Sex-specific genetic determinants of Asthma-COPD phenotype in middle-aged and older Canadian adults: An analysis of CLSA data

by

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science in Medicine (Clinical Epidemiology)

Division of Community Health and Humanities

Faculty of Medicine

Memorial University of Newfoundland

May 2023

St. John's, Newfoundland, and Labrador

Abstract

Asthma is a chronic respiratory disease affecting both children and adults, while chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease most often associated with smoking and is usually seen in middle-aged and older adults. Asthma-COPD phenotype is a newly found phenotype of chronic obstructive airway disease exhibiting features of both asthma and COPD. Patients with coexisting asthma and COPD are more likely than those with COPD or asthma alone to have poorer quality of life, more frequent exacerbation of respiratory symptoms, rapid lung function decline, higher mortality, and greater healthcare utilization. Many recent population studies have found that females have a higher prevalence of asthma-COPD phenotype and COPD than males. In addition, the prevalence of asthma is higher in males during childhood but increases in females in puberty and adulthood. Although it is unclear what causes sex differences in the risk of asthma-COPD phenotype, COPD, and asthma, it is widely believed that genetic, environmental risk factors, and gene-environmental interactions play an important role. This study used the Canadian Longitudinal Study on Aging (CLSA) data (Baseline Comprehensive and Genomic datasets) to examine sex-specific genetic risk factors for asthma-COPD phenotype, COPD, and asthma in middle-aged and older Canadian adults.

The study cohort consisted of 26,622 participants genotyped using the Affymetrix U.K. Biobank Axiom array from the Comprehensive Cohort of 30,097 adults aged 45 to 85. Participants were grouped into four mutually exclusive categories (asthma-COPD phenotype, COPD, asthma, and control). Asthma and COPD were defined as positive responses to the survey questions: "Has a doctor ever told you that you have asthma?"

and "Has a doctor told you that you have/had any of the following: emphysema, chronic bronchitis, chronic obstructive pulmonary disease (COPD), or chronic changes in the lungs due to smoking?" respectively. Participants who answered "yes" to self-reported physician-diagnosed asthma and COPD were classified as having asthma-COPD phenotype. The control subjects were those who responded "no" to a self-reported physician diagnosis of asthma and COPD. The association between genetic markers and the outcomes (asthma-COPD phenotype, COPD, and asthma) was assessed in 2 phases. First, A genome-wide SNP by sex interaction test was conducted using multivariate logistic regression under the additive inheritance model to identify SNPs with significant interaction with sex at the level of 10^{-5}. Second, a sex-stratified multivariate survey logistic regression was performed using SNPs with interaction p-values less than 10^{-5} to identify male and female-specific polymorphisms associated with asthma-COPD phenotype, COPD, and asthma.

A total of 416,562 SNPs were examined after GWAS quality control. Seven male-specific SNPs (rs11799559, rs3821479, rs77800494, rs11061082, rs926718, rs1884882, and rs1051169) in/near SMYD3, FHIT, ZNF608, RIMBP2, ZNF133, BPIFB1, and S100B respectively were significantly associated with asthma-COPD phenotype. No SNP was significantly associated with asthma-COPD phenotype in females.

Eight polymorphisms (rs13326145, rs56334611, rs6816344, rs17039240, rs6935314, rs13225543, rs12869252, and rs6090327) near MAGI1, COX18, OSTC, ELOVL5, C7orf72 FGF14, and NKAIN4 genes respectively were significantly associated with COPD in males. In addition, four polymorphisms (rs12025895, rs10931835, rs220806, and rs77625370) in/near CAMTA1, SATB2, PDE10A, and LINC00908 genes were

significantly associated with COPD amongst females. Five polymorphisms (rs6701638, rs17071077, rs254804, rs6013213, and rs2968822) in/near KIF26B, NMBR, PEPD, RTN4, and NFATC2 loci, were significantly associated with asthma in males. In contrast, three SNPs (rs2968801, rs2864052, and rs9525931) in/near RTN4 and SERP2 loci were significantly associated with asthma in females.

These results suggest a sexually dimorphic association between these polymorphisms and asthma-COPD phenotype, COPD, and asthma. It provides further evidence of the distinct pathogenesis of the three diseases since no overlapping SNP was identified. Further functional studies will help determine the roles these variants/genes play in the pathogenesis of asthma-COPD phenotype, COPD, and asthma, thus, potentially paving the way for better disease endotyping and precision medicine.

General Summary

Asthma is a common respiratory disease that affects both children and adults, whereas chronic obstructive pulmonary disease (COPD) is a smoking-related chronic respiratory disease that primarily affects middle-aged and older adults. A new obstructive airway disease known as the asthma-COPD overlap is a condition that combines the characteristics of both asthma and COPD. Patients with asthma-COPD overlap experience poorer quality of life, more severe symptom burden, frequent hospitalization, and medication usage than those with asthma or COPD. There are differences between sexes regarding the prevalence, disease presentation, and severity of these obstructive lung diseases. The underlying causes for these sex differences are not fully understood. However, genetic, environmental, and an interplay between genetic and environmental factors are thought to play key roles. This study found genetic markers in males and females that may confer sex-specific susceptibility to asthma-COPD overlap, COPD, and asthma. Sex-specific genetic biomarkers may provide information for improving precision medicine and designing preventative methods.

Co-Authorship Statement

I hereby affirm that U. O. wrote this thesis and all included manuscripts, performed data analysis, and interpreted the results under the supervision of Z. G and supported by A. S. and J. F.

Z.G., A.S., and J.F. conceptualized the study and were in charge of its overall planning and direction. The contribution of the co-authors was generally the provision of critical feedback, suggestions, editing, and revisions that helped shape the research and final version of the manuscripts.

Acknowledgments

I would like to thank my supervisor, Dr. Zhiwei Gao, for his patience, guidance, mentorship, and all-around support. I have greatly profited from your abundance of expertise and amazing research perspective. I am really glad that you accepted me as your student and have continued to believe in me throughout the years. My sincere thanks to my supervisory committee members, Professor. Ambikaipakan Senthilselvan and Dr. Jamie Farrell for their invaluable insights and perspectives throughout this process.

I would like to express my appreciation to the School of Graduate Studies, Memorial University of Newfoundland, CIHR, and the CLSA research team members for funding this project and providing data access.

I should not forget to mention my family, especially my wife, Deborah, children, parents, and siblings. Thank you for the emotional support and confidence reposed in me. The unparalleled support kept me motivated and upbeat throughout this process.

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List of Abbreviations

ACO	Asthma-chronic obstructive pulmonary disease overlap
AIC	Akaike information criterion
BPIFB1	BPI Fold Containing Family B Member
CAMTA1	Calmodulin Binding Transcription Activator 1
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CHR	Chromosome
CI	Confidence interval
CIHR	Canadian Institutes of Health Research
CLSA	Canadian Longitudinal Study on Aging
COPD	Chronic Obstructive Pulmonary Disease
COX18	Cytochrome C Oxidase Assembly Protein 18
C7orf72	Chromosome 7 Open Reading Frame 72
ELOVL5	Elongation Of Very Long Chain Fatty Acids Protein 5
EPHB1	EPH receptor B1
FEV_1	Forced expiratory volume in 1 second
FEV_1%	Forced expiratory volume in 1 second percentage predicted
FGF14	Fibroblast Growth Factor 14

FHIT	Fragile Histidine Triad Diadenosine Triphosphatase
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GOLD	Global Initiative for Obstructive Lung Disease
GWAS	Genome-wide association studies
KIF26B	Kinesin Family Member 26B
L.D.	Linkage disequilibrium
LINC00908	Long Intergenic Non-Protein Coding RNA 908
LLN	Lower Limit of Normal
MAF	Minor allele frequency
MAGI1	Membrane-Associated Guanylate Kinase Inverted 1
NFATC2	Nuclear Factor of Activated T cells 2
NKAIN4	Sodium/Potassium Transporting ATPase Interacting 4
NMBR	Neuromedin B Receptor
OR	Odds ratio
OSTC	Oligosaccharyltransferase Complex Non-Catalytic Subunit
PEPD	Peptidase D
PDE10A	Phosphodiesterase 10A

RIMBP2	RIMS Binding Protein 2
RAGE	Receptor for advanced glycation end products
RTN4	Reticulon 4
SATB2	Special AT-Rich Sequence-Binding Protein 2
SEM	Standard error of the mean
SERP2	Stress-Associated Endoplasmic Reticulum Protein 2 gene
SMYD3	SET And MYND Domain-Containing Protein 3
SNPs	Single nucleotide polymorphisms
S100B	S100 Calcium Binding Protein, Beta
ZNF133	Zinc Finger Protein 133
ZNF608	Zinc Finger Protein 608
ZPBP	Zona Pellucida Binding Protein

Chapter 1. Introduction

Background

Asthma and Chronic obstructive pulmonary disease (COPD) are the two most prevalent chronic respiratory diseases and are among the leading causes of morbidity and mortality worldwide. The Global Initiative for Asthma (GINA) defined asthma as a heterogeneous disease with a history of respiratory symptoms (e.g., wheezing, shortness of breath, chest tightness, and cough) that vary over time and in intensity, together with variable airflow limitation, which may later become persistent. [1] According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), COPD is a preventable and treatable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases.[2] These chronic respiratory diseases constitute major public health problems and are the most significant contributors to the global respiratory disease burden. The 2019 global burden of disease (GBD) reported a prevalence of 212 million cases of COPD. It is estimated that COPD caused 3.28 million deaths and 74.4 million disability-adjusted life years (DALYs) in 2019.[3] In the same year, the WHO global health estimates reported that COPD was the third leading cause of death globally.[4] On the other hand, asthma affected over 260 million individuals, resulting in 21.6 million DALYs and 461 000 deaths in 2019.[5] Asthma which is generally more prevalent in children, also affects adults; however, COPD is commonly seen in middle-aged and older individuals. In Canada, asthma and COPD remain among the leading cause of chronic respiratory disease. According to the 2018 Canadian Chronic Disease Surveillance System report, 3.8 million Canadians over the age of one and 2 million Canadians aged

35 and older, had asthma and COPD in 2012 and 2013, with an age-standardized prevalence rates of 10.8% and 9.5%, respectively.[6] The burden of asthma and COPD in Canada has increased substantially, putting a strain on healthcare costs, patients' quality of life, productivity, and social activities. Recent studies in Canada reported that the economic burden associated with asthma and COPD exacerbation ranged from $46 to $140 million and $646 to $736 million annually. [7, 8] In addition to individuals who have either asthma or COPD, some patients have coexisting asthma and COPD. Recently, a new obstructive airway condition called asthma-COPD overlap (ACO) was recognized within the continuum of asthma and COPD, with the first consensus document on its description, treatment, and management in place since 2015.[9] For this thesis, the terms asthma-COPD overlap (ACO) and asthma-COPD phenotype will be used interchangeably to represent this group of patients with coexisting asthma and COPD.

ACO represents a subset of patients with obstructive airway disease with clinical features of asthma and COPD. Previous studies have shown that patients with ACO have a faster decline in lung function, more respiratory symptoms, more frequent exacerbations, poor quality of life, a higher mortality rate, increased health care utilization, higher economic burden, and a higher prevalence of comorbidities than those with asthma or COPD. [10–20] The ACO prevalence reported in various epidemiological studies showed considerable heterogeneity due to varying definition criteria, differences in population characteristics, and study designs. A recent systematic review and meta-analysis estimated that the pooled global prevalence of ACO in the general population was 2.0%, 26.5% in asthma patients, and 29.6% in COPD patients.[21] The prevalence of ACO reported in Canadian population-based studies varies widely as well. In the Canadian

Cohort Obstructive Lung Disease study (CanCOLD), Barrecheguren et al.[22] reported that the prevalence of ACO ranged from 3.8% to 31% across seven different ACO definitions. Senthilselvan and Beach[23] found an ACO prevalence of 1.59% using the Canadian Health Measure Survey (CHMS), while a recent large population-based study on middle age and older Canadians estimated the prevalence of ACO to be 1.8%.[24] In addition to the variations in ACO prevalence across studies, reports of sex differences in the prevalence, incidence, severity, and presentation of asthma-COPD phenotype, COPD, and asthma have been documented in many studies.[24–34]

Sex differences in asthma-COPD phenotype, COPD, and asthma

Sex is a biological construct that impacts many human diseases and is regarded as one of the major risk modifiers of chronic diseases. Sex-specific differences have been noted among people with chronic obstructive lung diseases. Studies have shown that females are more likely to have asthma-COPD phenotype than males.[24–26, 35] Wheaton et al.[26] demonstrated that the prevalence of ACO in females was highest in current smokers and lowest in non-smokers. However, ACO's prevalence in males did not vary considerably by smoking status. Furthermore, Koleade et al.[25] reported that females who smoke daily had a twofold higher risk of having ACO than female non-smokers. Interestingly in that study, a similar trend was not observed in males. In a large population-based study, Veerasingam et al.[24] found that age modifies the effect of smoking on asthma-COPD phenotype in females but not in males. These imply that smoking may impact the severity of the disease in older females with an asthma-COPD phenotype.

COPD has long been thought to be a disease of older men, owing to the high frequency of smoking among men. The 2018 Canadian Chronic Disease Surveillance System report estimated that the sex-specific age-standardized prevalence of COPD amongst Canadians aged 35 and older in 2012 was 10% and 9.2% for males and females, respectively.[6] However, COPD prevalence has increased among women over the past few decades as cigarette smoking has grown progressively among women. Evidence suggests females develop COPD earlier[36], and tobacco smoke appears to make females more susceptible to severe COPD and faster lung function decline than males.[28, 29, 31] In a population-based study, Sørheim et al.[28] observed that women less than 60 years old had more severe COPD and lower FEV_1 percent-predicted with lower cigarette smoke exposure than their male counterparts. In a systematic review and meta-analysis, Gan and colleagues[29] found that female current smokers had a faster annual reduction in FEV_1 percent-predicted than male current smokers, despite females having lower cigarette smoke exposure. Furthermore, research using mice models exposed to chronic cigarette smoke suggests that sex hormones may increase females' susceptibility to COPD. Tam and colleagues[37] reported that female mice exposed to chronic cigarette smoke developed higher airway remodeling and peripheral airway obstruction, while male mice receiving the same dose of cigarette smoke exposure had emphysematous phenotype. Also, in the same study, ovariectomized mice developed an emphysema-like phenotype suggesting an estrogen-mediated involvement.

Sex disparity in asthma has been studied extensively. Asthma prevalence changes with age across the lifespan in both males and females. The burden of asthma is higher in adult females than males. In contrast, pre-pubertal boys are more frequently affected than

girls.[33] Studies have shown that variations in sex hormones have effects on the prevalence, incidence, and severity of asthma.[33, 38] Data from 564,896 and 83,084 reproductive-age women with asthma revealed that hormonal contraceptives decreased the risk of asthma incidence and severe asthma exacerbations in a 17-year population-based retrospective cohort study.[39, 40] The underlying mechanism for the sex-related disparity observed in these chronic obstructive lung diseases remains unclear. However, it is believed that genetic variations, environmental factors, hormonal influence, and gene-environmental interactions may play important roles.

Genetic and sex-related genetic risk factors for Asthma and COPD

There is evidence that genetic factors contribute to the development of asthma and COPD. Estimates of heritability for asthma, COPD, and COPD-related phenotype range from 35% to 95% for asthma, 37% for COPD status and lung function, and 25% for quantitative emphysema.[41, 42] Various genes and single nucleotide polymorphisms (SNPs) have been linked to asthma, from genetic linkage and positional cloning to candidate genes and genome-wide association studies (GWAS). Early asthma genetic studies through linkage and candidate gene approach have identified several asthma genes, including IL13, TNF, ADAM33, IL4RA, DPP10, PHF11, NPSR1, HLA-G, CYFIP2, IRAK3, COL6A5, OPN3/CHML, and TBXA2R.[43] Furthermore, GWAS of asthma has identified numerous loci replicated in various independent populations, including ORMDL3/GSDMB/ZPBP2 on 17q21 implicated in childhood asthma and severe asthma, and IL1RL1/IL18R1, TSLP, IL33, SMAD3, RORA, HLA-DQ, PYHIN1, IL2RB, SLC22A5, and IL13.[41, 43]

A well-known genetic risk factor for COPD, specifically emphysema, is SERPINA1 which encodes serine protease alpha-1-antitrypsin (A1AT) protein. Mutations in SERPINA1 cause alpha-1-antitrypsin deficiency, accounting for 1-3% of the COPD population.[44] A number of GWASs have identified and replicated SNPs of many genes associated with COPD, such as CHRNA3/CHRNA5/IREB2, HHIP, FAM13A, RIN3, TGFB2, MMP12, on chromosomes 15q25, 4q31, 4q22, 14q32, 1q41 and 11q22 respectively.[45]

Several studies have been conducted in an attempt to find sex-specific genetic markers associated with asthma and COPD. Polymorphisms of the TSLP gene have been shown to be associated with asthma in a sex-specific pattern. In a sex-stratified association analysis between SNPs and asthma in children with asthma in Costa Rica with subsequent replication in five independent cohorts, TSLP SNPs rs1837253 and rs2289276 specifically decreased the risk of asthma in males and females, respectively.[46] Furthermore, in a large GWAS using EVE Asthma Genetics Consortium, Six sex-specific asthma risk loci were found by Meyers et al.[47], including two male-specific SNPs near the IRF and RAB11FIP2 gene and four female-specific SNPs near the RAP1GAP2, C6orf118, ERBB4, and AK057517 genes.

Sex-specific genetic risk factors associated with COPD have been demonstrated in several studies. A GWAS of 11,529 current or former smoking individuals (6,260 COPD cases and 5,269 controls) identified SNP rs9615358 in the CELSR1 gene significantly associated with increased COPD risk in females but not in males.[48] Another sex-stratified GWAS using 12,958 males cases and 95,631 male controls, 11,311 female cases, and 123,714 female controls identified COPD-associated male-specific loci in the

C5orf56, CFDP1, TMEM170A, and CHST6 genes and female-specific loci in the ASTN2 and TRIM32 genes.[49]

Study Objectives

Few studies have explored sex-related genetic factors for asthma and COPD. However, the effects of the genetic variants identified through various genetic association studies have only accounted for a minority of the phenotypic variance. To date, sex-related genetic factors for asthma-COPD phenotype remain largely unknown. Identifying male and female-specific genetic variants for asthma-COPD phenotype, COPD, and asthma may serve as precursors for establishing sex-specific pathways for the pathogenesis of these diseases. The primary and secondary objectives of this thesis project are to use the Canadian Longitudinal Study on Aging (CLSA) baseline questionnaire and genomic datasets to identify;

(1) Primary Objective: sex-specific genetic markers associated with asthma-COPD phenotype amongst middle-aged and older Canadian adults.
(2) Secondary Objective: sex-specific genetic polymorphisms associated with COPD and asthma in middle-aged and older Canadian adults.

The description of the Canadian Longitudinal Study on Aging (CLSA)

The Canadian Longitudinal Study on Aging (CLSA) is a large population-based prospective cohort study of 51,338 participants from 10 Canadian provinces. All participants were between the ages of 45 and 85 at the time of enrollment and will be followed until 2033 or death.[50] A core set of information relevant to health and aging, including demographics, lifestyle/behavior, social, clinical/physical, psychological,

economic, health status, and health services utilization, was provided by 51,338 participants.

The cohort of 51,338 participants comprises two complementary cohorts: The Tracking cohort and the Comprehensive cohort. The "Tracking cohort" consists of 21,241 randomly selected participants who provided core information via computer-assisted telephone interview (CATI). The Comprehensive cohort of 30,097 participants was selected randomly in areas 25–50 km from the 11 data collection sites (DCSs) in seven provinces. Participants in the Comprehensive cohort provided a core set of information via computer-assisted personal interviews (CAPI), participated in in-depth physical assessments at DCSs, and provided blood and urine samples. A subset of 26,622 participants from the Comprehensive cohort had genotyping information obtained from DNA samples collected from blood.[51]

The studies presented in this thesis focused on this subset of 26,622 participants with in-depth genomic data. The genomic data from the 26,622 participants were linked to demographics, lifestyle/behavior, social, clinical/physical, psychological, economic, health status, and health services utilization measures. The CLSA data presents a unique opportunity to study sexual dimorphism in asthma-COPD phenotype, COPD, and asthma due to its large sample size and availability of several health-related measures for most genotyped participants.

CLSA Sampling weight

CLSA provided sampling weights (trimmed inflation and analytic weights). The inflation weight estimates the number of persons in each province (and across Canada) that each CLSA participant represents. Analytic weights are proportional to inflation weights and

have been rescaled so that the mean of the weights within the DCS parts of each province equals 1.[52] In the studies included in this thesis, inflation weight was used to estimate descriptive statistics of the study population, while analytic weight was employed for logistic regression. This approach was recommended by the CLSA sampling weight guide.[52]

Definition of Outcome Variables

The primary outcome variable for this thesis is asthma-COPD phenotype. The secondary outcome variables are COPD and asthma. Participants were categorized into four mutually exclusive groups: asthma-COPD phenotype, COPD, asthma, and healthy controls.

COPD was defined based on the positive response to the questions from the CLSA questionnaire: "Has a doctor told you that you have/had any of the following: emphysema, chronic bronchitis, chronic obstructive pulmonary disease (COPD), or chronic changes in the lungs due to smoking?" Positive responses to the following question from the CLSA questionnaire identified asthma: "Has a doctor ever told you had asthma?" Participants with positive responses to both self-reported physician-diagnosed asthma and COPD were classified as having the asthma-COPD phenotype. Finally, participants who responded "no" to self-reported physician diagnoses of asthma and COPD were regarded as the healthy controls.

Genotyping information and Genetic markers

CLSA provides whole-genome genotyping data on 794,409 genetic markers (Single Nucleotide polymorphisms) for 26,622 participants.[51] Genotyping was performed using the UK Biobank Axiom array (Affymetrix). The Genotyping quality control was

performed to remove samples and genetic markers with low call rates, genetic markers deviating from hardy Weinberg equilibrium, Samples with familial relatedness, outliers, discordant sex information, and the generation of principal components of genetic ancestry.

CLSA demographic, lifestyle, and socioeconomic variables

The following variables from the CLSA questionnaire data were considered: age, age group, biological sex, marital status, education, total personal income, total household income, province of recruitment, retirement status, smoking status, home ownership, and urban/rural dwelling. Several Epidemiological studies have shown that sociodemographic, environmental, lifestyle, and socio-economic factors are associated with asthma-COPD phenotype, COPD, and asthma. [26, 53–55] These factors were taken into account in the analysis.

Summary of thesis outline

This thesis is written in a manuscript-styled format and provides a comprehensive and methodical account of this research project. The research component of this thesis project is presented in chapters 3 and 4. These chapters are prepared in the format of journal articles, including an introduction, method, results, discussion, and conclusion sections. The Chapters of the thesis (chapters 1 to 5) have a separate list of references, and a unified bibliography of all cited work is provided at the end of the thesis's main text.

