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RESEARCH ARTICLE

Transcriptomics analysis of allergen-induced inflammatory gene expression in the Four-Core Genotype mouse model

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Abstract

Sex differences in allergic inflammation have been reported, but the mechanisms underlying these differences remain unknown. Contributions of both sex hormones and sex-related genes to these mechanisms have been previously suggested in clinical and animal studies. Here, Four-Core Genotypes (FCG) mouse model was used to study the inflammatory response to house dust mite (HDM) challenge and identify differentially expressed genes (DEGs) and regulatory pathways in lung tissue. Briefly, adult mice (8–10 wk old) of the FCG (XXM, XXF, XYM, XYF) were challenged intranasally with 25 μg of HDM or vehicle (PBS-control group) 5 days/wk for 5 wk ($n = 3/10$ group). At 72 h after the last exposure, we analyzed the eosinophils and neutrophils in the bronchoalveolar lavage (BAL) of FCG mice. We extracted lung tissue and determined DEGs using Templated Oligo-Sequencing (TempO-Seq). DEG analysis was performed using the DESeq2 package and gene enrichment analysis was done using Ingenuity Pathway Analysis. A total of 2,863 DEGs were identified in the FCG. Results revealed increased eosinophilia and neutrophilia in the HDM-treated group with the most significantly expressed genes in XYF phenotype and a predominant effect of female hormones vs. chromosomes. Regardless of the sex hormones, mice with female chromosomes had more downregulated genes in the HDM group but this was reversed in the control group. Interestingly, genes associated with inflammatory responses were overrepresented in the XXM and XYF genotypes treated with HDM. Sex hormones and chromosomes contribute to inflammatory responses to HDM challenge, with female hormones exerting a predominant effect mediated by inflammatory DEGs.

NEW & NOTEWORTHY Gene expression profiling helps to provide deep insight into the global view of disease-related mechanisms and responses to therapy. Using the Four-Core Genotype mouse model, our findings revealed the influence of sex hormones and sex chromosomes in the gene expression of lungs exposed to an aeroallergen (House Dust Mite) and identified sex-specific pathways to better understand sex disparities associated with allergic airway inflammation.

allergic inflammation; Four-Core Genotypes; lung inflammation; sex chromosomes; sex hormones

INTRODUCTION

Asthma is a chronic respiratory disease with known sex differences and an unclear etiology. This respiratory disease affects over 25 million people in the United States and over 300 million worldwide (1, 2), and its prevalence continues to increase in many countries (3, 4). In children, the prevalence of asthma is higher in males than in females (5), but the reverse occurs in adults (10.7% vs. 6.5%) from the age of 13 yr to about 65–70 yr (6). Because of this switch in prevalence observed at puberty, sex hormones have been suspected to play a role in asthma development (7). Both clinical and animal studies have established a strong link between the hypothalamic-gonadal-pituitary axis and lung function and immunity (8, 9). In addition to hormonal influences occurring at puberty, variations in genes of the X chromosomes as well as those of autosomes can contribute to the phenotypic differences seen in males and females during asthma (10, 11). Although asthma's genomic influence is thought to be sex-specific (12–14), there is a paucity of data on the mechanisms through which sex differences occur.

During asthma, infiltration of inflammatory cells occurs in the lung (15). These cells are known to play an essential role in driving inflammatory responses and releasing cytokines that result in airway inflammation and obstruction (15). Environmental exposures such as aeroallergens can influence the development and exacerbation of asthma (16). House dust mites (HDMs) are an example of aeroallergens that interact with the innate immune system and are therefore highly immunogenic (17). *Dermatophagoides pteronyssinus* and *Dermatophagoides farina* are HDM species that are among the primary sources of allergens in house dust globally (18). HDM can contain both protease and non-protease allergens and can cause the development of epithelial

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inflammation in the airway by secreting chemotactic factors (19). Some subtypes of *Dermatophagoides pteronyssinus* are proteases that degrade the vascular epithelium tight junction in the lungs, this allows the allergens to enter the body stream thereby leading to immune response stimulation (20). *Dermatophagoides farina* sensitivity has been linked to increased asthma hospitalization and total Immunoglobulin E (21). A linear-dose response relationship exists among dust mite, exacerbation of asthma, and increased medication (21). A study by Bracken et al. (22) showed that short (2 wk) and intermediate (5 wk) exposure to HDM induced an allergic airway disease phenotype in female C67BL/6 mice. Female mice exposed to 5 wk of HDM treatment displayed elevated eosinophilia, peribronchial and perivascular inflammation, and elevated airway resistance (22). More recently, Mostafa et al. (23) showed that HDM exposure (2 wk) induced sex- and strain-specific differences in eosinophilia, gene expression, and pro-inflammatory cytokine expression. However, how HDM exposures affect the asthma phenotype in males and females, and particularly the specific contributions of

