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Stephen John Onufrak

Asthma and Atherosclerosis

By

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Asthma and Atherosclerosis

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B.S. Pennsylvania State University, 1996
M.P.H. Emory University, 2003

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An Abstract of
A dissertation submitted to the Faculty of the Graduate School of
Emory University in partial fulfillment of
The requirements for the degree of
Doctor of Philosophy

Department of Epidemiology

2007

ABSTRACT

Asthma and atherosclerosis are clinically different diseases that share a common biological mechanism: inflammation. Thus, it is plausible that alterations in systemic inflammatory response among asthmatics could result in increased inflammation in the artery wall and predispose them to atherosclerosis. Asthma has also been associated with atherosclerotic disease in several studies, with some suggestion that the asthma-atherosclerosis association may be limited to or stronger among women. Although asthma-related inflammation is a biologically plausible mechanism of the observed association with atherosclerotic disease, no studies to date have examined this possibility. Furthermore, previous research in this area has generally ignored the increasingly accepted notion that asthma is not a single disease but a syndrome overlying a number of heterogeneous disease subtypes.

Three studies were performed for this dissertation. The first study assessed the cross-sectional association of asthma with coronary artery disease (CAD) among patients undergoing cardiac catheterization at Emory University Hospital and further examined whether haplotypes of genes that regulate leukotriene production were differentially associated with CAD according to asthma status and gender. Analysis indicated that although asthma was negatively associated with CAD in this highly-selected population, the association of ALOX5AP haplotypes with CAD was significantly modified by asthma status and gender whereby a positive association with CAD was observed only among asthmatic women.

The second and third studies of the dissertation utilized data from the Atherosclerosis Risk in Communities (ARIC) study and examined the association of the asthma age of onset subtypes with incident CAD, incident stroke, and carotid intima medial thickness, an indicator of subclinical atherosclerosis. The results of these studies suggest that adult onset asthma, but not child onset asthma, is associated with atherosclerotic disease, and that this association occurs only among women.

Taken together, the results of this dissertation suggest that asthma is associated with atherosclerotic disease among women but not men and that this association differs according to asthma subtype. In addition, our results support the role of leukotriene-mediated inflammation as a biologic mechanism underlying the asthma-atherosclerosis association.

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TABLE OF CONTENTS

Appendix

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Brief Overview

Asthma and atherosclerosis are clinically different diseases that share a common biological mechanism: inflammation (Canonica 2006; Ross 1999). Thus, it is plausible that alterations in systemic inflammatory response among asthmatics could result in increased inflammation in the artery wall and predispose them to atherosclerosis. Recent research connecting inflammatory leukotrienes, long associated with asthma pathogenesis (Votava 1984), with atherosclerosis (De Caterina and Zampolli 2004; Dwyer et al. 2004; Jala and Haribabu 2004) illustrate one of perhaps many inflammatory mechanisms involved in both diseases. Asthma has also been directly associated with myocardial infarction or stroke in several studies, with some suggestion that the asthma-atherosclerosis association may be limited to or stronger among women. Although asthma-related inflammation is a biologically plausible mechanism of the observed association with atherosclerotic disease, no studies to date have examined this possibility. Furthermore, previous research in this area has generally ignored the increasingly accepted notion that asthma is not a single disease but a syndrome overlying a number of heterogeneous disease subtypes. The proposed study examines the association of the general asthma subtypes, adult onset asthma and child onset asthma, with the atherosclerotic outcomes of myocardial infarction and stroke as well as with carotid atherosclerosis. In addition, the study assesses the role of leukotriene-regulating genes in the

association between asthma and angiographic coronary artery disease among patients undergoing coronary catheterization.

Research Purpose

Leukotrienes and the genes that regulate them are involved in the inflammatory process of both asthma and atherosclerosis (De Caterina and Zampolli 2004; Dwyer et al. 2004; Helgadottir et al. Association between the gene encoding 5-lipoxygenase-activating protein and stroke replicated in a Scottish population 2005; Zhao and Funk 2004). There is some evidence to suggest that asthma itself is a risk factor for myocardial infarction (MI), stroke, and atherosclerosis (Knoflach et al. 2005) and that this association may occur among women but not among men (Hubbard and West 2004; Iribarren, Tolstykh, and Eisner 2004; Schanen et al. 2005; Toren and Lindholm 1996). However, further research is required to investigate the association between asthma and atherosclerosis, the possible gender specificity of such association, and the role of asthma subtypes. Furthermore, no previous studies have addressed the role of leukotriene regulating 5-lipoxygenase pathway genes in the asthma-atherosclerosis association. If such genes are indeed implicated, this could provide evidence that the asthma-atherosclerosis association is causal (Keavney 2002).

The central hypothesis of this project is that asthma and atherosclerosis are linked by a common pathophysiological mechanism involving leukotriene-mediated

inflammatory pathways, and that women are particularly susceptible to this association. An additional aim is to examine whether this association is limited to particular asthma subtypes.

Scope of Dissertation

This study represents an effort to clarify and expand on previous reports that asthma is associated with increased incidence of atherosclerotic disease by examining the association among specific asthma subtypes. In addition to this, the study investigates the role of inflammation as a potential biologic mechanism by examining genes that regulate the production of inflammatory leukotrienes. Two components of this research utilize public-use data from the Atherosclerosis Risk in Communities (ARIC) study. This data was acquired through submission of a study proposal and approval letter from the Emory Institutional Review Board (IRB) to ARIC study personnel. The third component of the study utilizes data from an ongoing registry of catheterization patients at Emory University Hospital as well as genetic data from deCode genetics in Reykjavík, Iceland. Research work for the dissertation included providing assistance in questionnaire and database design and revision for an ongoing multi-departmental genetic registry of cardiac catheterization patients, maintaining a large database of questionnaire responses, linking questionnaire data to catheterization and coronary intervention data located in Emory clinical databases, creating algorithms for transforming raw catheterization data into meaningful outcome measures to be used by numerous researchers, assistance in validation of

outcome measurements, cleaning and verifying data, and computer entry of questionnaire data. Additional training for the dissertation included composing a successful pre-doctoral fellowship grant application to the American Heart Association, completing a course in genetic epidemiology, consulting with Emory cardiology faculty and genetic researchers, consulting with genetic researchers at deCode genetics, conducting presentations to Emory faculty and at American Heart Association fundraising events, and presenting results at national research conferences. All projects also required cleaning of data, assembling datasets for analysis, and use of SAS statistical packages for statistical analysis.

Study Responsibilities

All data collection for components of this dissertation involving ARIC data was performed by ARIC personnel and acquired through formal request by Stephen Onufrak to ARIC personnel. The remaining dissertation component is a sub-study of the Cardiogene Database, a registry of cardiac catheterization patients at Emory University Hospital and Emory Clinic. The principle investigator for the Emory Cardiogene Database is Dr. Arshed Quyyumi from the Division of Cardiology in the Emory School of Medicine. Dr. Viola Vaccarino from the Emory Program in Cardiovascular Outcomes Research and Epidemiology (EPICORE) is co-investigator. Fellows from the Division of Cardiology enroll patients undergoing cardiac catheterization, administer questionnaires, and collect blood samples from patients.

Genotyping of blood samples is conducted by deCode Genetics in Reykjavík, Iceland. Data management is performed by EPICORE.

Stephen Onufrak was supported in part by stipends from the Emory Graduate School of Arts and Sciences and by a pre-doctoral fellowship from the Southeast Affiliate of the American Heart Association.

Human Subjects Protection

The protocols for all components of this dissertation were approved by the Emory University Institutional Review Board (IRB) (see Appendices A and B). Stephen Onufrak and all co-investigators successfully completed the human subjects protection training through the Collaborative Institutional Training Initiative (CITI), which provides all such training to Emory researchers. No adverse events or human subjects complaints have occurred as a result of this study.

CHAPTER 2: LITERATURE REVIEW

Background on Atherosclerosis and Atherosclerotic Disease Outcomes

Atherosclerosis is the primary disease process leading to coronary artery disease and stroke. Collectively atherosclerotic diseases represent the leading cause of death among Americans and are responsible for as many deaths as all types of cancer combined (Smith 2005).

Atherosclerosis is generally thought to occur as a result of endothelial dysfunction, accumulation of lipids, and subsequent inflammatory response within the endothelium of medium and large arteries (Ross 1999). Factors potentially leading to endothelial dysfunction include hypertension, diabetes, smoking, and elevated plasma low density lipoprotein and triglycerides (Ross 1999). Endothelial dysfunction results in increased adhesiveness and permeability to leukocytes and low density lipoprotein cholesterol. The inflammatory response to endothelial injury and lipid accumulation involves migration of leukocytes, platelets, and smooth muscle cells to the site as well as the production of cytokines, vasoactive molecules, and growth factors (Ross 1999). As a result, the artery wall gradually thickens to eventually constrict the lumen area and the continued action of macrophages and lymphocytes results in further damage and necrosis creating a complicated plaque (Ross 1999). Complete obstruction of blood flow at atherosclerotic lesion sites may occur due to

lesion disruption, plaque rupture and subsequent clot formation (Libby and Theroux 2005).

If the obstruction site occurs in the coronary arteries supplying blood to the heart, myocardial infarction may occur. Similarly, an obstruction in arteries supplying the brain may result in an ischemic stroke.