Chapter 2 is a literature review manuscript titled "Current knowledge of Asthma-COPD overlap (ACO) genetic risk factors, characteristics, and prognosis." This manuscript was published in the Journal of Chronic Obstructive Pulmonary Disease in September 2021. Permission to use this journal article has been obtained, and it is included in **Appendix B**.

This review article x-rayed the genetic architecture of the asthma-COPD phenotype and its characteristics compared to asthma and COPD. It also offered suggestions for future research directions.

Chapter 3 includes a stand-alone manuscript titled "Sex-specific genetic determinants of Asthma-COPD phenotype and COPD in middle-aged and older Canadian adults: An analysis of CLSA data." This manuscript is currently being peer-reviewed in the Journal of Chronic Obstructive Pulmonary Disease. In this original research, we sought to identify and advance our understanding of sex-specific genetic variants associated with asthma-COPD phenotype and COPD by conducting genome-wide interaction testing between single nucleotide polymorphism (SNPs) and sex. Then we carried out male and female-specific analyses exploring the association between SNPs and the outcomes (asthma-COPD phenotype and COPD) while controlling for the participant's demographics, socio-economic and lifestyle behaviors.

Chapter 4 contains an original research manuscript titled "Identification of Sex-specific genetic polymorphisms associated with asthma in middle and older Canadian adults: An analysis of CLSA data." This work has been submitted to the Journal of Asthma and Allergy and is presently being peer-reviewed.

This original research focused on discovering male and female-specific genetic markers associated with the risk of asthma amongst middle-aged and older adults. We investigated the association by performing a genome-wide scan of SNPs and sex interaction amongst participants with asthma. Then, we conducted male and female-specific analyses to identify male and female-specific risk loci for asthma while adjusting

for potential confounders, such as demographic, socio-economic, and lifestyle behavior variables.

Chapter 5 cohesively summarizes all key findings and presents the study's strengths, limitations, and future directions for further research.

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Chapter 2. Current knowledge of Asthma-COPD overlap (ACO) genetic risk factors, characteristics, and prognosis

This manuscript has been published in a peer-review journal. The 'Accepted/Original Manuscript' was published by Taylor & Francis Group in the Journal of Chronic Obstructive Pulmonary Disease on the 24th of September 2021, available online: https://doi.org/10.1080/15412555.2021.1980870

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Authors contributions

UO reviewed the literature, wrote the original manuscript draft, and prepared tables. ZG, AS, and JF revised, edited, and suggested changes to the manuscript. All authors contributed to the article and approved the final version that was submitted.

Abstract

Asthma-COPD overlap (ACO) is a newly identified phenotype of chronic obstructive airway diseases with shared asthma and COPD features. Patients with ACO are poorly defined, and some evidence suggests that they have worse health outcomes and greater disease burden than patients with COPD or asthma. Generally, there is no evidence-based and universal definition for ACO; several consensus documents have provided various descriptions of the phenotype. In addition, the mechanisms underlying the development of ACO are not fully understood. Whether ACO is, a distinct clinical entity with its particular discrete genetic determinant different from asthma and COPD alone or an intermediate phenotype with overlapping genetic markers within asthma and COPD spectrum of obstructive airway disease remains unproven. This review summarizes the current knowledge of the genetic risk factors, characteristics, and prognosis of ACO.

Introduction

Asthma and chronic obstructive pulmonary disease overlap (Asthma-COPD overlap **(ACO)**) is an obstructive airway disease with a clinical presentation of features usually associated with both asthma and COPD.[1] Studies have shown that patients with ACO present with more frequent airflow obstruction symptoms, more exacerbation, lower quality of life, recurrent hospitalization, and more medical utilization than patients with COPD or asthma alone. [2–5] Alshabanat et al. [6] reported in a meta-analysis a pooled ACO prevalence of 27% and 28% amongst COPD patients from population and hospital-based studies, respectively. ACO patients were significantly younger, had a higher body mass index, increased healthcare utilization, and lower health-related quality of life than patients with COPD alone. [6] In 2019, the global prevalence of ACO was reported to be 2% in the general population. [7] In the same report, 26.5% and 29.6% of ACO patients were found in patients with previous diagnoses of asthma and COPD alone, respectively.[7] However, the prevalence of ACO was contingent upon the diagnostic criteria, definition, and management modalities applied in the various studies included in the reviews. Definitive treatment of asthma-COPD overlap is challenging because of clinical data's paucity from randomized controlled trials explicitly addressing treatment regimens for patients with overlap phenotype. Most COPD clinical trials generally exclude patients with asthmatic history, and most asthma clinical trials exclude patients with a past diagnosis of COPD or significant smoking history. [1, 8]

Genetics plays a crucial role in developing obstructive airway diseases. Many genetic variants associated with asthma and COPD alone have been identified from genetic association studies.[9–11] The identification and recognition of genetic variants are

important in understanding the underlying pathogenesis of diseases. In a recent review, Hall et al.[9] described remarkable advances made through genome-wide association studies in identifying genetic risk factors associated with asthma and COPD. Genetic loci of more than 58 genes, including CHRNA3/CHRNA5/IREB2, RIN3, MMP12, TGFB2, HHIP FAM13A, CYP2A6, MTCL1, SFTPD, SNRPF, PPT2, and AGER, have been associated with COPD. [12, 13] Likewise, loci of more than 108 genes (e.g., IL2RB, HLA-DQ, IL18R1, IL33, SMAD3, ORMDL3/GSDMB) have been associated with asthma. [13–16] However, few genetic association studies have been carried out to investigate genetic variants associated with asthma-COPD overlap. [17–19] Several other studies have recently investigated potential metabolomic profiles, specific immunological mediators, and inflammatory pathways for ACO compared to asthma and COPD alone. [20–33] This review's primary focus is **(1)** to present our current knowledge of ACO on definition, genetic risk factors, clinical characteristics, and prognosis; **(2)** to highlight areas that require further research, specifically in areas that would enable the discovery of genetic variants and other underlying biological mechanisms contributing to ACO development and inform the establishment of novel treatment modalities.

Description and definition of Asthma-COPD overlap

The definition of ACO varied between studies which restrict the comparison of results from different studies. Many population-based studies defined ACO as irreversible airflow obstruction (post-bronchodilator $FEV_1/FVC < 70\%$) with a combination of physician-diagnosed asthma or self-reported physician diagnosis of asthma or self-reported history of asthma or asthmatic symptoms and bronchodilator reversibility. [2, 6, 17, 19, 34–56] Currently, there is no validated definition for asthma-COPD overlap.

However, various consensus documents have been proposed to address the issue for better characterization of the "overlap phenotype."[1, 8, 57] The common similarity amongst the existing consensus documents is that the phenotypic term "asthma-COPD overlap" does not represent a single disease entity. These descriptive consensus documents, for operational purposes, serve the onerous objective of distinguishing the overlap phenotype from the classical definition of asthma or COPD alone.

The 2017 updated collaboration between GINA and GOLD project described "asthma-COPD overlap" as persistent airflow limitation with several diagnostic features associated with asthma and several features associated with COPD. According to the joint consensus document, ACO is identified by having an equal number of features from a checklist with both asthma and COPD syndromic characteristics. In addition to syndromic attributes, spirometric peculiarities of asthma, COPD and ACO are also included. Some of the diagnostic indicators proffered by the GINA/GOLD statement were: **(1)** A persistent yet reversible airflow limitation described as post-bronchodilator forced expiratory volume in one second to forced vital capacity ratio (FEV_1/FVC) less than 0.7; **(2)** A marked reversibility post-bronchodilator FEV_1 increase greater than 12% and 400ml from baseline; **(3)** A history of asthma diagnosed by a doctor, atopy, allergies, or exposure to noxious agents; **(4)** presence of either sputum neutrophilia or eosinophilia; **(5)** An age of onset of 40 years or older. However, the GINA and GOLD joint document's objectives were not to serve as a definition but rather to help clinicians identify, differentiate ACO from asthma and COPD, and make an interim clinical judgment on the best initial treatment. [1]

In 2017 Spanish Society of Pulmonology and Thoracic Surgery (SEPAR), through a joint effort of the Spanish COPD Guidelines (GesEPOC) and the Spanish Guidelines on the Management of Asthma (GEMA), developed a consensus document regarded as the unified criteria for defining and diagnosing ACO. [57] The definition of ACO was established based on the simultaneous existence of three factors: **(1)** chronic persistent airflow limitation; **(2)** significant current and past smoking history; **(3)** Previous diagnosis of asthma (according to GEMA criteria). [58] However, to confirm the diagnosis of ACO, these three essential factors apply to patients who are 35 years or older, with a current and past smoking history of at least ten pack-years, and confirmed post-bronchodilator forced expiratory volume in one second to a forced vital capacity ratio $<$ 0.7 after treatment with bronchodilators and inhaled corticosteroids. The unified consensus document further proposed that if an asthma diagnosis cannot be confirmed, ACO diagnosis will be established if positive bronchodilator response is substantial ($FEV_1 \geq 15\%$ and ≥ 400 ml from baseline) and an elevated- blood eosinophil count is ≥ 300 eosinophils/L. [57]

While the SEPAR unified consensus document for defining and diagnosing ACO has marked similarities with the 2017 GINA AND GOLD updated joint statement, there are apparent dissimilarities. A discrepancy exists in the age of onset in both consensus documents. While the age of onset for asthma-COPD overlap in the SEPAR document was reported as ≥ 35 years, the GINA and GOLD joint statement described this reference age as ≥ 40 years. Furthermore, there was a contrasting criterion for marked reversibility. In the GINA and GOLD statement, marked reversibility was regarded as a Post bronchodilator increase in $FEV_1 > 12\%$ and 400ml from baseline, a value divergent from

the SEPAR guideline's reversibility test reference value (FEV_1≥15% and ≥400 ml from baseline). An additional distinguishing diagnostic factor in the proposed systems is the inclusion of current and previous smoking history in the SEPAR guidelines as an essential element in ACO definition. Nonetheless, the input of environmental exposures (smoking) was not categorically expressed in the GINA/GOLD statement.

In a round table agreement held between 2015 and 2016, a committee of experts recommended using major and minor criteria to define and identify asthma-COPD overlap. [8] The committee suggested that patients who satisfy all three major criteria and at least one minor criterion be considered for an ACO diagnosis **(Table 1.1).**

Despite the heterogeneity and subtle limitations in proposed descriptive and diagnostic consensus statements, the documents currently serve as a provisional clinical recommendation for efficacious and safety-centered treatment of patients with asthma-COPD overlap. Nevertheless, it does not invalidate the need for evidence-based recommendations that will inform specific diagnostic criteria, clinical practice, and homogeneity of inclusion definition of overlap patients in future epidemiological studies.

Genetic risk factors of Asthma COPD overlap

Asthma and chronic obstructive pulmonary disease are heterogeneous complex entities that generally involve many genetic and environmental risk factors. Heterogenous genetic risk factors play intrinsic roles in the development of obstructive airway diseases. Many genomic loci that influence asthma and COPD susceptibility have been identified through family-based, candidate, and genome-wide association studies.[9–12, 14, 16, 59–61] However, no family base-genetic studies have been carried out on ACO. Furthermore, those genetic variants identified through genome-wide association studies cannot account

for the heritability of asthma and COPD. In a genome-wide association study of 15256 cases and 47936 controls, Hobbs et al. [59] identified a genetic correlation of 0.38 (p-value 6.25 x 10^{-5}) between asthma and COPD in European ancestry subjects. None of the genome-wide significant loci for COPD overlapped with known loci for asthma and asthma-associated traits in this study.

In a similar GWAS of 35735 cases and 222076 controls, Sakornsakolpat and colleagues[62] investigated genetic overlap using identified GWAS-associated loci for COPD and previous GWAS results in asthmatic subjects of European ancestry. The authors identified genetic segments near ADAM19, ARMC2, ELAVL2, and STAT6 shared by asthma and COPD. The discrepancy observed between the Hobbs et al. [59] and Sakornsakolpat et al. [62] studies could result from the differences in sample size and lack of considering many environmental factors. Notwithstanding, genome-wide association studies have not explicitly identified overlapping genetic loci for asthma and COPD. These could, to a reasonable extent, suggest the distinctness in the pathogenesis of both diseases. It is unclear whether specific genetic determinants associated with asthma-COPD overlap alone will be demonstrated in GWAS studies or if asthma-COPD overlap will share genetic variants with previously identified asthma and COPD GWAS-associated loci.

In a GWAS investigating genetic features of ACO in Non-Hispanic white and African Americans by comparing 450 patients with ACO and 3120 COPD patients, Hardin et al. identified rs11779254 in the CSMD1 gene and rs59569785 in the SOX5 gene on chromosomes 8 and 12, which are located in an intronic region as top hits nearing genome-wide significance of $5x10^{-8}$ for ACO compared to COPD patients in the Non-

Hispanic white population.[17] CSMD1 gene is a tumor suppressor that is important in lung squamous cell carcinomas. [63] Furthermore, variants near the CSMD1 gene were amongst the top hits associated with qualitative emphysema in a previous genome-wide association study.[64] SOX5 gene has been previously reported to be associated with COPD. Further investigation in a mouse model (SOX5 gene knocked out) demonstrated impaired embryonic lung development.[65]

Also, a SNP (rs2686829) in PKD1L1 gene on chromosome 7 approached the genome-wide significance threshold in the African American cohort.[17] The top hit SNPs from the Non-Hispanic white cohort associated with asthma-COPD overlap were not replicated in the African American population and vice versa. Further analysis in a meta-analysis combining the Non-Hispanic white and African American cohorts identified the most significant variant, rs6574978, in the GPR65 (G Protein-Coupled Receptor 65) gene on chromosome 14 ($p= 1.18 \times 10^{-7}$) for ACO compared to COPD subjects. [17] However, none of the top hits reached the pre-specified genome-wide significant threshold of 5×10^{-8}. Kottyan and colleagues[66] demonstrated that GPR65 has increased expression on eosinophils and asthmatic inflammation and showed that GPR65 knock-out mice had decreased airway eosinophilia.

Smolonska et al.[18] reported three loci associated with both asthma and COPD alone from a meta-analysis and replication in nine independent cohorts. SNPs (rs1477253) in DEAD-box polypeptide 1 (DDX1), (rs254149) in COMM domain containing 10 (COMMD10) and (rs9534578) in guanine nucleotide-binding protein, gamma 5 pseudogene 5 (GNG5P5) in chromosomes 2p24.3, 5q23.1, and 13q14.2, respectively were identified. Only SNP (rs9534578) in GNG5P5 reached the genome-wide

significance threshold after first phase replication and meta-analysis in 2 cohorts (p-value 9.96 $x10^{-9}$). However, None of the SNPs attained the genome-wide significance threshold in subsequent replication studies using seven other independent cohorts. [18]

As shown in **Table 1.2**, in a GWAS where asthma patients were compared with asthma-COPD overlap; No overlap was observed at the genome-wide significant threshold of $5x10^{-8}$ among the top ten SNPs associated with ACO compared to asthma alone.[19] In comparing ACO and normal subjects, three of the top ten SNPs (rs35614679, rs1677005, and rs1786253) associated with ACO were located in the intronic and downstream region of the TAF4B gene on chromosome 18. At the same time, five of the top ten SNPs (rs117733692, rs3772010, rs4601609, rs2149063, and rs34186721) were also located in the intronic region near MSRA, RNF144A, LINC01135, GPC5, and RAD51B) genes, respectively. Two SNPs (rs11665213 and rs12336157) located on chromosomes 18 and 9 from Kwon et al.[19] study had no gene-based annotation information.

Most recently, In a published conference abstract, Joo et al. carried out a GWAS using data from the U.K. biobank to identify genetic variants associated with asthma-COPD overlap (ACO) and investigate whether genetic loci associated with ACO overlap with known asthma or COPD loci. [67] The study identified 24 loci associated with ACO at a genome-wide significant level of $5x10^{-8}$, including well-known asthma and COPD loci such as ORMDL3, GSDMB, and HHIP [9], respectively. Interestingly, Joo et al. indicated that some loci (near HRNR and ID2 genes) associated with asthma-COPD overlap at the genome-wide significant threshold were not genome-wide significant for COPD and asthma. [67] These suggest that asthma-COPD overlap might have its specific associated genetic variants, despite the recognition of many overlapping clinical features

amongst ACO, COPD, and asthma. Furthermore, through candidate gene association studies, some genes have been associated with both asthma and COPD.[10, 68] These genes (ADAM33, TNFα, MMP9, TGFβ1, GSTM1, GSTP1), especially ADAM33 and genes from the GST family, have been linked to lung function growth as well as lung function reduction in childhood and adulthood, respectively.[10] SNPs from the ADAM33 gene have been associated with childhood asthma,[69] COPD, and lung function in tobacco smokers with greater than 20 pack-years smoking history.[70] It has been shown in a recent case-control study involving COPD (n=194) and asthma (n=150) cases compared to healthy control (n=220) that SNPs from ADAM33 gene (V4 [rs2787094], T1[rs2280091], S2[rs528557]) and AQP5 gene (rs3736309) were commonly associated with both COPD and asthma alone. [71] Furthermore, the association of these genes with airway remodeling and lung function decline in asthma and COPD has also been documented. [72–74] Therefore, polymorphisms in these genes could potentially play crucial roles in ACO pathogenesis.

The majority of previous genome-wide association studies investigating the genetic background of asthma-COPD overlap were underpowered to detect any significant association (**Table 1.2**). However, top hit SNPs identified in these studies should be regarded as variants of interest in future studies. In general, genetic association research on asthma-COPD overlap is still at an early stage; more genome-wide association studies, including environmental risk factors, with larger sample sizes that have undergone replication in at least one independent population, are needed to demonstrate and understand the genetic determinants of asthma-COPD overlap while seeking a plausible explanation to the pathobiology and pathogenesis of the disease.

Characteristics of ACO

Asthma-COPD overlap presents similar clinical features as other obstructive airway diseases. Many epidemiological studies have compared demographic features, symptoms burden, frequency of exacerbation, pulmonary functions, quality of life, and burden of comorbidities among patients with asthma-COPD overlap, asthma, and COPD alone.[2, 17, 19, 35–44, 46, 75–77] The Gina and Gold report highlighted features that could be used to differentiate the overlap phenotype from classical asthma and COPD (**Table 1.3**).

Demographic features (Age, Sex, smoking history, and BMI)

Various studies have reported that patients with ACO were younger than patients with COPD [2, 17, 36, 37, 40, 41, 43, 77, 78] and older than asthmatic patients.[19, 36–38, 41, 78] Some studies have shown that the mean age of COPD and ACO patients was similar and indistinguishable.[35, 44, 46, 52, 79, 80] In a population-based cross-sectional study using data from the Canadian Health Measures Survey (CHMS), the mean age of patients with ACO was between that of COPD patients and asthmatic patients alone. The authors reported that the observed difference in mean age amongst the groups was significant.[37] Hardin et al.[2, 17] also reported in two studies a significant difference in mean age between patients with ACO and COPD.

In two other studies of asthmatic and ACO patients, the mean age of patients in the ACO group was significantly higher than patients in the asthmatic group.[19, 38] Furthermore, epidemiological studies have reported conflicting sex distributions in ACO patients, with certain studies showing predominantly males [2, 36, 39, 43, 44, 77, 78] while others indicate that ACO is preponderant in females.[17, 19, 35, 41] In a CanCOLD study

comparing individuals with asthma-COPD overlap stratified by seven different definitions and COPD alone, Barrecheguren et al. showed that the overall patients with ACO were younger (66.6± 10.1 vs. 68.1± 9.3 years) and had more female sufferers (n=111 vs. 90) than COPD patients. [40] However, Chung et al. demonstrated a different sex distribution trend; In that study, 67% of ACO patients were males. [36]

Similar to COPD and asthma alone, smoking in ACO patients is well documented. Epidemiological studies have shown that compared to patients with COPD and asthma alone, patients with ACO were more likely to have lesser pack-years of smoking than COPD patients and greater pack-years than asthmatics. [2, 17, 19, 23, 27, 28, 35, 40, 43, 77, 81] However, some studies have reported no significant difference in smoking history between COPD and ACO subjects[39, 41, 78] and higher pack-years of smoking history in subjects with ACO than COPD.[24]

Chung et al. analyzed data of 9104 subjects from the Fourth Korea National Health and Nutrition Examination Survey of 2007–2009; 73% of ACO patients (n=210) were current and former smokers, while 65% and 54% were current and former smokers in COPD (n=700) and asthma patients (n=560) respectively.[36] Similarly, Senthilselvan and Beach [37] showed that the percentage of current smokers was higher in ACO (n=144) subjects than in COPD (n=200) and asthma (n=602) subjects (44.8% vs. 40.9% vs. 23.0%).

ACO patients have also been reported in some studies to have lower body mass index (BMI) than patients with asthma, and at the same time, higher BMI compared with patients with COPD.[17, 35, 36, 41, 43] One study found that greater percentage of ACO subjects (44.7%) were obese compared to asthma (32.1%) and COPD subjects (38.3%)

alone. [37] However, contrasting findings have been observed in some other studies, where mean BMI values were comparable and not significantly different amongst patients with asthma, ACO, and COPD.[2, 19, 38, 46, 78]

Symptom burden, Lung Function, Imaging features, and Quality of life

In a CanCOLD multicenter study, 264 individuals with ACO (ACO defined using 7 different definition criteria) had significantly worse lung functions in forced expiratory volume in 1 second (FEV_1[L], and FEV_1% predicted) than 258 COPD patients ([2.2 ± 0.8 vs. 2.4 ± 0.8] and [76.6 ± 18.3 vs. 83.9 ± 19.9] p-value < 0.001). In this study, the authors showed that a greater percentage of ACO patients (13.8%) had higher levels of dyspnea, with a score of 3 and above in the Medical Research Council Dyspnea Scale (MRC) in contrast to COPD patients (5.3%). Furthermore, ACO patients were reported to have significantly higher St George's Respiratory Questionnaire (SGRQ) scores, signifying a lower respiratory-specific quality of life than COPD patients. [40]

Also, in a multivariate analysis, Kauppi et al. [76] observed that ACO is associated with the poorest health-related quality of life (HRQoL) compared to COPD and asthma alone (OR:1.93, CI: 1.16–3.22, p-value 0.011). Furthermore, the mean score from the airway questionnaire 20 (AQ20) amongst subjects with ACO (8.8 ± 5.1) was significantly higher than COPD (7.4 ± 5.2) and asthma (6.8 ± 4.8).

