A Between-Sex Comparison of the Genomic Architecture of Asthma

To the Editor:

Significant sex differences exist in the prevalence and severity of asthma across the life span. Asthma, which affects 8% of the U.S. population, is more prevalent and severe in pubescent boys than girls but worse in girls and women after puberty (1). Population-based studies from the United States and the United Kingdom revealed significant associations between sex hormones and asthma (2, 3). This suggests that sex and sex hormones play an important role in asthma pathogenesis. The cumulative genetic burden of asthma susceptibility loci also appears to differ by sex (4). In this study, we identify sex-specific associations between germline genetic variation and asthma susceptibility to gain insight into mechanisms that drive disease-related sexual dimorphism.

We analyzed data from the UK Biobank, a large-scale biomedical database, which includes genomic data from 500,000 participants from the United Kingdom (5). We only included non-Hispanic white (~90%) participants because of small sample size in other ancestral populations. The diagnosis of asthma was made based on self-reported questionnaire information. Patients with other chronic lung diseases were excluded. We first conducted genotype quality control at both the individual level and the SNP level by omitting samples with mismatch between genetic and putative sex, excess heterozygosity, >5% missing genotypes, close relatedness (coefficient of relationship > 0.2), and ancestry outliers as determined using principal components and omitting variants with minor allele frequency < 0.01, >3% missing genotypes, or $P < 10^{-5}$ from an exact test of Hardy-Weinberg proportions. Y chromosome variants were excluded from comparison.

Sex-stratified genome-wide association analyses were then performed using logistic regression adjusted for age, body mass index, smoking status, pack-years smoking history, batch effect, and the first 10 principal components of ancestry as covariates. Genetic variants that were significantly associated with asthma ($P < 5 \times 10^{-8}$) in either sex-stratified genome-wide association analysis were identified. Of those 1,342 independent significant SNPs, 154 lead SNPs were mapped to Ensembl gene IDs using the biomaRt Bioconductor package (6). For each of these 154 lead SNPs, the odds ratio of asthma genetic risk susceptibility was then compared between women and men using the Wald test, where the *z*-score equals the difference in odds ratio between men and women divided by the square root of the sum of the squared SE in men and squared SE in women or $(\overline{x}_1 - \overline{x}_2)/(SE_1{}^2 + SE_2{}^2)$. *P* values were computed using R version 4.0.5 (R Project for Statistical Computing), and multiple testing was adjusted for using false discovery rate. Genes with significant sex differences in the effect size of the association with asthma were included in subsequent pathway enrichment analysis using Ingenuity Pathway Analysis (Ingenuity Systems) software. This research has been conducted using the UK Biobank Resource under Application Number 44578.

Inclusion criteria were met in 220,673 women and 187,584 men enrolled in the UK Biobank. Participants with asthma (26,198 women and 19,055 men) had a median age of 57.0 years (interquartile range, 49.0–63.1 yr), and 57.9% were females. Although age did not differ between both sexes ($P = 0.257$), women had a lower body mass index (median [interquartile range], 27.0 [24.0–31.1] vs. 27.4 [25.1–30.4]; $P < 0.001$) than men.

Sex-stratified genome-wide association analyses are demonstrated in Figure 1. Of the 803,113 genetic variants that passed the genotype quality control, 1,342 variants reached genome-wide significance at $P < 5 \times 10^{-8}$ in either male- ($n = 961$; $n = 301$ unique to male sex) or female- ($n = 1{,}041$; $n = 381$ unique to female sex) stratified genome-wide association analyses mapping to 161 unique genes. However, sex differences in the effect size of such associations were present in 76 genes ($P < 0.05$). Of those, sex differences were present in only eight genes (*HLA-DQA1*, *HLA-DQB1*, *IL1RL1*, *FLG-AS1*, *BTNL2*, *IL18R1*, *HLA-DPA1*, and *IRF4* [Interferon Regulatory Factor 4]) at a false discovery rate < 0.05 (colored in red in Figure 2). Pathway enrichment analysis highlighted several biological pathways, particularly T-helper cell type 1 (Th1) and Th2 activation pathway, antigen presentation pathway, glucocorticoid receptor signaling, and IL-4 signaling. Although these reached statistical significance, the difference in the effect size between genetic susceptibility and asthma association in men and women was small.

These data highlight that sex differences in asthma genetic susceptibility are strongly related to immunologic pathways, including Th1 and Th2 pathway activation, and differences in the immune response to viral infections. *HLA-DQA1* [Major Histocompatibility Complex, Class II, DQ Alpha 1], involved in Th cell signaling and Th17 differentiation, was highly associated with asthma in previous studies (7). *IRF4* (Interferon Regulatory Factor 4)—a transcription factor that drives dendritic cells to promote Th2 differentiation—has previously been linked to sex differences in the immune response to viral infections (triggering asthma exacerbations) (8).