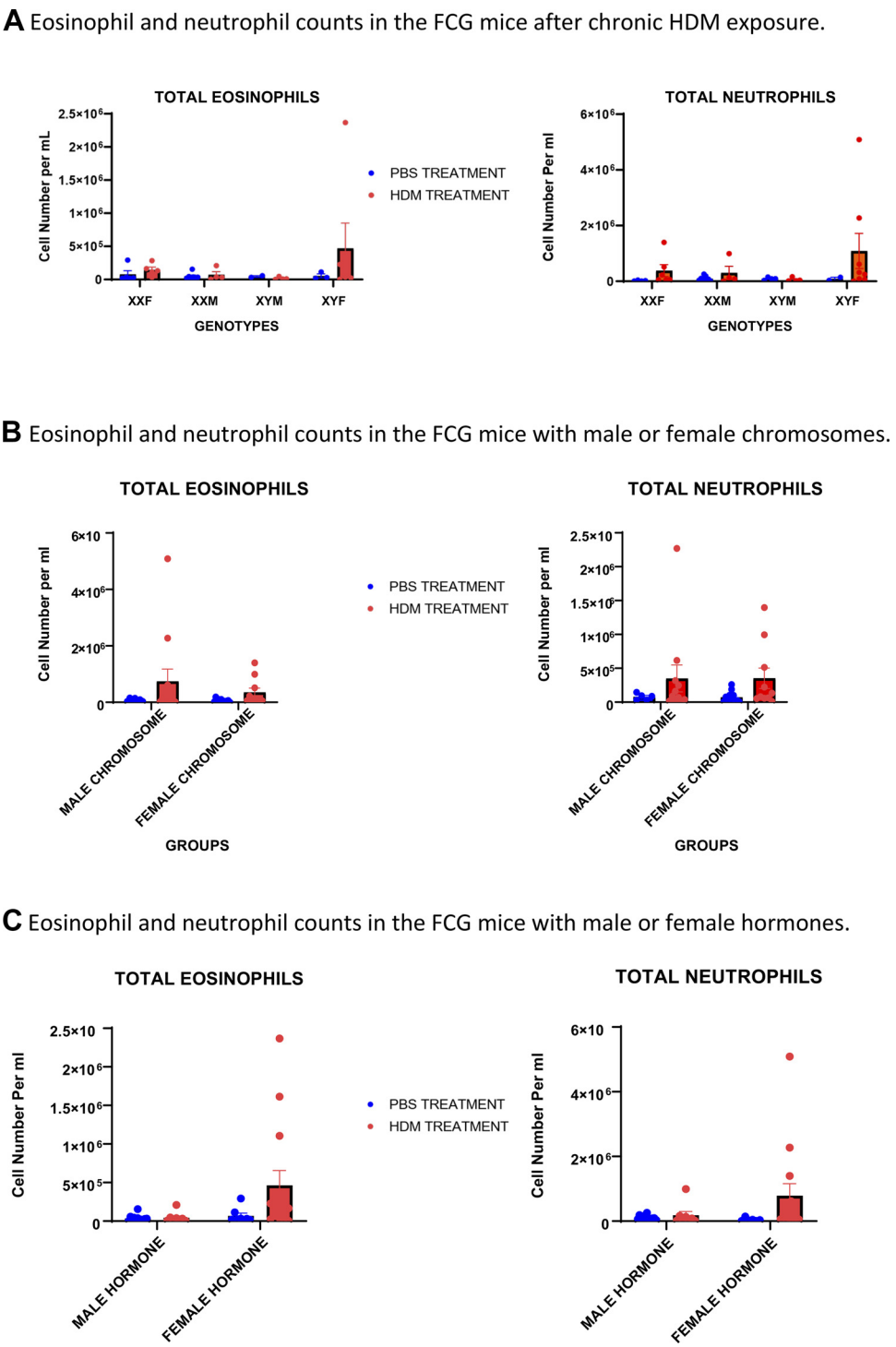

Figure 1. Eosinophil and neutrophil counts in the FCG mouse model of airway inflammation in the FCG mice following chronic exposure to HDM, in FCG mice with male or female chromosomes, and in the FCG mice with male or female hormones. Changes in the different groups were not statistically significant ($n = 7$–10, P value $<$ 0.05). *A*: nonsignificant increasing trend in eosinophil and neutrophil counts in the XYF genotype mice exposed to HDM compared with the other genotypes. *B*: nonsignificant increasing trend in eosinophils and neutrophils in the male and female chromosome HDM-induced mice compared with the control. *C*: nonsignificant increasing trend in eosinophil and neutrophil counts in the female hormone mice, induced with HDM compared with the male hormone mice. FCG, Four-Core Genotype; HDM, house dust mite.

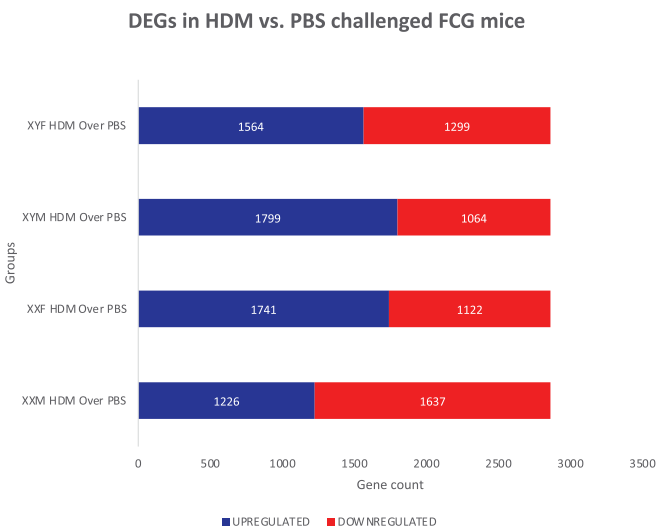

Figure 2. Numbers of DEGs in HDM-challenged mice over PBS-administered groups in the FCG mouse model ($N = 3$). Red part represents the upregulated DEGs, whereas the blue part represents the downregulated DEGs. More genes were upregulated in all the genotypes except in the XXM mice that have more genes downregulated (DESeq2 FDR < 0.05). DEG, differentially expressed gene; FCG, Four-Core Genotype; PBS, phosphate-buffered saline.

sex chromosomes and sex hormones to these phenotypes remain unknown. Therefore, to understand whether sex hormones or genes in sex chromosomes have a predominant role in asthma phenotypes, we evaluated the phenotypic effect of an allergen challenge using the Four-Core Genotypes (FCG) animal model (24). In this model, four groups of mice are genetically modified to have both male and female gonads irrespective of their biological sex producing "XX" or "XY" mice with male or female gonads (hence, the four cores are XXM, XXF, XYM, and XYF).

Based on the differences observed in males versus females before and after puberty, we hypothesized that sex hormones have a predominant role in allergen-induced inflammatory gene expression changes triggered by allergen exposure in females. Thus, by challenging the FCG model with HDM and assessing differentially expressed genes, we aimed to identified gene networks and predicted regulatory pathways in lung tissue that are influenced by sex hormones and chromosomes.

MATERIALS AND METHODS

Animals

Mice from the FCG model were obtained from Jackson Laboratories, Bar Harbor, ME (stock no 010905) and bred in-house according to protocol. The wild type was bred with XYM males of C57BL/6J background, and the resulting pups belong to one of the four genotypes (XXM, XXF, XYM, and XYF). After genotyping by genomic PCR to detect a control autosomal gene and the Y chromosome, *Sry* gene, mice from the FCG were used at 8–10 wk old (19–31 g). They were further divided into control and experiment groups of each genotype. These animals were treated according to guidelines of the National Institute of Health for the care and use of laboratory animals, and the protocol was approved by the Bloomington Institutional Animal Care and Use Committee (BIACUC) before the commencement of the study (Protocol 21-012).

