses were only performed on epithelial and blood cells because multiple gene expression datasets were needed for grouping (Table 1). The meta-analysis was carried out for each sex using the INMEX web tool by combining the effect size under the random effect models (34). Sex-specific DEGs were identified using two complementary approaches—sex-stratified analysis and pooled-control analyses (see Materials and Methods section for details).

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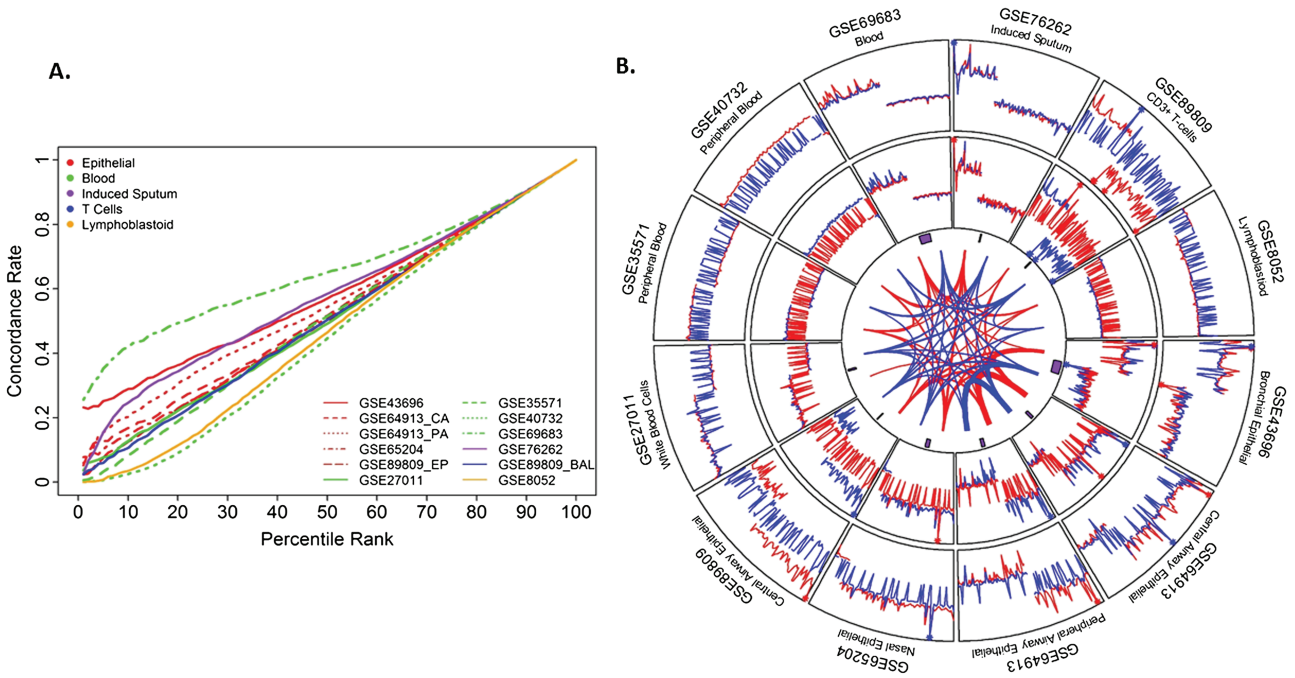

Figure 2. Comparison of sex-specific DEGs across individual datasets. A) Concordance Rate. The concordance rate is shown as the proportion of genes shared between males and females among the top r percentile genes ranked based on the p-values. Results from the datasets in the same tissue group are shown with the same color. **B) Top 100 differentially expressed genes by sex.** The circos plot displays the top 100 differentially expressed genes by sex. The outermost track compares the fold changes of the male-specific top 100 DEGs between males and females (red = male; blue = female). For each dataset, the genes were, first, separated into upregulated (+ve fold change) and down regulated (-ve fold change), then, genes with positive fold changes were plotted first followed by genes with negative fold changes sorted by the significance p-value. Graphs show the fold change within the range of -5 to 5 with fold-changes outside of this range are represented with asterisk (*). The following inner track compares fold changes of the female-specific top 100 DEGs in males and females. The width of the rectangles in innermost track shows the overlap between males and females within the same dataset. The lines connecting the datasets in the inner-circular space represent the overlap of DEGs between the connected datasets within same sexes (male = red, female = blue) and the width of the line is proportional to the number of overlapped genes.

Sex-stratified meta-analyses

Table 3 summarizes the number of samples used, the number of unique genes analyzed and the number of DEGs based on false discovery rate (FDR)-adjusted *P*-value obtained for each sex in each tissue type. In the epithelial tissue, we found a total of 315 and 69 DEGs as defined by an FDR-adjusted *P*-value < 0.05 in males and females, respectively. We only found 18 shared DEGs between sexes. Additionally, in the blood tissue, we identified 9 male- and 16 female-specific DEGs. In induced sputum, we identified a total of nine male- and two female-specific DEGs. No significant DEG was found in T cells and lymphoblastoid cells in either sex. For each sex, the tissue-specific DEGs were mostly unique with no cross-tissue overlap except the HN1 gene, which was differentially expressed in epithelial and induced sputum in males. Figure 3(A–E) illustrates differences in the expression profile of genes in males and females based on the association level of gene expression across all tissues. Genes were sorted based on the negative log transformation of the FDR-adjusted p-values for males ranging from most to least significant. The results showed that the genes with higher significance in either sex were not aligned well across the different tissue types analyzed. For instances, in epithelial cells, most highly significant DEGs in males were among the least significant DEGs in females and vice-versa Fig. 3(A–E). Similar differences in the association of gene expression in asthmatic males and females were observed across all tissue types studied. In total, the sex-stratified analyses identified 332 significant DEGs associated to asthmatic males and 87 significant DEGs associated to asthmatic females and only 19 genes (SYNE1, SLAMF7, CEACAM5, DOCK10, NTRK2, HLA-DMB, SLC39A8, HOMER2, YIPF1, LPGAT1, TCN1, LRRC8D, CXCR7, ST6GAL1, CCBL1, LY75, VNN2, RPS6KB2 and CCR6) were common between the sexes (Fig. 3F). There was no cross-tissue overlap of DEGs between males and females except SYNE1, which was significant in airways epithelial tissue among males and in blood among females. All overlapped genes showed effects in the same direction.

Pooled-control analysis. To further identify genes with expression level varying by sex, we performed sex interaction analysis using a pooled-control approach as described in the method. Meta-analyses were performed by combining multiple datasets within similar tissue types. In order to conceptualize the association signals that may arise from the pooled-control analysis, we showed three different representative scenarios of gene expression patterns, in lymphoblastoid cells (Supplementary Material, Fig. S1), which were significant in the pooled analysis but not in the sex stratified analysis.