In a similar study conducted in Australia, lung function, dyspnea, and St. George's Respiratory Questionnaire scores were compared between 60 ACO patients and 204 patients with COPD alone.[39] The mean pre-bronchodilator values of FEV_1% predicted and FVC % predicted were significantly lower in the ACO group than in the COPD group ([58.4 ± 14.3 vs. 67.5 ± 20.1] and [82.1 ±16.9 vs. 91.9 ± 17.2] p-value < 0.001

respectively). In contrast, there was no difference in post-bronchodilator spirometry values, quality of life scores assessed with SGRQ, and dyspnea evaluation using modified Medical council research council scale (mMRC) between ACO and COPD groups. [39]

Senthilselvan and Beach [37], in a population-based cross-sectional study, using data from the Canadian Health Measures Survey (CHMS), ACO subjects reported more cough, cough accompanied by phlegm and wheeze than in patients with asthma, COPD, and normal subjects. Compared with the healthy controls, the proportion of patients with poor general health in ACO subjects (21.3%) was higher than it in COPD (11.4%) or asthma alone (5.4%). [37]

In "Gene-Environment Interaction in Respiratory Diseases study" (GEIRD), de Marco et al. [42] observed a significantly higher prevalence of medical research council dyspnea score ≥ 3 in the ACO group (38.8%) than the COPD (20.8%) and asthma groups (9.3%). The authors also reported that the ACO group was more likely to have cough or phlegm, more wheezing, higher frequency of asthmatic attacks, greater use of anti-asthmatic medications, and increased hospitalization (3.1% vs. 2.5% vs. 1.1%) than COPD and asthma groups alone. [42]

In the PLATINO study, Menezes et al. analyzed data from 767 individuals; Subjects with ACO (n=89) had more respiratory symptoms (cough and phlegm), worse lung function (pre and post-bronchodilator FEV_1, FVC, and FEV_1/FVC in liters and % predicted), used more respiratory medication, and recorded worse general health status than asthma (n=84) and COPD (n=594) subjects. [41] Worse lung function parameters observed in ACO patients than in COPD and asthma patients were consistent with other studies.[19, 34–36, 38, 48]

In contrast, in the studies by Hardin et al. [2, 17], lung function parameters of (FEV_1, FVC % predicted, and FEV_1 in liters) of patients with ACO and COPD were comparable. Nevertheless, in Hardin et al. [17], post-bronchodilator FEV_1/FVC value in COPD patients was significantly lower than in patients with ACO despite comparable post-bronchodilator FEV_1 values (% predicted and in liters). Furthermore, In this study, subjects with ACO had worse BODE index (Body Mass Index, airflow obstruction, dyspnea, and 6 minutes exercise capacity) and SGRQ scores than COPD patients, suggesting worse disease severity and poorer quality of life, respectively.[17] Hardin and colleagues[17] also analyzed data from chest CT-Scan for COPD and ACO patients. The authors showed that patients with ACO had more airway wall thickness and less emphysema than COPD patients. This finding was consistent with three other studies.[40, 75, 81]

In a prospective longitudinal study using data obtained from stable COPD patients enrolled in Ishinomaki COPD Network Registry, Kobayashi et al. [43] reported that patients with ACO did not exhibit worse pulmonary functions. Neither did ACO patients display increased dyspnea symptoms than COPD patients. Park et al. [75], in a separate longitudinal study of 47 patients diagnosed with ACO from a cohort of 239 COPD patients, showed the post-bronchodilator FEV_1 (mL and predicted) were significantly higher in ACO patients than in COPD subjects (n=192). In contrast, baseline pre-bronchodilator FEV_1(mL and predicted values) were not significantly different between the two groups. They also observed that ACO subjects had a slower annual decline in pre-bronchodilator FEV_1 than patients with COPD alone over a median follow-up period of 5 years. Conversely, no significant difference was observed in the prevalence of

dyspnea and respiratory-specific quality of life between patients with ACO and COPD.[75]

Exacerbation

The mechanism underlying the aggravation of respiratory symptoms in ACO is unclear. However, exacerbations in asthma and COPD might result from external stimuli such as allergens, environmental pollutants, and microbial infections of the respiratory tract.[82, 83] Available data on the frequency of exacerbation in ACO patients compared to COPD have been Inconsistent, whereas in ACO patients compared to asthma, the exacerbation frequency has been consistently higher in patients with ACO. Patients with ACO have been shown to have more exacerbation in various studies than subjects with COPD [2, 17, 35, 40, 41, 45, 84] and asthma alone. [19, 34] Nonetheless, some other studies showed no significant difference in the exacerbation frequency between COPD and ACO patients. [27, 50, 51, 77, 80] As frequent and severe exacerbation of respiratory symptoms may increase lung function decline, mortality, and morbidity, more studies are needed to examine and understand how it changes the natural course of the disease.

Comorbidities

The burden of concomitant diseases in ACO complicates the natural course of the disease and leads to increased mortality and morbidity. Comorbidity profile in ACO has been reported in several studies. [37, 47, 76, 85–90] For instance, in a large case-control study in Germany using national data of statutory insured individuals, Atmatov et al.[89] identified the most prevalent top 20 comorbid diseases in ACO patients in the German population. The first 10 of the comorbid diseases were essential primary hypertension, disorders of lipoprotein metabolism, dorsalgia, type 2 diabetes mellitus, obesity,

depression, chronic ischemic heart disease, spondylosis, gastroesophageal reflux disease, and gonarthrosis. [89] Similarly, Leung and Sin [91], in a review, highlighted the burden of comorbidity in ACO in diseases such as gastroesophageal reflux disease, osteoarthritis, osteoporosis, depression, and anxiety.[91]

The extent of heterogeneity observed in the various reports of several studies describing the characteristics and clinical features of asthma-COPD overlap compared to asthma "alone" and COPD "alone" apparently stem from different diagnostic descriptions, eligibility criteria, and case definition criteria applied in the studies.

Prognosis

There are conflicting reports on the prognosis of patients with ACO compared to COPD and asthma patients. It has been reported that patients with ACO experience more frequent exacerbations, higher risk of hospital admission, poorer quality of life, more rapid decline in lung function, and higher mortality than patients with asthma or COPD alone.[2, 3, 35, 76, 92–94] Nevertheless, data from studies investigating the mortality rates in ACO and COPD subjects have been inconsistent.

Two studies found that mortality rates in ACO and COPD were indistinguishable. [79, 95] However, COPD subjects in one of the studies had a higher risk of all-cause mortality (HR:2.12, 95% CI: 1.71–2.63, $p < 0.001$) than ACO patients (HR:1.82, 95% CI:1.38–2.38, $p < 0.001$) when both diseases were compared to control subjects during a 15-year follow-up. [79]

Some studies reported higher mortality rates in ACO subjects[94, 96–99], while other studies revealed lower rates in patients with concomitant asthma and COPD with respect to COPD alone. [80, 84, 100, 101] For instance, an observational study using data from

NHANES III dataset where patients with a self-reported diagnosis of asthma(n=120), COPD (340), and ACO (n=126) were drawn from 4434 participants, Kumbhare and Strange [99] noted that death as a result of chronic lower respiratory disease was the highest in subjects with ACO (21.4%) than in patients with COPD (12.6%) and asthma (9.5%). In the same study, ACO subjects had a higher mortality rate of 3.2% due to influenza and pneumonia than COPD (2.1%) and asthma (1.6%).[99] Tkacova et al.[97] observed a two-fold increased risk of death (HR:2.38, 95% CI:1.38–4.11, p=0.002) due to respiratory cause in patients with ACO (defined as COPD with airway hyperactivity) than in COPD patients. [97]

Lange et al.[94], in a population-based study of participants from Copenhagen City Heart Study, demonstrated that ACO with late asthma onset had the worse prognosis in terms of respiratory mortality, all-cause mortality, and life expectancy than ACO with early asthma onset, COPD, Asthma, ever-smokers without disease, and never-smokers without the disease. Lange and colleagues quantified the reduction of life expectancy in ACO with late-onset asthma patients as 12.8 years, 10.1 years in COPD, and 9.3 years in asthma. These comparisons were made using subjects who were never smokers and without disease as the reference group. [94] Similarly, a study with an 18-year follow-up found that subjects with ACO had a higher risk of mortality than their COPD and asthma counterpart. The authors reported a hazard ratio of 1.45, 1.28, and 1.04 for ACO, COPD, and asthma patients, respectively, after adjusting for covariates such as age, sex, ethnicity, smoking status, education level, body mass index, respiratory disease, and lung function status. [96]

Contrastingly decreased mortality and improved survival in ACO have been reported in few studies compared to COPD. ACO patients (n=6279) were found to have in-hospital all-cause mortality of 2.3% opposed to 9.7% for COPD patients (n=4261).[100] Another study pointed out that in comparison to patients with COPD, ACO patients had better median survival years (9.1 vs. 7.9 years) and survival probability (0.35 vs. 0.25) during 15 years follow-up. [79]

While analyzing data from 65 ACO patients and 65 COPD patients, Bai and colleagues observed that despite more frequent exacerbations in the past 12 months (2.3 ± 2.2 vs. 1.4 ± 1.3 respectively), patients with ACO had a lesser number of deaths (3 deaths) and shorter days of hospitalization than COPD patients (13 deaths) during a median follow-up period of 45 months. [84] These might be due to better response to steroid treatments in patients with ACO than COPD.

Studies assessing lung function decline in ACO patients have reported conflicting results in comparison to COPD patients. However, one consistent trend observed in most studies except one [45] is the faster decline in FEV_1 seen in ACO patients than in patients with asthma. Marco et al. [45] reported similar changes in FEV_1 decline amongst young adults (age: 20-40 years) with asthma and ACO over nine years follow-up; However, ACO patients had a more favorable decline in FEV_1 than COPD patients within the same timeframe. Likewise, a longitudinal study of older patients with chronic obstructive airway disease (age: > 55 years) showed no significant difference amongst asthma, COPD, and ACO subjects in FEV_1 decline during four years of follow-up. [46]

In another longitudinal study evaluating long-term prognosis in asthma, COPD, and ACO subjects over an 18 – 22 years period, ACO subjects with late-onset asthma (defined as

asthma onset after 40 years of age) had the worst prognostic outcome with FEV_1 decline of 49.6mL/year. Patients with COPD, ACO with early-onset asthma, and asthma alone had FEV_1 decline of 39·5mL/year, 27·3mL/year, and 25·6mL/year, respectively. [94] Another study also reported a higher annual decline in FEV_1 in patients with ACO than in COPD patients (−49.6mL/year vs. -38.1mL/year).[40] ACO patients who were faster decliners in FEV_1 (defined as a reduction >40 mL/year) had a significantly higher decline in FEV_1 than patients with COPD (-81.1mL/year, 95% CI: [-116.5, -45.6] vs. -38.1mL/year, 95% CI:[-49.3,-26.9]). [40]

Contrarily, a more and faster annual decline in pre-bronchodilator FEV_1 was reported in COPD patients from the Korean Obstructive Lung Disease cohort. A cohort of 239 COPD patients showed that patients diagnosed with ACO (n=47) had a significantly lower annual pre-bronchodilator FEV_1 than patients with COPD alone (-13.9 mL/year vs. − 29.3 mL/year, p=0.042). [75] The result remained consistent after controlling for baseline age, body mass index, smoking status, exacerbation rate, and medication use (-13.61mL/year vs. -29.16mL/year, p=0.042). [75] The variations in the results could be attributed to the better response to ICS/LABA or ICS treatment in patients with ACO than patients in the non-ACO group.

Poorer prognosis in ACO patients appears to be influenced by the burden of comorbidities. ACO patients with three or more comorbid medical conditions have been shown to have poorer survival (n=81; median survival years: 3.7) and worse prognosis than in ACO patients with less than three comorbidities (n=48; median survival years: 6.0). [102] Furthermore, similar comorbidity profiles and causes of death, notably cardiovascular, malignant, gastrointestinal diseases, and diabetes, are plausibly present in

asthma, ACO, and COPD. [99, 101–103] The possible explanation for the observed variations in the findings of various studies evaluating the prognosis of ACO could be due to varying diagnostic criteria, the sample size of the cohorts, and disparities in obstructive airway disease severities of the cohorts.

Future direction

The recent pro-con debate and the non-inclusion of the term ACO in the GOLD 2020 report raise uncertainty on the utility of the term ACO to describe a subset of patients with shared COPD and asthmatic features.[104–106] Despite the non-inclusion of ACO as a term in the GOLD 2020 report, it acknowledged the coexistence of asthma and COPD in certain patients. There is some truth in that ACO is a blend of conditions as opposed to its entity. The absence of a globally unified definition, the heterogeneity in several diagnostic criteria, and the yet to be identified replicable specific genetic and biomarkers associated with the "overlap" phenotype could be the attributable factors for the exclusion. However, it will make further study of "a subset of patients with shared COPD and asthmatic features" harder. For example, identifying patients who will respond to specific treatments and those who are more likely to be harmed. It is a known clinical fact that COPD patients are not supposed to get Inhaled corticosteroids (ICS) alone, but it is the mainstay of treatment for asthma patients. What about patients with emphysema on high resolution computed tomography (HRCT) who also have high eosinophils or exhibit significant reversibility? Are they more or less likely to benefit or be harmed when treated with ICS? Are they more or less likely to manifest better response in FEV_1 when treated with ICS? Therefore, in order to advance our understanding of the "overlap phenotype," taking into consideration the heterogeneity

that exists in its presentation, just as asthma and COPD are evidently heterogenous, identification and a better understanding of both genetic and novel biomarkers may allow recognition of patients who would respond to specific pharmacotherapies rather than the current extrapolations of evidence from asthma and COPD studies.

Conclusion

ACO might be a distinct chronic obstructive lung disease phenotype with unique genetic risk factors or an intermediate phenotype with overlapping genetic architecture between asthma and COPD alone. Although the genetic risk factors associated with ACO may still be at a conjecture stage, the possibility that ACO might possess ACO-specific and shared genetic mechanisms of asthma and COPD should be considered. Therefore, future extensive genetic association studies need to identify genetic determinants associated with ACO, its molecular patterns, and the influence of environmental factors in developing the phenotypic trait. Therefore, it is necessary to explore if there are distinguishing pathogenetic factors that could differentiate ACO from classic asthma and COPD. A deep understanding of the genetic and inflammatory mechanisms associated with ACO will improve its characterization and the development of therapeutic options tailored toward managing treatable traits.

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Table 1.1 Criteria for ACO definition [8]

Major Criteria	• Persistent airflow limitation (post-BD FEV_1 /FVC <0.70 or LLN) in individuals 40 years of age or older. • At least ten pack-years of tobacco smoking or indoor or outdoor air exposure to pollutants • Asthma history prior to 40 years of age or BDR of >400 mL in FEV_1
Minor Criteria	• Documented history of atopy or allergic rhinitis • BDR ≥200 mL and 12% in FEV_1 from baseline values on two or more visits • Peripheral blood eosinophil count ≥300 cells·uL−1

LLN: Lower limit of normal; post-BD: post-bronchodilator; BDR: Bronchodilator response; FEV_1: Forced expiratory volume in 1 second; FVC: Forced vital capacity.

Table 1.*2.* SNPs identified through genome-wide association study for asthma-COPD overlap

Study	SNPs	Chr	Gene/nearest gene	Allele MA or R.A.*	p-value
Hardin etal[17]	rs6574978	14	GPR65	T*	1.18E-07
	rs8004567	14	GPR65	A*	1.18E-07
	rs1441809	14	GPR65	A*	1.58E-07
	rs3759712	14	GALC	C*	1.80E-07
	rs2686829	7	PKD1L1	T	4.03E-07
	rs11779254	8	CSMDI	G	1.57E-06
	rs59569785	12	SOX5	C	1.61E-06
Smolonska etal [18]	rs1477253	2	DDX1	T	7.28E-06
	rs254149	5	COMMD10	G	9.76E-06
	rs9534578	13	GNG5P5	A	9.96E-09
Kwon et al. [19]	rs117733692‡	8	MSRA	A	1.45E-05
	rs3772010‡	2	RNF144A	C	3.14E-06
	rs11665213‡	18	NR	T	7.12E-06
	rs1677005‡	18	TAF4B	C	8.40E-06
	rs35614679‡	18	TAF4B	A	8.46E-06
	rs4601609‡	1	LINC01135	C	9.69E-06
	rs2149063‡	13	GPC5	C	1.17E-05
	rs1786253‡	18	TAF4B	G	1.46E-05
	rs12336157‡	9	NR	G	1.96E-05
	rs34186721‡	14	RAD51B	A	2.06E-05
	rs11665213**	18	NR	T	1.15E-05
	rs7967352**	12	NR	G	1.23E-05
	rs1046706**	3	SLC9A9	T	1.31E-05
	rs78276030**	6	NR	G	1.93E-05
	rs8115801**	20	PAK7	T	2.02E-05
	rs7732758**	5	LHFPL2	C	2.15E-05
	rs4773144**	13	COL4A2	G	2.53E-05
	rs35614679**	18	TAF4B	A	3.17E-05
	rs6837266**	4	ATP10D	C	3.22E-05
	rs1786253**	18	TAF4B	G	3.52E-05

SNPs: Single nucleotide polymorphisms; Chr: Chromosome; MA: Minor allele; RA*: Reference allele; ** SNPs associated with ACO compared to asthma; ‡ SNPs associated ACO compared to normal subjects; NR: Not reported

Table 1.*3.* The characteristic feature of asthma, COPD, and ACO [1]

Features	Asthma	COPD	Asthma-COPD overlap
Age of onset	Usually in childhood	Usually, >40 years	Usually, ≥40 years but may have had earlier symptoms in early adulthood or childhood
Pattern of respiratory symptoms	May vary over time, often triggered by exposure to an allergen, exercise and emotions	Chronic, usually continuous symptoms, particularly during exercise	Symptoms persistent but may exhibit variability
Lung function	Current and/or historical reversible airflow obstruction	Post-BD FEV_1 /FVC ratio < 0.70 persist	Airflow obstruction not fully reversible, but often with variability
Lung function between symptoms	May be normal between symptoms	Persistent airflow obstruction	Persistent airflow obstruction
History or family history	History of allergies, personal or family history of asthma	History of exposure to noxious particles/ gases (mainly tobacco smoke and biomass fuel)	Frequently personal and family history of asthma and allergies, and history of noxious exposures (tobacco smoke)
Time course	Often improves spontaneously or with treatment	Slowly progressive over years despite treatment	Symptoms partly but significantly reduced by treatment. Progression is typical and treatment needs are high
Chest X-ray	Usually, normal	Severe hyperinflation and other changes	Similar to COPD
Exacerbations	Exacerbation risk reduced by treatment	Risk may be reduced by treatment; comorbidities may contribute to worse outcome	May be more common than in COPD but responsive to treatment. comorbidities may contribute to worse outcome
Airway inflammation	Characterized as Eosinophils ± neutrophils	Neutrophils ± eosinophils in sputum, may have systemic inflammation	Eosinophils ± neutrophils in sputum

Post-BD FEV_1 /FVC: Post-bronchodilator Forced expiratory volume in 1 second to Forced vital capacity ratio**.**

Chapter 3. Sex-specific genetic determinants of Asthma-COPD phenotype and COPD in middle-aged and older Canadian adults: An analysis of CLSA data

This manuscript was submitted to the Journal of Chronic Obstructive Pulmonary Disease and is currently under review.

Authors contributions

UO wrote the original manuscript draft, conducted data analysis, and prepared all figures and tables. ZG and AS provided critical feedback on statistical analysis and methodology. ZG and AS obtained ethics approval and provided access to the data. ZG, AS, and JF conceptualized the study and critically revised, edited, and suggested changes to the manuscript. All authors contributed to the article and approved the final version that was submitted.

Abstract

The etiology of sex differences in the risk of asthma-COPD phenotype and COPD is still not completely understood. Genetic and environmental risk factors are commonly believed to play an important role. This study aims to identify sex-specific genetic markers associated with asthma-COPD phenotype and COPD using the Canadian Longitudinal Study on Aging (CLSA) Baseline Comprehensive and Genomic data. There were a total of 1,415 COPD cases. Out of them, 504 asthma-COPD phenotype cases were identified. 20,524 participants without a diagnosis of asthma and COPD served as controls. We performed genome-wide SNP-by-sex interaction analysis. SNPs with an interaction p-value $< 10^{-5}$ were included in a sex-stratified multivariable logistic regression for asthma-COPD phenotype and COPD outcomes. 18 and 28 SNPs had a significant interaction term p-value $< 10^{-5}$ with sex in the regression analyses of asthma-COPD phenotype and COPD outcomes, respectively. Sex-stratified multivariable analysis of asthma-COPD phenotype showed that 7 SNPs in/near SMYD3, FHIT, ZNF608, RIMBP2, ZNF133, BPIFB1, and S100B loci were significant in males. Sex-stratified multivariable analysis of COPD showed that 8 SNPs in/near MAGI1, COX18, OSTC, ELOVL5, C7orf72 FGF14, and NKAIN4 were significant in males, and 4 SNPs in/near genes CAMTA1, SATB2, PDE10A, and LINC00908 were significant in females. An SNP in the ZPBP gene was associated with COPD in both males and females. Identification of sex-specific loci associated with asthma-COPD phenotype and COPD may offer valuable evidence toward a better understanding of the sex-specific differences in the pathophysiology of the diseases.

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory condition characterized by irreversible airflow limitation. It is a prevalent disease among middle-aged and elderly. In 2015, GINA and GOLD collaboration described a new chronic obstructive airway phenotype known as asthma-COPD overlap.[1] The Asthma-COPD phenotype was characterized as a chronic obstructive airway phenotype with persistent airflow limitation and a clinical presentation of features associated with asthma and COPD. A number of studies have shown that patients with the overlap phenotype present with more frequent airflow obstruction symptoms, more exacerbation, lower quality of life, recurrent hospitalization, and more medical utilization than patients with classic COPD or asthma alone.[2–7] In addition, the lack of clinical data from randomized controlled trials explicitly addressing treatment regimens for patients with the overlap phenotype makes definitive treatment difficult.

Many epidemiological studies have found significant sex differences in the risk of asthma-COPD phenotype and COPD, with females having a higher prevalence than males.[8–12] COPD was initially considered a disease of middle-aged and older men; however, its prevalence and hospitalization rates in women have increased in the last 20 years. [13–15] Although the etiology of sex differences is not fully understood, it is commonly believed that both environmental and genetic factors play an important role.