Intranasal Instillation of PBS or HDM Challenge

The experimental group was challenged by administering 50 μL of HDM solution (25 μg of HDM extract from two species, *Dermatophagoides pteronyssinus, and* Dermatophagoides *farinae,* Citeq biologics, Groningen, The Netherlands) five times a week for 5 wk. The control group was challenged intranasally by administering 50 μL of phosphate-buffered saline (PBS) solution while using the same route of administration. The 50 μL HDM or PBS was intranasally administered to the FCG mice using 20- to 200-μL pipette tips after a light anesthesia with 5% isoflurane using the SomnoSuite device (Kent Scientific). At 72 h after the last exposure, lung tissues were harvested for the differential gene expression analysis.

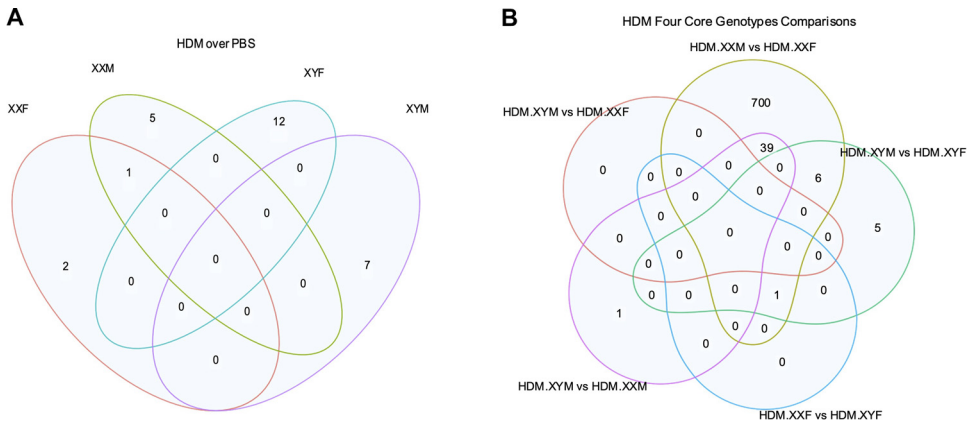

Figure 3. *A*: numbers of significantly expressed genes in the HDM-challenged mice over the control. Venn diagram shows the number of genes that were significantly expressed comparing the HDM-challenged groups with the control mice in all the genotypes ($N = 3$). The diagram also shows the interception of these genes among the group. Only the XXF and the XXM had one gene, GM20831, in common while the XYF had the most significantly expressed genes. *B*: numbers of significantly expressed genes in the HDM-challenged mice. Venn diagram shows the number of genes that were significantly expressed in the HDM-challenged mice ($N = 3$). The different genotypes were paired and the HDM-challenged mice in XXM>XXF had the highest number of significantly expressed genes compared with the other pairs. Some genes were intercepted between the XXM vs. XXF and XYM vs. XXM pairs and with the XXM vs. XXF and XYM vs. XYF pairs. HDM, house dust mite.

Bronchoalveolar Lavage Fluid

The lungs were lavaged with a mix of 1 mM EDTA/PBS (2.5 mL per mouse). The trachea of the mice was cannulated and 0.5 mL of the mix was injected into the lungs and the chest cavity massaged. The mix was withdrawn and placed into a collecting tube; the step was repeated about five times to retrieve up to 2.5 mL of lavage fluid. This is then spun down at 1,300 rpm for 10 min at 4°C, and the supernatant is removed. The pellet was resuspended in 500 μL of 1 mM PBS/EDTA mix, and 100 μL of the resuspended cells were used for differential cell counts. Cytospin slides were prepared, air-dried, and stained. A differential cell count was determined in a minimum of 100 cells.

RNA Extraction and Gene Expression Profile

Lung tissues were harvested and pulverized, and RNA was extracted using the Direct-zol RNA Miniprep plus kit (Zymo Research Corporation, Irvine, CA). A Nanodrop ND-2000 spectrophotometer (Thermo Scientific, Wilmington, DE) was used to analyze the concentration and quality of the RNA extracted in the different groups. Using targeted sequencing-based RNA expression, the mouse RNA (n = 3/group) was analyzed, and the result was generated from TempO-Seq assays (Arctoris, Ltd., Oxfordshire, UK).

Data Analysis

The eosinophils and neutrophils were counted by three blind counters, and the average of the count was collated, using the GraphPad prism 10.0.2, data were expressed in means ± SE. The means of the different groups were compared using the two-way ANOVA and a P value of <0.05 was taken as statistically significant. For TempO-Seq, differential expression analysis was performed using the *DESeq2* package (version 1.40.2) in *R/Bioconductor* (R version 4.3.1) (25). Heatmaps were generated using the R Bioconductor package *heatmap* (version 1.0.12). Significant differentially expressed genes were determined using a P adj. < 0.05. Venn diagrams were generated using the R package *Venn Diagram* (version 1.2.2). *UpSet* plots were generated using *Complex Upset* (version 1.3.3) and *UpSetR* (version 1.4.0).

Ingenuity Pathway Analysis

Networks of differentially expressed genes were constructed with the use of QIAGEN Ingenuity Pathway Analysis (IPA) (QIAGEN, Inc., https://digitalinsights.qiagen.com/IPA) (26). This was used to visualize the genes' associations