First, EIF1AY is ubiquitously expressed in asthmatic and control males but not expressed in females and consequently not significant in the sex-stratified analysis (Supplementary Material, Fig. S1A). However, this gene is significant in both sex-specific interaction analyses (Supplementary Material, Table S2) with the pooled-control approach, which suggests spurious associations. Second, ETV4 was significantly expressed in the male-specific interaction analysis but not in other analyses. ETV4 is expressed equally among females and control males but has increased expression in asthmatic males (Supplementary Material, Fig. S1B). Finally, EMR1 showed a similar expression

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Table 2. Results of the main interaction analysis with INVEX

GSE Dataset	Tissue type	Number of distinct genes used for analysis	Range of logfc (min, max)		Top gene correlation[a]	Overall correlation
Epithelial cells						
GSE43696	Bronchial epithelial cells	17 798	Females Males	(−2.539, 3.698) (−3.124, 2.955)	0.6262 (1413)	Control: 0.9915; case: 0.9922
GSE64913_CA	Central airway epithelium	12 041	Females Males	(−2.796, 3.048) (−1.766,3.176)	0.4846 (620)	Control: 0.9787; case: 0.9758
GSE89809_EP	Endobronchial epithelial brushings	17 555	Females Males	(−2.295, 2.862) (−4.548, 2.993)	−0.0631 (4137)	Control: 0.968; case: 0.940
GSE65204	Nasal epithelial cells	12 406	Females Males	(−1.643, 3.260) (−1.514, 3.520)	0.6561 (371)	Control: 0.991; case: 0.991
GSE64913_PA	Peripheral airway epithelium	12 324	Females Males	(−1.986, 2.730) (−2.328, 2.981)	0.6231 (574)	Control: 0.9768; case: 0.9774
T cells						
GSE89809_BAL	CD3+ (bronchoalveolar bavage)	17 500	Females Males	(−2.188, 2.829) (−4.717, 2.688)	−0.1752 (4581)	Control: 0.972; case: 0.943
Blood						
GSE69683	Blood	17 393	Females Males	(−0.699, 1.107) (−0.971, 1.741)	0.9581 (65)	Control: 0.999; case: 0.999
GSE27011	White blood cells	17 990	Females Males	(−1.663, 1.607) (−1.054, 1.356)	0.4182 (241)	Control: 0.9954; case: 0.9953
GSE40732	Peripheral blood mononuclear cells	18 213	Females Males	(−0.970, 1.256) (−1.020, 0.951)	−0.7622 (657)	Control: 0.990; case: 0.986
GSE35571	Peripheral blood cells	16 919	Females Males	(−0.613, 0.948) (−0.516, 0.666)	−0.1745 (48)	Control: 0.994; case: 0.993
Induced sputum						
GSE76262	Induced sputum	17 441	Females Males	(−2.215, 2.795) (−1.689, 2.284)	0.8037 (2394)	Control: 0.979; case: 0.987
Lymphoblastoid						
GSE8052	Lymphobastoid cell line	17 929	Females Males	(−0.458, 0.551) (−0.705, 0.625)	-0.2534 (49)	Control: 0.996; case: 0.997

[a]Total number of genes with an absolute fold change of 1.5 or more in males and females.

Table 3. Description of datasets used for gene expression analyses stratified by tissue and sex

Tissue	Sex	Number of samples		Number of distinct genes used for analysis	DEGs (FDR < 0.05)
		Asthma	Controls		
Blood	Males	190	146	15 762	9
	Females	230	119		16
Epithelial	Males	47	60	12 401	315
	Females	61	50		69
T cells	Males	3	11	17 500	0
	Females	5	3		0
Lymphoblastoid	Males	163	58	16 931	0
	Females	105	78		0
Induced sputum	Males	18	15	17 441	9
	Females	31	6		2
	Females	31	39		26

pattern in asthmatic females and showed significant association in female-specific pooled analysis (Supplementary Material, Fig. S1C).

Table 4 summarizes the pooled-control analyses along with the number of DEGs for each sex and the number of shared DEGs between males and females. This approach of sex interaction analysis using pooled controls discovered a new set of sex-specific DEGs across different tissues in both sexes. In epithelial tissue, we identified 54 and 68 genes differentially expressed in males and females, respectively. Similarly, in blood, 40 genes were differentially expressed in males and 107 were differentially expressed in females. Although sex-stratified analyses of lymphoblastoid cell did not exhibit DEGs in either sex, the interaction analyses identified 37 DEGs in females and 60 DEGs

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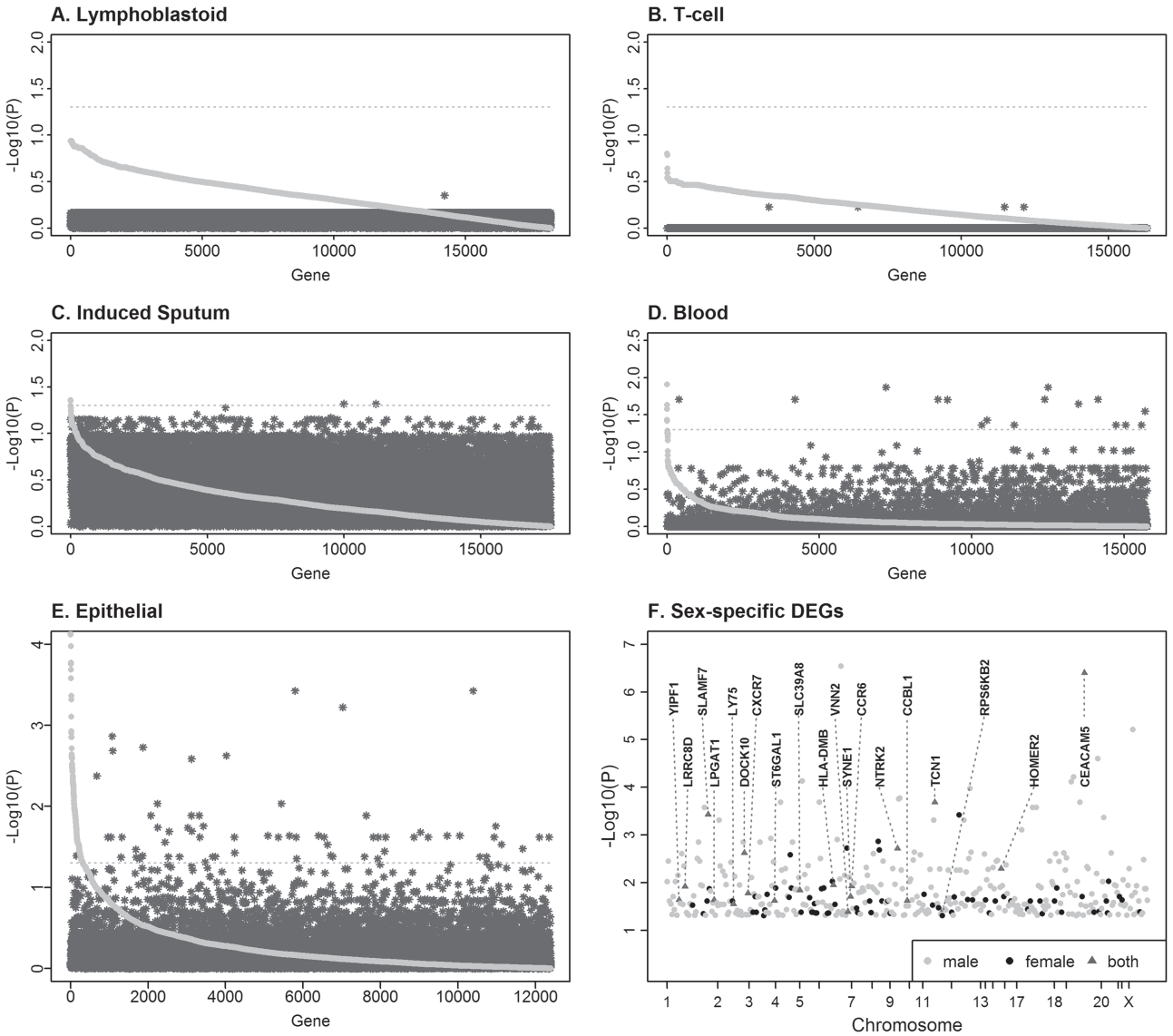