Cigarette smoking is the most consistent risk factor for COPD, especially in developed nations; exposure to biomass and noxious pollutants impacts COPD risk in developing countries.[16, 17] Apart from the strong influence of environmental factors on COPD risk, various genetic risk factors have been associated with COPD susceptibility.[18–22]

As genetic and environmental risk factors play crucial roles in COPD development, studies have revealed that COPD's clinical presentation and susceptibility vary by gender.[23–31] Women who were ex-smokers and current smokers had a higher risk of airflow obstruction than men who were ex-smokers and current smokers exposed to the same dose of tobacco.[31] A study observed that females with early-onset COPD and < 20 pack-years of smoking had a greater reduction in lung function (FEV_1% predicted) and more severe disease than males.[24] Females with COPD have been reported to have more dyspnea, less expectoration, worse exercise capacity, and more frequent diagnosis of concomitant asthma than males.[29] Whereas in another study, males with COPD were reported to have more emphysema and lesser airway remodeling than females.[28] Epidemiological studies have linked several socio-demographic, socio-economic, and lifestyle factors to asthma-COPD phenotype risk.[8, 9] In a study of the risk of asthma-COPD phenotype in Aboriginal people, daily smoking, working more than 40 hours per week, and being separated, widowed, or divorced were associated with the risk of asthma-COPD phenotype in females but not in males.[9] In addition, Aboriginal women who smoked at home compared to those who did not were almost three times more likely to be associated with asthma-COPD phenotype risk. However, this trend was not observed in men.[9] Similarly, in another population-based study, women who smoked cigarettes for ten years or more had a higher prevalence of asthma-COPD phenotype than men.[12] Nevertheless, the underlying mechanisms for the sex-specific differences in asthma-COPD phenotype and COPD remain unclear. Although many genetic loci have been associated with COPD and asthma-COPD phenotype,[18–22, 32–34] no genetic association studies have considered these socio-demographic, socio-economic, and

lifestyle factors in their analyses.[35, 36] Using the Canadian Longitudinal Study on Aging (CLSA) baseline questionnaire and genomic dataset, this study aimed to explore sex-specific genetic differences in asthma-COPD phenotype and COPD while accounting for various socio-demographic, lifestyle, socio-economic, and environmental factors.

Materials and Method

Study design and Population

The Canadian Longitudinal Study on Aging (CLSA) is a population-based cohort of 51,338 Canadians aged 45 to 85 years old. CLSA's study design and methodology have previously been published.[37, 38] It is made up of two complementary cohorts: the Tracking cohort, which consisted of 21,241 people who were questioned over the phone, and the Comprehensive cohort, which consisted of 30,097 people who provided baseline data via an in-person home interview as well as other questionnaires, tests, physical measurements, and biospecimen (blood and urine) collected at the data collection sites.[38] Our study focused on 26,622 subjects from the Comprehensive Cohort who were genotyped using the Affymetrix UK Biobank Axiom array.[39]

Outcomes

We defined COPD and asthma from the positive responses to the questions, “Has a doctor told you that you have/had any of the following: emphysema, chronic bronchitis, chronic obstructive pulmonary disease (COPD), or chronic changes in the lungs due to smoking?" and “Has a doctor ever told you had asthma?”, respectively. Participants with positive responses to both self-reported physician-diagnosed asthma and COPD were categorized as asthma-COPD phenotype. Control subjects were those who responded

"no" to self-reported physician diagnoses of asthma and COPD. Participants who self-reported a physician diagnosis of only asthma and those with missing responses were excluded from each study group. Asthma-COPD phenotype cases were also excluded from the COPD case-control group. On the other hand, COPD cases were excluded from the asthma-COPD phenotype case-control group **(Figure 1.1).**

Covariates

The CLSA questionnaire contains a wide range of socio-demographic and socio-economic data, information on lifestyle and health behaviors, as well as clinical and physical data. Our study considered the following potential confounders such as age group (45 to 54 years, 55 to 64 years, 65 to 74 years, and 75 years and above), biological sex, marital status (single or never married, married or in a common-law relationship, widowed/divorced/separated), education level (less than post-secondary education, post-secondary but not university education, and university education/others), total personal income and total household income (Less than $20,000, $20,000 to less than $50,000, $50,000 to less than $100,000, $100,000 or more), province of recruitment (Prairies, British Columbia, Eastern provinces, Ontario and Quebec), retirement status (Retired completely and not retired/partly retired), smoking status (current, never, and former smokers), homeownership (owned and rented/others), and urban/rural dwelling.

Genotyping and Quality Control

Genotyping for 794,409 genetic markers was done using Affymetrix UK Biobank Axiom array. Quality control of genetic markers and samples was adequately described in another paper.[39] SNPs and Samples that failed the QC metrics were removed. All genomic positions were in reference to the human genome build GRCh37/hg19.[39]

Using PLINK v1.90b6.25,[40, 41] we excluded samples with discordant sex information and more than 5% genotype missingness. Autosomal SNPs were extracted, SNPs with less than 99% call rate, minor allele frequency less than 1% (MAF < 0.01), and SNPs deviating from the Hardy Weinberg equilibrium threshold of 1e-10 were excluded. In order to obtain SNPs that are in approximate linkage equilibrium with each other, we generated a subset of LD pruned using PLINK's Indep-pairwise command (Indep-pairwise 50, 5, 0.5). Following quality control, we had 504 asthma-COPD phenotype cases, 911 COPD cases, 20,524 controls, and 416,562 SNPs for genome-wide SNP-by-sex interaction analysis. (**figures 1.1 & 1.2).**

Genetic and Statistical Analysis

In order to investigate sex-specific variants for asthma-COPD phenotype and COPD outcomes, genome-wide SNP-by-Sex interaction was examined in logistic regression for the asthma-COPD phenotype and COPD, respectively, after adjusting for age, sex, smoking status, and the first four principal components of genetic ancestry using PLINK 1.9 v1.90b6.25.[40, 41] Those SNPs with an interaction p-value less than 10^{-5} were further examined by sex-stratified analysis for asthma-COPD phenotype and COPD outcomes using survey-specific logistic regression (*Proc Surveylogistic)* in SAS version 9.4. The survey-specific SAS procedure allows us to apply sampling weights, incorporate complex survey designs, and control for potential confounders. For SNPs in high linkage disequilibrium (D'>0.80), the SNP with the highest polymorphism information content (PIC) value was chosen.

For descriptive statistics of continuous and categorical variables, mean and standard error and frequency and percentage were used to describe the study population. CLSA's

trimmed inflation and analytic weights (CLSA Sample Weights Version 1.2) were used for descriptive and regression analysis, respectively. In order to identify potential confounders to be included in the final sex-stratified analysis, a univariate logistic regression model was used to identify significant risk factors. The covariates with p-values ≤ 0.20 from the univariate analysis were entered into an interim multivariate model. The least significant variable was then removed one at a time until only variables with significant p-values ($p \leq 0.05$) and clinically important factors remained in the model.

To identify sex-specific genetic risk factors, sex-stratified analysis was carried out by survey logistic regression using sex as a domain factor. For the SNPs with an interaction term p-value less than 10^{-5}, we included one SNP at a time and the first four principal components into the final model while controlling for potential environmental, demographic, and socio-economic risk factors.

Three inheritance models (dominant, recessive, and additive) for each variant were considered. The model with the lowest Akaike information criterion values (AIC) was considered the best-fitting model. The results from the best-fitted model were presented.

In order to account for multiple comparisons, the Bonferroni correction was applied in the sex-stratified analyses of asthma-COPD phenotype and COPD based on the number of SNPs with a significant interaction term with the sex variable. The strength of association was reported as odds ratios with 95% confidence intervals.

Results

Characteristics of Study Population

Table 2.1 depicts the characteristics of the study population stratified by sex. A total of 26622 subjects (13,343 males and 13,279 females) represented 3,295,958 participants in the weighted sample (1,670,060 males and 1,625,898 females).

In males and females, the prevalence of asthma-COPD phenotype was 1.4% and 2.3%, respectively. The prevalence of COPD, however, was 2.6 % for males and 3.0% for females.

Males and females differed significantly in the distribution of age, age groups, smoking status, marital status, urban or rural dwelling, homeownership, total household income, total personal income, retirement status, province of recruitment, and highest education status.

Approximately 52.4% of males were smokers (current smokers: 10% and former smokers:42.4%), whereas, in females, 48.6% had a positive smoking history (current smokers: 8.5% and former smokers: 40.1%). In comparison to males, there were more females in the age groups of 55 to 64 (30.2% vs. 29.8%), 65 to 74 (18.3% vs. 15.8%), and 75 and over (11.7% vs. 10.2%). In contrast, more males (44.3%) than females (39.7%) were in the age group 45-54. A significantly higher proportion of females (21.6%) were widowed/divorced/separated than males (9.6%).

Males were more likely than females to have a total household income (49.1% vs. 38.0%) and a total personal income (23.6% vs. 8.2%) of $100,000 or more. In contrast, more females than males earned less than $20,000 (5.6% vs. 3.4%) and less than $50,000 (22.2% vs. 15.1%) for total household income. Similarly, for total personal income, a

higher proportion of females than males earned less than $20,000 (22.9% vs. 7.8%) and less than $50,000 (39.9% vs. 27.9%). Females (38.0%) tended to be retired more than males (29.0%). In contrast, for the highest education status, males (61.4%) were more likely to have post-secondary education at the university level than females (56.8%).

Genome-wide SNP-by-Sex Interaction

In the SNP-by-sex interaction analysis, we examined, after quality control, 416,562 SNPs, 21435 subjects for COPD (cases: n=911, controls: n=20524) and 21028 subjects for the asthma-COPD phenotype (cases: n=504, controls: n=20524) **(Figures 1.1 and 1.2)**.

There were 18 and 28 distinct SNPs from the SNP-by-sex interaction terms with p-values less than 10^{-5} for asthma-COPD phenotype and COPD, respectively. **(Tables 2.2 and 2.3).** No indication of population stratification based on the genomic inflation factor was observed in the genome-wide interaction results for both phenotypes**(Figures 1.3 and 1.4)**.

SNP rs926718, an intronic variant in the ZNF133 gene, had the lowest interaction p-value ($p=2.18 \times 10^{-5}$) for the asthma-COPD phenotype **(Table 2.2).** Regarding COPD outcome, the SNP with the lowest p-value was an intronic variant [rs73838466 ($p=9.35 \times 10^{-6}$)] in the OSTC gene **(Table 2.3)**.

Sex stratified analysis for Asthma-COPD phenotype

Out of the 18 SNPs with a significant SNP-by-sex interaction term, p-values less than 10^{-5} for asthma-COPD phenotype, the polymorphisms rs61140467, which was in high LD (D'=0.99) with rs11799559, was excluded from the analysis due to its lower polymorphic information content. **Table 2.4** demonstrates the results of significant SNPs

from the sex-stratified multivariate analysis of asthma-COPD phenotype after Bonferroni correction ($p \leq 0.003 = 0.05/17$). 7 SNPs were significant in males, and no SNP was significant in females **(Table 2.4 and Tables S2.3 and S2.4)**.

The top significant SNP amongst the males was rs3821479 in the FHIT gene **[OR=0.55, p= <0.0001, 95% CI (0.41 - 0.73)]**. The polymorphisms rs926718 in the ZNF133 gene, which had the lowest SNP-by-sex interaction p-value, was significantly associated with asthma-COPD phenotype amongst the males **[OR=0.58, p= 0.0005, 95% CI (0.43 - 0.79)]**. Howbeit, the effects of these variants were protective **(Table 2.4)**.

As illustrated in **Table 2.4**, SNP **rs11061082** in/near the RIMBP2 gene **[OR=2.15, p= 0.0009, 95% CI (1.37 - 3.37)]** and SNP **rs1884882** near BPIFB1 gene **[OR=2.24, p= 0.0029, 95% CI (1.32 - 3.81)]** under the recessive inheritance model were significantly associated with asthma-COPD phenotype risk in males. While in the additive model, SNPs **rs11799559 [OR=1.56, p= 0.0021, 95% CI (1.18 - 2.07)]** in the SMYD3 gene, **rs1051169 [OR=1.56, p= 0.0019, 95% CI (1.18 - 2.06)]** in S100B gene and rs77800494 **[OR=2.08, p= 0.0025, 95% CI (1.30 - 3.34)]** near ZNF608 gene were significantly associated with asthma-COPD phenotype risk in males.

Sex-stratified analysis for COPD

Out of the 28 SNPs with a significant SNP-by-sex interaction term, p-values less than 10^{-5} for COPD, the polymorphism rs73838466, which was in high LD (D'=0.99) with rs17039240, was excluded from the analysis due to its lower polymorphic information content. **Table 2.5** displays the results of significant SNPs from the sex-stratified multivariate analysis of COPD after Bonferroni correction ($p \leq 0.002 = 0.05/27$).

As illustrated in **Table 2.5, and supplementary tables (S2.1 and S2.2)**, eight variants (rs13326145, rs56334611, rs6816344, rs17039240, rs6935314, rs13225543, rs12869252, and rs6090327) mapped in/near MAGI1, COX18, OSTC, ELOVL5, C7orf72 FGF14, and NKAIN4 genes respectively were significantly associated with COPD in males. Whereas four SNPs (rs12025895, rs10931835, rs220806, and rs77625370) in/near CAMTA1, SATB2, PDE10A, and LINC00908 genes were significantly associated with COPD amongst females. The SNP rs1911770 in gene ZPBP was associated with COPD for both males **[OR=1.64, p= 0.0005, 95% CI (1.24 - 2.16)]** and females **[OR=0.76, p= 0.002, 95% CI (0.64 - 0.91)]**; however, its effect increased COPD risk in males while conferring a protective effects in females.

Out of the eight male-specific SNPs, an intergenic SNP rs17039240 near the OSTC gene was significantly associated with COPD risk in males **[OR= 2.48, p= < 0.0001, 95% CI (1.62 - 3.79)]** but not in females. The top female-specific SNP was rs12025895 in the CAMTA1 gene **[OR=0.72, p= <0.0001, 95% CI (0.61 - 0.84)]**. All the variants significantly associated with COPD amongst females had protective effects, with ORs ranging from 0.45 to 0.76.

Discussion

This study found 18 and 28 distinct signals for a genome-wide SNP-by-Sex interaction on asthma-COPD phenotype and COPD outcomes, respectively. The SNPs with the lowest SNP-by-Sex interaction p-values for asthma-COPD phenotype and COPD outcomes were found in the intronic regions of the ZNF133 and OTSC genes at 20p11.23 and 4q25 cytogenetic positions, respectively.

We found seven male-specific variants in or adjacent SMYD3, FHIT, ZNF608, RIMBP2, ZNF133, BPIFB1, and S100B genes that were significantly associated with asthma-COPD phenotype. Five of the seven SNPs (rs11799559, rs77800494, rs11061082, rs1884882, and rs1051169) were associated with an increased risk, with ORs ranging from 1.56 to 2.24, while two other SNPs (rs3821479 and rs926718) showed protective effects, with OR of 0.55 and 0.58.

A total of eight male-specific variants (rs13326145, rs56334611, rs6816344, rs17039240, rs6935314, rs13225543, rs12869252, and rs6090327) in/near the MAGI1, COX18, OSTC, ELOVL5, C7orf72 FGF14, and NKAIN4 genes, and 4 female-specific variants (rs12025895, rs10931835, rs220806 and rs77625370) in/near the CAMTA1, SATB2, PDE10A, and LINC00908 genes were significantly associated with COPD. One SNP, rs1911770 in the ZPBP gene, was significantly associated with an increased risk of COPD in males and a reduced risk of COPD in females.

To the best of our knowledge, this is the first study to look at sex-specific genetic risk factors for asthma-COPD phenotype and COPD while also considering environmental, lifestyle, socio-economic, and socio-demographic factors. Previous genetic and gene-based association studies have only demonstrated sex-specific genetic effects on COPD and asthma.[35, 36, 42] However, the influence of socio-demographic and socio-economic factors was not considered in these studies.

In this study, the strongest male-specific associations for asthma-COPD phenotype risk were from SNPs (rs1884882, rs11061082 rs77800494, rs11799559, rs1051169) in/near BPIFB1, RIMBP2, ZNF608, SMYD3, and S100B genes. The BPIFB1 gene on

chromosome 20q11.21 encodes a protein secreted by goblet cells in the airway epithelium, trachea, submucosal glands of airways, and nasal cavities.[43] It is thought to be involved in innate immunity against inhaled toxins and pathogens. BPIFBI is upregulated in several respiratory diseases. For example, after a segmental allergen challenge, higher levels of BPIFB1 were found in bronchoalveolar lavage fluid in asthmatic patients.[44] In addition, BPIFB1 levels in COPD smokers' sputum were significantly higher than in smokers and non-smokers without COPD.[45] De Smet and Colleagues[46] observed that the mRNA expression levels of BFIFB1 amongst COPD subjects were positively correlated with disease severity and that smokers with COPD had higher BPIFB1 mRNA and protein expression levels in lung tissue and airway epithelium than non-smokers and smokers without COPD. BPIFB1 levels have also been found to be significantly inversely correlated to FEV_1% predicted, FEV_1 /FVC ratio, and diffusing capacity of the lung for carbon monoxide (DLCO), all of which are proxies for COPD disease severity and emphysema.[45, 46] In addition, human and animal studies have demonstrated that males are more prone to emphysematous changes than females.[28, 47] Polymorphisms of RIMBP2, FHIT, and ZNF608 genes on 12q24.33, 3p14.2, and 5q23.2 cytogenetic positions have been associated with testosterone levels.[48] Several studies have suggested the influence of sex hormones on lung diseases and inflammatory responses of the lungs to pathogens and inhaled toxins, including cigarette smoke and pollutants.[49, 50] Androgen (testosterone) is thought to have anti-inflammatory effects, which are mediated by interaction with androgen receptors (AR) and control the expression of transcriptions.[51] For example, one study found that testosterone reduced pulmonary epithelial inflammation in rats with COPD.[52] As

androgens declines with advancing age, the binding of androgen and AR complexes to transcriptions might be attenuated. These might lead to altered expressions of genes, increased pro-inflammatory cytokines, and chronic inflammatory diseases.

The S100B gene on chromosome 21q22.3 belongs to the S100 family of proteins, which regulates calcium balance, cell apoptosis, migration, proliferation, differentiation, energy metabolism, and inflammation. Studies have shown that S100B is one of the major ligands of the receptor for advanced glycation end products (RAGE), a pattern recognition receptor, highly expressed in alveolar type I epithelial cells, bronchiolar epithelium, alveolar type II epithelial cells, and alveolar macrophages.[53, 54] S100 protein interaction with RAGE activates NF-κB, causing the production of pro-inflammatory cytokines and the migration of neutrophils, monocytes, and macrophages.[55] For instance, In an in-vitro study, S100B was shown to stimulate the secretion of TNF-alpha and IL-6 in Alveolar Type-I (AT-I) derived cells from the pulmonary tissue of male fetuses of Han-Wistar rats.[56] The SMYD3 gene, a protein-coding gene known for its histone methyltransferase activity, is more expressed in males' dorsolateral frontal cortex and anterior cingulate cortex of the brain than in females.[57] Other histone-encoding genes have been shown to be more expressed in males than females in the heart, kidney, and colon.[57]

This study also identified the strongest male-specific SNP (rs17039240) for COPD. This SNP is an intergenic variant near the OSTC gene. OSTC plays a critical role in the generation and processing of amyloid-beta peptides (Aβ) from the amyloid precursor protein (APP).[58] APP and Aβ have not been adequately explored in lung diseases; however, studies have shown that APP and Aβ from human monocyte-derived

macrophages regulate pro-inflammatory and anti-inflammatory mediators.[59] Increased levels of amyloid-beta peptides have been observed in the serum and lungs of COPD patients compared to controls. In addition, higher serum Aβ negatively correlated with worse lung function in COPD patients.[60, 61] Studies have shown that androgens regulate amyloid beta-peptides.[62–64] Gillett et al. [62] demonstrated that lower androgen levels were associated with increased plasma amyloid beta-peptide in older men with dementia. Furthermore, low total testosterone has been associated with worse pulmonary function in men with COPD.[65] Given that a significant proportion of COPD patients are middle-aged and older men, the age-related decline in androgens (low testosterone) in males, as well as the proteolytic action of OSTC protein on amyloid precursor protein, may result in elevated levels of amyloid beta-peptide and might be indicative of the role of OSTC gene polymorphisms in increasing COPD risk in males.

We also observed intronic and intergenic SNPs (rs1911770 and rs13225543) in/near ZPBP and C7orf72 genes associated with COPD in males. ZPBP, a Zona Pellucida Binding Protein implicated in adult fertility, is expressed in the testis and ovary.[66, 67] This intronic SNP (rs1911770) in the ZPBP gene had opposite effect on the risk of COPD in males and females (increasing COPD risk for males but protective for females). In a previous GWAS, variants in/near the ZPBP gene approached genome-wide significance for an association with pulmonary function amongst smokers (FEV_1 and FEV_1/FVC ratio).[68] Furthermore, this locus (ZPBP) is an important paralog to a locus on chromosome 17q21.1 that contains ZPBP2. ZPBP2 has been implicated in various studies as one of the genes associated with asthma and childhood asthma.[69–73] Naumova et al. [74] found sex-specific differences in the DNA méthylation of the ZPBP2

gene between males and females in relation to asthma susceptibility. In that study, males had lower average methylation than females in the ZPBP2 gene promoter region, suggesting that hypo-methylation of the ZPBP2 gene increases asthma risk in males. Furthermore, ZPBP/ZPBP2 deletion in a mouse model produces sperm abnormalities and infertility in men but not females.[66] The intergenic SNP (rs13225543) near the C7orf72 gene was found to be male-specific. C7orf72 gene, a spermatogenesis-associated protein, has been linked to spermatogenesis.[75] In male hamsters, pulmonary emphysema has been shown to influence the spermatogenesis mechanism and reproductive organs' morphophysiological changes through oxidative stress and hormonal imbalance.[76] This suggests that polymorphisms or mutations in ZPBP/ZPBP2 and C7orf72 could have a greater impact on males regarding disease development.

Other male-specific associations, albeit protective in the direction of effect, were found within or near the MAGI1, COX18, ELOVL5, FGF14, and NKAIN4 genes in the 3p14.1, 4q13.3, 6p12.1, 13q33.1, and 20q13.33 cytogenetic bands, respectively. Variants of the FGF14 gene, which belongs to the fibroblast growth factor family, have been associated with post-BD FEV1 in children with asthma.[77] Members of the FGF family have been associated with lung development and respiratory disease.[78, 79] For instance, polymorphisms in the FGF7 gene have been reported to be associated with COPD.[78] FGFs 1,2,8,9 and 10 have been implicated in various levels of lung development.[79] Interestingly, FGF10 expression was higher in males than females in a study that looked at the expression profile of androgen-regulated genes in murine fetal developing lungs.[80] In our study, an SNP (rs12869252) in FGF14 was significantly associated with

COPD in males and may, in combination with sex hormones, potentially play a sexually dimorphic role in COPD susceptibility.