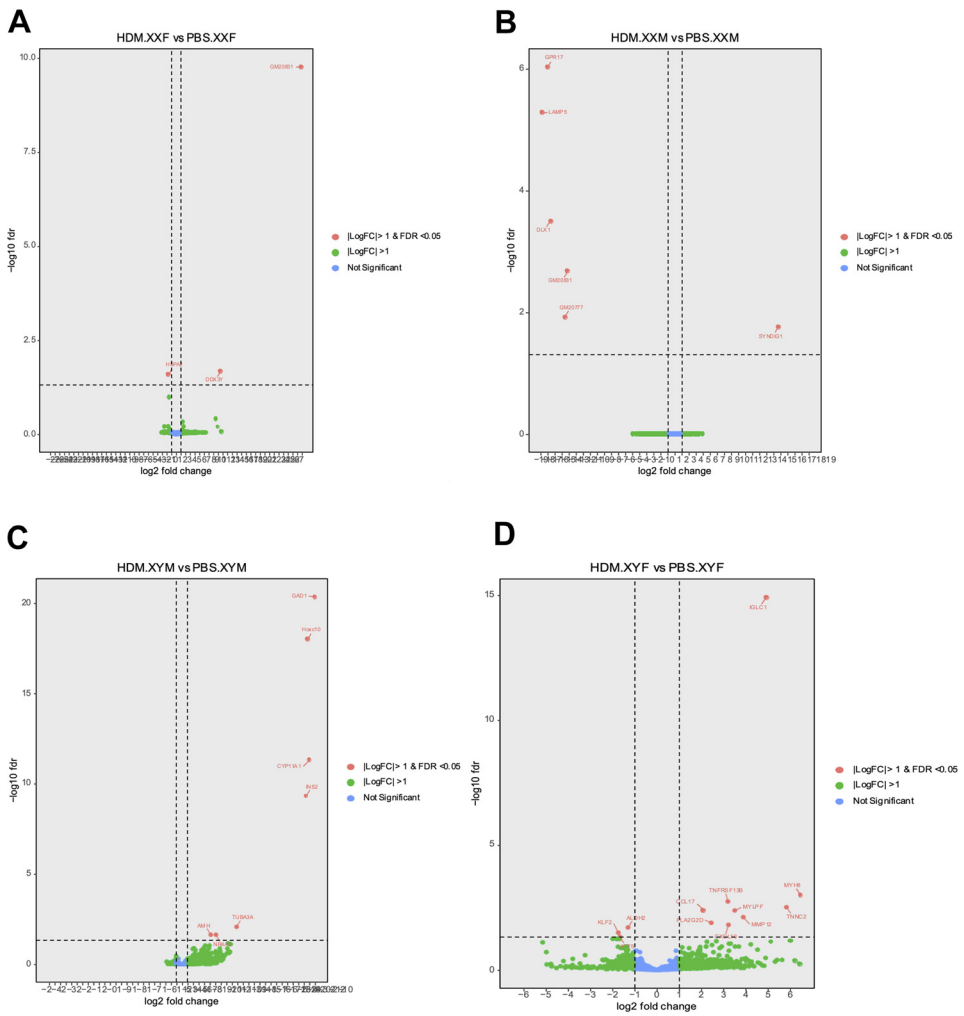

Figure 4. Volcano plots identifying the significantly expressed genes in the HDM-challenged over the control mice in the four core genotypes. *A*: significantly expressed genes in the HDM-challenged over the control mice in the XXF genotype. Volcano plot shows three genes that were significantly expressed in the XXF HDM-challenged mice compared with the control group ($N = 3$). One of the genes was downregulated while the other two were upregulated. *B*: significantly expressed genes in the XXM genotype. Volcano plot shows the 6 genes that were significantly expressed in the XXM HDM-challenged mice compared with the control group ($N = 3$). Only one of the genes was upregulated while the others were downregulated. *C*: significantly expressed genes in XYM genotype. Volcano plot shows the 6 genes that were significantly expressed in the XYM HDM-challenged mice compared with the control group ($N = 3$). All the significantly expressed genes in this genotype were upregulated. *D*: significantly expressed genes in the XYF genotype. Volcano plot shows the 6 genes that were significantly expressed in the XYF HDM-challenged mice compared with the control group ($N = 3$). Many of the significantly expressed genes in this genotype were upregulated while only three of them were downregulated. HDM, house dust mite.

with canonical pathways implicated in asthma and lung inflammation.

RESULTS

Effect of Chronic HDM Exposure on Neutrophils and Eosinophils in the FCG Mouse Model

FCG mice were exposed to PBS or HDM intranasally for 5 days/5 wk to induce allergic airway inflammation in the lungs. We analyzed the neutrophils and eosinophil counts in the PBS and HDM-exposed mice in the different genotypes. The analysis showed a trend toward increase in neutrophil and eosinophil counts in the HDM-induced group compared with the PBS group mostly in the XYF genotype (Fig. 1*A*) and slightly in the XXF and XXM genotypes, but the increase was not statistically significant at P value < 0.05. Although we observed a trend toward an increase in counts, there was no statistically significant change in eosinophils and neutrophils when comparing animals with male and female chromosomes exposed to HDM versus PBS (Fig. 1*B*). Similarly, the HDM-treated mice with female gonads showed elevated eosinophil and neutrophil counts when compared with the corresponding male mice, but this increase was also not statistically significant (Fig. 1*C*).

Differentially Expressed Genes in FCG Model Mice After HDM Exposure

A comprehensive gene expression analysis using the Tempo-Seq was performed in the lungs of the control and the HDM-challenged FCG mice to identify differentially expressed genes (DEGs). The total number of upregulated and downregulated DEGs in individual comparisons are shown in Fig. 2 The responses to HDM versus PBS treatment in each genotype are also shown in Fig. 2, whereas differences among the FCG are shown in Supplemental Fig. S1. When comparing the HDM-challenged groups with the PBS control groups, mice from all genotypes displayed more than 50% of DEGs upregulated, except for XXM mice (Fig. 2). The DEGs that remained significant afteradjusting for multiple comparisons (adj. P values ≤ 0.05) are shown in Fig. 3 (Venn diagrams) and Fig. 4 (volcano plots), as well as in Table 1. Among these genes, 2 were upregulated and 1 downregulated in the XXF group,