Figure 3. Tissue-based meta-analysis. (A–E). Graphs show the results from the tissue-specific meta-analysis of differential gene expression for males (light gray) and females (dark gray). Genes were sorted based on the negative log of the FDR-adjusted P-values. The horizontal dashed line marks the significance level of FDR = 0.05. **F) Sex-specific differentially expressed genes from sex-stratified analyses.** The plot shows the unique and overlapped differentially expressed genes in males and females. DEGs from the sex-stratified analyses for each sex are merged from the five tissues analyzed as shown in panels A to E. Genes are plotted along the X-axis ordered uniformly from Chromosome 1 to Chromosome X. Y-axis shows the negative log of the FDR adjusted p-values. For the shared genes, the minimum of the p-values were used.

in males. Similar analyses of induced sputum identified 26 genes in females, but only 1 gene was differentially expressed in males. There were no DEGs identified in T cells.

By merging the DEGs from the sex-stratified and pooled-control gene expression analyses, we were able to identify tissue-specific DEGs in males and females in all tissue groups. Supplementary Material, Table S3 shows all the DEGs identified in males and females separated by tissue type. We found that the epithelial tissue group yielded the highest numbers of DEGs among males when compared to the other tissue groups (325 DEGs in epithelial tissue, 47 DEGs in blood, 60 DEGs in lymphoblastoid and 10 DEGs in the induced sputum). In females, we found 118, 118, 37, and 28 DEGs in epithelial, blood, lymphoblastoid, and induced sputum tissues, respectively. There were 23 shared genes between males and females in the epithelial tissue, and there was 1 shared gene between males and females in the blood group. There were no shared DEGs in the induced sputum and lymphoblastoid tissues between males and females. In all, we obtained 439 unique DEGs in males and 297 unique DEGs in females from the four different tissues (Supplementary Material, Table S3). Thirty-two genes were shared between males and females across all tissues. Eight of these genes had contrasting expressions in males and females. Our results showed that airway epithelial tissues yielded the largest share of significant DEGs in asthmatics.

Genetic influence on sex-specific gene expression

We extracted genome-wide significant genetic variants (single nucleotide polymorphisms, SNPs) with P-value < 5E-8 from the published GWAS catalog database (http://www.ebi.ac.uk/gwas/, as of May 2018) (23). From the GWAS catalog, we

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Table 4. Summary of meta-datasets for gene expression-sex interaction analysis and the number of DEGs

Tissue	Sex	Number of samples		Number of distinct genes used for analysis	DEGs (FDR < 0.05)
		Asthma cases	Pooled controls		
Blood	Males	190	495	15 762	40
	Females	230	455		107
Epithelial	Males	47	171	11 495	54
	Females	61	157		68
T cells	Males	3	19	17 513	0
	Females	5	17		0
Lymphoblastoid	Males	163	241	16 927	60
	Females	105	299		37
Induced sputum	Males	18	52	17 407	1
	Females	31	39		26

identified 402 genes that mapped to 284 asthma-associated SNPs with p-value < 5E-8. Among these, 278 genes were represented in at least one gene expression meta-analysis. Figure 4 shows the negative log transformation of the FDR-adjusted P-values from the tissue-specific meta-analysis of males (light gray) and females (dark gray) of the GWAS-mapped genes plotted in linear order along the x-axis based on the position of the GWAS SNP mapped to the genes. There were 17 GWAS genes with significant sex-specific expression differences (Table 5). Eight GWAS genes were significant in male-specific analyses (IL18R1, IL5RA, ITPR3, ALOX15, FBXL7, B4GALT3, ACTR1A and RAD51B), and seven genes (HLA-DQA1, AIF1, HCP5, KYNU, MANBA, RUNX3 and TRAF3) were significant in female-specific analysis. Only two genes (JAK2 and CCBL1) were shared between males and females (Table 5). We observed overlap between GWAS genes and sex-specific DEGs across all four tissue types except T cells, which had no significant DEGs.

Next, we mapped the sex-specific DEGs from airways epithelial tissues and blood to lung and blood eQTL data from GTEx (35). The overlap resulted in 147 male-specific DEGs and 53 female-specific DEGs with significant eQTL association in lung tissue. Similarly, the overlap in blood eQTL data resulted in 21 male- and 54 female-specific DEGs with significant eQTL associations. An overlap among the epithelial DEGs, lung eGenes and GWAS genes resulted in three male-specific DEGs (FBXL7, ITPR3 and RAD51B) and one female-specific DEG (HLA-DGA1). Similar overlap among the blood DEGs, whole blood eGenes and GWAS genes yielded a single male-specific gene (ALOX15). We compared gene expression patterns of these five genes in asthmatic and control across different datasets (Supplementary Material, Fig. S2). Genes RAD51B and HLA-DQA1 were downregulated, whereas genes ITPR3, FBXL7 and ALOX15 were upregulated (Supplementary Material, Fig. S2). Supplementary Table S4 lists all the differentially expressed eGenes in the lung and blood separated by sexes. This result suggests a strong genetic influence in the expression of sex-specific genes. Multiple variants may influence gene expression through regulatory and epigenomic mechanisms in a sex- and tissue-specific manner though such variants are not directly associated to the phenotype.

Functional enrichment, pathways and network analysis of DEGs

In an effort to understand potential mechanisms driving sex differences in asthma, we compared the enriched KEGG pathways and gene ontology (GO) terms using the Consensus Path DB (36) in males and females. After pooling DEGs from the tissue-level meta- and interaction analyses, we obtained 439 DEGs in males and 297 DEGs in females with 32 shared genes (Supplementary Material, Table S5). Because the difference in the number of genes discovered across tissues and sexes affect the number of pathways discovered and FDR adjustment, we used unadjusted P-value < 0.05 to identify and compare the significant pathways between males and females. From the pooled set of genes, we identified 45 and 26 pathways that were significantly enriched (P-value < 0.05) with male- and female-specific DEGs, respectively, with only 10 shared pathways (Fig. 5). Cytokine–cytokine receptor interaction, leishmaniasis and malaria pathways were the most significantly enriched pathways with P-value < 0.01 shared between males and females (Fig. 5). Hypoxia-inducible factor 1 (HIF-1) signaling pathway, PI3K-Akt signaling pathway and hematopoietic cell lineage were among the male-specific enriched pathways, while interleukin 17 (IL-17) signaling, chemokine signaling and NOD-like receptor signaling pathways were induced in females. Interestingly, the IL17 signaling pathway overlapped with a total of eight DEGs in females (CXCL1, CXCL6, CSF3, MUC5AC, CXCL2, MUC5B, PTGS2 and TRAF3) but did not share any gene with male-specific DEGs. HIF-1 signaling pathway was identified as male specific with 10 male-specific DEGs. There were 17 male-specific DEGs in the cytokine–cytokine receptor interaction pathway versus 13 female-specific DEGs in the same pathway, and only three genes (CCR6, CXCR7 and CCL26) were shared between the sexes (Supplementary Material, Table S5).