Diagnosis and Measurement of Atherosclerotic Disease

Diagnosis of atherosclerotic disease may occur after an individual has experienced an atherosclerotic outcome event such as myocardial infarction or ischemic stroke. Alternatively, diagnosis may occur prior to the occurrence of events based upon a combination of symptoms, risk factor profile, and diagnostics tests. Symptoms of coronary artery disease, that is atherosclerosis of the coronary arteries of significant magnitude to impede blood flow to the myocardium, often include chest pain, called angina, and shortness of breath, called dyspnea, in response to episodes of physical exertion. Transient Ischemic Attacks (TIA's) are self-resolving symptoms of cerebrovascular disease preceding stroke. They may present in a number of different ways depending on the location within the brain where blood flow is interrupted. Examples of TIA symptoms include sudden numbness on one side of the body, acute changes in vision or hearing, loss of balance, memory, or speaking ability. Risk factors for atherosclerotic disease include advanced age, male gender, smoking, hypertension, and diabetes.

Many diagnostic methods exist to measure the presence and burden of atherosclerotic disease among individuals. Noninvasive methods for atherosclerotic coronary artery disease include electrocardiography, echocardiography, various computer imaging methods, and exercise stress testing. Noninvasive diagnostic tests for cerebrovascular disease include ultrasound and computer imaging methods. Catheterization imaging methods are invasive tests to determine the presence of atherosclerotic blockages in the coronary or carotid arteries or the major blood vessels of the brain. Because it is invasive, catheterization is only indicated in the event of positive results for noninvasive tests or when it is believed that a patient is currently experiencing or has recently experienced an acute myocardial infarction or stroke.

Carotid Intima-Media Thickness

The intima and media are the two inner most layers of an artery. At the site of an atherosclerotic lesion, the intima and media layers will thicken as the lesion progresses, even before the lumen of the vessel is occluded (Ross 1999). Carotid intima-media thickness (cIMT) is determined through ultrasound imaging of the carotid arteries using a handheld device pressed upon the neck. It is accepted as a safe, noninvasive, inexpensive, measurement of subclinical atherosclerosis (Greenland et al. 2000). Furthermore, it has been validated as a predictor of stroke and myocardial infarction (Chambless et al. 2000; Chambless et al. 1997; O'Leary et al. 1999). For these reasons, cIMT is widely used as an outcome measurement in studies of atherosclerotic disease.

Cardiac Catheterization and Coronary Angiography

During cardiac catheterization, a catheter is most commonly inserted into the femoral artery of the leg and directed through the artery until it reaches the coronary arteries. Once the catheter is in place, contrast dye which appears opaque when viewed with x-ray imagining, is released through the catheter to flow through the coronary arteries. Using this dye, blood flow through the coronary arteries is visible in digitally amplified moving images using imaging software allowing identification of stenosis sites caused by atherosclerotic lesions. Features of the catheterization imaging software also provide a quantitative estimate of the percent of stenosis. If significant stenosis is detected, the catheter can also be used to perform angioplasty which relieves the stenosis and restores blood flow to the myocardium during the same catheterization episode. Cardiac catheterization is considered a minimally invasive procedure, but has a serious adverse event rate of approximately one per thousand procedures. As such, it is only performed when coronary artery disease is suspected due to patient symptoms, risk factor profile, and results of noninvasive tests.

Genetic Background and Genetic Association Studies

A gene is a region of a chromosome that performs a single function, usually the coding of a specific protein. Depending on the specific genes, the coded proteins may constitute enzymes involved in various physiological pathways. Variations in a specific gene coding for a particular enzyme could affect the biological activity of that enzyme or alter the amount of enzyme produced.

Genetic information on each chromosome is coded by DNA, which is composed of four different nitrogenous bases: adenosine, cytosine, guanine, and thymine, each joined by ester bonds to a backbone of sugar and phosphate groups. The specific sequence of nucleotide bases determines the function of the gene. DNA is double stranded and as such each person has two copies of each gene, one copy is inherited from the person's father and one from the mother. There is less than 0.1% variation in base sequence among humans. One type of variation that is observed is a single nucleotide polymorphism (SNP), in which there are two different observed nucleotide bases at a specific locus of a gene. Within a single gene there may be many observed SNPs. However, geographically adjacent SNPs are often not independent of one another and can be inherited together as a group. As a result, even though a given region of a particular gene might contain N observed SNPs in a certain population, there will often be far fewer observed combinations of the SNPs than the 2^N combinations expected if the SNPs were inherited independently of one another. In this way, the heterogeneity of a gene region within a specific population is

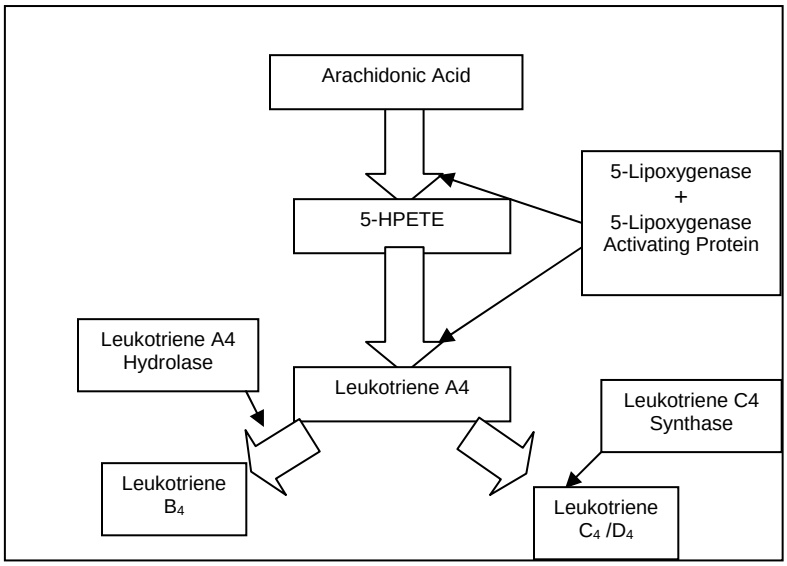
often better described by the observed combination of multiple SNPs rather than by the individual SNPs themselves. These combinations of SNPs are known as haplotypes.

Haplotypes may be used in genetic association studies to determine if a particular haplotype is associated with a phenotype of interest such as a disease. Such studies closely resemble classic epidemiologic case-control studies. If the haplotype is observed more or less frequently among cases than among controls then that haplotype is said to be associated with the disease. A significant association may indicate that the haplotype under consideration is the functional genetic variant; that is the haplotype changes either the protein structure or promotion of protein production for a particular gene. Alternatively, an association may indicate that the observed haplotype is in linkage disequilibrium with the true functional genetic variant. Under this scenario, the haplotype under consideration is likely to be inherited along with the true functional genetic variant associated with increased risk of the disease.

Atherosclerosis and Leukotrienes

Recent research suggests that a class of inflammatory mediators known as leukotrienes may play a role, among other mediators, in the inflammatory response of atherosclerosis (Lotzer, Funk, and Habenicht 2005). Leukotrienes are produced through the 5-lipoxygenase arachidonic acid cascade by leukocytes (figure 2.1).

Figure 2.1: The 5-Lipoxygenase Arachidonic Acid Cascade



At least three genes involved in this cascade have been associated with atherosclerosis or atherosclerotic outcomes in population studies. First, haplotypes HapA and HapB of the arachidonate 5-lipoxygenase-activating protein (ALOX5AP) gene, which is involved in leukotriene regulation by encoding the 5-lipoxygenase-activating protein, have been associated with MI and stroke in several European cohorts (Helgadottir et al. Association between the gene encoding 5-lipoxygenase-activating protein and stroke replicated in a Scottish population 2005; Helgadottir et al. 2004). deCode researchers also reported a significant reduction in leukotriene B_4 and C-reactive protein levels in a randomized trial of a ALOX5AP inhibitor drug (Hakonarson et al. 2005). Subsequent studies attempting to replicate results of the genetic association studies have been mixed. Japanese researchers found that alleles of these haplotypes were rare in their Japanese study population but identified an

additional two-SNP haplotype, HapAC, that was associated with MI in their study population (Kajimoto et al. 2005). Girelli et al. found a significant association between HapB and a related Haplotype called HapC with angiographic CAD but did not find an association with HapA (Girelli et al. 2007). Kaushal et al. reported an association of stroke with a SNP featured in HapA but did not examine the HapA haplotype itself (Kaushal et al. 2007). Zee et al. reported no association between HapA and HapB with either MI or stroke in a nested case-control study using data from the Physicians' Health Study (Zee et al. 2006).

Second, carriers of two variant alleles of the 5-lipoxygenase promoter gene (ALOX5) have been found to have increased atherosclerosis as measured by carotid intima-media thickness (Dwyer et al. 2004). Third, a single haplotype (HapK) of the gene LTA4H, which encodes an enzyme involved in the leukotriene cascade called leukotriene A4 hydrolase, has also been associated with MI, particularly among African Americans (Helgadottir et al. A variant of the gene encoding leukotriene a4 hydrolase confers ethnicity-specific risk of myocardial infarction 2005). Finally, animal and human invitro studies have shown increased expression of 5-lipoxygenase and LTA4H in atherosclerotic lesions and an association with plaque instability (Qiu et al. 2006).