Table 1. *List of DEGs identified by IPA*

Groups	IPA Terms	Genes
XXM	Proliferation of lymphocytes	Upregulated: *CCL4, TNFRSFR1, GJA1, PECAM1, CXCR4, IL1A, EIF4E, GATA2, CD83, DCLRE1C, MMP9, ATADS* Downregulated: *NOS1, HNF1A, GNAS, CHRNB2, HNF4A, FKBP1A, LAPTM5, MAPK14, HOXA10, DRD2*
	Proliferation of immune cells	Upregulated: *CCXC4, IL1A* Downregulated: *ENPP3, MAPK14, LAPTM5, HOXA10, NOS1, GNAS, LAPTM5, DRD2, EIF4E, DCLRE1C, ATAD5, HNF1A, HNF4A, FKBP1A, ESR1*
	Pituitary cell proliferation	Upregulated: IL1A Downregulated: GNAS, CGA, DRD2
	Proliferation of gonadal cell lines	Upregulated: CAST Downregulated: *NOS1, KIF2C, AGT, ESR1, and FGF4*
	Proliferation of endocrine cells	Upregulated*: CCL3, IL1A* Downregulated: *HNF1A, CGA*
	G protein-coupled receptor signaling	Upregulated: *ADGRL4, MYL3, PIK3C2A,* and *TTN* Downregulated: *ADGRB3, CELSR3, CREB3L3, DRD2, GDPD3, GNAS, GPRI7, HTR5A, MAPK14, MTNR1A, PAK5, PAK6, PDE6B, RG-S7, RHO*
	Inflammatory response	Upregulated: CCL4, CCL3, GJA1, PECAM1, CXCR4, S100A4, IL1A, CCN4, LPL, BDNF, MMP9, SELP Downregulated: NOS1, GNAS, HNF4A, FKBP1A, CREB3L3, LECT2, PDE6B, MAPK14, ENPP3, AGT, ESR1, NR1H4
XXF	Inflammation of respiratory system	Upregulated: CCL22, H2-Q6, F5 Downregulated: ATP1B1, SERPINE1, IKBKG, ALDH2, SQSTM1, SERPING1, POSTN, FOXO3, CAV1, TIMP3, CD36, IGFBP3
	Recruitment of leukocytes	Upregulated: CCL22, ITGB2, ALOX5AP Downregulated: SERPINE1, CAT, CRYAB, CAV1, CTNNB1, APP, KDR, CD36, JUN
XYM	Inflammation of lungs	Upregulated: *CXCL3, IGF1, TLR4, TNF, GATA3, CCL11, PTGER3, CCL22, SELP, ELAVL1, AR, IL10, NR1H3, IL11, F11, TK1, IL27ARA, BSG* Downregulated: SELE
	Immune-mediated inflammatory disease	Upregulated: *CYP26B1, CXCL3, STEAP4, GABRA4, NR4A3, USP7, OPRL1, IGF1, BCL2, PTGER3, AR, IL10*
	Chronic inflammatory disorder	Upregulated: *CYP11A1, CXCL3, STEAP4, GABRA4, NR4A3, USP7, PTGER3, OPRL1, IGF1, TYK2, FGF2, NTRK1*
XYF	Chronic inflammatory disease	Upregulated: *ENPP3, CCL22, BSG, MMP12, ELAVL1, IL11, MMP9, IL10, IL27RA, IL4, CXCL3, PLA2G2D, CXCL1, FPR2, NOS2, FPR2, CTSS, TNF, TSLP, TK1, FCER1A, TRPC6, CCL2, IGFBP3, FOXO$_3$, LPAR2, ABL1, ATF3, MMP12, ELAVL1, IL11, MMP9, IL10, TK1, BSG, CCL22, ENPP3, IL27RA, IL4, CXCL3, CXCL1, FPR2, NOS2, CTSS, TNF, TSLP, PLA2G2D, CXCL10, FCER1A* and *IGFBP3*
	Inflammation of lungs	Upregulated: *MMP12, CXCR2, GABRA4, IL4, CRP, ELAVL1, CDKN2A, CXCL3, PLA2G2D, IL11, MMP9, CXCL1, IMPDH2, PGLYRP1, EPR2, IL10, NOS2, IL27RA, ENPP3, CCL22, BSG*

DEGs in lung tissue of HDM-challenged FCG mice compared with control (PBS) with a threshold of −Log (P-value)>1.2 at adj P value < 0.05. These genes were related to inflammation. DEG, differentially expressed gene; FCG, Four-Core Genotype; HDM, house dust mite; IPA, Ingenuity Pathway Analysis; PBS, phosphate-buffered saline.