Although the sexual dimorphism of asthma is well described, little is known about the effect of sex or sex hormones on the biological mechanisms underlying asthma and severe asthma (SA) (1). Testosterone attenuates both Th2-induced and IL17A-associated allergic inflammation (9), whereas estradiol and progesterone increase IL-17A production from TH17 cells, providing potential mechanisms for sex differences in asthma (10). In the National Heart, Lung, and Blood Institute SARP (Severe Asthma Research Program), an ongoing multicenter program to investigate the pathogenesis of SA (1), we demonstrated that increasing serum androgens, but not estradiol concentrations, were associated with better lung

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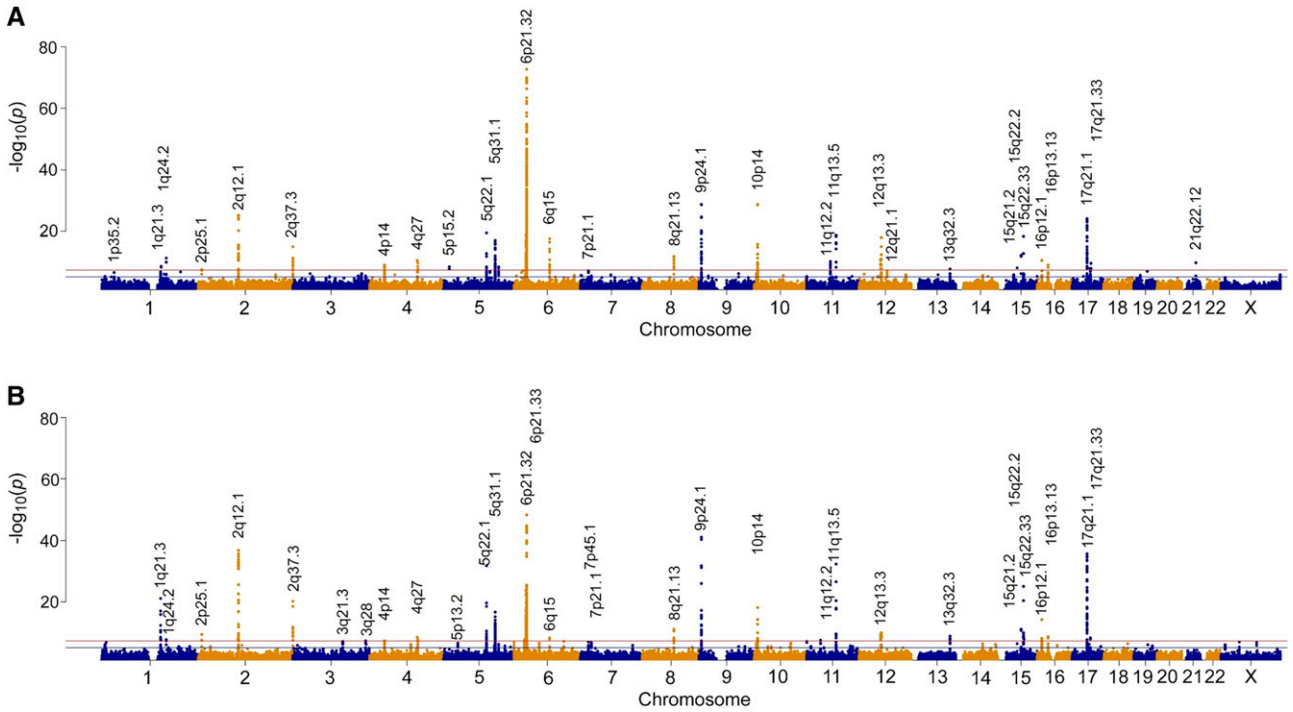

Figure 1. Manhattan plot of results of genome-wide association testing are shown for (*A*) female, and (*B*) male UK Biobank participants. The horizontal line indicates the stringent genome-wide significance threshold ($P < 5 \times 10^{-8}$).