Background on Asthma

Asthma is a disease characterized by hyperreaction of airway tissues to external or internal stimuli resulting in temporary reduction in breathing function. During an asthmatic episode, airways are obstructed due to smooth muscle contraction, secretions, and bronchial inflammation (Moseby 2001). According to the 2003 National Health Interview Survey, 9.7% of all adults over the age of 18 were told at some point during their life that they have asthma by a health professional and 6.4% of all adults currently have asthma (National Center for Health Statistics 2003). Women have a higher prevalence of ever being diagnosed with asthma than men (11.3% verses 8.0%) and a higher prevalence of current asthma than men (8.1% verses 4.6%) (National Center for Health Statistics 2003).

Asthma Subtypes

Asthma can be classified according to many epidemiologically or biologically distinct subtypes (Bel 2004; Miranda et al. 2004; Wenzel 2006). It is unclear whether these subtypes represent unique diseases that present as a common syndrome or whether the various subtypes represent different manifestations of the same disease (Bel 2004). One subtype differentiation is that of intrinsic asthma versus extrinsic asthma (Rackemann 1947). In extrinsic (or atopic) asthma, the stimuli which induce hyperreactive response are allergens such as pet dander, plant pollens, mold, arthropods, and house dusts (Moseby 2001). Patients with extrinsic asthma produce

allergen specific immunoglobulin E (IgE) in response to one or more of these specific allergens (Moseby 2001). In intrinsic asthma, asthma episodes are induced through nonallergic stimuli including cold air, exercise, chemicals, drugs (especially aspirin related drugs) and smoke (Moseby 2001).

Asthma can also be subtyped according to childhood or adult age of onset (Bel 2004). In general, childhood onset asthma is most often extrinsic and occurs more commonly among males than among females. While most childhood asthma subsides before adulthood, some cases may persist and may be considered as a separate category from either childhood or adult onset asthma. Generally, adult onset asthma occurs more commonly among females and is more likely to be intrinsic (Bel 2004). There is evidence that inflammatory mechanisms differ among patients with child or adult onset asthma (Hsu et al. 2004; Miranda et al. 2004); overall conclusions suggest a larger role of leukotriene-mediated inflammatory response in adult onset asthma. Although prevalence of allergen specific immunoglobulin E (IgE) decreases with increasing age of asthma onset (Hsu et al. 2004), adult onset asthmatics have higher bronchial tissue eosinophil counts (Hsu et al. 2004) and twice as high urinary leukotriene E4 levels compared to child onset asthmatics (Miranda et al. 2004). The number of polymorphonuclear (PMN) cells in bronchial secretions, which produce leukotrienes, is also higher among adult onset asthmatics while child onset asthmatics have a greater number of CD3 (+) lymphocytes (Miranda et al. 2004)

Asthma and Sex Hormones

It has long been recognized that susceptibility to developing asthma and exacerbation of existing asthma can be affected by changes in female sex hormones (Forbes 2005). Asthma incidence among females rises sharply at the onset of puberty (de Marco et al. 2000) and then again during the perimenstrual shift preceding menopause (Balzano et al. 2001). Asthma incidence is also increased among users of estrogen replacement therapy (Lange et al. 2001). Perimenstrual asthma is a special subtype of asthma that occurs among women and is characterized by a worsening of asthma symptoms during the premenstrual or menstrual phase of the menstrual cycle and affects 30-40% of women who are seen at asthma/allergy clinics (Vrieze, Postma, and Kerstjens 2003). The biological mechanisms behind perimenstrual asthma are not known, but an effect of hormonal fluctuations on inducing leukotriene-mediated inflammation is one of the suspected mechanisms (Vrieze, Postma, and Kerstjens 2003). This concept is supported by the observation that asthma severity is decreased among asthmatic women taking oral contraceptives, which reduce hormonal fluctuation (Salam, Wenten, and Gilliland 2006). The human uterus is capable of synthesizing leukotrienes and uterine leukotriene release peaks during menstruation (Rees et al. 1987) . Furthermore, leukotriene levels have also been associated with severity of dysmenorrhea (Harel et al. 2000; Nigam et al. 1991). Recent studies also suggest that estrogens modulate the release of proinflammatory cytokines from activated monocytes, macrophages (Kramer, Kramer, and Guan 2004), and vascular

cells (Miller et al. 2004; Xing et al. 2007) and also regulate the production of leukotrienes from mast cells (Zaitsu et al. 2007).

Asthma and Leukotrienes

Leukotrienes are also implicated in the pathogenesis of asthma, and drugs which act as either leukotriene inhibitors or antagonists have proven effective in the treatment of asthma (Ramires et al. 2004). The effectiveness of leukotriene inhibitors has been shown to vary according to the number of mutant alleles present on the ALOX5-promoter genotype (Drazen et al. 1999). The same mutation has also been linked to severity of aspirin sensitive asthma (Kim et al. 2005), asthma severity among children (Kalayci et al. 2006), and asthma exacerbation rates (Lima et al. 2006). Haplotypes of the ALOX5 gene and a SNP of the LTA4H gene have also been associated asthma exacerbation rates (Lima et al. 2006). However, recent population studies have failed to replicate earlier research linking polymorphisms of the ALOX5AP gene with asthma (Kedda et al. 2005; Koshino et al. 1999; Sayers et al. 2003).

Previous Studies of The Association of Asthma and Atherosclerosis

At least eight studies have examined the association between asthma and risk of atherosclerotic outcomes, (Iribarren, Tolstykh, and Eisner 2004; Knoflach et al. 2005; Liss et al. 2000; Musk et al. 1987; Schanen et al. 2005; Soriano et al. 2005;

Toren and Lindholm 1996; Zureik et al. 2004) although many of these studies have limitations such as not adjusting for potential confounders, and not examining potential differences by gender. All of these studies reported positive associations except one that reported no association between asthma and coronary artery disease but found an association between asthma and stroke (Schanen et al. 2005). Four studies reported standardized (for age, gender, and time period) mortality ratios, rate ratios, or crude t-tests (Musk et al. 1987; Toren et al. 2004; Toren and Lindholm 1996; Zureik et al. 2004), and four performed multivariate survival analysis or logistic regression (Iribarren, Tolstykh, and Eisner 2004; Knoflach et al. 2005; Musk et al. 1987; Schanen et al. 2005). Moreover, four of the eight presented gender-specific analysis (Iribarren, Tolstykh, and Eisner 2004; Schanen et al. 2005; Toren et al. 2004; Zureik et al. 2004). Three of these four studies suggested a stronger association of asthma and atherosclerotic outcome risk among women (Iribarren, Tolstykh, and Eisner 2004; Schanen et al. 2005; Toren and Lindholm 1996) while one very small study of asthma and carotid intima-media thickness suggested a stronger association among men and no association among women but did not present multivariate analysis (Zureik et al. 2004).

The first study compared causes of death among patients treated in an outpatient facility for asthma and reported age, gender, and calendar year standardized mortality ratios (SMR) of 1.8 (95% CI: 1.3 to 2.1) for all vascular disease and 1.9 (95% CI: 1.4 to 2.4) for ischemic heart disease (Toren and Lindholm 1996). Stratification by gender suggested stronger associations between asthma and

cardiovascular disease among women (All Vascular Disease SMR = 1.9, 95% CI: 1.3 to 2.4; Ischemic Heart Disease SMR = 2.5, 95% CI: 1.7 to 3.3) than among men (All Vascular Disease SMR = 1.6; 95% CI: 1.1, 2.1; Ischemic Heart Disease SMR = 1.4; 95% CI: 0.8 to 2.0). That study did not perform multivariate analysis and did not test for interaction between gender and cardiovascular outcomes.

The second study examined the incidence of various comorbidities including MI and angina among patients (including children) with newly diagnosed asthma or chronic obstructive pulmonary disease but did not perform analysis stratified by gender or present multivariate analysis (Soriano et al. 2005). The rate of MI and angina among newly diagnosed asthmatics of all ages did not differ from nonasthmatic counterparts. However, the investigators reported that among subjects over 65 years of age, the rate for MI and angina were 1.33 and 1.30 times (95% CI or p-value not reported) that of nonasthmatics. Because cases had newly diagnosed asthma, the association detected among older subjects only lends evidence to the notion that adult age of asthma onset may be more strongly associated with atherosclerosis than child onset.

The third was an Australian study which reported an elevated SMR (SMR = 1.3, 95% CI and p-value not reported) for ischemic heart disease among asthmatic patients over 65 years of age but did not present multivariate or gender specific analysis (Musk et al. 1987). In the fourth study, researchers examined a cohort of Canadian workers and reported an elevated risk of cardiovascular disease among

those with occupational asthma claims compared to those with injury claims (HR = 1.4, 95% CI: 0.9 to 2.0) and a slightly elevated risk of ischemic heart disease (HR = 1.2, 95% CI: 0.7 to 2.1) but again did not present analysis stratified by gender (Liss et al. 2000). This study was adjusted for period of birth, sex, and period of accident.

The fifth study was a cohort study that investigated rate of coronary artery disease death or hospitalization among asthmatic patients in a large managed care association (Iribarren, Tolstykh, and Eisner 2004). Patients were classified as asthmatic based upon either self-report of physician-diagnosed asthma or on inpatient admission due to asthma. That study reported multivariate adjusted relative rates of CAD of 1.22 (95% CI: 1.14 to 1.31) among asthmatic women and 0.99 (95% CI: 0.93 to 1.05) among asthmatic men. Although this study included multivariate adjustment for many variables including smoking status, the authors were unable to adjust for physical activity or asthma medication use.