MAGI1 is widely expressed in lung epithelial cells, where it functions as a scaffolding protein at intercellular junctions and maintains epithelial barrier function.[81] The airway epithelial lining serves as the first line of defense against environmental insults such as cigarette smoke. MAGI1 gene has been implicated as a surfactant regulator with increased expression in the fetal lung of males compared to females.[80] Cigarette smoke, a significant risk factor for COPD, adversely affects surfactants and airway epithelial architecture.[82–84] In one study, the expression of the MAGI1 gene in the airway epithelium was significantly downregulated in smokers with COPD and healthy smokers compared to non-smokers.[84] This suggests that cigarette smoke compromises the integrity of airway epithelial cell-cell junction. With males and females having differential susceptibility to cigarette smoke, the distribution and population of MAGI1 proteins in airway epithelial cells' tight junctions may play an important role in COPD pathogenesis in males and females.

SNPs (rs56334611 and rs6816344) near the COX18 gene, which encodes a cytochrome c oxidase assembly protein involved in mitochondrial biogenesis, MT-CO2/COX2 maturation, and regulation of mitochondrial respiratory chain complex IV, were found to be significantly associated with COPD in males. Oxidative stress due to excessive reactive oxygen species in COPD patients has been linked to mitochondrial damage, reduced mitochondrial biogenesis, and mitochondrial homeostasis.[85, 86] Sex disparities in oxidative stress and reactive oxygen species generation have been reported, with males having more oxidative stress, more reactive oxygen species, and lower antioxidant

capacity than females.[87] ELOVL5 gene, widely expressed in the brain, lung, testis, adrenal gland, and prostate, also regulates mitochondrial functions and reactive oxygen species production.[88, 89] ELOVL5 has also been found to be overexpressed in prostate cancer. For instance, one experimental study found that ELOVL5 was significantly more expressed in prostate cancer cells than in normal/benign prostatic hyperplasia cells and that this upregulation was mediated via androgen receptors.[88]

Variants (rs12025895, rs10931835, rs220806 and rs77625370) within/near the CAMTA1 SATB2 PDE10A and LINC00908 genes on chromosomes 1p36.31, 2q33.1, 6q27, and 18q23, respectively, showed female-specific associations for COPD affection status. In a large GWAS of lung function using the UK biobank data, SNPs in SATB2 have been associated with an increase in FEV_1 and FVC.[32] In an experimental study using ovariectomized rats, Wu et al. [90] demonstrated that the bone marrow stromal cells (BMSCs) of ovariectomized rats experienced weaker SATB2 expression, reduced bone formation capacity, and increased senescence. On the other hand, estrogen increased SATB2 expression, slowed down cellular aging, and increased the osteogenicity of bone marrow stromal cells. Estrogen deficiency has been associated with osteoporosis during post-menopause.[91] Osteoporosis is a major comorbid condition in females with COPD.[92] It is probable that the SATB2 gene's expression might decrease with the decline of estrogen in menopausal and post-menopausal females with COPD, which might lead to lung function decline.

CAMTA1 gene, another female-specific association with COPD in our study, has been associated with lung function and COPD.[93, 94] Kang and colleagues suggested that CAMTA1 gene plays a regulatory role in the nuclear factor of activated T cells

pathway.[95] The nuclear factor of activated T cells (NFAT), identified in activated T-cells, regulates the expression of IL-2, IL-4, and IL-5.[96, 97] T-cells play a central role in adaptive immune response. Also, Innate and adaptive immune responses differ between sexes. Females have been reported to have a greater number of activated T-cells and T-cell proliferation than males.[98] Studies have shown that the increased number of T cells in the lungs and airways of patients with COPD correlates with disease severity,[99] and that inflammatory response in the airways is higher in female smokers than in male smokers.[27] These suggest that the CAMTA1 gene may modulate inflammatory mechanisms differently for males and females with COPD.

An SNP (rs220806) in the PDE10A gene was one of the female-specific loci for COPD affection status. This gene's protein belongs to the cyclic nucleotide phosphodiesterase family (PDEs), which plays an important role in controlling intracellular cyclic nucleotide by hydrolyzing cAMP and cGMP second messengers involved in regulating airway smooth muscle function.[100] PDE10A plays an essential role in lung inflammation by promoting macrophage activation and neutrophil infiltration.[101] PDE10A knockout mice exhibited reduced IL-1b, MCP-1, IL-6, and TNF-alpha protein levels in lung tissues than in PDE010-WT mice after exposure to lipopolysaccharide.[101] Sexual dimorphism in the PDE10A gene has been demonstrated in an experimental animal study. PDE10A knockout mice were confirmed to have decreased body weight compared to their wild-type counterparts, with females being more affected than males.[102]

In our study, the identified sex-specific loci associated with asthma-COPD phenotype and COPD may have direct or indirect sexually dimorphic roles suggesting that the complex

interplay between these sex-specific gene signatures and sex hormones or lifestyle factors, such as cigarette smoking, may influence the varying expression and pathobiological functions of these genes in males and females susceptibility to asthma-COPD phenotype and COPD.

Our study had some limitations. The identification of COPD and asthma-COPD phenotype cases as self-reported physician-diagnosed COPD and concomitant diagnosis of COPD and asthma without objective spirometry measurements are subject to misclassification. However, self-reported obstructive airway disease diagnosed by physicians has been widely used by large GWAS and population-based studies to identify genetic and clinical characteristics.[11, 12, 71, 103–105] The sample size for our study was moderately large. However, it appeared relatively underpowered to identify variants (rare or common) with interaction p-values significant at the genome-wide significance threshold. Genome-wide interaction studies, in general, necessitate a much larger sample size and more statistical power than standard GWAS. Due to the high proportion of missing values in the spirometry variables, we decided not to use spirometry criteria to define our outcomes. Our study lacked an independent replication cohort to validate our findings. Future replication of our findings in subsequent studies could improve generalizability.

Conclusion

Our study identified novel sex-specific loci associated with asthma-COPD phenotype and COPD. These findings are potential precursors to deepening our understanding of sex-related genetic differences in asthma-COPD phenotype and COPD pathology. Future

research identifying these loci's general and functional sex-specific roles would improve disease endotyping in individuals with asthma-COPD phenotype and COPD.

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Table 2.1. Descriptive characteristics of the study population

Variables	Groups	Males (%) (n=13,343)	Females (%) (n= 13,279)	p-value
Asthma+COPD	Yes	1.4	2.3	<.0001
COPD	Yes	2.6	3.0	<.0001
Age, mean ± SEM		59.1 ± 0.1	59.9 ± 0.1	<.0001
Age group	45 to 54 55 to 64 65 to 74 75 and above	44.3 29.8 15.8 10.2	39.7 30.2 18.3 11.7	<.0001
Smoking Status	Current Smoker Never Smoker Former Smoker	10.0 47.6 42.4	8.5 51.4 40.1	<.0001
Marital status	Single/never married Married/Living in common law Widowed/Separated/Divorced	8.1 82.3 9.6	8.2 70.1 21.6	<.0001
Urban or Rural Dwelling	Urban core Rural/not Urban core	86.6 13.4	85.0 15.0	0.0028
Homeownership	Owned Rented/Others	86.6 13.2	84.9 15.0	0.0029
Total household income	Less than $20,000 $20,000 to less than $50,000 $50,000 to less than $100,000 $100,000 or more	3.4 15.1 32.5 49.1	5.6 22.2 34.2 38.0	<.0001
Total personal income	Less than $20,000 $20,000 to less than $50,000 $50,000 to less than $100,000 $100,000 or more	7.8 27.9 40.7 23.6	22.9 39.9 28.9 8.2	<.0001
Retirement Status	Retired completely Not retired/partly retired	29.0 71.0	38.0 62.0	<.0001
Province of recruitment	Prairies British Columbia Eastern province[a] Ontario Quebec	22.2 28.9 5.2 13.5 30.2	19.1 30.0 6.2 13.6 31.1	<.0001
Highest Education Status	No post-secondary education Non-university post-secondary education	7.4 31.4	8.4 34.8	<.0001

	University post-secondary education	61.4	56.8	

Number of participants in the unweighted sample: 26622 (13,343 males and 13,279 females) represent 3,295,958 participants (1,670,060 males and 1,625,898 females) in the weighted sample
[a]: Newfoundland and Labrador, Nova Scotia; COPD: Chronic obstructive pulmonary disease; Asthma+COPD: Asthma-COPD overlap; SEM: Standard error of the mean

Table 2.2. 18 signals of the SNP-by-sex interactions with p-value less than 10^{-5} for asthma-COPD phenotype

Chr	SNP	A1/ A2	MAF	OR (95% CI)	Interaction p-value	Gene/ Nearest Gene	Consequence
1	rs619928	A/G	0.387	0.57 (0.43, 0.75)	5.74E-05	SPSB1	Intron variant
1	rs6666629	T/C	0.061	2.77 (1.67, 4.60)	8.47E-05	HSD52	Intron variant
1	rs12062324	G/A	0.059	2.99 (1.73, 5.16)	8.93E-05	RPS6KC1	Intergenic variant
1	rs61140467*	A/G	0.285	1.76 (1.33, 2.35)	9.78E-05	SMYD3	Intron variant
1	rs11799559*	A/G	0.458	1.68 (1.29, 2.17)	8.55E-05	SMYD3	Intron variant
2	rs77602394	G/A	0.064	2.96 (1.76, 4.97)	3.99E-05	RAD51AP2	Intergenic variant
3	rs3821479	C/T	0.430	0.57 (0.43, 0.74)	4.51E-05	FHIT	Intron variant
4	rs72901492	A/C	0.107	0.40 (0.26, 0.62)	3.83E-05	NDST4	Intergenic variant
5	rs77800494	T/G	0.043	3.84 (1.98, 7.43)	6.73E-05	ZNF608	Intergenic variant
7	rs7782108	C/T	0.190	0.48 (0.33, 0.69)	8.00E-05	CARD11	Intron variant
7	rs34331963	C/T	0.159	2.05 (1.45, 2.89)	4.52E-05	SDK1	Intergenic variant
8	rs6986253	A/G	0.254	1.81 (1.34, 2.43)	9.60E-05	LRP12	Intron variant
12	rs11061082	A/G	0.399	1.71 (1.32, 2.22)	6.16E-05	RIMBP2	Intron variant
14	rs2582577	T/C	0.199	1.88 (1.38, 2.57)	7.47E-05	C14orf79	Intergenic variant
20	rs926718	A/G	0.374	0.55 (0.41, 0.72)	2.18E-05	ZNF133	Intron variant
20	rs1884882	C/T	0.282	1.82 (1.38, 2.40)	2.66E-05	BPIFB1	Intergenic variant
20	rs6013630	T/C	0.122	2.34 (1.56, 3.51)	4.26E-05	TSHZ2	Intron variant
21	rs1051169	C/G	0.329	1.72 (1.31, 2.26)	9.53E-05	S100B	Synonymous variant

SNP-by-Sex interaction GWAS on asthma-COPD phenotype affection status controlling for age, sex, smoking status, and top 4 principal components

*: SNP **rs11799559** was in high linkage disequilibrium (D'=0.99) with **rs61140467**
Chr: Chromosome; SNP: Single Nucleotide Polymorphism; MAF: Minor allele frequency; OR: Odds ratio; A1/A2: Minor allele/Major allele

Genes contain variants or are located within 500kb of variants

Table 2.3. 28 signals of the SNP-by-sex interactions with p-value less than 10^{-5} for COPD

Chr	SNP	A1/A2	MAF	OR (95% CI)	Interaction p-value	Gene / Nearest Gene	Consequence
1	rs12025895	C/T	0.44	1.48 (1.22, 1.79)	6.74E-05	CAMTA1	Intron variant
1	rs6429144	T/C	0.24	1.63 (1.29, 2.04)	3.17E-05	CHRM3	Intron variant
2	rs10931835	C/T	0.43	1.52 (1.25, 1.86)	3.01E-05	SATB2	Intergenic variant
2	rs7557339	T/C	0.39	0.65 (0.53, 0.79)	1.85E-05	SLC19A3	Intergenic variant
3	rs13326145	T/C	0.05	0.32 (0.19, 0.55)	3.86E-05	MAGI1	Intron variant
3	rs12633737	A/G	0.31	1.57 (1.28, 1.93)	2.00E-05	ALCAM	Intergenic variant
4	rs56334611	G/A	0.27	0.63 (0.51, 0.78)	3.69E-05	COX18	Intergenic variant
4	rs6816344	T/A	0.28	0.62 (0.49, 0.77)	1.65E-05	COX18	Intergenic variant
4	rs73838466*	T/C	0.15	1.86 (1.41, 2.44)	9.35E-06	OSTC	Intron variant
4	rs17039240*	T/C	0.26	1.57 (1.26, 1.96)	5.84E-05	OSTC	Intergenic variant
4	rs11947441	C/T	0.03	0.19 (0.08, 0.44)	9.65E-05	FRG1	Intergenic variant
5	rs76189059	A/G	0.06	0.38 (0.23, 0.61)	8.89E-05	NA	Intergenic variant
6	rs6935314	C/T	0.33	0.64 (0.52, 0.79)	3.93E-05	ELOVL5	Intergenic variant
6	rs117896837	T/C	0.02	4.80 (2.19, 10.54)	9.10E-05	FAM46A	Intergenic variant
6	rs220806	T/C	0.31	1.58 (1.28, 1.95)	2.78E-05	PDE10A	Intron variant
7	rs59794319	G/A	0.08	0.48 (0.33, 0.69)	9.09E-05	NXPH1	Intergenic variant
7	rs10488084	C/A	0.08	0.48 (0.33, 0.69)	9.95E-05	PLEKHA8	Intron variant
7	rs1911770	T/C	0.43	1.51 (1.24, 1.83)	4.81E-05	ZPBP	Intron variant
7	rs13225543	T/C	0.41	0.67 (0.55, 0.81)	5.48E-05	C7orf72	Intergenic variant
8	rs112593560	T/C	0.03	3.69 (1.93, 7.07)	8.14E-05	KCNQ3	Intron variant
9	rs4076140	G/A	0.08	2.18 (1.51, 3.16)	3.39E-05	TRPM3	Intron variant
9	rs74964803	G/T	0.05	0.39 (0.24, 0.62)	7.95E-05	MED27	Intergenic

							variant
13	rs66468120	A/G	0.05	2.64 (1.65, 4.22)	5.02E-05	RNF219-AS1	Intron variant
13	rs12869252	G/A	0.24	0.64 (0.51, 0.80)	9.73E-05	FGF14	Intron variant
14	rs12879329	G/A	0.07	2.24 (1.53, 3.29)	3.85E-05	NPAS3	Intron variant
18	rs77625370	T/C	0.05	2.75 (1.67, 4.53)	7.03E-05	LINC00908	Intergenic variant
20	rs1891778	G/A	0.48	1.52 (1.25, 1.84)	2.60E-05	CDH4	Intron variant
20	rs6090327	C/T	0.17	0.59 (0.45, 0.77)	8.39E-05	NKAIN4	3 prime UTR variant

SNP-by-Sex Interaction GWAS on COPD affection status controlling for Age, Sex, smoking status, and top 4 principal components

* : SNP rs17039240 was in high linkage disequilibrium (D'=0.99) with rs73838466,

Chr: Chromosome; SNP: Single Nucleotide Polymorphism; MAF: Minor allele frequency;

OR: Odds ratio; NA: No annotation; A1/A2: Minor allele/Major allele

Genes contain variants or are located within 500kb of variants

Table 2.4. Result of sex-stratified analysis for asthma-COPD phenotype

Chr	**Gene /Nearest Gene**	**SNP (A1/A2)**	**Male**		**Female**	
			OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
1	SMYD3	rs11799559 (A/G)†	1.56 (1.18 - 2.07)[A]	0.0021*	PM: 0.69 (0.50 - 0.94)[D] WT**:** 1	0.0185
3	FHIT	rs3821479 (C/T)	0.55 (0.41 - 0.73)[A]	<0.0001*	PM: 0.79 (0.57 - 1.08)[D] WT: 1	0.141
5	ZNF608	rs77800494 (T/G)	2.08 (1.30 - 3.34)[A]	0.0025*	0.42 (0.20 - 0.86)[A]	0.0178
12	RIMBP2	rs11061082 (A/G)	PM: 2.15 (1.37 - 3.37)[R] WT: 1	0.0009*	PM: 0.75 (0.48 - 1.16)[R] WT: 1	0.199
20	ZNF133	rs926718 (A/G)	0.58 (0.43 - 0.79)[A]	0.0005*	1.32 (1.07 - 1.64)[A]	0.0101

20	BPIFB1	rs1884882 (C/T)	PM: 2.24 (1.32 - 3.81)[R] WT: 1	0.0029*	0.93 (0.73 - 1.20)[A]	0.5823
21	S100B	rs1051169 (C/G)	1.56 (1.18 - 2.06)[A]	0.0019*	PM: 0.74 (0.55 - 1.01)[D] WT: 1	0.0562

Sex-stratified multivariate logistic regression of top interaction single nucleotide polymorphisms for asthma-COPD phenotype affection status, adjusting for age, smoking status, marital status, dwelling ownership/ rent status, total household income, education level, retirement status, and first four principal components

†: SNP **rs11799559** was in high linkage disequilibrium (D'=0.99) with **rs61140467;** hence **rs61140467** was removed from sex-stratified analysis due to lower polymorphic information content (PIC)
*: statistically significant after adjusting for multiple comparisons ($p \leq 0.003$)
[A]: Additive; [D]: Dominant; [R]: Recessive; PM: Polymorphism; WT: Wild Type; Chr: Chromosome;
SNP: Single Nucleotide Polymorphism; A1/A2: Minor allele/Major allele; OR: Odds ratio; CI: Confidence Interval;
Genes contain variants or are located within 500kb of variants

Table 2.5. Result of sex-stratified analysis for COPD

Chr	Gene /Nearest Gene	SNP (A1/A2)	Male		Female	
			OR (95% CI)	p-value	OR (95% CI)	p-value
1	CAMTA1	rs12025895 (C/T)	PM: 1.29 (0.99 - 1.68)[D] WT: 1	0.0622	0.72 (0.61 - 0.84)[A]	<0.0001*
2	SATB2	rs10931835 (C/T)	PM: 1.20 (0.92 - 1.57)[D] WT: 1	0.1761	PM: 0.64 (0.50 - 0.81)[D] WT: 1	0.0002*
3	MAGI1	rs13326145 (T/C)	0.38 (0.22 - 0.65)[A]	0.0005*	PM: 0.61 (0.08 - 4.58)[R] WT: 1	0.6267
4	COX18	rs56334611 (G/A)	0.69 (0.57 - 0.84)[A]	0.0002*	PM: 1.37 (1.09 - 1.73)[D] WT: 1	0.0073
4	COX18	rs6816344 (T/A)	0.71 (0.58 - 0.85)[A]	0.0003*	1.26 (1.04 - 1.52)[A]	0.0176

4	OSTC	rs17039240 (T/C)†	PM: 2.48 (1.62 - 3.79)^{R} WT: 1	<0.0001*	0.83 (0.69 - 1.01)^{A}	0.0571
6	ELOVL5	rs6935314 (C/T)	0.74 (0.61 - 0.89)^{A}	0.0017*	PM: 1.30 (1.03 - 1.65)^{D} WT: 1	0.0276
6	PDE10A	rs220806 (T/C)	PM: 1.19 (0.93 - 1.51)^{D} WT: 1	0.1624	0.70 (0.58 - 0.84)^{A}	0.0001*
7	ZPBP	rs1911770 (T/C)‡	PM: 1.64 (1.24 - 2.16)^{R} WT: 1	0.0005*	0.76 (0.64 - 0.91)^{A}	0.002*
7	C7orf72	rs13225543 (T/C)	PM: 0.65 (0.51 - 0.83)^{D} WT: 1	0.0005*	PM: 1.36 (1.05 - 1.77)^{D} WT: 1	0.0196
13	FGF14	rs12869252 (G/A)	PM: 0.68 (0.53 - 0.87)^{D} WT: 1	0.002*	1.24 (0.99 - 1.56)^{A}	0.0194
18	LINC00908	rs77625370 (T/C)	1.61 (1.12 - 2.30)^{A}	0.0094	0.45 (0.28 - 0.74)^{A}	0.0014*
20	NKAIN4	rs6090327 (C/T)	0.65 (0.51 - 0.84)^{A}	0.001*	1.38 (1.11 - 1.71)^{A}	0.0038

Sex-stratified multivariate survey logistic regression of top interaction single nucleotide polymorphisms for COPD affection status, adjusting for age, smoking status, province of recruitment, marital status, homeownership, total personal income, retirement status, and first four principal components.