We also compared the GO terms enriched for the sex-specific DEGs in males and females. The GO terms have hierarchical structure, and to make the comparison simpler, we limited our comparison to the level 2 GO-BP terms using the Consesus-PathDB web tool. Using the combined list of genes on Consesus-PathDB, we found 50 significant GO-BP terms in males and 22 terms in females, which shared a total of 20 terms at the significance level of P-value < 0.01 (Supplementary Material, Table S6). The top three GO-BP shared terms were related to cell activation, immune response and leukocyte activation. Among the top GO-BP terms for males were cell death, establishment of localization and cell communication. The large number male-specific DEGs may have resulted in the large number of significant GO terms for males in comparison to females, but the results indicates that there is a large overlap in the biological mechanism between male and female asthmatics, but the shared mechanisms may likely be induced by different set of sex-specific genes.

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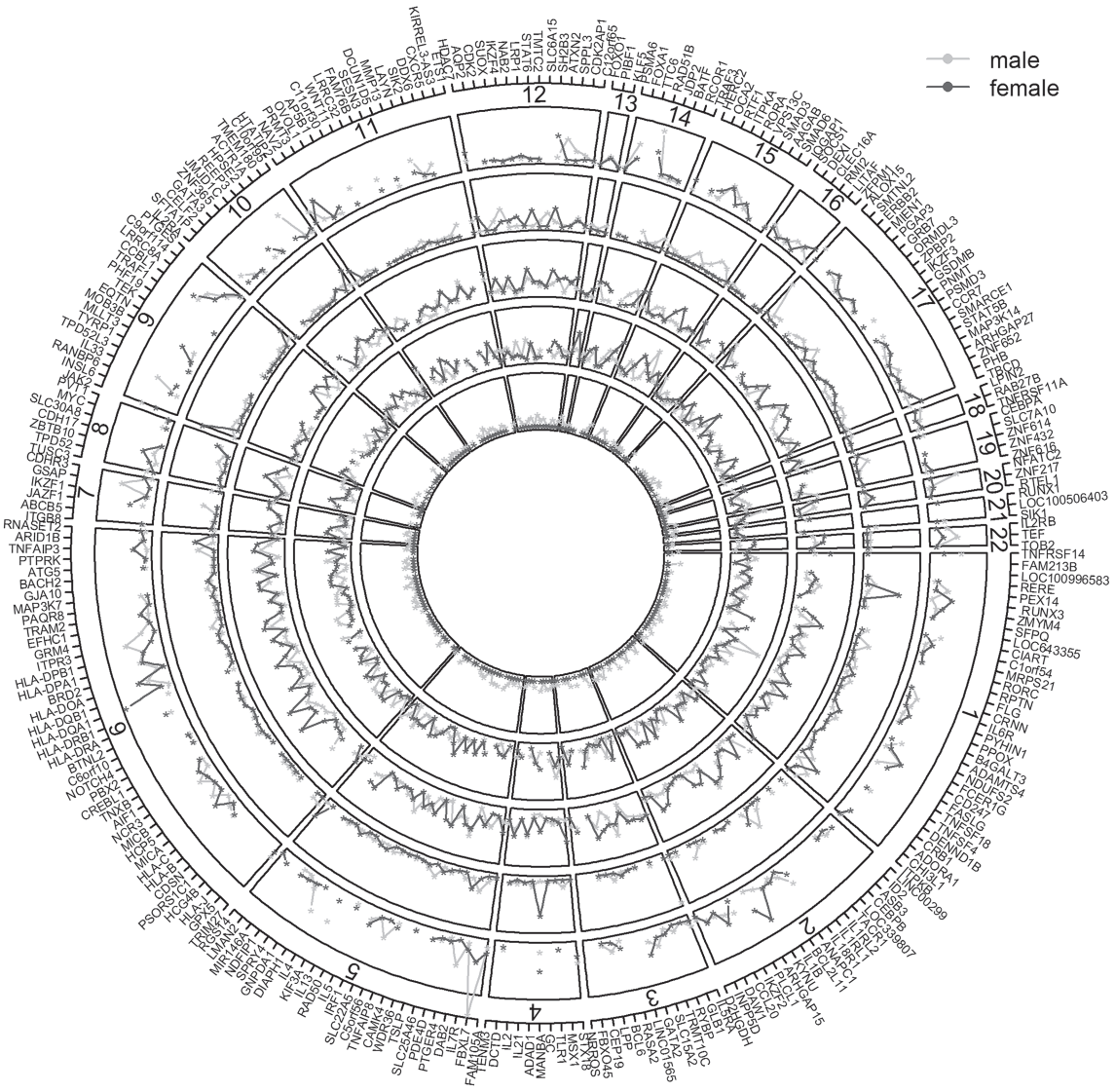

Figure 4. Differential expression of genes mapped to GWAS. The circle plot compares the differences in gene expression between males and females for genes mapped to asthma-associated GWAS variants. Starting from the outermost track, the plots show the results from (i) epithelial, (ii) blood, (iii) induced sputum, (iv) lymphoblastoid and (v) T-cells (CD3+ cells). Results from the sex-stratified and pooled analyses were merged with genes assigned to the strongest signal from either analyses. The y-axis represents the negative log of FDR-adjusted *P*-values from the tissue-specific meta-analysis for males (light gray) and females (dark gray) and ranges from 0 to 2. Genes are plotted along the x-axis by chromosome, with genes placed in uniform order based on the positional order GWAS-SNP mapped to the gene. Gaps in the plots reflect genes missing from the gene expression (meta-) analyses of the corresponding tissue.

We further refined the pathway level comparison between males and females to determine tissue-specific expression. The comparison is carried out at the significance level of *P*-value < 0.01 (Supplementary Material, Table S7). In epithelial cell, we identified 11 and 11 different significant pathways consisting of at least two DEGs in males and females, respectively, with only 1 shared pathways (hematopoietic cell lineage). The hematopoietic cell lineage pathway overlapped seven DEGs (HLA-DMB, ITGA6, ITGAM, CD36, CD44, CD9 and IL7) in males and four DEGs (HLA-DMB, HLA-DQA1, SF3, and CSF1R) in females with only one shared gene (HLA-DMB). HIF-1 signaling pathway was the most significantly enriched pathway in males but not in females. IL-17 signaling pathway was the most significant pathway in females but not in males. Although there was very little overlap at a significance level of *P*-value < 0.01, we have found several asthma- and immune-related pathways that were shared between males and females and likely regulated by the sex-specific DEGs. For example, cytokine–cytokine receptor interaction pathway shared 9 DEGs (IL7, CCR6, CCL26, BMP7, CXCR7, CXCR6, CX3CL1, CSF2RB and CCL5) from males and 10 DEGs (CXCL1, CXCL2, CXCL6, CSF3, CXCR7, IL18, CSF1R, CCR6, IL20RB and CCL28) from females but only 2 DEGs were shared between males and females (CCR6 and CXCR7). Similarly, chemokine signaling pathway shared six DEGs from males and six DEGs from females, but only one gene (CCR6) was common between males and females. Cytokine–cytokine receptor and chemokine signaling pathways were significantly enriched in females but not in males. Tissue-level pathway analysis for the blood group identified three and one significantly enriched pathways for males and females, respectively. However, none of these were shared between the sexes. NOD-like receptor signaling pathway was the only significant pathway enriched

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Table 5. GWAS Catalog genes significant in sex-specific DEGs