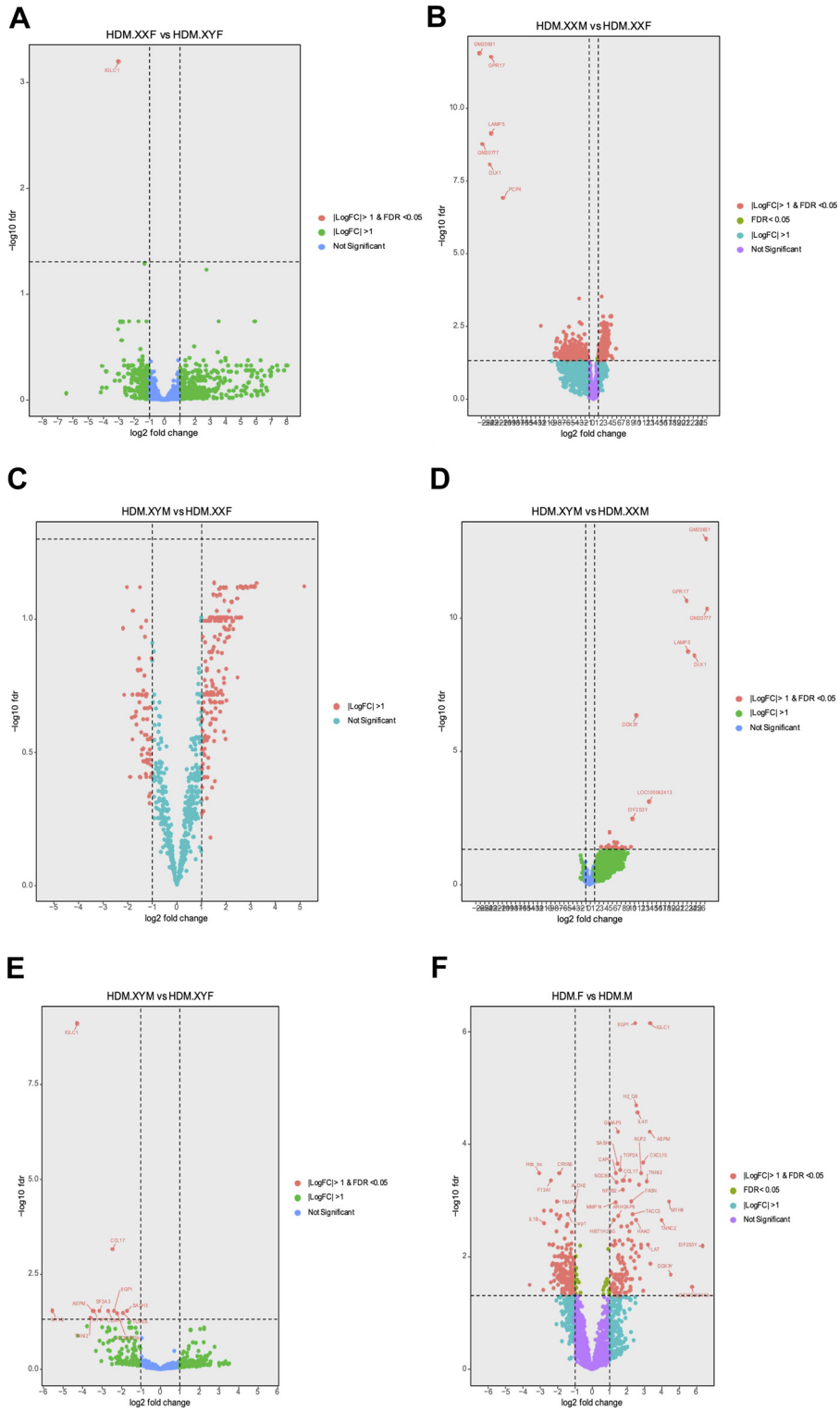

Figure 5. Volcano plots identifying the significantly expressed genes in the HDM-challenged mice when comparing the four core genotypes. *A*: significantly expressed DEGs in HDM-challenged XXF vs. XYF mice. Volcano plot shows the only gene, *IGLC1*, significantly downregulated in the HDM-challenged mice of the XXF vs. XYF genotypes ($N = 3$). *B*: significantly expressed DEGs in HDM-challenged XXM vs. XXF mice. This pair had the most significantly expressed genes (746 genes) with about one-third upregulated. *C*: DEGs in the HDM-challenged XYM vs. XXF mice. Numerous genes were identified but they were not statistically significantly expressed (DESeq2 FDR $\leq$ 0.05). *D*: DEGs in HDM-challenged XYM vs. XXM mice. Plot shows 40 DEGs in the HDM-challenged mice of the XYM and XXM genotypes (DESeq2 FDR $\leq$ 0.05). *E*: significantly expressed DEGs in HDM-challenged XYM vs. XYF mice. Plot shows 12 DEGs (downregulated). *F*: significantly expressed DEGs in the HDM-challenged female vs. male mice. Volcano plot shows the genes that were significantly expressed in the female and male mice after HDM exposure. Most of the genes were upregulated. In previous studies, some of the genes that were known to be anti-inflammatory were significantly downregulated in female mice. DEG, differentially expressed gene; HDM, house dust mite.

1 gene was upregulated and 5 were downregulated in the XXM group, 9 genes were upregulated and 3 downregulated in the XYF group, and 7 genes were upregulated in the XYM group (Table 1). We also identified differences among the four genotypes in HDM-challenged mice (Fig. 5) and the control mice (Supplemental Fig. S2).

Differences in Genes Expressed Between the Genotypes With the Same Sex Gonads or Chromosomes in PBS and HDM Exposure Conditions

The animals were further divided into different groups using the sex chromosomes and gonads. The numbers of

Table 2. *DEGs significantly expressed in HDM-induced FCG mice*

Genotype	Upregulated Gene	Adj. *P* Value	Log Fold Change	Downregulated Gene	Adj. *P* Value	Log Fold Change
XXF	*GM20831*	1.71E-10	26.614	*HSPA2*	0.026	−1.739
	DDX3Y	0.02	9.41			
XXM	*SYNDIG1*	0.017	14.640	*GPR17*	9.45E-07	−18.1145
				LAMP5	5.14E-06	−18.8956
				DLX1	0.0003	−17.672
				GM20831	0.0021	−15.264
				GM20777	0.0118	−15.615
XYF	*IGLC1*	1.32E-10	4.887	*ALDH2*	0.020	−1.287
	MYH8	0.001	6.425	*KLF2*	0.032	−1.724
	TNFRSF13B	0.0018	3.206	*ATF5*	0.047	−1.656
	TNNC2	0.003	5.84			
	CCL17	0.004	2.0606			
	MYLPF	0.004	3.516			
	MMP12	0.008	3.900			
	PLA2G2D	0.013	2.458			
	CXCL10	0.016	3.233			
XYM	GAD1	4.67E-21	23.955			
	HOXC10	9.66E-19	22.733			
	CYP11A1	4.99E-12	22.982			
	INS2	4.77E-10	22.362			
	TUBA3A	0.009	9.853			
	AMH	0.025	5.217			
	NR4A3	0.025	6.152			

DEGs that were statistically significant in the HDM-induced group compared with the control (PBS) with a threshold of −Log (*P*-value)>1.2 at adj *P* value < 0.05. DEG, differentially expressed gene; FCG, Four-Core Genotype; HDM, house dust mite; IPA, Ingenuity Pathway Analysis; PBS, phosphate-buffered saline.

DEGs identified in the different groups are shown in Supplemental Fig. S1. There were more downregulated genes in the XX HDM-challenged mice regardless of the sex hormone present when compared with the XY HDM-challenged mice (1,073 vs. 1,790). In the XX versus XY HDM-challenged mice, none of the identified genes showed significant differences. However, when comparing F- versus M HDM-challenged groups, 339 genes were differentially expressed (Fig. 5) out of which, 136 were upregulated (top 8: *TRP53, C1QC, CRELD2, DMTF1, COMT, USP18, SLC22A17, ALDH841*) and 203 downregulated (top 6: *KPNB1, NUP88, BPNT1, BSG, SPINT1, YTHDF1*). Interestingly, only *DDX3Y* was downregulated in the XX versus XY control group while the four significantly expressed genes (*SATB1, H2-Q6, CD8B1, PRL2C3*) in the F versus M control group were all upregulated.

Differential Expression Pathways Across Genotypes vis Ingenuity Pathway Analysis

IPA revealed significant top upstream regulators in the different groups (Table 2) and Fig. 6. The top canonical pathways and associated diseases were identified in all the genotypes in the control and HDM-challenged mice as shown in Table 2. IPA also revealed canonical pathways that were enriched in the HDM-challenged groups versus PBS (Table 1). The glucocorticoid receptor signaling pathway was enriched in the XYM and XXF groups, whereas Agranulocyte adhesion and diapedesis were enriched in the XXM and XYF groups. Most importantly, the FXR/RXR activation pathway was revealed to be enriched only in the XYM group. The diseases or disorders associated with the FCG mice when comparing animals of the same sex chromosomes or sex hormones were inflammatory responses and immunological diseases in all the groups (Table 1).