The sixth study on the association between asthma and atherosclerosis was performed using data from the Atherosclerosis Risk in Communities Study (Schanen et al. 2005). That study compared rates of coronary artery disease and stroke among study participants according to self-reported asthma status. The authors reported no significant elevation in CAD risk among either asthmatic men or women but found significantly elevated rate of stroke among asthmatics compared to nonasthmatics (Hazard Ratio = 1.5; 95% CI: 1.04 to 2.15). Although the authors reported that effect modification on the asthma/stroke association by gender was statistically

nonsignificant, the rate ratios differed substantially among men (HR = 0.72, 95% CI: 0.26 to 1.95) and women (HR = 2.20, 95% CI: 1.25 to 3.90). That study controlled for smoking status, physical activity, and other covariates but not for asthma medication use.

The seventh study compared carotid artery intima-media thickness (IMT) according to bronchial hyperresponsiveness in a French population and included sub-analysis according to asthma history (Zureik et al. 2004). The study found that bronchial hyperresponsiveness was associated with increased IMT among men but not among women after multivariate analysis. In contrast to other research, this study also found that asthma was associated in crude analysis with increased IMT men but not among women. The study population, however, included only six asthmatic men and twenty asthmatic women and did not present multivariate analysis for asthma. This is the only study to report an association between asthma and atherosclerosis in men but not in women.

The eighth study examined the association between asthma, allergic rhinitis, IgE level and atherosclerosis in two separate European study cohorts (Knoflach et al. 2005). In the first cohort, researchers compared the five-year progression of atherosclerotic plaques of the carotid arteries in Italian men and women ages 40 to 79 according to asthma and/or allergic rhinitis status. Patients with significant increase in atherosclerosis were more likely to have allergic rhinitis or asthma (multivariate adjusted Odds Ratio[OR]=3.9; 95% CI: 1.3 to 11.5) and higher IgE (multivariate

OR=1.7, 95% CI 1.1 to 8.0). Gender stratified results were not presented. The second cohort in this study consisted of a small group of male army recruits that included only two subjects with asthma due to military physical health requirements. The investigators reported that recruits having at least one carotid or femoral artery IMT measurement site exceeding the 90th percentile were more likely to have allergic rhinitis or asthma (multivariate OR=3.0, 95% CI: 1.1 to 7.9). IgE did not significantly differ according to IMT status in this cohort. This study is the first to suggest that allergic rhinitis may be associated with atherosclerosis.

Rationale and Significance of the Proposed Studies

Asthma is a common disease affecting approximately 10% of the United States population some time during their lives (National Center for Health Statistics 2003). Furthermore, atherosclerotic diseases represent the leading cause of death among Americans (Smith 2005). Therefore, the detection of an independent association between asthma and atherosclerotic disease could help to identify a very large number of individuals with increased risk of cardiovascular disease. Because the proposed studies investigate the relationship between atherosclerosis, leukotriene genotypes, asthma phenotypes, and gender, these data will help to elucidate a biologically plausible pathway and causal link between asthma and atherosclerosis.

At least three studies suggest that the association is either limited to or stronger among women but the biological mechanism behind this gender specificity is

unknown. Our study seeks to confirm the observed gender specificity and further examine whether alleles of leukotriene-regulating genes are differentially associated with CAD according to gender and asthma status. These will be important data for the understanding of the pathophysiology of CAD in women.

Because asthma is a heterogeneous disease with many subtypes that differ by gender, analysis of atherosclerotic risk associated with specific asthma subtypes may provide insight into the biological mechanism and the gender specificity of the association. Furthermore, research of specific asthma subtypes would help in the identification of individuals at increased risk of atherosclerosis.

Primary Research Hypotheses

1. Asthma age of onset subtypes are associated with MI and stroke.
    a. Adult onset asthma is associated with incident MI and stroke.
    b. Child onset asthma is associated with incident MI and stroke.
    c. There is interaction between asthma subtypes and gender on MI and stroke risk.

2. Asthma age of onset subtypes are associated with carotid intima-media thickness (cIMT).
    a. Adult onset asthma is associated with cIMT
    b. Child onset asthma is associated with cIMT
    c. There is interaction between asthma subtypes and gender on cIMT

3. Asthma is associated with angiographic coronary artery disease (CAD).
    a. There is interaction between asthma and gender on prevalence of CAD.
    b. There is interaction between asthma and ALOX5AP or LTA4H genotype on prevalence of CAD.
        1. There is interaction between asthma and the HapA haplotype of the ALOX5AP gene on prevalence of CAD.

2. There is interaction between asthma and the HapB haplotype of the ALOX5AP gene on prevalence of CAD.
3. There is interaction between asthma and the HapK haplotype of the LTA4H gene on prevalence of CAD.

c. There is interaction between asthma, gender, and ALOX5AP or LTA4H genotype on prevalence of angiographic CAD.

CHAPTER 3: METHODOLOGY

Overview of Dissertation Projects

This dissertation consists of three projects designed to investigate the association between asthma and atherosclerosis. The primary project involves data collection, genotyping, and cross-sectional analysis of patients undergoing coronary catheterization at Emory University Hospital and the Emory Clinic. Two additional projects involve acquisition, formatting, and analysis of existing data from the Atherosclerosis Risk in Communities (ARIC) Study to examine the association of asthma age of onset subtypes with clinical atherosclerotic outcomes and subclinical atherosclerosis.

Project One

Overview of Project One

Project One is a two-year cross-sectional study of the role of asthma history on the presence of coronary artery disease (CAD) among patients undergoing cardiac catheterization at Emory University clinical sites. Patients were currently being enrolled as part of a larger, ongoing Emory study (the Emory Cardiogene Database Study) which is a collaborative project with deCode Genetics. deCode genetics is a multinational biopharmaceutical company headquartered in Reykjavik, Iceland that

performs population-based genetic disease research. The Emory Cardiogene Database Study was approved by the Emory University Institutional Review Board. Approximately 1000 patients had already been enrolled as of December, 2005. Data collection continued for the purposes of this study until April 10, 2007, at which time 2305 patients were enrolled. All patients undergoing cardiac catheterization at Emory University Hospital and the Emory Clinic were eligible to enroll in the study. Patients who agreed to participate were interviewed at the time of catheterization by trained study personnel and information was collected on history of asthma, age of asthma onset, smoking history, physical activity, and basic demographic information such as age, gender, and race. Information on other comorbidities, CAD risk factors and other medical history, such as hypertension, diabetes, hyperlipidemia, and body mass index, was obtained through self-report and confirmed by patient hospital records. A history of coronary interventions, such as angioplasty and bypass graft surgery, that would affect outcome measurement was also determined through self-report and hospital records. Blood samples were collected for analysis of genetic factors and inflammatory markers; these samples were shipped overnight in batches to deCode genetics, who performed genotyping. The study was approved by the Emory University Institutional Review Board.

Patient Protection and Confidentiality Procedures for Project One

All study participants in this project were participants in the Emory Cardiogene Database Study, an ongoing registry approved by Emory University's

Institutional Review Board. This study adhered to the same procedures for minimizing risks as those employed by the Emory Cardiogene Database Study. Those risks and procedures are summarized below.

Study participants were patients undergoing coronary catheterization at Emory University Hospital. All patients were referred for catheterization by their personal physicians and cardiologists because of suspected or known coronary artery disease or other cardiovascular disease. Under no circumstances were patients asked to submit to catheterization merely for research purposes. The main risks from participating in the study were: the potential for release of confidential information and associated problems, such as insurance and employment discrimination; possible risk of bleeding, bruising, infection, and inflammation from the blood draw procedure..

Informed consent was obtained from all study subjects by trained, IRB-approved study personnel. All information was kept private. All scientific reports, publications, or presentations written using information from this study do not identify patients by name or any other individual identifiers. All informed consent forms, study questionnaires, and other study documents containing sensitive information are kept in confidential folders in locked file cabinets in the study personnel office. Access to study information in computer databases is restricted by a database manager and is password protected. A unique study ID number was assigned to each study subject in the dataset used for analysis. This number is not associated

with any personal identifiers such as social security number. The only individuals who can see patient identifiers such as name, social security number, date of birth, or address are the principal investigator, research coordinators, and database managers. Blood samples are identifiable only by study ID number.

Blood samples were obtained by nurses or physicians trained to minimize the risk of infection, bruising, and inflammation. The research investigators for this study and all future studies are forever prohibited from any attempt to use collected blood materials to attempt to clone a human being. All information on participant genotypes will be kept confidential. Any papers or publications resulting from this study will not contain individually identifiable genetic information. Theoretically, DNA testing could lead to information that could affect employability and/or insurability. However, the genetic tests done in this study were experimental, and do not have clinical applications. It is our policy not to disclose such information.

Population and Exclusions for Project One

Emory University Hospital is a leading regional center for interventional cardiology treatments and routinely conducts coronary catheterizations on a racially diverse patient population from Georgia, North Carolina, South Carolina, and Alabama. Approximately 75% of patients are white or Caucasian, 20% are African American, and 5% have other races. Approximately 60% are male and 40% are female. Individuals are not excluded from the study on the basis of race or gender.

The response rate so far has been greater than 95%. For the present study, patients will be excluded if they have a history of heart transplant because of the difficulty of interpreting coronary catheterization results among these patients.

Data Collection Procedures for Project One

Each day at Emory University Hospital and the Emory Clinic, a trained research assistant approached all patients undergoing scheduled coronary catheterization. After informed consent was obtained, the research assistant led the patient through a series of questionnaires for approximately 20 minutes (Appendix D). Using questions from populations studies, such as NHANES and ARIC, the questionnaires addressed a variety of factors such as patient medical history (including history of asthma), family history of disease, and health behaviors (smoking and physical activity).