GWAS catalog gene	Associated SNPs	Chr. position	GWAS *P*-value	Sex	Tissue types
IL18R1	rs3771166	2q12	8.5229	Males	Induced sputum
IL5RA	rs9815663	3p26.2	7.699	Males	Epithelial, Blood
FBXL7	rs10044254	5p15.1	7.699	Males	Epithelial
HLA-DQA1	rs34004019	6p21.3	51.398	Female	Epithelial
ITPR3	rs10947428	6p21.31	12.398	Males	Epithelial
JAK2	rs16922576	9p24.1	9.5229	Males, female	Epithelial, blood
CCBL1	rs12551834	9q34.11	8.5229	Males, female	Epithelial
ACTR1A	rs10883723	10q24.32	7.699	Males	Epithelial
RAD51B	rs3784099	14q24.1	7.699	Males	Epithelial
ALOX15	rs71368508	17p13.2	8.699	Males	Blood
B4GALT3	rs4233366	1q23.3	14.301	Males	Lymphoblastoid
AIF1	rs28895016	6p21.33	15.0458	Female	Induced sputum
HCP5	rs2855812	6p21.33	11.0458	Female	Induced sputum
KYNU	rs74847330	2q22.2	8.699	Female	Epithelial
MANBA	rs227275	4q24	10.3979	Female	Blood
RUNX3	rs760805	1p36.11	12.2219	Female	Blood
TRAF3	rs9989163	14q32.32	7.6989	Female	Lymphoblastoid

in females with five DEGs (AIM2, VDAC1, IFNAR1, CARD16 and IRAK4), but this pathway did not respond to asthma in males. On the other hand, human papilloma virus infection was the most significant pathway in males with five DEGs (PDGFRB, COL9A2, PSMC1, THBS4 and PRKCI). In the lymphoblastoid cell, we identified five and one significantly enriched pathways for males and females, respectively, with no shared common pathway. NOD-like receptor signaling pathway was the only significant pathway in females with three DEGs (TRAF3, CASP1 and RANSEL). Cytokine–cytokine receptor pathway was identified to be significant in males with four DEGs (IL10, PDGFA, TNFRSF17 and LTA). There were no significant pathways identified in the induced sputum at the significance level of *P*-value < 0.01. All significantly enriched pathways from all the tissue groups can be found in Supplementary Material, Table S7.

Discussion

Asthma is a complex disease where males and females differ in prevalence and progression (1,10). While the DNA sequence among individuals may be similar, the gene expression landscape varies significantly depending on their sexes. These variations may contribute to distinct cellular processes (37) and to the development of sexually dimorphic phenotypes (38). Our approach to analyze gene expression data stratified by sex (males versus females) instead of a combined analysis using sex as a covariate is to get a better insight into how males and females with asthma differ in gene expression and how these differences play a role in pathways related to sexual dimorphism in asthma. Until recently, however, direct comparison of sex-differentiated gene expression profiles across multiple tissue types was not possible due to the limited number of gene expression data derived from different cells and the small sample size in each study. The availability of multiple data from GEO (39) has now provided an opportunity to perform combined sex- and tissue-based comparative analysis. In a recent candidate gene association study stratified by sex, we demonstrated that >55% of the genetic variants identified in sex-stratified analyses were not present in combined analysis (1). The same study found that 31% of DEGs in the sex-specific analysis were not found in the sex-combined analysis. Furthermore, sex differences between asthmatics were not primarily due to sex chromosomes, hormonal differences and/or meiotic recombination but due to genetic variations in autosomal chromosomes (1,22). Gershoni and Pietrokovski analyzed the GTEx data and found that more than 6500 protein-coding genes with significant sex-dependent differential expression in multiple tissues. This finding indicates that the autosomal transcriptome landscape varies considerably between sexes (37,38,40).

In our study, four important findings were evident. First, we identified distinct landscapes of sex-related differential gene expression in male and female asthmatics. We showed that males and females differ in the magnitude and, to a certain extent, in the direction of gene expression as demonstrated by low concordance rate among the top-ranked DEGs. Several of these genes are involved in various cellular processes that are vital to asthma pathogenesis, such as cell migration, airway remodeling and mucus production (41). The identification of sex-specific genes and pathways, which predispose individuals to asthma, will facilitate the targeting of novel therapeutics and treatment strategies in development. In addition, males and females differ in pathways that regulate immune responses, which could potentially drive sexual dimorphism in the prevalence and severity of asthma. To our knowledge this is the first study that analyzed tissue-specific gene expression in asthma using relatively big publicly available data stratified by sex (males versus females).

Second, we investigated the tissue specificity of sexual dimorphism in asthmatics gene expression. We showed that gene expression results from the airways epithelial cells showed consistent expression in both sexes compared to gene expression results from the blood group. Induced sputum and epithelial cells, which are the first contact of airborne microbe and allergens, exhibited similar gene expression profiles in males and females. Large proportion of DEGs in males were found in airways epithelia cells (epithelial, 325; blood, 47; lymphoblastoid, 60), whereas in females, epithelial and blood cells had a similar number of DEGs (epithelial, 118; blood, 118; lymbhoblastoid, 37) (Supplementary Material, Table S3). Based on this study, airways epithelial tissues harbored the majority of sex-specific biological pathways enriched with DEGs. We suggest that epithelial tissues can be used as a model system for asthma. These results suggest that sex-specific differences in gene expression are related to tissue-specific differences in

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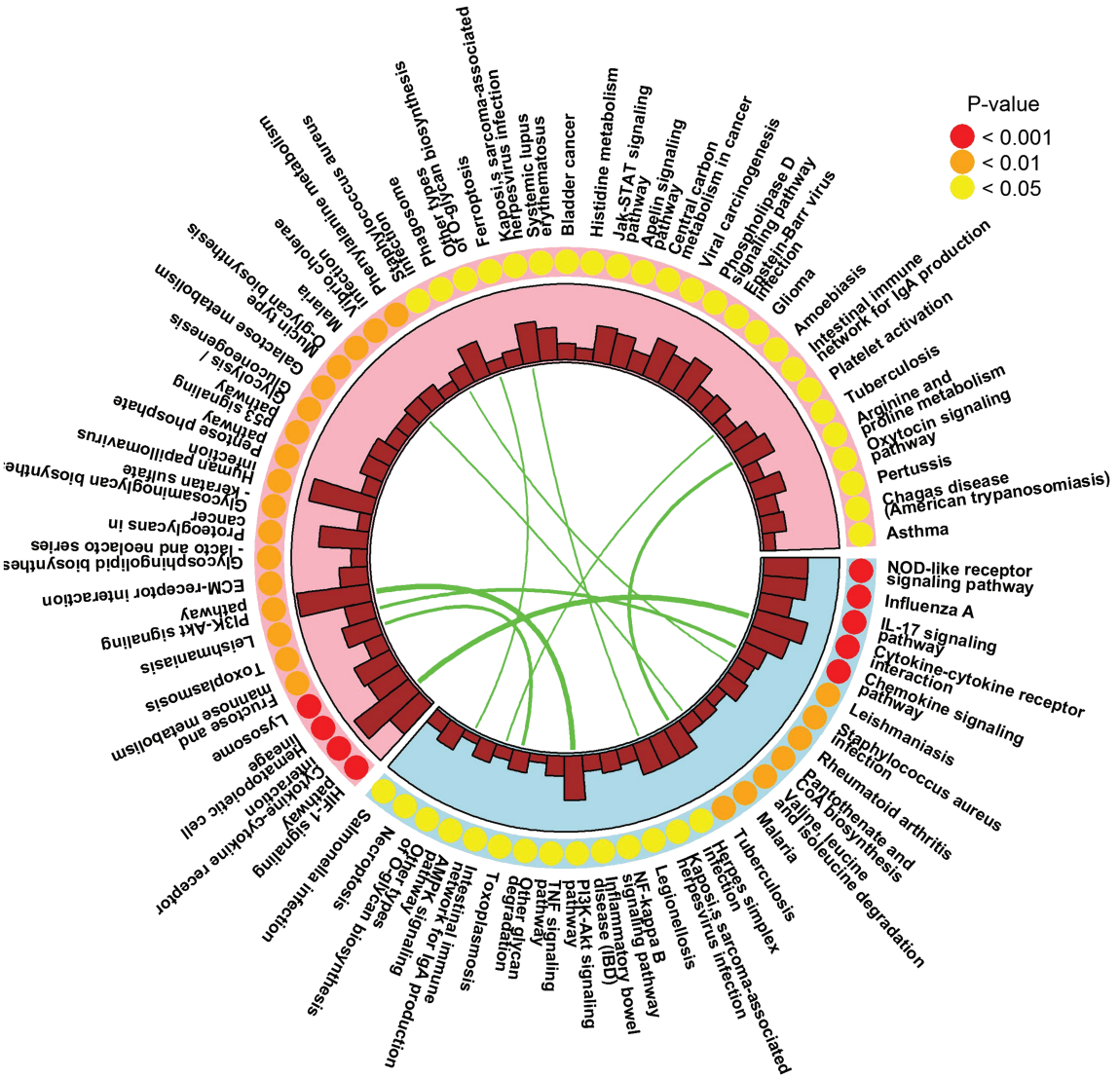