†: SNP **rs17039240** was in high linkage disequilibrium (D'=0.99) with **rs73838466;** hence **rs73838466** was removed from sex-stratified analysis due to lower polymorphic information content (PIC)

*: statistically significant after adjusting for multiple comparisons ($p \leq 0.002$)

‡: significant in both males and females

^{A}: Additive; ^{D}: Dominant; ^{R}: Recessive; PM: Polymorphism; WT: Wild Type; Chr: Chromosome; SNP: Single Nucleotide Polymorphism; A1/A2: Minor allele/Major allele; OR: Odds ratio; CI: Confidence Interval;

Genes contain variants or are located within 500kb of variants

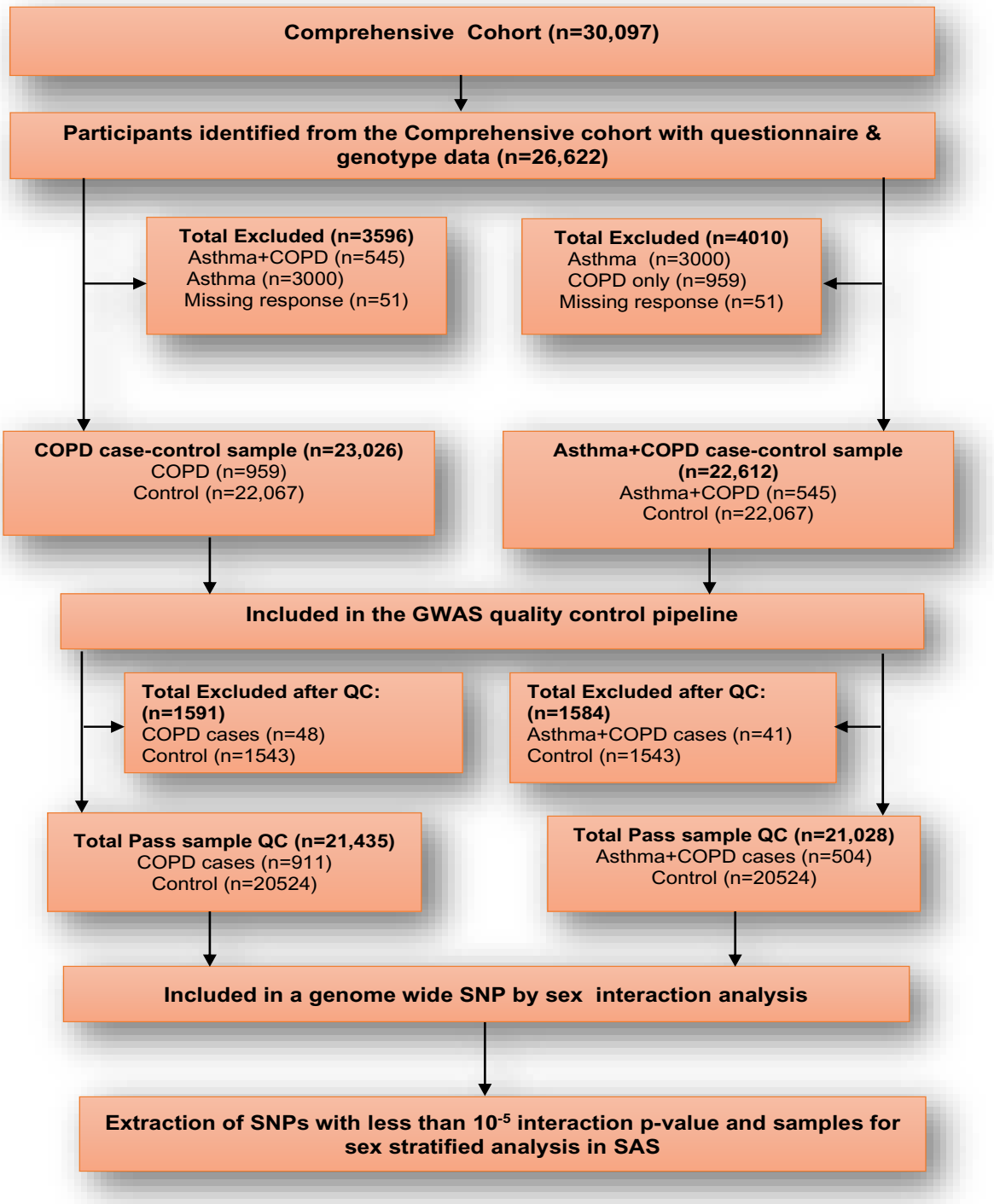

Figure 1.1 Study flowchart

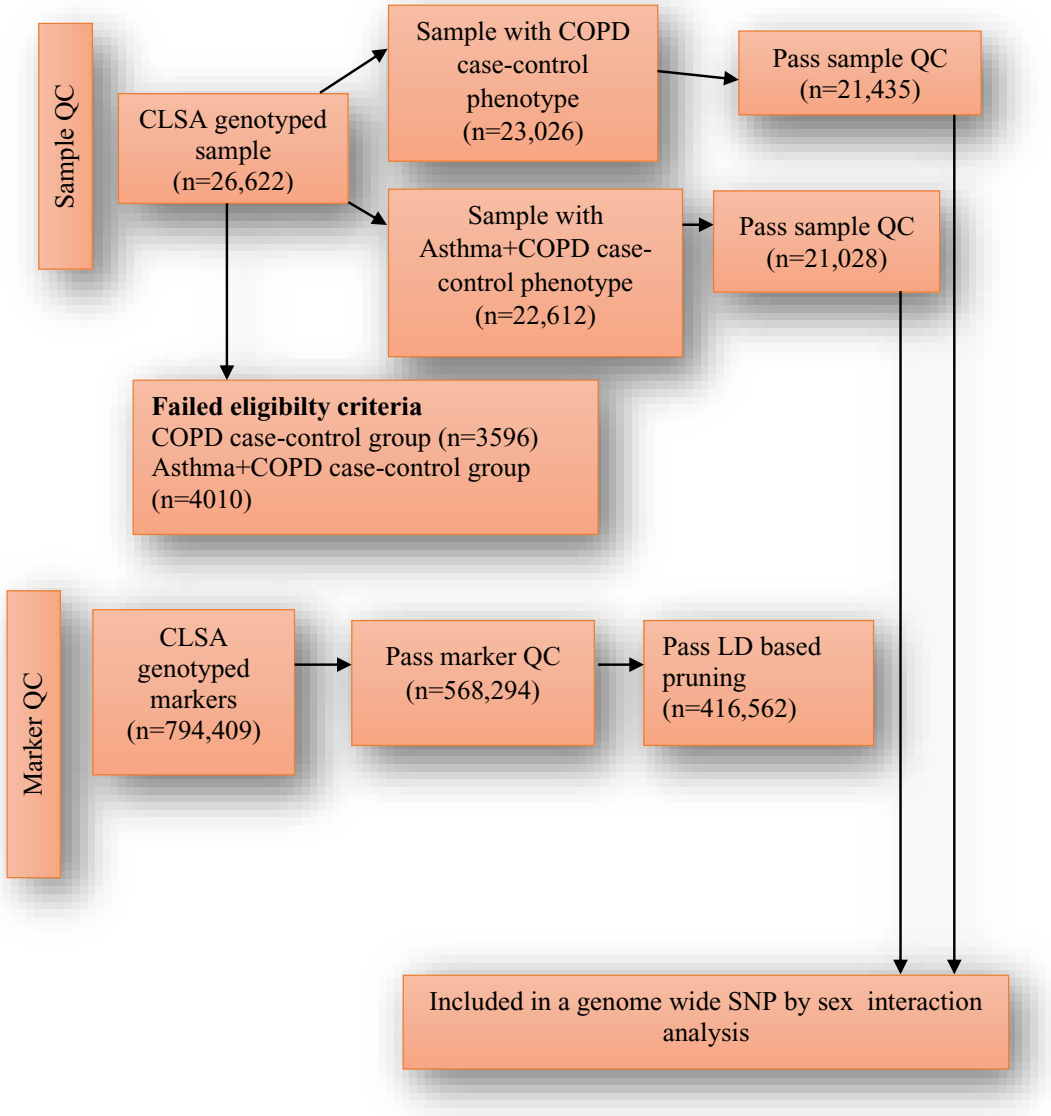

Figure 1.2. Flow diagram of sample and marker quality control for genome-wide SNP-by-sex interaction of asthma-COPD phenotype and COPD

SNP-by-sex interaction testing Q-Q plot for asthma-COPD phenotype

λ=1.00

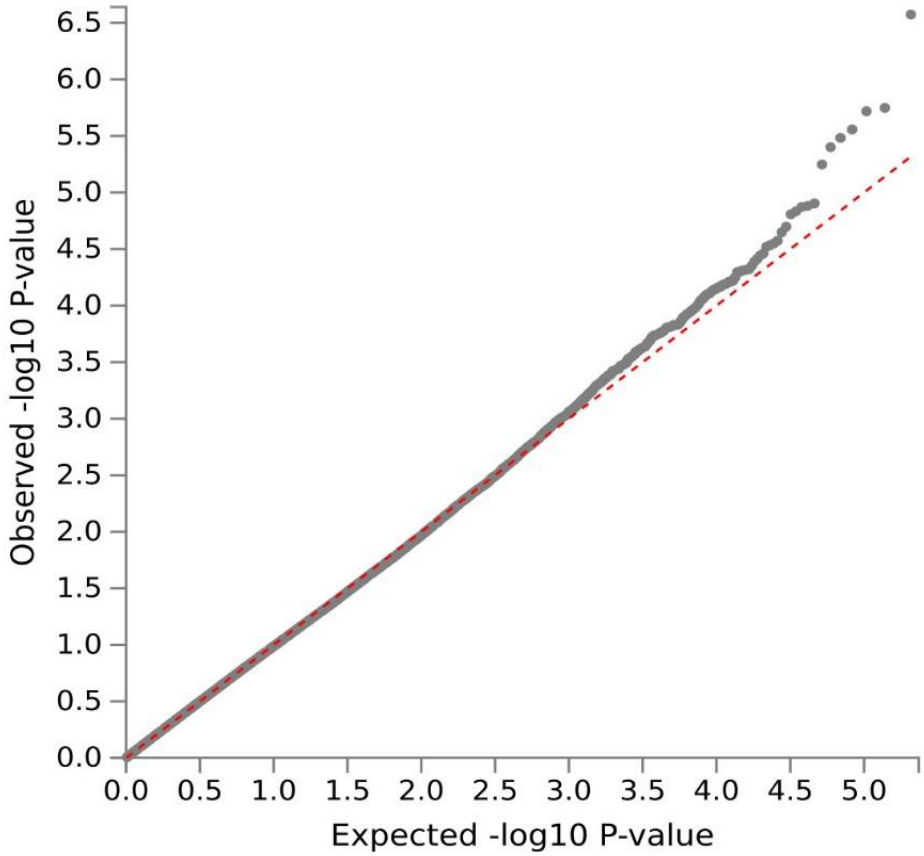

Figure 1.3. Quantile-Quantile plot for genome-wide SNP-by-sex interaction analysis for asthma-COPD phenotype showing the distribution of observed vs. expected p-values

SNP-by-sex interaction testing Q-Q plot for COPD

λ=1.00

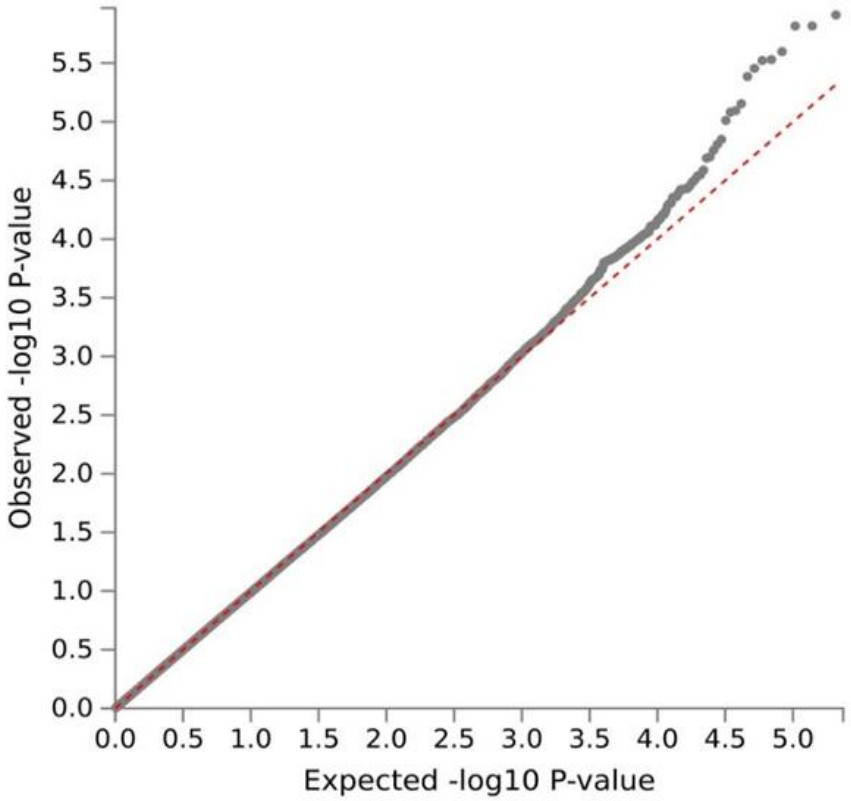

Figure 1.4. Quantile-Quantile plot for genome-wide SNP-by-sex interaction analysis for COPD showing the distribution of observed vs. expected p-values

Table S2.1. Result of sex-stratified analysis of 27 polymorphisms with interaction p-value less than 10^{-5} for COPD outcome amongst males

Males					
CHR	SNP	Gene/Nearest Gene	Best Genetic Model	OR(95% CI)	p-value
1	rs12025895	CAMTA1	Dominant	1.288 (0.987 - 1.680)	0.0622
1	rs6429144	CHRM3	Recessive	1.592 (1.054 - 2.406)	0.0271
2	rs10931835	SATB2	Dominant	1.203 (0.920 - 1.573)	0.1761
2	rs7557339	SLC19A3	Dominant	0.707 (0.555 - 0.901)	0.0051
3	**rs13326145**	**MAGI1**	**Additive**	**0.378 (0.219 - 0.651)**	**0.0005**
3	rs12633737	ALCAM	Recessive	1.660 (1.177 - 2.341)	0.0038
4	**rs56334611**	**COX18**	**Additive**	**0.693 (0.570 - 0.841)**	**0.0002**
4	**rs6816344**	**COX18**	**Additive**	**0.705 (0.584 - 0.852)**	**0.0003**
4	**rs17039240**	**OSTC**	**Recessive**	**2.479 (1.623 - 3.786)**	**<0.0001**
4	rs11947441	FRG1	Additive	0.337 (0.111 - 1.020)	0.0543
5	rs76189059	NA	Additive	0.537 (0.314 - 0.919)	0.0232
6	**rs6935314**	**ELOVL5**	**Additive**	**0.737 (0.609 - 0.891)**	**0.0017**
6	rs117896837	FAM46A	Additive	2.020 (1.257 - 3.246)	0.0037
6	rs220806	PDE10A	Dominant	1.188 (0.933 - 1.512)	0.1624
7	rs59794319	NXPH1	Dominant	0.675 (0.467 - 0.974)	0.0356
7	rs10488084	PLEKHA8	Dominant	0.801 (0.551 - 1.166)	0.2468
7	**rs1911770**	**ZPBP**	**Recessive**	**1.640 (1.243 - 2.164)**	**0.0005**
7	**rs13225543**	**C7orf72**	**Dominant**	**0.648 (0.507 - 0.828)**	**0.0005**
8	rs112593560	KCNQ3	Recessive	20.490 (1.753 - 239.5)	0.0161
9	rs4076140	TRPM3	Dominant	1.342 (0.987 - 1.827)	0.061
9	rs74964803	MED27	Dominant	0.581 (0.344 - 0.980)	0.0418
13	rs66468120	RNF219-AS1	Additive	1.624 (1.167 - 2.261)	0.004
13	**rs12869252**	**FGF14**	**Dominant**	**0.675 (0.526 - 0.866)**	**0.002**
14	rs12879329	NPAS3	Recessive	4.277 (1.621 - 11.282)	0.0033
18	rs77625370	LINC00908	Additive	1.606 (1.123 - 2.296)	0.0094
20	rs1891778	CDH4	Additive	1.206 (1.019 - 1.427)	0.0289
20	**rs6090327**	**NKAIN4**	**Additive**	**0.653 (0.506 - 0.842)**	**0.001**

Rows highlighted in bold are significant SNPs after Bonferroni correction ($p \leq 0.002$)

Table S2.2. Result of sex-stratified analysis of 27 polymorphisms with interaction p-value less than 10^{-5} for COPD outcome amongst females

Females					
CHR	SNP	Gene/Nearest Gene	Best Genetic Model	OR(95% CI)	p-value
1	**rs12025895**	**CAMTA1**	**Additive**	**0.719 (0.613 - 0.843)**	**<0.0001**
1	rs6429144	CHRM3	Additive	0.807 (0.658 - 0.991)	0.0406
2	**rs10931835**	**SATB2**	**Dominant**	**0.636 (0.502 - 0.805)**	**0.0002**
2	rs7557339	SLC19A3	Dominant	1.264 (0.986 - 1.620)	0.0645
3	rs13326145	MAGI1	Recessive	0.605 (0.080 - 4.578)	0.6267
3	rs12633737	ALCAM	Dominant	0.861 (0.684 - 1.085)	0.204
4	rs56334611	COX18	Dominant	1.372 (1.089 - 1.730)	0.0073
4	rs6816344	COX18	Additive	1.257 (1.041 - 1.517)	0.0176
4	rs17039240	OSTC	Additive	0.832 (0.688 - 1.006)	0.0571
4	rs11947441	FRG1	Dominant	1.460 (0.917 - 2.324)	0.1105
5	rs76189059	NA	Additive	1.389 (0.962 - 2.005)	0.0797
6	rs6935314	ELOVL5	Dominant	1.302 (1.030 - 1.646)	0.0276
6	rs117896837	FAM46A	Additive	0.473 (0.226 - 0.991)	0.0474
6	**rs220806**	**PDE10A**	**Additive**	**0.695 (0.577 - 0.836)**	**0.0001**
7	rs59794319	NXPH1	Dominant	1.257 (0.935 - 1.692)	0.1302
7	rs10488084	PLEKHA8	Dominant	1.570 (1.173 - 2.101)	0.0024
7	**rs1911770**	**ZPBP**	**Additive**	**0.763 (0.642 - 0.906)**	**0.002**
7	rs13225543	C7orf72	Dominant	1.362 (1.051 - 1.766)	0.0196
8	rs112593560	KCNQ3	Additive	0.441 (0.217 - 0.896)	0.0236
9	rs4076140	TRPM3	Additive	0.644 (0.458 - 0.904)	0.0109
9	rs74964803	MED27	Additive	1.687 (1.208 - 2.355)	0.0021
13	rs66468120	RNF219-AS1	Additive	0.609 (0.406 - 0.915)	0.0169
13	rs12869252	FGF14	Additive	1.235 (1.035 - 1.475)	0.0194
14	rs12879329	NPAS3	Dominant	0.743 (0.505 - 1.092)	0.1302
18	**rs77625370**	**LINC00908**	**Additive**	**0.451 (0.277 - 0.735)**	**0.0014**
20	rs1891778	CDH4	Additive	0.837 (0.709 - 0.986)	0.0338
20	rs6090327	NKAIN4	Additive	1.377 (1.109 - 1.711)	0.0038

Rows highlighted in bold are significant SNPs after Bonferroni correction ($p \leq 0.002$)

Table S2.3. Result of sex-stratified analysis of 17 polymorphisms with interaction p-value less than 10^{-5} for asthma-COPD phenotype outcome amongst males

Males					
CHR	SNP	Gene/Nearest gene	Best Genetic Model	OR(95% CI)	p-value
1	rs619928	SPSB1	Dominant	0.720 (0.495 - 1.049)	0.0873
1	rs6666629	HSD52	Dominant	1.740 (1.109 - 2.731)	0.016
1	rs12062324	RPS6KC1	Dominant	1.685 (1.005 - 2.823)	0.0477
1	**rs11799559**	**SMYD3**	**Additive**	**1.560 (1.176 - 2.070)**	**0.0021**
2	rs77602394	RAD51AP2	Additive	1.492 (0.935 - 2.380)	0.093
3	**rs3821479**	**FHIT**	**Additive**	**0.546 (0.408 - 0.729)**	**<.0001**
4	rs72901492	NDST4	Additive	0.613 (0.378 - 0.993)	0.047
5	**rs77800494**	**ZNF608**	**Additive**	**2.079 (1.295 - 3.338)**	**0.0025**
7	rs7782108	CARD11	Additive	0.625 (0.420 - 0.930)	0.0204
7	rs34331963	SDK1	Dominant	1.753 (1.192 - 2.578)	0.0044
8	rs6986253	LRP12	Additive	1.219 (0.892 - 1.665)	0.2132
12	**rs11061082**	**RIMBP2**	**Recessive**	**2.148 (1.370 - 3.368)**	**0.0009**
14	rs2582577	C14orf79	Additive	1.478 (1.085 - 2.011)	0.0131
20	**rs926718**	**ZNF133**	**Additive**	**0.578 (0.425 - 0.787)**	**0.0005**
20	**rs1884882**	**BPIFB1**	**Recessive**	**2.239 (1.316 - 3.809)**	**0.0029**
20	rs6013630	TSHZ2	Dominant	1.237 (0.812 - 1.886)	0.3222
21	**rs1051169**	**S100B**	**Additive**	**1.555 (1.176 - 2.056)**	**0.0019**

Rows highlighted in bold are significant SNPs after Bonferroni correction ($p \leq 0.003$)

Table S2.4. Result of sex-stratified analysis of 17 polymorphisms with interaction p-value less than 10^{-5} for asthma-COPD phenotype outcome amongst females

Females					
CHR	SNP	Gene/Nearest Gene	Best Genetic Model	OR(95% CI)	p-value
1	rs619928	SPSB1	Recessive	1.616 (1.110 - 2.353)	0.0123
1	rs6666629	HSD52	Additive	0.558 (0.333 - 0.934)	0.0263
1	rs12062324	RPS6KC1	Additive	0.529 (0.272 - 1.028)	0.0605
1	rs11799559	SMYD3	Dominant	0.687 (0.502 - 0.939)	0.0185
2	rs77602394	RAD51AP2	Dominant	0.493 (0.272 - 0.894)	0.0199
3	rs3821479	FHIT	Dominant	0.788 (0.574 - 1.082)	0.141
4	rs72901492	NDST4	Additive	1.550 (1.137 - 2.114)	0.0056
5	rs77800494	ZNF608	Additive	0.415 (0.201 - 0.859)	0.0178
7	rs7782108	CARD11	Dominant	1.319 (0.963 - 1.806)	0.0844
7	rs34331963	SDK1	Additive	0.818 (0.595 - 1.124)	0.2147
8	rs6986253	LRP12	Additive	0.711 (0.553 - 0.914)	0.0078
12	rs11061082	RIMBP2	Recessive	0.750 (0.483 - 1.164)	0.199
14	rs2582577	C14orf79	Recessive	0.331 (0.093 - 1.184)	0.0892
20	rs926718	ZNF133	Additive	1.324 (1.069 - 1.639)	0.0101
20	rs1884882	BPIFB1	Additive	0.933 (0.729 - 1.195)	0.5823
20	rs6013630	TSHZ2	Additive	0.603 (0.412 - 0.881)	0.009
21	rs1051169	S100B	Dominant	0.741 (0.545 - 1.008)	0.0562

Rows highlighted in bold are significant SNPs after Bonferroni correction ($p \leq 0.003$)

Chapter 4. Identification of sex-specific genetic polymorphisms associated with asthma in middle and older Canadian adults: An analysis of CLSA data.

This manuscript was submitted to the Journal of Asthma and Allergy on January 14, 2023 and is currently under review.

Authors contributions

UO wrote the original manuscript draft, conducted data analysis, and prepared all figures and tables. ZG and AS provided critical feedback on statistical analysis and methodology. ZG and AS obtained ethics approval and provided access to the data. ZG, AS, and JF conceptualized the study and critically revised, edited, and suggested changes to the manuscript. All authors contributed to the article and approved the final version that was submitted.

Abstract

Asthma is a chronic heterogeneous respiratory disease resulting from a complex interplay between genetic variations and environmental exposures. There are sex disparities in the prevalence and severity of asthma in males and females. Asthma prevalence is higher in males during childhood but increases in females in adulthood. The mechanisms underlying these sex differences are not well understood; nevertheless, genetic variations, hormonal changes, and environmental influences are thought to play important roles. This study aimed to identify sex-specific genetic variants associated with asthma using CLSA genomic and questionnaire data. First, a genome-wide SNP-by-sex interaction analysis was carried out after quality control. 416,562 single nucleotide polymorphisms (SNPs) were examined. There were 49 SNPs with an interaction p-value less than 10^{-5}. Out of the 49 SNPs, a sex-stratified survey logistic regression showed that five male-specific SNPs (rs6701638, rs17071077, rs254804, rs6013213, and rs2968822) in/near KIF26B, NMBR, PEPD, RTN4, and NFATC2 loci, and three female-specific SNPs (rs2968801, rs2864052, and rs9525931) in/near RTN4, and SERP2 loci were significantly associated with asthma after Bonferroni correction. An SNP (rs36213) in the EPHB1 gene was significantly associated with an increased risk of asthma in males [OR=1.35, 95% CI (1.14, 1.60)] but with a reduced risk of asthma in females [OR=0.84, 95% CI (0.76, 0.92)] after Bonferroni correction. In conclusion, we found novel sex-specific genetic markers that could potentially shed light on the sex differences in asthma susceptibility in males and females. Future mechanistic studies are required to understand better the underlying sex-related pathways of the identified loci in asthma development.