Information on angiographic results and intervention history was prospectively collected on all patients undergoing cardiac catheterization at Emory. Data were entered by the examining clinical house staff in an electronic database managed by the Emory Heart Center Information Services and available for use in clinical studies (after obtaining specific IRB approval). After merging with the patient questionnaire and genetic data, this clinical information was utilized for analysis in our study.

Blood Collection Procedures

A nurse or physician collected 60 milliliters of blood from each patient at the time of catheterization. The samples were processed at the Emory General Clinical Research Center (GCRC) Core Laboratory where the plasma and the buffy coat were separated and aliquoted. The GCRC also stored the samples until they were periodically shipped to deCode genetics for DNA analysis.

Role of Candidate in Data Collection

The candidate designed questions regarding asthma status and also aided in revision and updating of the study questionnaire. These revisions included the addition of new modules as well as the identification and correction of existing questions, based on pilot data, for potentially ambiguous questions and misunderstood skip patterns. The candidate was also responsible for managing data entry, assisting in database maintenance and revision, linking questionnaire data to catheterization results and interventional history data, data cleaning, and extensive programming to derive useful and relevant exposure and outcome variables for all potential users of data from the Emory Cardiogene Databank.

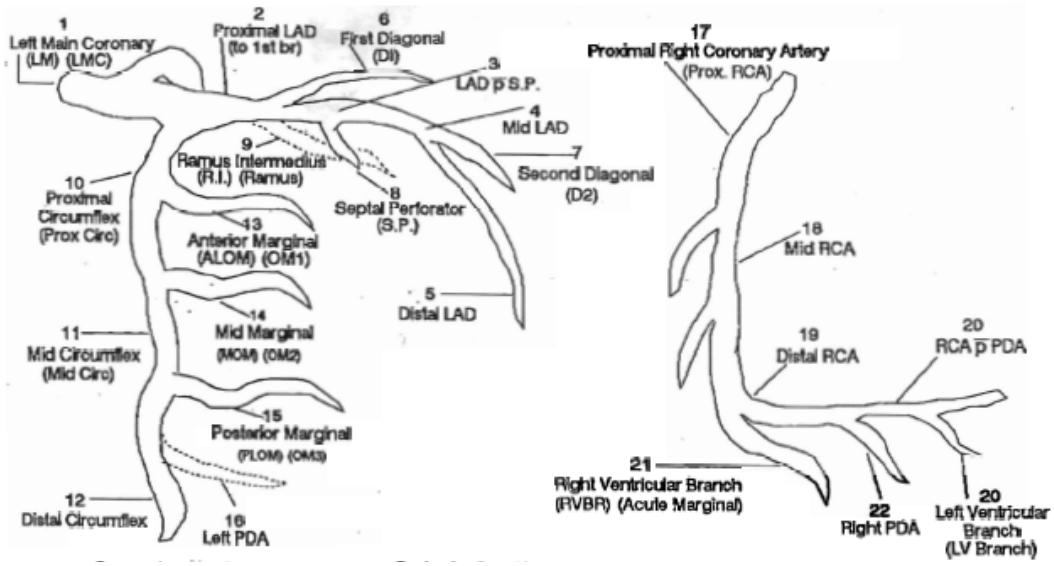
Data Collection Variables for Project One

Asthma Status. Asthma (self-reported) was examined in two ways. First, asthma was classified as a dichotomous exposure according to self report of ever being diagnosed with asthma or not. Asthma was also further measured as a three-level exposure factor according to either child onset (age<21 years), adult onset (age≥21 years), or no history of asthma.

Study Outcome. Cardiac catheterization results were recorded by Emory physicians at the time of catheterization. For each atherosclerotic lesion detected, physicians reported both the lesion severity, expressed according to percent luminal occlusion at the lesion site, as well as the anatomical location within the left or right coronary arteries (see figure 3.1). These anatomical locations were coded according to an Emory institutional standard (see Appendix E). Because the catheterization population features patients with previous coronary disease, a combined CAD outcome of current coronary angiography and history of coronary disease was used as the study outcome. Patients were classified as CAD positive if they had at least one ≥50% stenosis as determined through catheterization, or a positive history of MI, percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass surgery (CABG). Derivation of angiographic CAD status was performed through processing of raw catheterization result data through a computer algorithm designed primarily by the applicant. A sample of CAD scores derived by the computer

algorithm was compared to scores obtained by a trained physician scoring recorded catheterization films.

Figure 3.1: Anatomy of Coronary Arteries



(Source: Emory University)

Genetic Methods for Project One

Genotyping was performed by deCode genetics; this work had already been done in a preliminary sample of 713 subjects from our institution (Helgadottir et al. 2005) and was continued in the full sample. To initially identify SNPs and potential causal variants in LTA4H, deCode personnel sequenced across the gene region (42 kb) in 93 Icelandic MI patients and controls(Helgadottir et al. 2005). No coding sequence variants that lead to amino acid substitutions were found. However, from this effort 8 SNPs were selected, which together with 2 public SNPs in the 5´ region

of the gene, were genotyped. The genotyped SNPs extend 11.9 kb upstream and 1 kb downstream of LTA4H (NM_000895) and were selected to capture all haplotypes above 2% frequency across the gene region.

deCode personnel also sequenced ALOX5AP in 93 Icelandic MI patients and controls to identify SNPs (Helgadottir et al. 2004). The sequenced region covers 60 kb containing ALOX5AP, including the five known exons and introns, the 26-kb region 5′ to the first exon and 7-kb region 3′ to the fifth exon. Of the 144 SNPs identified, 96 were excluded from consideration because of low allele frequency or complete collinearity with other SNPs. For the present study, we will examine the HapK haplotype of the LTA4H gene and the HapA and HapB haplotypes of the ALOX5AP (FLAP) gene.

The HapA haplotype of this gene is defined by the SNPs SG13S25 (G), SG13S114 (T), SG13S89 (G), and SG13S32 (A). The HapB haplotype of this gene is defined by the SNPs SG13S377 (A), SG13S114 (A), SG13S41 (A), and SG13S35 (G). The HapK haplotype can be discerned by the SNPs rs1978331 (A), rs17677715 (T), rs2540482 (C), rs2660845 (G), rs2540475 (G), rs22448570 (T), and rs2660898 (T).

Statistical Procedures for Project One

The principal independent variables include:

1. Asthma history (initially classified as a dichotomous variable of ever being diagnosed with asthma or not, and further classified as either child onset if onset age<21 years or adult onset if onset age≥21 years)

2. HapA and HapB haplotypes of 5-lipoxygenase activating protein (FLAP) gene and Hap K haplotype of leukotriene A4 hydrolase (LTA4H) gene.

3. Demographic variables (e.g. age, race, gender)

4. Comorbidity variables and CAD risk factors (e.g. diabetes, hypertension, body mass, etc.)

5. Behavioral variables (e.g. physical activity, smoking status)

The dependent variable is prevalent coronary artery disease (CAD) as indicated by one or more of the following:

1. ≥50% stenosis of at least one of the three main coronary arteries during catheterization at time of enrollment

2. History of myocardial infarction prior to enrollment

3. History of CABG or PTCA procedure prior to enrollment

Baseline covariates were compared according to gender and asthma history using chi-square and t-tests. We also evaluated the bivariate association of HapK, HapA, and HapB haplotypes of the ALOX5AP and LTA4H genes with asthma and CAD phenotypes.

We first performed crude analysis of the asthma-CAD relationship. We then fit multivariable logistic models to control for potential confounding and allow assessment of effect modification (interaction) of asthma phenotype with gender. In addition to the asthma variable, the multivariable models contained age, gender, race, smoking, physical activity, hypertension, diabetes, and hyperlipidemia Analyses were also repeated with asthma classified according to age of onset.

Using a subset of subjects for whom genetic data was available, we then fit multivariable models that included terms for genotypes of interest to determine the extent to which the asthma-CAD association is explained by these genes. These models included the HapA, HapB, and HapK haplotypes. We tested interaction terms for asthma history and gender, asthma history and genotype, and three-way interactions of asthma, gender, and genotype using likelihood ratio tests and Wald tests. Final models were assessed for collinearity.

Power and Sample Size for Project One

Prior to commencement of the study, the following power calculations were performed to ensure that the study was adequately powered given the expected number of subjects enrolled during the timeframe of the study.

At the time of the analysis, 2305 subjects were available enrolled in the Emory Cardiogene Database. Of these, 247 subjects were excluded because of missing data on asthma status, 326 subjects were excluded because of unavailable catheterization results, 96 subjects were excluded due to history of heart transplant because of difficulty interpreting angiographic results for these patients, and 40 were excluded due to unknown history of myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass surgery (CABG). This left 1594 subjects (1078 men and 516 women) available for analysis. After these exclusions, the study population contained 63 men and 82 women reporting history of asthma. Prevalent CAD was present in 71.1% of all patients (77.9% of men and 56.8% of women).

Given these estimates, we should have 80% power to detect an asthma-CAD prevalence ratio (PR) of at least 1.2 among all participants. Stratifying by gender, we should have 80% power to detect a PR of at least 1.3 among women and at least 1.2 among men.