The DEGs analyzed in the XXM HDM-challenged group compared with the control group were known to be related to the proliferation of lymphocytes, pituitary, endocrine, immune, and gonadal cell lines. Furthermore, all but one of the DEGs associated with the proliferation of the gonadal cell lines were upregulated in this group. The majority of the DEGs revealed in the XXF HDM-challenged group compared with the control group, which was known to decrease inflammation, were downregulated, *KRT18, IKBKG, KLF2, GPX4, SQSTM1, LPIN1, FOXO3, CAV1, CTNNB1*, and *TIMP3*, whereas those that were known to increase inflammation were upregulated (*ITGB2* and *ZBP1*). Several DEGs revealed in the XYM HDM challenged group are known to affect chronic inflammation in an unspecified manner, while very few of them, known to decrease inflammation, were downregulated. The DEGs identified in the animals with female chromosomes challenged with HDM (XXF vs. XXM) were related to inflammation of the respiratory system and components including the lungs, immune-mediated inflammatory diseases, cell movement of leukocytes, quantity of lymphocytes (T-lymphocytes), and immune cell trafficking. Many of them specifically play roles in the migration/movement, accumulation, cellular infiltration, and activation of leucocytes, lymphocytes, and phagocytes, whereas, in the group with male chromosomes (XYM vs. XYF), many of the DEGs identified were downregulated but performed similar physiological functions as the group with the female chromosome.

In the group with female gonads (XXF vs. XYF), cell death and survival, cellular function and maintenance, cellular development, cellular growth and proliferation, and cellular movement were the molecular and cellular functions revealed to the associated with the DEGs identified but in the group with male gonads (XXM vs. XYM), majority of the DEGs known to increase inflammation, were downregulated.

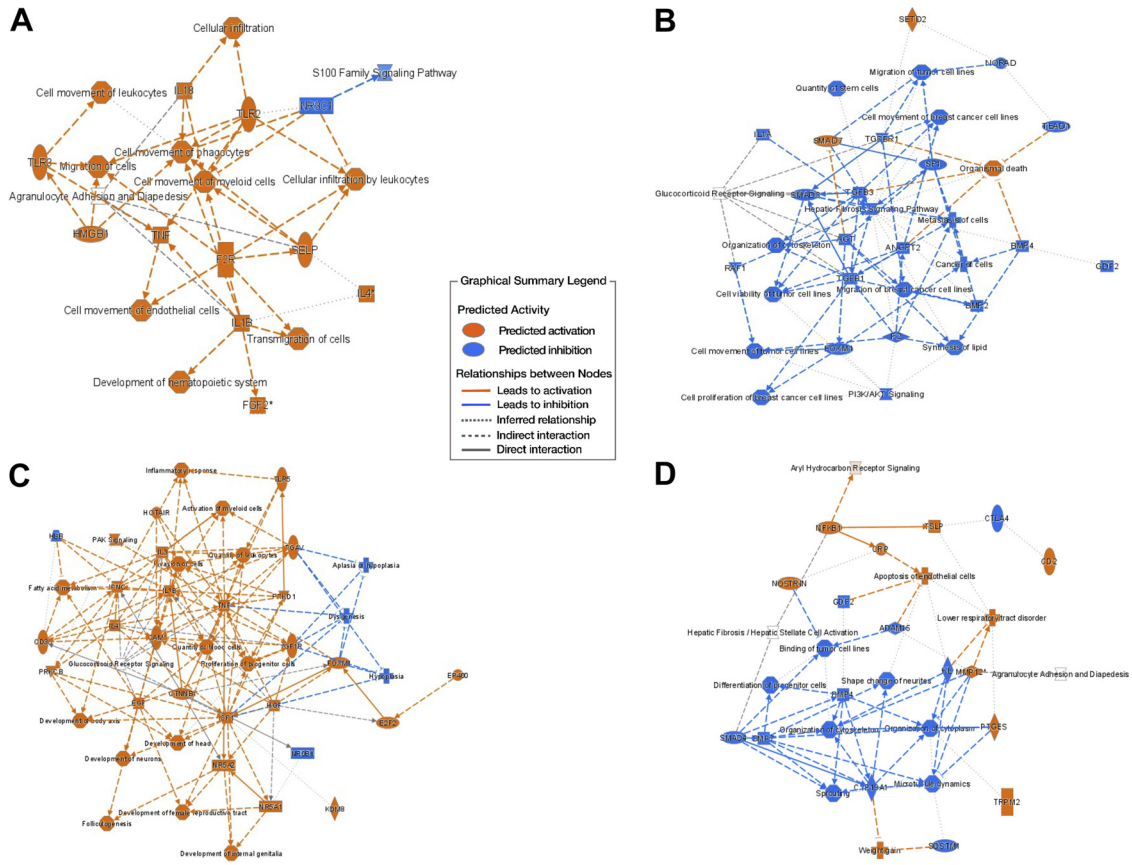

Figure 6. Graphic summary of the pathways identified by IPA to be activated or inhibited relating to the DEGs of FCG mice model following HDM induction. *A*: identified pathways to be activated or inhibited in the XXM genotype exposed to HDM. *B*: identified pathways to be activated or inhibited in the XXF genotype exposed to HDM. *C*: identified pathways to be activated or inhibited in the XYM genotype exposed to HDM. *D*: identified pathways to be activated or inhibited in the XYF genotype exposed to HDM. DEG, differentially expressed gene; FCG, Four-Core Genotype; HDM, house dust mite; IPA, Ingenuity Pathway Analysis.

DISCUSSION

Asthma is a heterogeneous inflammatory lung disease that is influenced by sex and hormones. The mechanisms underlying airway inflammation in asthma in both sexes can help classify the severity of the disease (27) and help improve individual management. In the present study, we performed a comprehensive gene expression analysis using the FCG mouse model of allergic airway inflammation via HDM sensitization. We identified gene expression networks and pathways that are influenced by sex hormones, sex chromosomes, or both, in response to allergen challenge, contributing to our understanding of sex-specific mechanisms underlying lung allergic inflammation.