Figure 5. Functional pathway comparison. The circle plot represents enriched functional pathways in males and females with $P < 0.05$ cutoff. DEGs from stratified and pooled analyses across different tissues for each sex are combined together for the enrichment analysis (see the main text for detail). The outer track represents the enriched functional pathway, ordered from the most to the least significant, in the clockwise direction. The inner track shows bars (brown) representing the number of DEGs overlapped in each pathway. The green lines connect enriched pathways shared between males and females with width proportional to the number of shared DEGs. Track background color: light red, male; light blue, female.

asthmatics. Furthermore, sex–tissue interactions may be the major determinants in the development of asthma.

Third, we established sex-specific expression of asthma SNPs from GWAS catalog databases. By leveraging lung and blood eQTL annotation data from the GTEx and linking the DEGs and GWAS catalogs, we were able to map four male-specific genes (FBXL7, ITPR3 and RAD51B from epithelial tissue and ALOX15 from blood) and one female-specific gene (HLA-DQA1 from epithelial tissue). The overlap between GWAS-based genes with sex-specific gene expression should be given high priority. Often, these genes do not show strong associations in any one study, but the consistent evidence for association in different studies suggest that these are most likely relevant associations of interest (42).

Fourth, instead of focusing on identifying individual genes whose expressions are associated with sex-specific roles in asthma, we aimed to identify biological pathways influenced by sex-specific variants. HIF-1 signaling pathway is significantly enriched in male-specific DEGs. HIF-1 is a transcription factor that functions as a master regulator of oxygen homeostasis. HF-1 is essential for recruitment and aggregation of myeloid cell at the site of inflammation (43). Several studies have linked HIF-1 to allergic airway inflammation and asthma. Higher levels of HIF-1 alpha are found in lung tissue and bronchial fluid in asthmatic patients (44). Expression of HIF-1alpha also increases hypoxia-induced apoptosis of primary alveolar epithelial cells (45). HIF-1alpha subunit is also upregulated in alveolar macrophages and lung parenchyma during asthma (46). Studies in mice have shown that knockout HIF-1alpha subunit in myeloid cells reduces eosinophil infiltration to the airway epithelium and treatment with the antagonist YC-1 decreased airway hyper-responsiveness (47). In our study, genes in this pathway were upregulated in males suggesting that, in males, overexpression of HIF-1 would trigger eosinophil infiltration

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to the airways causing hyper-responsiveness in response to allergen exposure. Overexpression of HIF-1 signaling pathways in asthmatic males, especially in the airways epithelial cells, when compared to females may compensate for an estrogen deficiency in males while activating the immune system in the presence of allergens. IL-17 cytokines are precursors of TH17 cells and are associated with airway inflammation, hyper-responsiveness and severity of asthma (48). The IL-17-mediated asthma represents a distinct endotype, called neutrophilic asthma, characterized by neutrophilic inflammation of airways (49). Estrogen and progesterone, the predominant sex hormones in females, are responsible for increased production of IL-17 cytokine in females (50).

Although the role of IL-17 in asthma severity specifically in females is unknown, our study shows that IL-17 signaling pathway is enriched in asthmatic females but not in males, which could partly explain the prevalence of severe asthma in post-pubertal females. The relationship between IL-17 receptor gene and the risk of asthma has been reported by several studies (51–54). Meanwhile males and females share the cytokine–cytokine receptor pathway. Interestingly, most of the genes in this pathway were upregulated in males and downregulated in females. Furthermore, in males, most of the genes in this pathway cluster around the IL-4-like cytokine regulation genes. IL-4 is a cytokine that regulates the production of IgE, and it helps in the differentiation of naive T-cells into T-helper 2 cells (55). Over-production of IL-4 creates a hyper-response cascade in the airways. In females, the genes in the cytokine–cytokine receptor pathway cluster around the CXC-subfamily of chemokines. Cytokines are essential proteins that act as regulators and mobilizers of cells engaged in innate and adaptive inflammatory response (56). This may imply that, although males and females share a common pathway, the mechanism of action is different in each sex.

Functional enrichment analyses of the top DEGs in both males and females revealed an overrepresentation of pathways related to immunity and regulation of inflammation. We have found several immune-related pathways uniquely overrepresented in males and females, which could be potential targets for sex-specific asthma prognosis. Perhaps the greatest finding from sex- and tissue-specific analysis of gene expression is not the differential expression of individual genes but the identification of sex-specific pathways where these genes act. It should be noted that the female sex hormone estrogen is pro-inflammatory and plays a major role in activating innate and adaptive immune systems and myeloid cell development (57). Estrogen is produced in larger amounts in females than males (58). The disparity in the level of estrogen in males and females may lead to activation or inhibition of sex-specific immune regulatory mechanisms, especially in the presence of microbes and allergens, which could explain biological pathways enriched for sex-based DEGs (59).

Although our study is powered with large sample size and systematic statistical approaches, it has important limitations. The majority of DEGs in sex-stratified analysis were sex-specific with very low or no overlapping genes. Thus, it is possible that our approach missed sex interaction effects. However, our sex-stratified analysis inherently accounts for the interaction effects without requiring to model the main effects. Such an approach has the potential to avoid canceling effects and increase the power of detecting DEGs (1,22). In addition, sex-stratified analyses were further complemented by 'pooled analyses', identifying genes with sex-specific marginal effects not initially identified. The pooled analysis is optimal to identify the sex interaction effects but has low power in detecting the main effects (22). On the other hand, distinct biological pathways enriched with sex-differentiated DEGs indicate differences in asthma pathogenesis between males and females. Although asthma is primarily an autosomal trait (60), the female sex hormone estrogen affects immune-regulatory cells and induces pro-inflammatory reactions and increases allergy and asthma susceptibility among females during post-puberty (61,62). It is possible that hormonal changes, dictated by genetic and epigenetic factors, may contribute to the sex bias in immune response and hence contribute to differences in prevalence and severity of asthma and other autoimmune responses in males and females (63). A future study incorporating genetic, epigenetic, hormone and environment on sex-stratified analyses can further elucidate their roles in sexual dimorphism on asthma.