For the analysis of leukotriene genes, data for genetic markers was available for 1173 subjects. Prevalence of the HapK and HapA haplotypes are both approximately 15%. The HapB haplotype is mutually exclusive of HapA and has a prevalence of approximately 8%. Among women, power is approximately 80% to detect haplotype-CAD prevalence ratios of 1.5 for haplotypes HapA and HapK and 1.9 for HapB. Among men, power is approximately 80% to detect haplotype-CAD prevalence ratios of 1.3 for HapA and HapK and 1.5 for HapB.

Strengths and Limitations of Project One

Strengths

- This appears to be the first study to examine the role of 5-lipoxygenase genes in the association between asthma and atherosclerosis.

- By examining the role of this genotype, we were able to gain insight into the causal role of asthma-related inflammatory response in the etiology of CAD.

- By examining asthma age of onset subtypes, this study helps to better identify individuals at increased risk of atherosclerotic disease and help to elucidate the gender specificity of the asthma-atherosclerosis association that has been observed in previous studies.

- The study population is diverse in regards to race and gender.

Limitations

- The use of self-reported asthma history may have resulted in misclassification of exposure and may include cases of chronic obstructive pulmonary disease and other respiratory disorders. However, self-reported measures of asthma

are typically employed in large epidemiologic studies, due to feasibility issues.

- The study population is not a random sample of the general population. As such, non-asthmatics in the study population may be different in regard to comorbidities and CAD risk when compared to non-asthmatics in the general population. This may affect the generalizability of the findings.

- Sample size may result in inadequate power to detect gene-asthma or gene-asthma-gender interactions in multivariate models. However, since this is the first study to look at such interactions, this study may be considered exploratory in this area.

- Because the study is cross-sectional, measurement of CAD is limited to prevalent disease, which may make the results susceptible to selection bias due to selective survival. Also, the prevalent CAD outcome includes self-reported history of myocardial infarction, which may introduce misclassification of CAD.

Projects Two and Three

Overview of Projects Two and Three

The second and third projects of this dissertation utilize public-use data from the Atherosclerosis Risk in Communities (ARIC) Study to assess the association between child and adult onset asthma with incident coronary artery disease (CAD), stroke, and carotid intima-media thickness (cIMT). The study of incident CAD and stroke follows a prospective cohort study design and utilizes survival analysis methods. The study of cIMT features a cross-sectional study design and uses linear regression analysis methods.

Patient Protection and Confidentiality Procedures for Projects Two and Three

All subjects in the ARIC study provided informed consent for collection of medical information, contact for follow-up, access to medical records, and sharing of non-personal information with other researchers. Data for the present studies was received from ARIC study personnel on a data disk. The dataset contains no personal identifiers and all subjects are identified only by a unique cohort identification number that cannot be traced back to the participant. The study protocol was approved by the Emory University IRB. All manuscripts prepared using ARIC data were reviewed by ARIC personnel to further ensure that patient confidentiality is maintained.

Population and Exclusions for Projects Two and Three

The Atherosclerosis Risk in Communities (ARIC) study is a prospective study of the etiology of atherosclerotic, cardiovascular, and cerebrovascular disease in four communities in North Carolina, Mississippi, Minnesota, and Maryland. The study population of 15,792 men and women ages 45 to 64 years includes both black and white participants. Subjects were enrolled and underwent baseline examination including measurement of cIMT between 1987 and 1989. Follow-up for cardiovascular and cerebrovascular events is ongoing.

For the study of incident MI and stroke, 47 subjects were excluded because follow-up data was not available and 159 subjects were excluded because of missing data for asthma status. We also excluded 320 subjects with a self-reported history of stroke and 692 subjects with prevalent coronary artery disease, defined as a history of MI, silent MI, or revascularization surgery at baseline. This left 14,567 subjects for analysis.

For the study of cIMT, 167 subjects were excluded because of missing asthma history data; 1154 subjects were excluded because of missing carotid IMT data; and 81 subjects were excluded due to history of endarectomy because IMT thickness may have been altered in these subjects. Carotid angioplasty and stenting were not yet being performed at the time IMT was measured in these subjects. An additional 763 subjects were excluded because of missing data for smoking, hypertension, diabetes,

body mass index (bmi), physical activity, and lipid profile. This left 13,627 subjects available for analysis.

Data Collection Procedures for Projects Two and Three

Data was collected by trained ARIC study personnel according to ARIC protocols. Participants were enrolled between 1987 and 1989 at four study sites: Washington County, Maryland; Jackson, Mississippi; Suburban Minneapolis, Minnesota; and Forsyth County, North Carolina. Households within each area were selected using probability sampling. All members of selected households between the ages of 45 and 64 years were invited to participate in the study. Following enrollment, participants were interviewed in their home by an ARIC interviewer with questions covering five general areas: health status and risk factors; family medical history; smoking; employment; and education. During the home interview, participants were also scheduled for a visit to the ARIC field center for clinical examination. Components of clinical examination included measurement of sitting blood pressure, review of medications taken by study participants, anthropometry, electrocardiography (ECG), pulmonary function, collection of fasting blood sample, brief physical examination of systems, interview for medical history and dietary habits, and ultrasound measurement of carotid intima-media thickness.

Participants were contacted by mail and telephone annually within one month of the anniversary of their clinical examination to ascertain any medical events that

occurred during the past year and to verify physical address. Medical records were reviewed by ARIC personnel for any events reported during annual follow-up. Vital status of subjects was also ascertained through systematic review of death certificates and obituaries for the four study communities. Medical records were reviewed for deaths occurring in the hospital. For deaths occurring out of the hospital, family members and physicians of the participant were contacted and death certificates are reviewed to determine cause of death. All medical events or deaths among cohort members were systematically reviewed according to established criteria by members of the ARIC Morbidity and Mortality Classification Committee to determine outcome classification of coronary artery disease or cerebral vascular disease. Participants also underwent three subsequent clinical examinations occurring every three years after the initial examination. The content of these examinations was generally similar to the initial examination.

Data Collection Variables for Projects Two and Three

Asthma Status. Classification of asthma status was based upon self-report of physician diagnosis and self-reported age of asthma onset. Asthma (self-reported) was measured as a three-level exposure factor according to either child onset (age<21 years), adult onset (age≥21 years), or no history of asthma.

Study Outcomes. The first study utilizing ARIC data uses the study outcomes of incident CAD and stroke. Incident CAD includes definite or probable MI or death

attributed to coronary artery disease. Potential non-fatal CAD events in cohort members were identified through the annual follow-up interview of subjects and surveys of area hospital discharge lists for the names of study participants. When potential events were detected through follow-up interview or when discharge summaries featured diagnosis codes for cardiovascular disease, diabetes, stroke, or included stroke-related keywords, hospital records were abstracted by trained ARIC study personnel. Subsequent classification of definite or probable MI was based upon a combination of chest pain, elevation in cardiac enzyme levels, and ECG changes. Classification of definite or probable CAD events in subjects presenting with chest pain required either a positive ECG finding or abnormal cardiac enzyme levels. Classification of CAD events in subjects without chest pain required both a positive ECG finding and the presence of abnormal cardiac enzyme levels. For nonfatal strokes, information was abstracted from medical records and classified according to stroke type based upon presenting symptoms and diagnostic test results. Classification of stroke was performed using computer algorithm and independently verified by a member of the ARIC Stroke Morbidity and Mortality Classification Committee.

Deaths among cohort members were identified through review of state health department lists of deaths corresponding to each study site as well as systematic review of death certificates, annual follow-up interviews, obituary notices, review of hospital records and other means. Death certificates were reviewed for all deaths identified among cohort members. Classification of fatal CAD or stroke was based

upon chest pain symptoms, cause of death from the death certificate, and available hospital information and medical history, including ARIC clinic visits. Deaths occurring outside the hospital were investigated by death certificates, interviews with one or more next of kin, physician questionnaire, and coroner or autopsy reports.

The second study utilizing ARIC data uses carotid intima-media thickness (cIMT) measured during the first clinic visit as a study outcome. For measurement of cIMT, the far wall of the common carotid artery, the bulb and the internal carotid artery were measured bilaterally (6 measurements) and averaged for each individual subject. The measurements were obtained positioning the subjects in a lateral decubitus and with the use of a caliper to guarantee a reproducible imaging angle. If one or more of the 6 cIMT measurements were missing, they were imputed by ARIC statisticians. A weight was assigned to the mean far wall cIMT for individual subjects, to account for imputed cIMT measurements. It was calculated by dividing the number of far wall sites available for each subject by six (the maximum number of sites). Thus, more weight was given to subjects whose mean cIMT was based on more sites compared to those whose mean cIMT was determined by fewer measurements.

Statistical Procedures for Projects Two and Three

The principal independent variables include:

1. Asthma history classified as child onset if onset age<21 years or adult age of onset if onset age≥21 years

2. Demographic and cardiovascular risk factor variables (e.g., age, BMI, black race, smoking status, diabetes, hypertension, education level, low and high density lipoprotein levels, and physical activity)

3. Pulmonary variables (lung function, chronic bronchitis, emphysema)

The principal dependent variables are:

1. Incident CAD

2. Incident stroke

3. Carotid intima-media thickness

For both studies, baseline covariates were compared according to gender and asthma history using chi-square and t-tests. Analysis of the association between

asthma history and incident CAD and stroke was performed using Cox proportional hazards models. All variables used in the final model were tested to ensure that they satisfied the proportional hazards assumptions by plotting log-log survival curves, testing the significance of time-dependent variables, and using goodness of fit tests (Kleinbaum 1996). Asthma-gender interactions for incident CAD and stroke were assessed using Wald chi-square tests. Analysis of asthma history and cIMT was performed using linear models to compare adjusted least square mean cIMT according to asthma history. We first tested the interaction of asthma history and gender using the likelihood ratio test and then fit separate models for each gender. Final models for both studies were tested for multi-collinearity.