Specifically, we identified DEGs in the FCG mice following 5 wk of HDM challenge compared with the control group that had PBS instillation for the same period. The 5-wk HDM-exposed mouse model of allergic airway inflammation showed an increase in eosinophil and neutrophil counts in the XYF HDM-induced mice compared with the other genotypes. These neutrophilia and eosinophilia were also observed in HDM-treated mice with male chromosomes and female sex hormones. This increase in the eosinophil and neutrophil counts is expected considering the phenotype of severe asthma, usually associated with inhaled allergens, and it is consistent with other researchers' reports (23, 28, 29). However, the increase was not statistically significant ($P < 0.05$), which was consistent with the recent findings of Srinivasan et al. (30), which showed that HDM-induced asthmatic phenotypes display a time-of-day response and sex-based differences. Since all our exposures were done in the morning, our data agrees with those observed by Srinivasan et al.

We were able to study the phenotypic effect of two major factors potentially contributing to asthma: sex chromosomes (XX vs. XY), and gonadal hormones (F vs. M). These have been previously implicated in the sex disparities observed in asthma across the life span (31). Our study results identified a high number of DEGs and showed that different genes were expressed in the lungs of FCG mice in response to HDM exposure when compared with the control. *GPR17* was one of the genes that was downregulated in XXM HDM challenged and has been reported by Maekawa et al. (32) to regulate HDM-induced immune pulmonary inflammation. In addition, it is known to be a negative regulator of *CYSLTR1,* a broncho-constrictor in clinical studies (33). *LAMP5* was also downregulated in the same group of animals and the mice deficient in this gene were reported to have aggravated airway resistance after ovalbumin sensitization (34).

The immunoglobulin lambda constant 1 gene (IGLC1) is the only gene that was significantly expressed in the XYF versus XXF HDM-challenged group. This gene is expressed primarily in the lungs, blood microparticles, and exosomes. It is also predicted to aid antigen and immunoglobulin receptor binding, and it is involved in several processes such as the activation of immune response, phagocytosis, and defense responses in organisms. However, its role in allergic airway inflammation remains unknown. Our data supports a hypothesis of a sex chromosome-related effect in airway inflammation.

For comprehensive insight into the biological processes and functions of the sex chromosomes and sex hormones in the sex disparities that occur in asthma, we applied the IPA. IPA exposed a highly significant overrepresentation of gene set families that are related to inflammation of the lungs, inflammatory responses, chemokine and cytokine activities immune-mediated diseases, and chronic respiratory disease responses. We identified the overrepresentation of ontology sets that are related to cancer, organismal injury, and abnormality in the groups with male gonads (XXM and XYM), whereas the sets corresponding to inflammatory responses and immunological diseases were strongly enriched in the groups with female gonads (XXF and XYF). We took advantage of the unbiased method of ranking gene expression offered by IPA, and it showed that pathways involved in glucocorticoid receptor signaling had the strongest correlation in the XXF and XYM groups. In contrast, the pathway set related to the G protein-coupled receptor signaling strongly correlated with the XXM group and agranulocyte adhesion, and the diapedesis pathway only correlated with the XYF group. Interestingly, the FXR/RXR Activation pathway was one of the top enriched canonical pathways, only in the XYM group. Farnesoid X receptor (FXR) is a bile acid-activated receptor that binds with the retinoid X receptor (RXR) (35). It is known to play a role in the maintenance of organ integrity. It also plays a physiological and pathological role in the respiratory system (36) and it is believed to possess an anti-inflammatory effect in asthma (37).

The top upstream regulators in the XXM, XYF, and XYM following HDM exposure were β-estradiol and tumor necrosis factor (*TNF*). β-Estradiol plays an important role in allergic asthma as ER-β knockout mice showed exacerbated airway hyperresponsiveness and remodeling (38) and the *TNF* is a proinflammatory cytokine that has been implicated in the modulation of inflammation in asthma (39). It provides immediate host defense before the activation of adaptive immunity (40). Transforming growth factor β 1 (*TGFB1*) was exposed as the top upstream regulator in the XXF, XYF, and XYM groups of mice exposed to HDM. It is a major mediator of tissue remodeling in asthma (41). It is a profibrotic cytokine that is produced by many cells, including fibroblasts, eosinophils, epithelial cells, and macrophages. It is highly expressed in severe asthma (42). However, *TP53,* a gene that codes for *P53,* was revealed to be an upstream regulator in the XXF and the XYM group. The *P53* is known as a tumor suppressor protein that helps to prevent the development of tumor, as evidence abound that it has a protective effect in lung inflammatory reaction (43). Its deficiency has been associated with severe respiratory disorders (44).

In conclusion, we used an animal model of HDM-challenged airway inflammation to characterize the contributions of sex hormones and sex chromosomes in the changes in lung gene expression and inflammatory phenotypes. We demonstrated that sex hormones and sex chromosomes influence asthma-related genes and phenotypes, as the FCG mice expressed different genes. The understanding of genes associated with the sex disparities that occur in asthma may help to identify important biological pathways for sex-specific therapeutic intervention. These findings contribute to the development of novel therapies for the sex-specific management of this heterogeneous disease. Further studies will be necessary to validate these findings and investigate the underlying mechanisms.

Perspectives and Significance

The present study investigated the contribution of sex hormones and sex chromosomes in the genes expressed in the lungs following aeroallergen exposure using the FCG mouse model. We identified differentially expressed genes in all the genotypes (XXF, XXM, XYM, and XYF). Most importantly, we revealed DEGs that play a role in inflammatory responses. Further studies are needed to specifically identify the role of the revealed genes and pathways to serve as targets for sex-specific therapeutic interventions in the management and treatment of allergic asthma to help eliminate the sex disparities associated with this disease.

DATA AVAILABILITY

Gene expression raw data files can be found here: https://github.com/exposurelabiu/Transcriptomics-analysis-of-allergen-induced-inflammatory-gene-expression-in-the-Four-Core-Genotype-.git.

SUPPLEMENTAL DATA

Supplemental Figs. S1 and S2: https://doi.org/10.6084/m9.figshare.24195354.v1.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

P.S. conceived and designed research; C.D.E., R.A., D.R., M.B., S.S., S.C., and P.S. performed experiments; C.D.E., D.R., A.B., D.B.R., and P.S. analyzed data; C.D.E., D.R., M.B., A.B., and P.S. interpreted results of experiments; C.D.E., A.B., and D.B.R. prepared figures; C.D.E. drafted manuscript; C.D.E., R.A., D.R., M.B., S.S., S.C., A.B., D.B.R., and P.S. edited and revised manuscript;

C.D.E., R.A., D.R., M.B., S.S., S.C., A.B., D.B.R., and P.S. approved final version of manuscript.

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