Another approach to analyze gene–sex interaction was to perform a case-only study comparing asthmatic males versus females after excluding genes with functions inherently associated with sex. We performed the case-only analyses by tissue types comparing asthmatic male versus asthmatic female. We excluded the significant genes from sex chromosomes and other genes ubiquitously expressed in males or females as these genes may likely be functionally associated with sex. We further matched the significant genes from the case-only analyses with the DEGs from the sex-specific analyses. This analysis produced 33 and 9 new significant genes in blood group and lymphoblastoid, respectively, but no unique genes were found in epithelial, induced sputum and T cell (Supplementary Material, Table S8). These genes may represent tissue–sex interaction of genes, which the sex-specific analyses may not have accounted for and could be explored in further studies. However, the new genes from the case-only analyses could not be assigned to male- or female-specific gene set as these genes were not significant in the sex-specific analyses.

In summary, a comprehensive sex-stratified analysis of multiple gene expression datasets from distinct human tissues/cells revealed a not previously recognized involvement of sex-specific genes and pathways in asthma. Our results highlight the importance of sex-stratified analysis in tissue-specific manner when analyzing gene expression data. We suggest that sex-specific gene expression analysis should become a standard framework for interpreting the wealth of disease-related omics data and for enhancing our understanding of the etiologies of asthma. Future analysis should implement sex and tissue-specific analysis to establish personalized medicine in asthma. More research is needed to prospectively validate and ultimately develop sex-specific treatments for asthma. By integrating sex-specific DEGs with GWAS catalog and GTEx eQTL, we identified biologically relevant overlapping asthma genes, which should be given high priority for follow up analysis. Linking omics data intuitively represents an important step for interpreting the biological and clinical relevance of DEGs and facilitates future sex-specific precision medicine.

Materials and Methods

Gene expression datasets for asthma

To investigate whether the association between gene expression and asthma differs between male and female, we used gene expression datasets on asthma available from the National Center for Biotechnology Information GEO database (39). We searched asthmatics versus healthy controls using the following

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criteria: publication date between January 1, 2000, and March 31, 2018; asthma diagnosis; and organism human. The inclusion criteria for the datasets were (a) dataset must compare asthmatic individuals and healthy controls (non-asthmatics), (b) dataset must contain male and female samples in the asthmatic and control groups, (c) dataset must include a tissue label and (d) dataset must compare multiple individuals in each group. The following information was extracted from each study: (1) GEO accession numbers, (2) tissue type, (3) gene expression platform, (4) number of asthmatic and healthy individuals, (5) sex and (6) gene expression values. Information about the datasets including tissue types are summarized in Table 1. We used the normalized gene expression data from GEO for downstream analyses.

Statistical analysis

Gene expression analysis of individual datasets. To evaluate differences in gene expression between males and females, we first stratified each dataset by sex and used *t*-test based on the limma package (64) to analyze the sex-stratified datasets comparing asthmatic males versus control males and asthmatic females versus control females. The analysis was carried out using INVEX, an online tool for gene expression analysis (34). We used average gene expression to combine multiple probes mapped to the same gene and dropped 5% of the genes with the lowest variation. We used concordance rate and correlation to compare gene expression similarity between males and females across different databases.

Concordance rate: the concordance rate measures the proportion of shared genes among the top ranked DEGs between males and females in the sex-stratified analysis of a database. Genes were ranked using the unadjusted *P*-value from the most to the least significant in males and females, separately. If **m** is the number of overlapped genes within the top **t** percentile ($t = 1, 2, 3 \ldots 100$) DEGs in males and females and **N** is the total number of genes analyzed, then the concordance rate (ζ) is defined as

$$\zeta = \frac{100m}{tN}.$$

Correlation: we used the Pearson correlation coefficient to compare correlation of gene expression between males and females. The Pearson's correlation coefficient between random variables *x* (females) and *y* (males) is defined as

$$r = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}},$$

where x_i and y_i are the ith value of *x* and *y*, respectively; $\bar{x}$ and $\bar{y}$ are the average of *x* and *y*, respectively. The correlation coefficient (*r*) ranges from −1 to 1, with *r* closer to 1 or −1 indicating a monotonically increasing or decreasing relationship and *r* closer to 0 signifying weak or no relationship. We performed correlation analysis on average gene expression values between males and females, separately for cases and controls respectively. Additionally, we further computed correlation on the fold change differences using all genes with fold change ≥1.5 in either sexes. In the fold change correlation analysis, if there were fewer than 50 genes together with fold change >1.5, the correlation coefficient was computed using the top 25 genes.

Tissue-based sex-specific meta-analysis

To identify the tissue-specific gene expression differences in males and females, we performed the sex-specific gene expression analyses on data grouped by tissue. Data were grouped into five distinct tissue groups—epithelial, whole blood, T cells, induced sputum and lymphoblastoid cell line from asthmatics and controls as shown in Table 1. Epithelial and blood groups consisted of multiple datasets, and we performed gene expression meta-analyses on the two tissue groups. The meta-analysis was performed by combining the effect size (*d*), which is based on the *t*-statistics from limma and is defined as

$$d = \frac{t_{\text{limma}}}{\sqrt{\bar{n}}},$$

where t_{limma} is the moderated test statistics based on limma and $\bar{n} = n_1 n_2/(n_1 + n_2)$ with n_1 = sample size in asthmatic and n_2 = sample size in control group in each data set. For each gene, the statistic *d* is computed across all datasets within the tissue group, and the meta-analysis based on the *d*-statistics is performed under random effect model with correction for batch effect. The combined test statistics based on the effect size (*d*) and variance estimation were done as described previously (65).

We performed two complementary approaches of sex-specific gene expression analyses—sex stratified and pooled control. In the sex-stratified approach, gene expression (meta) analyses were carried out independently for male and female on the stratified data for each tissue/cell type. In the pooled-control approach, we performed sex-specific analysis by comparing the cases of one sex to a pooled control group that consist of the cases of the opposite sex plus the controls of both sexes. The pooled-control analysis was performed at the tissue level followed by meta-analyses by combining multiple datasets within same tissue group. Significant DEGs were identified at tissue-level with an FDR-adjusted *P*-value < 0.05. The pooled control provides a larger sample size and hence increased power in identifying the DEGs with sex interaction effects and complements the sex-stratified analyses described above. We used the INMEX tool to run gene–sex interaction analysis (34).

Genetic influence on asthma transcriptome

To explore whether GWAS catalog genes showed differential expression between sexes in different tissues, asthma-associated SNPs with genome wide significance cutoff of *P*-value < 5E-8 were extracted from the published GWAS catalog database (http://www.ebi.ac.uk/gwas/, as of May 2018) (66). There were 37 different GWAS studies with 284 asthma-associated SNP variants mapped to 402 genes that included the nearest upstream and downstream genes for intergenic SNP variants. These genes were mapped to sex-specific significant DEGs across the five tissue types. To further explore the influence of genetic variation on gene expression, we used the GTEx eQTL datasets to identify genes with eQTL association (35). We used the lung eQTL data for mapping DEGs from airways epithelial tissue and the whole blood eQTL data for mapping DEGs from the blood tissue. The pairing of tissues (airways epithelial versus lung and blood versus blood) in the eQTL-DEG mapping reciprocated the primary asthma-relevant tissue types in the respective studies.