For the study of CAD and stroke outcomes, missing data for covariates was imputed using multiple imputation methods (Barnard and Meng 1999). This was performed using the SAS multiple imputation procedure. First, missing values for variables were imputed that reasonably followed the joint distribution of available non-missing data values. This step was repeated five times to create five sets of complete data that included non-missing and imputed values. Then the data for each was analyzed for each imputed dataset. Finally the SAS multiple imputation analyze procedure (PROC MIANALYZE) was used to combine estimates obtained over the five imputed sets into a single set of parameter estimates that reflects the uncertainty introduced by the imputation of missing values.

Power and Sample Size for Projects Two and Three

The ARIC population features 15,792 subjects. Among ARIC participants, prevalence of adult onset asthma is 2% among men and 3% among women. Child onset asthma occurs in 3% of men and 2% of women.

Incident CAD and Stroke Study. After exclusions, 14,567 subjects were available for the study of incident CAD and stroke. Mean follow-up time for subjects was 12.5 years. Incidence rates of CAD were approximately 8.0 events per 1000 person-years among men and 3.5 among women. Rates of stroke were approximately 3.5 events per 1000 person-years among men and 2.5 among women. Assuming these rates and using the formula Risk = $1 - e^{(-\text{Rate x time})}$, the 12.5 year risk of CAD was 9.5% among men and 4.3% among women; the 12.5 year risk for stroke was 3.5% among men and 3.1% among women. Under these assumptions, the minimum detectable risk ratios for the associations of asthma with CAD and stroke among men and women with 80% power are shown in table 3.1.

Table 3.1: Minimum detectable risk ratios under 80% power for the association of adult and child onset asthma with incident CAD and stroke among men and women in the ARIC study cohort.

	CAD		Stroke	
	Adult Onset Asthma	Child Onset Asthma	Adult Onset Asthma	Child Onset Asthma
Men	1.9	1.7	2.7	2.3
Women	2.0	2.4	2.2	2.8

Carotid Intima-media Thickness Study. After exclusions, 13,627 subjects were available for the study of cIMT. Among male ARIC participants mean cIMT was 0.780mm with a standard deviation (SD) of 0.167mm. Among women mean cIMT was 0.680mm with SD of 0.134mm. Under these assumptions, the minimum detectable differences in cIMT at 80% power according to gender are shown in table 3.2.

Table 3.2. Minimum detectable difference in cIMT with 80% power among men and women comparing adult and child onset asthmatics to non-asthmatics.

	Adult Onset Asthma	Child Onset Asthma
Men	0.040mm	0.035mm
Women	0.024mm	0.028mm

Strengths and Limitations of Projects Two and Three

Strengths

- These are the first studies to examine the association of specific asthma subtypes with atherosclerotic disease outcomes.

- The ARIC cohort is large and features high quality, prospectively measured data for covariates and well-defined adjudicated outcome measurements.

- The study population is diverse in regards to race and gender and is representative of the general population.

- Data is available on asthma medications, spirometry measurements, and pulmonary comorbidities.

Limitations

- The use of self-reported asthma history may result in misclassification of exposure and may include cases of chronic obstructive pulmonary disease and other respiratory disorders. However, self-reported measures of asthma are typically employed in large epidemiologic studies, due to feasibility issues.

- Power may be insufficient among certain asthma subtype/gender subgroups to detect weak or moderate associations with CAD and stroke.

- Information on asthma medications is limited to point prevalence at baseline and may not be reflective of long term usage. Furthermore, new types of asthma medications, i.e. leukotriene agonists, have been developed during the study period which may have a different effect on atherosclerotic risk than medications commonly used at baseline.

CHAPTER 4

THE ASSOCIATION OF ALOX5AP GENOTYPE WITH CORONARY ARTERY DISEASE IS MODIFIED BY ASTHMA AND GENDER

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Abstract

Asthma has been associated with atherosclerotic disease in the general population, with the suggestion that the association may be limited to women. Although systemic inflammation related to asthma has been proposed as a potential mechanism for this association, no studies have examined this hypothesis. In this study, we examined whether haplotypes of the Arachidonate 5-Lipoxygenase Activating Protein (ALOX5AP) and Leukotriene A4 Hydrolase (LTA4H) genes were differentially associated with coronary artery disease (CAD) according to asthma status and gender among patients referred for cardiac catheterization.

Genotyping and haplotyping was performed by DeCode Genetics using the program Nemo to impute haplotype probabilities. Asthma classification was based upon self-report of physician diagnosis. CAD was classified according to angiographic finding of at least one ≥50% stenosis, history of myocardial infarction, angioplasty, or coronary artery bypass surgery (CABG). We fit logistic regression models to assess the association of genotype with CAD and to test interactions with asthma and gender.

The association of HapA and HapB haplotypes of ALOX5AP with CAD was significantly modified by asthma status and gender (p-value for gene-asthma-sex interaction = 0.03) whereby one copy of HapA or HapB haplotype increased the risk of CAD among asthmatic women (OR=4.70, 95%CI: 1.73 to 12.75) but not

among asthmatic men or nonasthmatic men or women. The significant interaction of ALOX5AP genotype with asthma and sex on CAD suggests that leukotriene inflammation may be a mechanism behind previously reported associations of asthma with atherosclerotic disease among women.

Introduction

Recent papers have suggested that asthma is a risk factor for atherosclerotic vascular disease among women (Iribarren, Tolstykh, and Eisner 2004; Onufrak S 2007; Schanen et al. 2005; Toren and Lindholm 1996). Inflammation has been proposed as a possible mechanism because of its central role in the pathogenesis of both asthma and atherosclerosis. In particular, leukotrienes are important inflammatory mediators in the pathogenesis of both asthma (Votava 1984) and atherosclerosis (Jala and Haribabu 2004). However, no studies to date have specifically examined this hypothesis.

In the present study, we examine whether haplotypes of genes involved in the production of cysteinyl leukotrienes are differentially associated with coronary artery disease (CAD) according to asthma status and gender among patients undergoing cardiac catheterization at Emory University Hospital and The Emory Clinic in Atlanta, Georgia. Specifically, we examined HapA and HapB haplotypes of ALOX5AP, which codes for 5-lipoxygenase activating protein, and the HapK haplotype of LTA4H, which codes for leukotriene A4 hydrolase. We also assess the associations of these haplotypes with asthma and the association of asthma with CAD.

Materials and Methods

Study population

The Cardiogene Data Base is an ongoing prospective registry of patients undergoing coronary catheterization in the Emory University Hospital, The Emory Clinic, and Emory Crawford Long Hospital beginning in May, 2004. The Emory Healthcare System is the largest in the state of Georgia and serves as a regional referral center for cardiology patients from throughout Georgia as well as Alabama, North Carolina, and South Carolina. Patients are enrolled at the time of catheterization, at which time a blood sample is collected and subjects are administered a questionnaire to collect data on demographic and behavioral factors, health history, and other variables. Further baseline data on medical history is obtained through the Emory Cardiac Database. This analysis is based on the 2,305 patients included in the Cardiogene Data Base as of April 10, 2007. The response rate for this period was 96.3%. For the present study, 247 subjects were excluded because of missing data on asthma status, 326 subjects were excluded because of unavailable catheterization results, 96 subjects were excluded due to history of heart transplant because of difficulty interpreting angiographic results for these patients, and 40 were excluded due to unknown history of myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass surgery (CABG) . This left 1,594 subjects (1078 men and 516 women) available for

analysis. For the analysis of leukotriene genes, data for genetic markers was available for 1,173 subjects.

Baseline assessment of asthma status and other covariates

Asthma status was determined by self report of prior physician diagnosis during interview. Subjects were further classified as having "adult-onset asthma" if age of onset was 21 or above or "childhood-onset asthma" if onset was before age 21. Patients were also classified as current asthmatics if they reported current treatment for asthma by a physician.

Smoking was measured by self-reported smoking status (former, current, or never) and packyears were calculated based upon self-reported smoking history. Physical activity was classified based upon self-reported number of hours per week of strenuous physical activity performed prior to the onset of current cardiovascular disease symptoms and dichotomized according to whether or not the subject performed at least two hours of physical activity weekly. This cutoff approximately corresponds to American Heart Association physical activity recommendations for primary and secondary prevention of atherosclerotic disease (Fletcher 1997). Education level was classified according to the number of years of school completed (<12 years, 12-16 years, or >16 years). Classification of health history including prior myocardial infarction (MI), stroke, diabetes, hypertension, obesity, and hypercholesterolemia was based on self-report through interview. We

calculated body mass index using self-reported height and weight and categorized subjects with BMI ≥ 30 as obese in accordance with National Heart, Lung, and Blood Institute Guidelines. Depression data was collected by means of the PHQ-9 depression questionnaire. Subjects were classified as depressed if their PHQ-9 score was 10 or higher.

Self-reported health history data for MI, stroke, diabetes, and hypertension from a sample of subjects (N=181) was validated against available data from the Emory Cardiac Database collected by Emory clinical care givers during patient cardiology visits prior to enrollment. Sensitivity and specificity of these self-reported comorbidities ranged from 77% to 96% with mean sensitivity and specificity both 88%.