Introduction

Asthma is a chronic inflammatory respiratory disease that affects both children and adults. It is characterized by reversible airflow obstruction, airway remodeling, hyperresponsiveness, shortness of breath, wheezing, and mucus production. In 2019, there were more than 262 million asthma cases globally, and the age-standardized point prevalence was highest in the high-income North American region.[1] In Canada, between 2011 and 2012, an estimated 3.8 million people one year and older lived with asthma.[2] Asthma prevalence and incidence differ in childhood and adulthood, and sex differences in prevalence, incidence, susceptibility, and severity have long been recognized.[3–5] During childhood, the prevalence of asthma is higher in males than in females, while in puberty and adulthood, females are more likely to develop asthma than males.[6]

Sexual dimorphism has been reported to influence the severity, pathogenesis, pathophysiology, progression, and susceptibility of various human diseases and treatments, including asthma.[7–10] Women with asthma tend to have higher mortality and morbidity,[11] greater perception of symptom bothersomeness, and poorer quality of life than men with asthma.[12, 13] Studies using antigen-challenged mouse asthma models have shown that female mice had greater levels of antigen-specific serum IgE, eosinophils, and Th2 cytokine than male mice in the lung tissue and bronchoalveolar lavage fluid.[14, 15] Furthermore, airway hyperresponsiveness has been reported to be greater in male mice than in female mice at six weeks of age and reduced at twelve weeks of age.[16] Although the underlying biological mechanism of respiratory diseases and sex differences in risk are not fully understood, it is commonly believed that anatomical

differences, genetic predisposition, hormonal changes, environmental exposures, and gene and environment interactions play an important role.[4, 17–19] Genetic predisposition plays a vital role in asthma pathogenesis. More than a hundred independent genetic loci have been associated with asthma.[20, 21] Studies have shown that several gene polymorphisms and gene expressions associated with asthma are sex-specific.[22–25] For instance, a large genome-wide association study (GWAS) using EVE Asthma Genetics Consortium found six sex-specific asthma risk loci, with two male-specific SNPs in/near IRF and RAB11FIP2 genes and four female-specific SNPs in/near RAP1GAP2, C6orf118, ERBB4, and AK057517.[25] Other sex-specific polymorphisms in many genes have been identified, e.g., a male-specific SNP in the LCORL gene was significantly associated with childhood asthma[22], and a female-specific polymorphism in the COX-2$_{-765}$ gene was significantly associated with bronchial asthma.[26] Most GWAS of asthma generally include limited information on social and environmental factors and do not have enough sample size to investigate sex-specific genetic effects; this study aims to identify the sex-specific genetic polymorphisms significantly associated with asthma in middle-aged and older Canadian adults using the Canadian Longitudinal Study on Aging (CLSA) data (Baseline Comprehensive and Genomic datasets).

Methods and Materials

Study Population

This study included 26,622 individuals with complete genotyping information from the Canadian Longitudinal Study for Aging (CLSA) comprehensive cohort.[27] The CLSA comprehensive cohort comprises over 30,000 middle-aged and older Canadian adults

between the ages of 45 and 85 years. Baseline data on a wide range of variables, including socio-demographic and socio-economic factors, information on lifestyle and health behaviors, physical measurements including height, weight, pulmonary function test, and biospecimens (blood and urine), were obtained through in-person home interviews and visits to data collection sites (DCS).[28, 29]

Definition of asthma

A positive response to the following question from the CLSA questionnaire determined the presence of self-reported physician-diagnosed asthma, "Has a doctor ever told you that you have asthma?" However, we excluded the following participants from this study: participants with missing responses, participants who self-reported a physician diagnosis of COPD, and participants who self-reported a physician diagnosis of asthma and COPD.

Covariates

The following potential confounders were considered: age and principal components of genetic ancestry were included as continuous variables. Categorical variables included were age groups (45 to 54 years, 55 to 64 years, 65 to 74 years, and over 75 years), biological sex (male vs. female), smoking status (current, never, and former smokers), marital status (single/never married, married/common-law relationship, widowed/divorced/separated), education level (less than post-secondary education, post-secondary but not university education, and university education/others), total personal income and total household income (Less than $20,000, $20,000 to less than $50,000, $50,000 to less than $100,000, $100,000 or more), province of recruitment (Prairies, British Columbia, Eastern provinces, Ontario and Quebec), retirement status (Retired

completely vs. retired/partly retired), homeownership (owned vs. rented/others), and urban/rural dwelling (urban core vs. rural/not urban core).

Genotyping, Sample, and Marker Quality Control

Genotyping of 794,409 genetic markers was undertaken using Affymetrix UK Biobank Axiom array.[27] Detailed sample and markers quality control have been described previously.[30] Samples and markers that failed the quality control requirements were excluded. Samples with inconsistent sex information (discrepancy between reported sex and sex determined from genetic data) and high rates of genotype missingness (> 5%) were removed. SNPs in sex chromosomes, SNPs with low genotype call rates (< 99%), SNPs with minor allele frequency < 1% (MAF < 0.01), and SNPs deviating from the Hardy Weinberg equilibrium threshold of 1e-10 were excluded. Using PLINK's Indep-pairwise command (Indep-pairwise 50, 5, 0.5), we generated a subset of SNPs in approximate linkage equilibrium. After conducting quality control, we had 2,799 asthma cases, 20,524 controls**,** and 416,562 SNPs for genome-wide SNP-by-sex interaction analysis.

Statistical Analysis

Descriptive statistics, mean (SE), and frequency (%) for continuous and categorical variables were presented to describe the study population. Characteristics between male and female participants were compared using chi-square tests and Student's t-test**.** Trimmed inflation and analytic weights provided in the CLSA data (CLSA Sample Weights Version 1.2) were used for descriptive and regression analyses.

In order to identify sex-specific SNPs associated with asthma, first, we performed a multivariate logistic regression using PLINK 1.90b6.2 [31, 32] to identify significant

interaction terms between a sex variable and each of the SNPs in the regression analysis of asthma after controlling for age, sex, smoking status, and the first four principal components of genetic ancestry. Second, we performed a sex-stratified analysis for those SNPs with an SNP-by-sex interaction p-value less than 10^{-5} using survey-specific logistic regression (*Proc Surveylogistic*) in SAS 9.4 version. This approach allowed us to include sampling weights, complex survey design variables and adjust for potential confounders.

A purposeful model selection method was used to identify potential confounders for inclusion in the final model of the sex-stratified analysis. The covariates with a p-value $\leq$ 0.20 from the univariate analysis were entered into an interim multivariate model. The least significant covariate was then removed one at a time until only covariates with significant p-values ($p \leq 0.05$) and clinically important factors remained in the model. The "sex variable" was included as a domain factor in the sex-stratified multivariate survey logistic regression to examine one SNP at a time after controlling for the first four principal components, age, smoking status, province of recruitment, marital status, total personal income, and retirement status.

Three inheritance models (dominant, recessive, and additive) for each variant were evaluated. However, only the inheritance model with the smallest AIC value (the best-fitted model) was selected and presented. Bonferroni correction was applied to control for multiple comparisons. The association's strength was reported as an odds ratio with 95% confidence intervals. All statistical analysis was performed using plink 1.90b6.2[31] and SAS 9.4 (SAS Institute Inc, Cary, NC).

Results

Population Characteristics

Table 3.1 compares the characteristics of the study population between males and females. Female subjects were more likely to have self-reported physician-diagnosed asthma than males (14.0% vs. 10.6%). The mean age of females was significantly greater than that of males (60.0 vs. 59.1, $p \leq 0.0001$). The distribution of smoking status, marital status, urban or rural dwelling, homeownership, total household, personal income, retirement status, province of recruitment, and highest education status also differed significantly between males and females. The proportion of current and former smokers was significantly greater in males than in females. The percentage of widowed, separated, or divorced females (21.6%) was significantly higher than that of males (9.6%).

Results of SNP-by-Sex Interaction analysis

After quality control, 416,562 SNPs were included in the GWAS of SNP by sex interaction analysis. As indicated in **Table 3.2**, 49 variants showed a p-value less than 10^{-5} for the interaction term. The polymorphisms of rs7676077 and rs6701638 in/near the SLC34A2 and KIF26B genes (interaction p-values = 3.84×10^{-7} and 7.88×10^{-7} respectively) were close to reaching the genome-wide significance p-value threshold. There was no evidence of population stratification, as indicated by the genomic inflation factor (lambda) in the Q-Q plot shown in **figure 2.1.**

Results of Sex stratified analysis

Table 3.3 presents the significant sex-specific SNPs from the sex-stratified multivariate survey logistic regression. After adjusting for multiple comparisons using Bonferroni correction at $\alpha \leq 0.001$ (0.05/49) significance level, we identified five male-specific SNPs

(rs6701638, rs2968822, rs17071077, rs254804, and rs6013213) in/near KIF26B, RTN4, NMBR, PEPD, and NFATC2 loci and three female-specific SNPs (rs2968801, rs2864052 and rs9525931) in/near RTN4, and SERP2 genes. The polymorphism rs36213 in the EPHB1 gene was significantly associated with an increased risk of asthma [OR=1.35, p= 0.0004, 95% CI (1.14, 1.60)] in males and a reduced risk of asthma [OR=0.84, p= 0.0003, 95% CI (0.76, 0.92)] in females.

Out of the five male-specific SNPs, three polymorphisms (rs6701638, rs254804, and rs268822) in/near KIF26B, PEPD, and RTN4 were significantly associated with increased risk of asthma, whereas two polymorphisms (rs17071077 and rs6013213) in/near NMBR and NFATC2 were significantly associated with reduced risk of asthma. The three female-specific SNPs (rs2968801, rs2864052, and rs9525931) in/near RTN4 and SERP2 and genes were significantly associated with reduced risk of asthma.

Discussion

In this study, we identified sex-specific SNPs associated with asthma among middle and older Canadians. Five male-specific SNPs (rs6701638, rs17071077, rs254804, rs6013213 and rs2968822) and three female-specific SNPs (rs2968801, rs2864052 and rs9525931) were significantly associated with asthma. Three of the five male-specific SNPs were associated with an increased risk of asthma, with an odds ratio ranging from 1.22 to 1.27. The other two male-specific SNPs were associated with a reduced risk of asthma (OR=0.79 and OR=0.77). All three female-specific SNPs were associated with a reduced risk of asthma, with ORs ranging from 0.77 to 0.80. The polymorphism rs36213 was significantly associated with an increased risk of asthma (OR=1.35) in males but with a reduced risk of asthma (OR=0.84) in females. In previous studies, male and female-

specific genetic markers have been associated with asthma.[22, 25] However, these studies did not consider the influence of socio-environmental and lifestyle factors.

The five male-specific polymorphisms, i.e., rs6701638 in the KIF26B, rs254804 near the PEPD, rs17071077 near the NMBR, rs6013213 in the NFATC2, and rs2968822 near the RTN4 genes are located at 1q44, 19q13.11, 6q24.1, 20q13.2 and 2p16.1 genomic regions, respectively. The three female-specific polymorphisms, i.e., rs2968801 near the RTN4, rs2864052 in the RTN4, and rs9525931 near the SERP2 genes, are located at 2p16.1 and 13q14.11 cytogenetic regions.

The polymorphism rs36213 (minor allele: G), which was significantly associated with an increased risk of asthma in males and a decreased risk of asthma in females, is an intronic variant in the EPH receptor B1 gene (EPHB1). EPHB1 is a receptor for Ephrin-B ligands (Ephrin-B1, Ephrin-B2, Ephrin-B3). EPH receptors are the largest family members of receptor tyrosine kinase (RTK), which are expressed in various immune cells, including $CD4^+$ and $CD8^+$ T cells, lymphocytes, monocytes, and granulocytes.[33–35] Furthermore, the EPH receptor-ephrin ligand interaction has been linked to immune cell activation, T cell differentiation, proliferation, and migration.[34, 35] Previous research has linked allergic rhinitis and asthma to EPH receptors, especially the EPHB2 gene, an important paralog of the EPHB1 gene.[36] Recently, animal studies have illustrated that sex hormones augmented the sex-specific effect of EPHB receptors and Ephrin-B ligands.[37–39] Wang et al. [37] found that the deleting EPHB4 from vascular smooth muscle cells of mice resulted in hypotension in males but not females. In a different experiment, female but not male Ephrin-B3 null mice had higher blood pressure and increased vascular smooth muscle cell contractions than wild-type.[38] In addition,

estrogen enhanced increased vascular smooth muscle contraction in female Ephrin-b3 null mice compared with wild type, whereas testosterone reduced it.[38] These suggest that sex hormones may modulate ephrin-Eph receptor functions in males and females and, as such, may play crucial sex-specific role in asthma susceptibility.

This study also found three variants in/near the RTN4 gene, SNPs rs2968822 (minor allele: T), rs2968801 (minor allele: G), and rs2864052 (minor allele: A), associated with asthma in males and females, respectively. While the SNP rs2968822 indicated an increased risk of asthma in males, SNPs rs2968801 and rs2864052 showed a protective effect against asthma risk in females. RTN4 (Reticulon 4), commonly known as a neurite outgrowth inhibitor (Nogo) with three major isoforms, most notably NOGO-B (RTN4B), has previously been shown to be expressed in the lung tissue and airway epithelium.[40, 41] Numerous animal and human studies have examined the role of RTN4 in asthma and immune responses.[41–45] In an experimental study using mice, Wright et al.[41] demonstrated that RTN4 inhibits Th2-mediated inflammation in the lungs, airway epithelium, and smooth muscles by showing that eosinophils levels and Th2 cytokines (IL-13, IL-4, and 1L-5) were higher in RTN-A/B knockout mice when compared to wild-type mice.[41] In addition, RTN4B has also been shown to enhance the production of the inflammatory cytokine after the stimulation of various nucleic acid-sensing toll-like receptors, including TLR9, TLR3, and TLR7, in macrophages and serum.[43] Studies have shown that males produce higher TNF-α (a pro-inflammatory cytokine) and IL-10 (anti-inflammatory cytokine) than females after the stimulation of TLR3 and TLR9 or viral infection in peripheral blood mononuclear cells (PBMCs).[46] However, a review article reported that older men or men with androgen deficiency express higher pro-

inflammatory cytokines and lower anti-inflammatory cytokines than older or menopausal women.[47] These suggest that sex hormones may distinctively impact RTN4-mediated expressions of Th2 immune responses, toll-like receptors, and the production of inflammatory and suppressive cytokines in males and females.

An intergenic SNP rs9525931 near the Stress-Associated Endoplasmic Reticulum Protein 2 gene (SERP2) had a female-specific association with asthma. Variants near the SERP2 gene have previously been associated with FEV_1 and FEV_1/FVC ratio in a GWAS of lung function and COPD.[48] Furthermore, CpGs and SNPs mapped to the SERP2 gene have been reported to be associated with Alzheimer's disease in females. [49]

An intron variant rs6013213 (minor allele: A) in the NFATC2 gene was protective against the risk of asthma in males only. NFATC2, a member of the nuclear factor of activated T cells (NFAT) family, regulates Th cell immune response, differentiation, and the expression of induced cytokines such as IL-2, IL-3, IL-4, IL-10, and TNF-alpha.[50] Previous genome-wide association studies have associated several variants in the NFATC2 gene with asthma and allergic disease, including hay fever, eczema, and allergic rhinitis.[51–55] NFATC2 mRNA levels in PBMCs of allergic asthmatics were higher than in healthy non-asthmatic controls.[56] Furthermore, NFATC2 mRNA has been demonstrated to correlate positively with IL-5-induced eosinophils in asthma.[56] Animal and human studies have shown that estrogen and cigarette smoke exposure increased NFAT mRNA expression in the airways of females.[57, 58] Furthermore, NFAT has been reported to have a regulatory function in estradiol-mediated MUC5AC mRNA and protein expression in airway epithelial cells.[58] MUC5AC is a marker for mucus production in the airway epithelial cells. Thus, it is reasonable to suggest that

NFATC2 may influence sex-hormone-induced mucus secretion differently in male and female asthmatic bronchial epithelial cells.

The intergenic variant rs17071077 (minor allele: G) near the Neuromedin B Receptor (NMBR) gene exhibited a significant protective effect against the risk of asthma in males. NMBR is a G protein-coupled peptide receptor that binds with the regulatory neuropeptide Neuromedin B (NMB).[59] NMB/NMBR, widely expressed in the lungs, broncho epithelial cells, pulmonary neuroendocrine cells, brain, and testis at the protein and mRNA levels,[59–61] has been shown to promote fetal lung development.[62] Several studies have shown that NMBR plays an innate immune defense role against respiratory viral infection by enhancing IFN-alpha and reducing the expression of IL-6.[63] Females have been shown to have greater Type I IFN responses and are less susceptible to viral infections than males.[64] We reasonably infer that any abnormality or polymorphic alterations in the NMBR gene may have a higher impact on males.

In our study, males with the polymorphism rs254804 near the PEPD gene had a significantly higher risk of asthma susceptibility. PEPD is a gene that encodes prolidase, an enzyme involved in collagen metabolism, wound healing, inflammation, angiogenesis, and cell growth.[65] Numerous studies have shown that airway remodeling in asthma is largely due to the deposition of extracellular matrix protein, including collagen fibers, around the airway smooth muscle layers. [66, 67] This suggests that polymorphisms of the PEPD gene may contribute to airway remodeling. Patients with mutations in the PEPD gene and dysfunctional prolidase enzyme have been reported to have asthma and asthma-like airway disease.[68, 69] Studies have shown that serum prolidase activity was associated with bronchial asthma.[70, 71] Higher serum prolidase levels appear to be

associated with higher oxidative stress and lower antioxidant levels.[72, 73] The homeostatic balance between the oxidant and antioxidant systems is impaired in asthma,[74] with oxidative stress increasing tissue damage, triggering the production of pro-inflammatory mediators, and exacerbating airway inflammation.[74]Studies have shown that males exhibit higher levels of oxidative stress, oxidative stress biomarkers, reactive oxygen species, and lower antioxidant capacity than females.[75]

In our current study, the SNP rs6701638 in the Kinesin Family Member 26B gene (KIF26B) exhibited a significantly increased risk of asthma susceptibility in males but not females. Several variants of the genes in the 1q43-q44 region have been associated with asthma and atopic asthma.[76] White et al. demonstrated that a variant in the KIF26B gene within the 1q43-44 region was associated with atopic asthma.[76] However, the association's p-value was not significant after multiple testing adjustments. KIF26B is a target of the Wnt5a-Ror signaling pathway.[77] Studies have revealed that non-canonical Wnt5a-Ror signaling activation reduces KIF26B protein expression levels. Susman et al. [77] demonstrated that the KIF26B protein expression level increased in embryonic fibroblast of Wnt5a null mice compared to the wild type. Dysregulation of Wnt5a signaling has been implicated in disrupted alveologenesis and the development of chronic lung disease, including asthma.[78] Furthermore, loss of Wnt5a in male mice resulted in abnormal reproductive organ development.[79] Taking together, we suggest that the alteration of the Wnt5a-Ror-KIF26B signaling pathway may have a deleterious impact in males and could play a crucial role in sex-specific asthma pathogenesis.

In our study, most sex-specific polymorphisms associated with asthma susceptibility may directly or indirectly interact with sex hormones in modulating immunoregulation,

immune cell population, airway remodeling, oxidative stress, and lung function differently in males and females.

This study has strengths and limitations. A major strength was the size of the study population and the inclusion of lifestyle, socio-economic and socio-demographic factors in our analysis. Regarding the limitations, we used all asthma cases in our analysis without stratifying asthma by the age of onset (childhood onset and adulthood onset); this could potentially mask the identification of sex-specific loci associated with childhood and adulthood onset asthma. We reckon that misclassification of asthma is possible with self-reported physician-diagnosed asthma. However, self-reported physician diagnosis of asthma has been used in genome-wide association and population-based studies to identify sex-specific genetic and clinical characteristics of asthma.[22, 80] Our genome-wide SNP-by-sex interaction testing found no variants that met the genome-wide significance p-value criterion ($p \leq 5 \times 10^{-8}$). There was no independent replication cohort in our study to corroborate our findings. Our work lacks functional follow-up and enrichment analysis of sex-specific genetic variants associated with asthma; future experimental investigations can address the underlying biological processes for the sex differences.

In conclusion, we found evidence of sex-specific polymorphisms associated with asthma susceptibility in/near the KIF26B RTN4 EPHB1 NMBR SERP2 PEPD NFATC2 gene. Most of the identified loci may potentially play direct or indirect roles in the immune modulatory mechanism, oxidative stress, and airway remodeling. Future studies aimed at finding sex-specific expressions of these loci and the related pathways of action would give functional insights into their sex-specific involvement in asthma pathogenesis.