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GO and pathway enrichment analysis

The functional annotation of gene sets can identify the most pertinent biological processes represented by the sex-specific DEGs. In an effort to understand the underlying mechanisms driving the difference in asthma prevalence between males and females, we identified and compared enriched biological pathways and GO terms. Using the ConsensusPathDB web tool, we obtained significantly enriched GO biological process terms and KEGG pathways (42). We considered enriched functional classes with P-value < 0.01 as significant.

Supplementary Material

Supplementary Material is available at *HMG* online.

Conflicts of Interest statement. None declared.

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Supplementary Figures

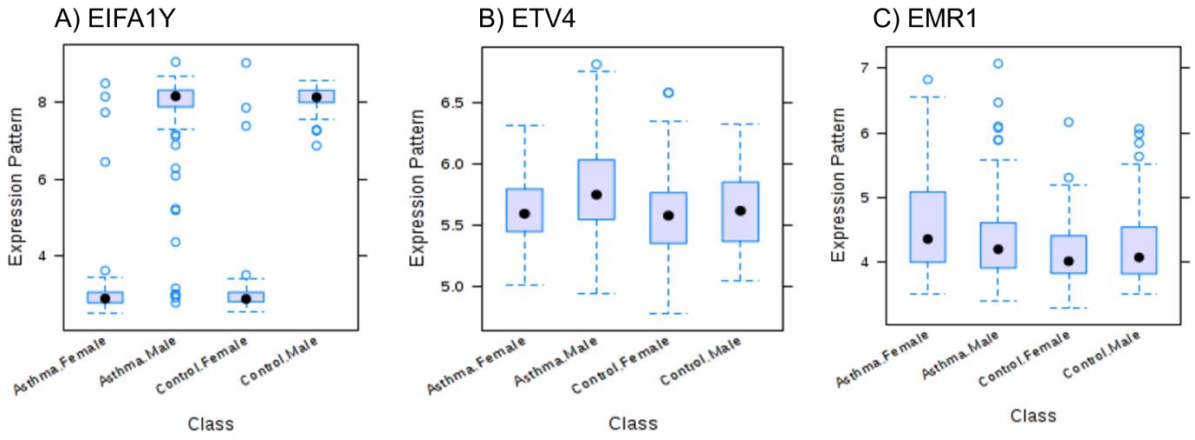

Figure S1. Examples of gene-expression - sex interaction analysis. Figure compared the gene expression patterns in the four subgroups of samples: asthmatic female, asthmatic male, control female, and control male. Normalized gene expression data for the selected genes (EIFA1Y, ETV4, and EMR1) from the dataset GSE8052 (lymphoblastoid cell line) were used to generate the box plots. The solid circles in the box represent the average gene expression in the respective subgroups.

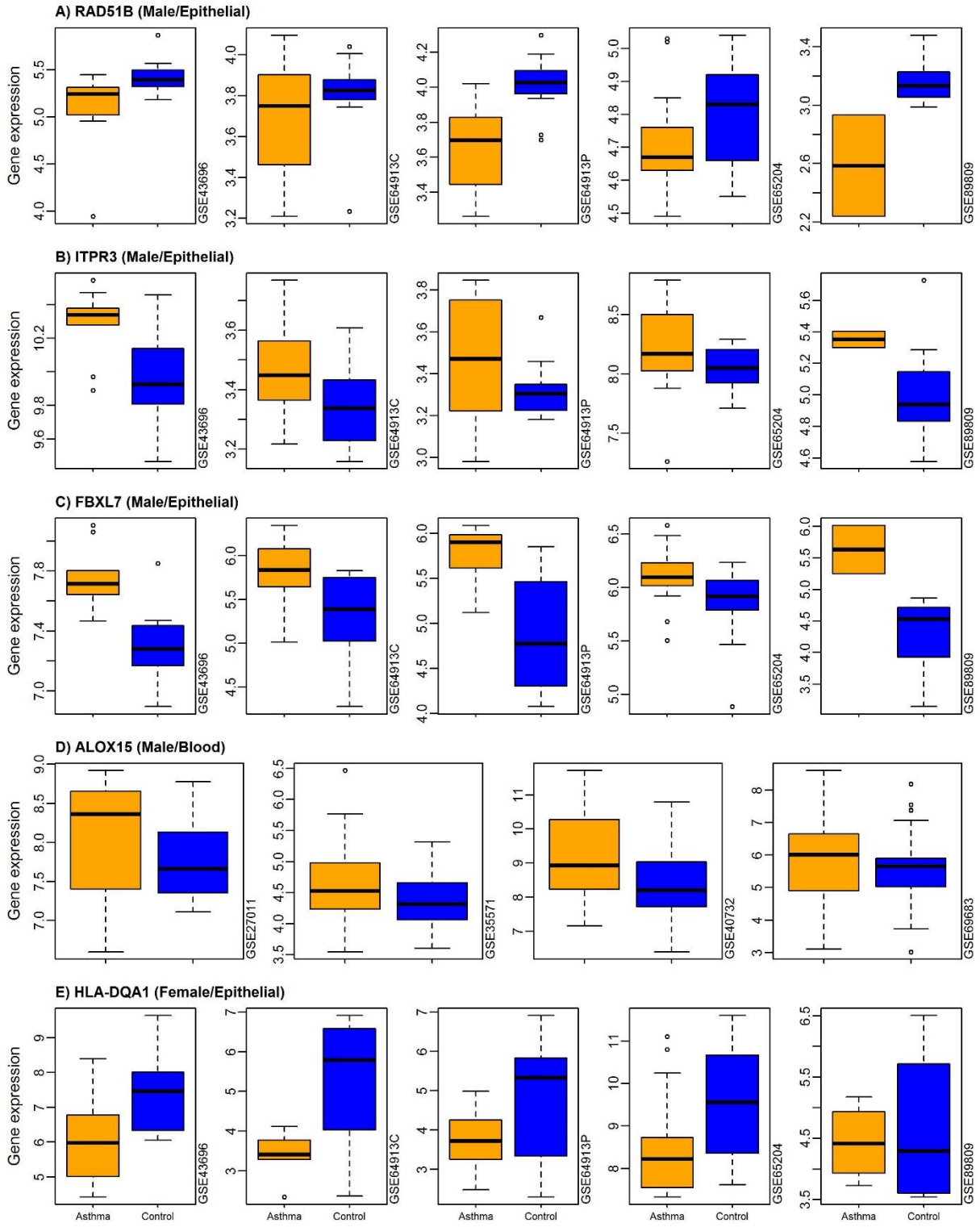

Figure S2. Gene expression of DEG-GWAS-GTEx overlapped genes. Figure shows the distribution of the gene expression of the selected sex- and tissue- specific differentially expressed genes among asthmatic and control group across different data bases. Genes were selected based on the overlapped among the GWAS genes, GTEx eGenes, and sex-specific

DEGs (see details in the method section). Panels A), B), and C): normalized expression of RAD51B, ITPR3, and FBXL7, respectively, among asthmatic and control males across five different databases within epithelial group. Panel D): normalized expression of ALOX15 among asthmatic and control males across the four different databases within the blood group. Panel E): normalized expression of HLA-DQA1 among asthmatic and control females across the five different databases within the epithelial group. GSE ID of the database was shown at the bottom-right corner of each plot.