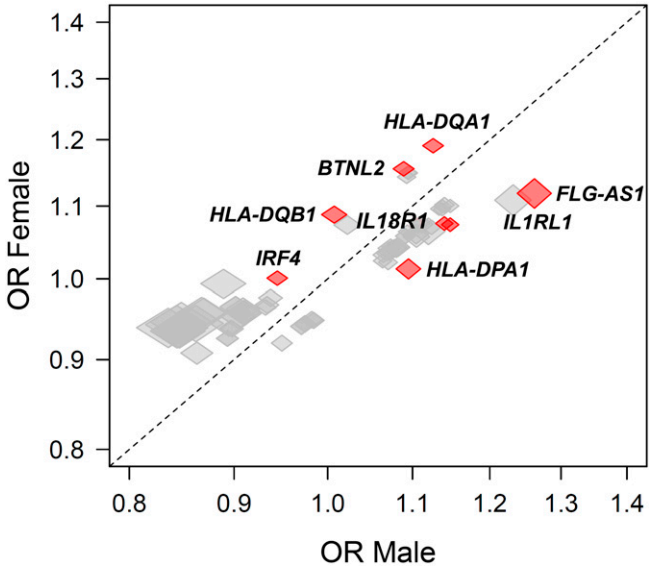

Figure 2. Estimated effect sizes stratified by sex. The size of each marker reflects confidence intervals (with height reflecting the confidence interval along the *y*-axis and width reflecting the confidence interval along the *x*-axis). Comparisons reaching $P < 0.05$ are labeled and colored in red. *BTNL2* = Butyrophilin Like 2; *FLG-AS1* = Filaggrin Antisense RNA 1; *HLA-DPA1* = Major Histocompatibility Complex, Class II, DP Alpha 1; *HLA-DQA1* = Major Histocompatibility Complex, Class II, DQ Alpha 1; *HLA-DQB1* = Major Histocompatibility Complex, Class II, DQ Beta 1; *IL18R1* = Interleukin 18 Receptor 1; *IL1RL1* = Interleukin 1 Receptor Like 1; *IRF4* = interferon regulatory factor 4; OR = odds ratio.

function (11). Similarly, our data suggested that the androgen receptor signaling pathway plays an important role in asthma pathogenesis. In fact, airway androgen receptor expression was lower in patients with asthma than in control subjects in both sexes, and androgen receptors negatively correlated with nitrosative airway inflammation (11, 12).

Our results add to the existing body of evidence highlighting the role of sex differences in asthma susceptibility. Yet, the effect of sex on the genomic drivers of asthma is marginal, suggesting that other mechanisms, such as gene regulation, gene-by-gene interaction, and gene-by-environment interactions (such as the hormonal milieu or outdoor exposures) may also play an important role. Although understanding the pathobiology of asthma and SA has been a public health priority (1), defining key sex-specific hormonal and genomic drivers of asthma risk and severity is critical to implementing personalized, hormone-based risk stratification to refine treatment approaches in women and men with asthma and SA. ■

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Joe G. Zein, M.D., Ph.D.*
Peter Bazeley, M.D.
Cleveland Clinic
Cleveland, Ohio

Deborah Meyers, Ph.D.
Eugene Bleecker, M.D.
University of Arizona
Tucson, Arizona

Benjamin Gaston, M.D.
Indiana University School of Medicine
Indianapolis, Indiana

Bo Hu, Ph.D.
Amy Attaway, M.D.
Cleveland Clinic
Cleveland, Ohio

Victor Ortega, M.D., Ph.D.
Mayo Clinic
Phoenix, Arizona

*Corresponding author (e-mail: zeinj@ccf.org).

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Use of the Iron Chelator Deferiprone to Restore Function in BAL Fluid Macrophages in Smoking and Chronic Obstructive Pulmonary Disease

To the Editor:

Alveolar macrophages (AMs) from individuals with chronic obstructive pulmonary disease (COPD) have impaired function, a phenomenon that may be closely linked to mitochondrial dysfunction in these cells (1–3). The trigger for this mitochondrial dysfunction is unknown. We have previously demonstrated a mechanistic role for mitochondrial iron overload in COPD and showed that systemic administration of deferiprone, a membrane-permeable iron chelator, slowed disease progression in a murine cigarette smoke exposure model (4). Clinically, lung iron overload is a well-recognized feature in individuals with COPD, with high levels of iron and iron-binding proteins in the lung extracellular space and within AMs (5–8). This iron accumulation is biologically relevant and may be connected to impaired AM function in COPD and consequently contribute to the recurrent respiratory infections that drive COPD exacerbation events (7). AM iron is therefore potentially a novel therapeutic target in COPD, but whether an iron-directed agent like deferiprone can relieve the iron burden in AMs and improve their immune function is unknown.

In this study, we collected BAL fluid (BALF) macrophages (hereafter referred to as AMs) from healthy nonsmoker study participants ($n = 19$), smokers ($n = 18$), and study participants with COPD ($n = 10$) as previously described (3, 9) (Table 1). Smokers and participants with COPD were significantly older than nonsmoker participants ($P < 0.007$ and $P < 10^{-6}$, respectively), and participants with COPD were older than smokers ($P < 0.002$). Smokers were more likely to be current smokers than were participants with COPD ($P < 0.0005$). Following an adherence purification step (95–98% purity of AMs), as previously described (3, 9, 10), we measured the amount of total elemental iron and total heme using graphite furnace atomic absorption spectrometry (7) and a fluorescent heme assay (11), respectively (Figure 1A). Iron levels in AMs from smokers and participants with COPD trended toward being higher than those from control participants, whereas AM heme levels did not differ between any of the subgroups (Figures 1B and 1C and Table 1). We tested AM iron levels for associations with mitochondrial function (previously measured oxygen consumption rates) in AMs from matched study subjects (3) and found that higher AM total iron was significantly associated with higher basal respiration ($r^2 = 0.34$,

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This letter has a data supplement, which is accessible from this issue's table of contents at www.atsjournals.org.