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Table 3.1. Baseline characteristics of the study population

Characteristics	Males (n=13,343) (%)	Females (n= 13,279) (%)	p-value*
Asthma			
Yes	10.6	14.0	<0.0001
Age, mean ± SEM	59.1 ± 0.10	60.0 ± 0.10	<0.0001
Lifestyle and Health Behavior			
Smoking Status			<0.0001
Current Smoker	10.0	8.5	
Never Smoker	47.6	51.4	
Former Smoker	42.4	40.1	
Socio-Demographic and Economic Factors			<0.0001
Marital status	8.1	8.2	
Single/never married	82.3	70.2	
Married/Living in common law	9.6	21.6	
Widowed/Separated/Divorced			
Urban or Rural Dwelling			0.003
Urban core	86.6	85.0	
Rural/not Urban core	13.4	15.0	
Homeownership			0.0008
Owned	86.7	85.0	
Rented/Others	13.3	15.0	
Total household income			<0.0001
Less than $20,000	3.4	5.6	
$20,000 to less than $50,000	15.0	22.2	
$50,000 to less than $100,000	32.5	34.2	
$100,000 or more	49.1	38.0	
Total personal income			<0.0001
Less than $20,000	7.8	22.9	
$20,000 to less than $50,000	27.9	39.9	
$50,000 to less than $100,000	40.7	28.9	
$100,000 or more	23.6	8.2	
Retirement Status			<0.0001
Retired completely	29.0	38.0	
Not retired/partly retired	71.0	62.0	
Province of recruitment			<0.0001
Prairies	22.2	19.1	
British Columbia	28.9	30.0	
Eastern province[a]	5.2	6.2	
Ontario	13.5	13.6	
Quebec	30.2	31.1	

Highest Education Status			<0.0001
No post-secondary education	7.2	8.4	
Post-secondary (non-university)	31.4	34.8	
University	61.4	56.8	

*p values for overall comparison between males and females; [a]: Newfoundland and Labrador, Nova Scotia

Table 3.2. 49 signals of the SNP-by-sex interactions with p-value less than 10^{-5} for asthma

CHR	SNP	A1/A2	MAF	Gene/Nearest Gene	OR (95% CI)	Interaction p-value
4	rs7676077	T/C	0.308	SLC34A2	0.73 (0.641, 0.821)	3.84E-07
1	rs6701638	G/A	0.493	KIF26B	1.33 (1.188, 1.492)	7.88E-07
2	rs2968822	T/C	0.403	RTN4	1.33 (1.184, 1.489)	1.24E-06
2	rs2968801	G/A	0.297	RTN4	1.35 (1.190, 1.521)	2.14E-06
14	rs10467731	G/A	0.014	SLC38A6	0.27 (0.158, 0.473)	3.52E-06
20	rs11699160	C/T	0.353	RPS21	0.76 (0.671, 0.852)	4.40E-06
18	rs4800396	C/T	0.169	CTAGE1	0.70 (0.602, 0.817)	4.94E-06
18	rs3911730	A/C	0.079	RTTN	0.62 (0.503, 0.766)	8.95E-06
2	rs2006878	A/G	0.271	EML6	1.33 (1.173, 1.510)	9.06E-06
1	rs3737247	G/A	0.384	SMYD3	0.77 (0.687, 0.869)	1.53E-05
1	rs7519461	T/C	0.331	KIF26B	1.30 (1.154, 1.468)	1.84E-05
13	rs117190915	C/T	0.034	LINC00408	0.48 (0.338, 0.669)	1.94E-05
21	rs34026679	A/C	0.041	APP	1.88 (1.405, 2.506)	1.99E-05
20	rs79496646	C/T	0.038	KIF16B	1.87 (1.403, 2.504)	2.12E-05
7	rs62442915	T/C	0.039	MAD1L1	1.88 (1.406, 2.520)	2.13E-05
6	rs17071077	G/A	0.278	NMBR	0.76 (0.664, 0.860)	2.21E-05
5	rs75073573	A/C	0.199	FGF18	0.73 (0.631, 0.845)	2.37E-05
13	rs9525931	G/A	0.245	SERP2	1.33 (1.166, 1.523)	2.50E-05
13	rs628778	T/C	0.066	FARP1	0.61 (0.481, 0.767)	2.78E-05

19	rs254804	G/T	0.181	PEPD	1.37 (1.180, 1.578)	2.83E-05
10	rs72804487	T/C	0.018	BICC1	0.38 (0.238, 0.595)	2.85E-05
5	rs61027918	G/A	0.173	TENM2	0.72 (0.622, 0.842)	3.00E-05
3	rs35644024	A/G	0.073	TGFBR2	0.61 (0.487, 0.772)	3.07E-05
13	rs74438452	G/T	0.019	DACH1	2.67 (1.680, 4.237)	3.21E-05
16	rs111933452	A/G	0.049	CDH5	1.75 (1.342, 2.272)	3.31E-05
8	rs73191549	A/C	0.087	MSRA	1.52 (1.243, 1.848)	3.93E-05
7	rs4718733	G/A	0.317	NA	1.29 (1.141, 1.457)	4.51E-05
6	rs1416037	A/G	0.086	HACE1	1.54 (1.250, 1.887)	4.51E-05
15	rs16974494	C/G	0.231	SEC11A	1.32 (1.155, 1.509)	4.64E-05
21	rs382583	T/C	0.109	RBM11	0.68 (0.562, 0.818)	5.08E-05
3	rs1488342	T/G	0.368	LOC101927394	0.78 (0.694, 0.881)	5.27E-05
3	rs36213	G/C	0.487	EPHB1	1.26 (1.128, 1.414)	5.43E-05
5	rs6893959	A/G	0.439	CD180	1.26 (1.128, 1.416)	5.63E-05
16	rs7200747	C/G	0.396	ZNF598	0.79 (0.700, 0.884)	5.74E-05
20	rs6013213	A/G	0.445	NFATC2	0.79 (0.704, 0.886)	6.06E-05
2	rs10205651	C/T	0.131	NA	0.71 (0.605, 0.841)	6.10E-05
2	rs2864052	A/G	0.239	RTN4	1.31 (1.147, 1.493)	6.11E-05
7	rs59889022	T/C	0.122	LOC100507642	0.69 (0.582, 0.831)	6.53E-05
2	rs3821261	C/A	0.075	TGFA	0.64 (0.515, 0.799)	7.35E-05
20	rs117107888	T/C	0.054	RALGAPA2	1.68 (1.300, 2.178)	7.67E-05
16	rs4513116	G/A	0.019	RBFOX1	0.37 (0.226, 0.606)	7.92E-05
2	rs1527243	C/T	0.160	TSN	0.73 (0.629, 0.856)	8.18E-05
15	rs837132	C/T	0.262	MEIS2	1.29 (1.137, 1.467)	8.30E-05
13	rs7998573	T/C	0.012	SLITRK6	0.29 (0.163, 0.545)	8.38E-05
1	rs868376	T/C	0.279	PBX1	0.77 (0.679, 0.879)	8.56E-05
3	rs73877940	T/A	0.033	ARL14	0.49 (0.348, 0.704)	8.92E-05

16	rs72799714	G/T	0.054	NA	0.58 (0.436, 0.759)	9.11E-05
6	rs6926101	T/C	0.057	SLC18B1	0.61 (0.477, 0.782)	9.25E-05
20	rs6041383	T/C	0.029	LOC101929486	0.46 (0.314, 0.681)	9.47E-05

SNP-by-Sex Interaction GWAS on asthma controlling for age, sex, smoking status, and top 4 principal components.
Chr: Chromosome; SNP: Single Nucleotide Polymorphism; MAF: Minor allele frequency; OR: Odds ratio;
A1/A2: Minor/Major allele; NA: No Annotation;
Genes contain variants or are located within 500kb of variants

Table 3.3. Result of sex-stratified analysis for asthma

Chr	Gene /Nearest Gene	SNP (A1/A2)	Male		Female	
			OR (95% CI)	p-value	OR (95% CI)	p-value
1	KIF26B	rs6701638 (G/A)	1.24 (1.11, 1.38)[A]	0.0001*	0.91 (0.83, 1.00)[A]	0.0503
6	NMBR	rs17071077 (G/A)	0.79 (0.70, 0.91)[A]	0.0006*	PM: 1.05 (0.92, 1.19)[D] WT: 1	0.5095
19	PEPD	rs254804 (G/T)	1.27 (1.11, 1.45)[A]	0.0005*	PM: 0.89 (0.77, 1.03)[D] WT: 1	0.1167
20	NFATC2	rs6013213 (A/G)	0.77 (0.69, 0.86)[A]	<0.0001*	PM: 1.04 (0.89, 1.19)[D] WT: 1	0.6293
2	RTN4	rs2968822 (T/C)	1.22 (1.09, 1.36)[A]	0.0004*	PM: 0.85 (0.74, 0.97)[D] WT: 1	0.0174
2	RTN4	rs2968801 (G/A)	1.12 (0.99, 1.25)[A]	0.0587	PM: 0.79 (0.69, 0.91)[D] WT: 1	0.0007*
2	RTN4	rs2864052 (A/G)	1.09 (0.96, 1.23)[A]	0.1843	PM: 0.77 (0.67, 0.89)[D] WT: 1	0.0003*

13	SERP2	rs9525931 (G/A)	1.07 (0.95, 1.21)[A]	0.2716	0.80 (0.72, 0.89)[A]	<0.0001*
3	EPHB1	rs36213 (G/C)	PM: 1.35 (1.14, 1.60)[R] WT: 1	0.0004*	0.84 (0.76, 0.92)[A]	0.0003*

Sex-stratified multivariate survey logistic regression of top interaction single nucleotide polymorphisms for asthma, adjusting for age, smoking status, province of recruitment, marital status, total personal income, retirement status, and first four principal components.

*: statistically significant after adjusting for multiple comparisons ($0.05/49 = p \leq 0.001$)
[A]: Additive; [D]: Dominant; [R]: Recessive; PM: Polymorphism; WT: Wild type;
Chr: Chromosome; SNP: Single Nucleotide Polymorphism; A1/A2:Minor/Major Allele;
OR: Odds ratio; CI: Confidence Interval;
Genes contain variants or are located within 500kb of variants

SNP-by-Sex interaction testing Q-Q plot for asthma

λ=1.025

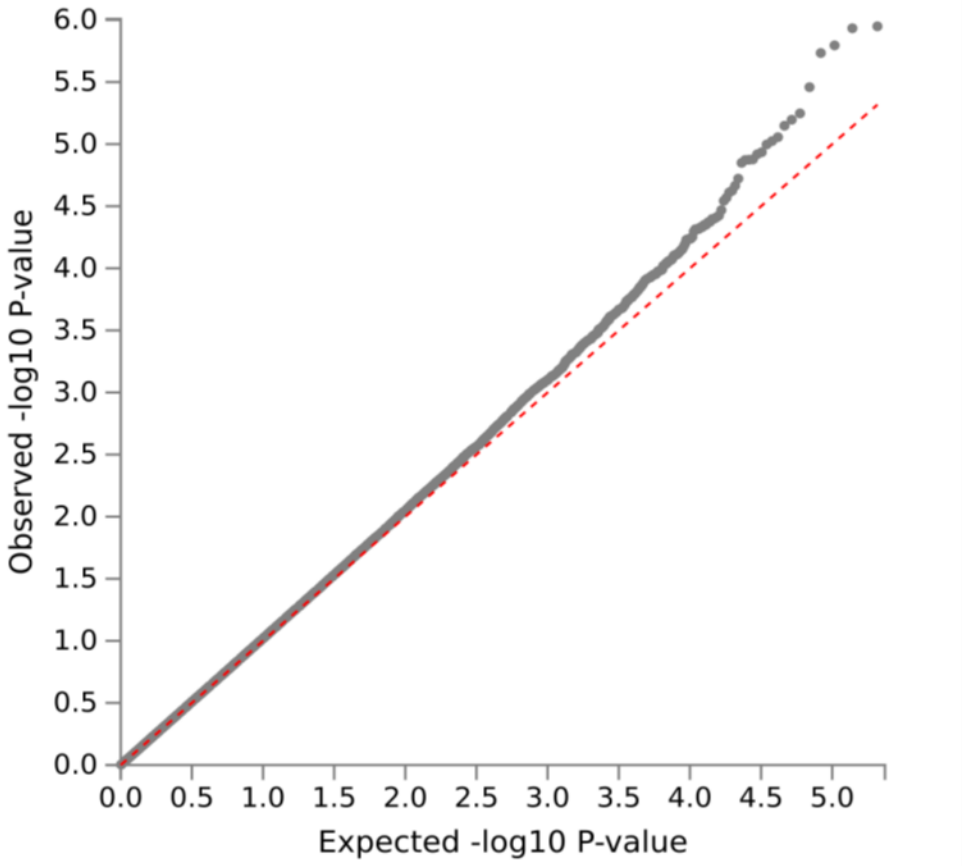

Figure 2.1. Quantile-Quantile plot for the genome-wide SNP-by-Sex interaction analysis for asthma showing the distribution of observed vs. expected p-values

Chapter 5. Summary of key findings and Conclusion

Sex-related differences in the prevalence, susceptibility, disease severity, and presentation of the asthma-COPD phenotype, COPD, and asthma have been widely reported in epidemiological studies.[1–6] The biological mechanisms underlying these sex differences remain unclear; sex hormones, environmental exposures, genetic factors, and gene-environment interactions may play influential roles.[7] Genetic association studies have also identified genetic loci associated with asthma-COPD phenotype, COPD, and asthma.[8–11] Few genetic association studies have explored the intrinsic variation in genetic risk factors between males' and females' susceptibility to COPD and asthma.[12–15] However, there has been no study on sex-related genetic differences for an asthma-COPD phenotype. This thesis addressed this issue with the following specific primary and secondary objectives:

1)To identify sex-specific genetic variants associated with asthma-COPD phenotype in middle-aged and older Canadian adults

2) To identify sex-specific genetic polymorphisms associated with COPD and asthma in middle and older Canadian adults.

Summary of key findings

This study identified sex-specific genetic markers associated with asthma-COPD phenotype, COPD, and asthma using the CLSA baseline comprehensive and genomic datasets. First, we conducted a genome-wide SNP-by-sex interaction in 504 asthma-COPD phenotypic cases, 911 COPD cases, 2,799 asthma cases, and 20,524 controls, examining 416,562 SNPs after quality control. Second, SNPs with interaction p-value < 10^{-5} were further analyzed for sex-specific association with asthma-COPD phenotype,

COPD, and asthma in a sex-stratified analysis. The purpose of conducting two-stage analyses was: **(1)** to identify SNPs significantly modified by sex for each outcome. **(2)** to determine which of these SNPs were significantly associated with asthma-COPD phenotype, COPD, and asthma in males and females.

Our main findings for the primary objective showed that seven male-specific polymorphisms (rs11799559, rs77800494, rs11061082, rs1884882, rs3821479, and rs926718) in/near the genes SMYD3, FHIT, ZNF608, RIMBP2, ZNF133, BPIFB1, and S100B were significantly associated with asthma-COPD phenotype. In females, however, no polymorphism was significantly associated with the asthma-COPD phenotype. Five of the seven polymorphisms (rs11799559, rs77800494, rs11061082, rs1884882, and rs1051169) conferred an increased risk of asthma-COPD phenotype in males (ORs: 1.56 to 2.24), whereas two other variants (rs3821479 and rs926718) exhibited protective effects (ORs: 0.56 and 0.58).

For the secondary objectives, sex-specific significant associations with COPD were observed in eight male-specific polymorphisms (rs13326145, rs56334611, rs6816344, rs17039240, rs6935314, rs13225543, rs12869252, and rs6090327) in/near the genes MAGI1, COX18, OSTC, ELOVL5, C7orf72 FGF14, and NKAIN4, and four female-specific variants (rs12025895, rs10931835, rs220806 and rs77625370) in/near CAMTA1, SATB2, PDE10A, and LINC00908 loci. The polymorphism rs17039240 near the OSTC gene in the 4q25 cytogenetic location increased the risk of COPD in males (OR= 2.48). All other male-specific polymorphisms reduced the risk of COPD, with ORs ranging from 0.38 to 0.74. All four female-specific variants associated with COPD demonstrated protective effects, with ORs varying from 0.45 to 0.72.

Five polymorphisms (rs6701638, rs17071077, rs254804, rs6013213, and rs2968822) in/near KIF26B, NMBR, PEPD, RTN4, and NFATC2 were significantly associated with asthma in males. Three SNPs (rs2968801, rs2864052, and rs9525931) in/near RTN4 and SERP2 loci were significantly associated with asthma in females alone. Three male-specific polymorphisms (rs6701638, rs254804, and rs268822) in/near KIF26B, PEPD, and RTN4 loci were significantly associated with an increased risk of asthma. In contrast, polymorphisms (rs17071077 and rs6013213) in NMBR and NFATC2 attenuated the risk of asthma in males. However, in females, asthma risk was considerably decreased by all three female-specific SNPs (rs2968801, rs2864052, and rs9525931) in/near RTN4 and SERP2 genes.

Additionally, our data also identified signals (rs1911770 and rs36213) corresponding to genes ZPBP and EPHB1, which were significantly associated with COPD and asthma in both males and females. SNPs rs1911770 and rs36213 had opposite effects on the risk of COPD and asthma in males and females, displaying an increased risk of COPD and asthma in males and attenuating the risk in females (rs1911770: OR=1.64 vs. 0.76, rs36213: OR= 1.35 vs. 0.84). These findings provide further evidence of the distinctiveness of the three diseases since no overlapping SNP was identified. Therefore, it is reasonable to suggest that these three diseases should be regarded as distinct phenotypes.

Strengths and Limitations

The strength of this study was the utilization of CLSA data, which offers a large sample size of individuals' data on genotyping information, physical measures, socio-demographic, socio-economic, lifestyle, and behavior variables to study sex-specific

genetic differences of asthma-COPD phenotype in middle-aged and older Canadian adults. To the best of our knowledge, this is the first study to look at sex-specific genetic markers associated with asthma-COPD phenotype, COPD, and asthma while also considering the influence of environmental, lifestyle, socio-economic, and socio-demographic factors. Incorporating environmental, socio-demographic, socio-economic, and lifestyle variables in our analysis ensured the control of potential confounding variables or other risk factors for asthma-COPD phenotype, COPD, and asthma. Sex-specific genetic susceptibility for COPD and asthma have been shown in previous studies.[12–16] However, these studies did not consider the influence of socio-demographic and socio-economic factors. Our study is limited due to the lack of an independent replication cohort to validate our findings. There is the possibility of misclassification since asthma-COPD phenotype, COPD, and asthma cases were identified based on self-reported physician diagnoses of coexisting asthma and COPD, asthma alone, and COPD alone, respectively. We did not employ spirometry variables to determine our outcome because of the high proportion of missing values in pulmonary function variables and the lack of post-bronchodilator spirometry data. However, self-reported physician diagnosis of chronic lung diseases, including asthma and COPD, has been used in genome-wide association and many population-based studies to identify these phenotypes' genetic and clinical characteristics.[17–21, 2, 3]

Future Direction

Genome-wide association studies have usually examined autosomal variants' association with asthma-COPD phenotype, COPD, and asthma while excluding sex-chromosome variants. Including X and Y chromosome polymorphisms in sex-specific studies of these

chronic lung diseases could partly explain some of the phenotypic variability and possibly identify more sex-specific biomarkers for these complex diseases. The polygenic risk score is another area in genetic association studies generating much interest. Considering that the effect size of each variant identified via genome-wide association studies is usually small, aggregating the effects of many variants into a weighted genetic risk score may be used to estimate an individual's genetic susceptibility to disease. Hence incorporating polygenic risk scores in sex-specific genetic association studies could help predict males and females at higher genetic risk of developing asthma-COPD phenotype, COPD, and asthma. This study's findings have great implications. The identification of sex-specific genetic markers for respiratory diseases can be used for disease prevention and control by enabling early identification of at-risk individuals, developing personalized prevention strategies, improving disease management, tailoring treatment, and improving drug development. By understanding an individual's genetic makeup, healthcare professionals can develop a more personalized approach to treatment and monitoring, leading to better health outcomes. Moreover, this knowledge can be used by public health officials to develop targeted interventions to prevent and control respiratory diseases in populations that are at a higher risk.

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Appendices

Appendix A

Ethics Approval

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

Research Ethics Office
Suite 200, Eastern Trust Building 95 Bonaventure Avenue
St. John's, NL A1B 2X5

May 06, 2019

Suite 4M130, Medical Education Building
Memorial University, 300 Prince Phillip Drive
St. John's, NL
A1B 3V6

Dear Dr. Gao:

Researcher Portal File # 20193089
Reference # 2019.072

RE: Sex-specific environmental and genetic determinants of COPD in middle-aged and older Canadian adults: An analysis of Canadian Longitudinal Study on Aging (CLSA) data

Your application was reviewed by the Chair under the direction of the HREB and the following decision was rendered:

X	Approval
	Approval subject to changes
	Rejection

Ethics approval is granted for one year effective May 6, 2019. This ethics approval will be reported to the board at the next scheduled HREB meeting.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

Doc / Agreement	Version Date	Status	Description
Budget		Acknowledged	Budget

Informed Consent Form		Acknowledged	CLSA_Concent_Form
List of Variables		Acknowledged	CLSA_Data Biospecimen_App_Catalyst Grant 2018
Other	2019/05/02	Acknowledged	CLSA GWAS
Research Proposal/Protocol		Approved	Proposal

Please note the following:

This ethics approval will lapse on May 6, 2020. It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date.

- This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approvals.
- Modifications of the study are not permitted without prior approval from the HREB. Request for modification to the study must be outlined on the relevant Event Form available on the Researcher Portal website.
- Though this research has received HREB approval, you are responsible for the ethical conduct of this research.
- If you have any questions please contact info@hrea.ca or 709 777 6974.

The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), ICH Guidance E6: Good Clinical Practice Guidelines (GCP), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

We wish you every success with your study.

Sincerely,

[redacted]

From: administrator@hrea.ca [mailto:administrator@hrea.ca]
Sent: March-08-22 10:00 AM
To: Gao Zhiwei(Principal Investigator)
Cc: Senthilselvan Ambikaipakan(Co-Principal Investigator); Hreaadministrator
Subject: Renewal (proposed modifications) 565265

Researcher Portal File #: 20193089

Dear Dr. Zhiwei Gao:

This e-mail is to inform you that your renewal event for study HREB # 2019.072 - Sex-specific environmental and genetic determinants of COPD in middle-aged and older Canadian adults: An analysis of Canadian Longitudinal Study on Aging (CLSA) data - was reviewed, and the following revisions have been requested:

- As there is no longer any active data collection, recruitment is closed, and data transfer is complete, this study can be closed. If you believe a renewal is warranted, please provide a rationale to explain whether a renewal is needed in lieu of a closure.

To make revisions to this event, please revise the current event form in the Researcher Portal.

If the requested revisions result in changes to supporting documentation (e.g., consent forms, recruitment posters, etc.) please re-upload the revised documents to the Attachments tab of your application in the Researcher Portal. Please remove all previous versions of updated documents. Finally, please reflect any changes to updated supporting documentation in a tracked change version of the document, as well as including a final clean copy for HREB review.

If you would like to speak to someone regarding the requested changes, please do not hesitate to contact the Research Ethics Office by telephone or e-mail.

Thank you,

Research Ethics Office

From: administrator@hrea.ca [mailto:administrator@hrea.ca]
Sent: March-17-22 2:46 PM
To: Gao Zhiwei(Principal Investigator)
Cc: Hreaadministrator
Subject: Closure (approved or acknowledged) 565265

Researcher Portal File #: 20193089

Dear Dr. Zhiwei Gao:

This e-mail is to inform you that your closure event – Event No. [565265] - for study HREB # 2019.072 – Sex-specific environmental and genetic determinants of COPD in middle-aged and older Canadian adults: An analysis of Canadian Longitudinal Study on Aging (CLSA) data - was reviewed by the [Chair] and has been approved and/or acknowledged (as indicated in the Researcher Portal).
You may view this decision by logging into the Researcher Portal.

It is your responsibility to seek the necessary organizational approval from the Regional Health Authority (RHA) or other organization as appropriate. You can refer to the HREA website for further guidance on organizational approvals.

Thank you,

Research Ethics Office

Appendix B

Journal Permission

Good Afternoon, Ugochukwu Odimba

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