Data on angina severity, physical limitation, and quality of life were collected with the Seattle Angina Questionnaire (SAQ) and each scale was scored on a 0-100 scale and each SAQ subscale score was measure on a 0-100 scale with a score of 100 representing lowest angina severity, least physical limitation, and best quality of life, respectively for each scale. Dyspnea severity was determined according to patient self report of dyspnea during either washing or dressing, walking on level ground at patient's own pace, walking with people of same age on level ground, or hurrying on level ground or walking up a slight hill during the 4 week prior to interview. Patients reporting no dyspnea during any activity were

scored as one while patients reporting dyspnea during all four activities were scored as a four.

History of coronary intervention procedures, including percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft (CABG) surgery was ascertained through Emory admission records and when unavailable, through self-report.

Genotyping and Haplotype Estimation

Blood samples were centrifuged and the buffy coat was extracted on site by Emory study personnel. Samples were stored at Emory University until they were shipped to DeCode Genetics in Reykjavík, Iceland for genotyping.

To initially identify SNPs and potential causal variants in LTA4H, DeCode personnel sequenced across the gene region (42 kb) in 93 Icelandic MI patients and controls(Helgadottir et al. A variant of the gene encoding leukotriene a4 hydrolase confers ethnicity-specific risk of myocardial infarction 2005). No coding sequence variants that lead to amino acid substitutions were found. However, from this effort 8 SNPs were selected, which together with 2 public SNPs in the 5′ region of the gene, were genotyped. The genotyped SNPs extend 11.9 kb upstream and 1 kb downstream of LTA4H (NM_000895) and were selected to capture all haplotypes above 2% frequency across the gene region. DeCode personnel also sequenced

ALOX5AP in 93 Icelandic MI patients and controls to identify SNPs(Helgadottir et al. 2004). The sequenced region covers 60 kb containing ALOX5AP, including the five known exons and introns, the 26-kb region 5′ to the first exon and 7-kb region 3′ to the fifth exon. Of the 144 SNPs identified, 96 were excluded from consideration because of low allele frequency or complete collinearity with other SNPs. Haplotype imputation was performed by DeCode genetics using the program NEMO, which estimates haplotype frequency using expectation-maximization algorithm. For the present study, we examined the HapK haplotype of the LTA4H gene and the HapA and HapB haplotypes of the ALOX5AP (FLAP) gene. These haplotypes were chosen because they have been previously associated with atherosclerotic outcomes (Helgadottir et al. Association between the gene encoding 5-lipoxygenase-activating protein and stroke replicated in a Scottish population 2005; Helgadottir et al. A variant of the gene encoding leukotriene a4 hydrolase confers ethnicity-specific risk of myocardial infarction 2005; Helgadottir et al. 2004). The HapA haplotype of this gene is defined by the single nucleotide polymorphisms (SNPs) SG13S25 (G), SG13S114 (T), SG13S89 (G), and SG13S32 (A). The HapB haplotype of this gene is defined by the SNPs SG13S377 (A), SG13S114 (A), SG13S41 (A), and SG13S35 (G). The HapK haplotype can be discerned by the SNPs rs1978331 (A), rs17677715 (T), rs2540482 (C), rs2660845 (G), rs2540475 (G), rs22448570 (T), and rs2660898 (T).

We used haplotype probabilities as haplotype dose variables to account for uncertainty in subjects with unknown phase (Haiman et al. 2003). Each haplotype

dose variable takes on continuous values between 0, indicating that the haplotype is not possible based upon the subject's observed genotype, and 2, indicating that the subject is homozygous for the particular haplotype. Non-integer values between 0 and 1 indicate the probability that an individual carries one copy of the haplotype based upon observed genotype and population haplotype frequencies. A value of exactly one indicates that the individual is a phase- known heterozygote. One minus non-integer values between 1 and 2 indicate the probability that an individual with at least one copy of the haplotype is actually homozygous.

Ascertainment of Coronary Artery Disease Status

Coronary artery disease (CAD) status was classified as a dichotomous variable according to the presence or absence of one or more vessel stenosis of at least 50% during catheterization or a positive history of MI, PTCA, or CABG. Stenosis status was computed by applying a computer algorithm to catheterization results including location and percent stenosis of lesions detected during catheterization. This algorithm was validated in 12 subjects for whom a blinded reading and scoring of catheterization images by a trained cardiologist. CAD status was concordant for all 12 subjects.

Analysis

Analysis was completed using SAS version 9. Missing values for covariates were imputed using multiple imputation methods (Barnard and Meng 1999), first in the primary analysis sample population and then again in the subset of subjects that had data available for at least one haplotype. Baseline covariates were compared by asthma history among men and women separately using chi-square tests, Fisher exact tests, and pooled or unpooled t-tests.

We then compared CAD prevalence according to asthma status and calculated crude and adjusted prevalence odds ratios. All multivariate models were adjusted for age, catheterization lab facility, Black race, smoking status, diabetes, heart failure, current depression, hypertension, hyperlipidemia, shortness of breath, and physical activity. We tested for interaction between asthma and gender in crude and multivariate models using Wald chi-square tests. We also repeated the analysis with asthma classified according to adult or child onset and with asthma classification restricted to current asthma. Finally, we also repeated the analysis among neversmokers and again among subjects free of previous MI, PTCA, or CABG using ≥50% stenosis of at least one vessel as the outcome measure.

For the genetic analysis, we included all subjects with genetic data for at least one of the three haplotypes (n=1,173). Missing haplotype and covariate data within this subpopulation was imputed using multiple imputation methods (Barnard

and Meng 1999). Allele frequencies of HapA, HapB, and HapK haplotypes were compared according to asthma and CAD status by dividing the mean haplotype dose for each group by two and then comparing the results using t-tests.

We then assessed the association of the haplotypes with CAD by fitting logistic regression models and testing gene-asthma-sex and gene-asthma interactions. All models used in this genetic subanalysis were adjusted for age and race. Interactions were tested in hierarchically well formulated models starting with a full model including interaction terms for asthma-gene-sex interactions and all associated two-way interactions. Subsequent models eliminated non-significant three-way and then non-significant two-way interactions until a final interaction model remained (Kleinbaum DG 2002).

Because HapB is a rare haplotype, we combined HapA and HapB into a single variable to increase power to detect associations and interactions. The combined HapA/HapB variable is simply the sum of HapA and HapB dose variables because HapA and HapB are on the same gene and are mutually exclusive. Specifically, HapA features thymine at SG13S114 but HapB features adenine. To verify a uniform effect among HapA and HapB, we also estimated the individual associations of each haplotype with CAD according to asthma status and sex.

Results

After exclusions, there were 1,078 men and 516 women ages 22 through 93 years (median age = 63 years); approximately 85% were white and 13% were African American. Asthma prevalence was 5.8% among men and 15.9% among women. Table 4.1 displays baseline characteristics among men and women according asthma status. Among both genders, asthmatics were younger and tended to have higher prevalence of obesity, diabetes, hypertension, hypercholesterolemia, and depression. Asthma was associated with more smoking among women but less among men. Among both men and women, asthmatics tended to have lower (worse) angina frequency, quality of life, and physical limitation scores compared to nonasthmatics. Asthmatic men and women also tended to experience shortness of breath more frequently and reported shortness of breath as a reason for catheterization more frequently.

CAD was present in 71.1% of all patients (77.9% of men and 56.8% of women). Asthma prevalence was lower in patients with CAD compared to those without, both among men (71.4% vs. 78.3%, Crude Prevalence Odds Ratio [POR]: 0.69; 95% Confidence Interval [CI] :0.39 to 1.32) and women (50.0% vs. 58.1%, POR=0.72; 95% CI: 0.45 to 1.16). Controlling for age, catheterization lab facility, Black race, smoking status, diabetes, heart failure, current depression, hypertension, hyperlipidemia, shortness of breath, and physical activity, asthma remained negatively associated with CAD prevalence among both men (POR=0.70; 95% CI:

0.38 to 1.31) and women (POR=0.59; 95% CI: 0.0.34 to 0.98) (figure 4.1). The test of asthma-gender interaction was non-significant (p=0.64) Results were similar when we repeated analysis only among neversmokers and when asthma status was restricted to current asthma. Results were also unchanged when we assessed the association of asthma with current angiographic stenosis among patients without prior MI, PTCA, or CABG.

Asthma Age of Onset Subtypes

Mean age of asthma onset was 26.9 years among men and 39.0 years among women; there were 21 subjects who reported history of asthma but did not report age of asthma onset. The distribution of asthma age of onset according to gender among study subjects is shown in figure 4.2. The prevalence of adult onset asthma was 2.5% among men and 10.5% among women, while child onset asthma prevalence was 2.3% among men and 3.8% among women.

Adult onset asthma was not significantly associated with prevalence of coronary disease, with a tendency for patients with asthma to have lower likelihood of CAD, among men (Crude POR=0.55, 95%CI: 0.25 to 1.25) as well as women (POR=0.70, 95%CI: 0.39 to 1.23). For child onset asthma, the crude prevalence odds ratio among men was 0.88 (95%CI: 0.35 to 2.22) and among women was 0.80 (95%CI: 0.32 to 2.02). After adjustment, both adult onset asthma (POR=0.61, 95%CI: 0.33 to 1.14) and child onset asthma (POR=0.60, 95%CI: 0.21 to 1.70)

were similarly but not significantly negatively associated with CAD among women. Adjusted results were similar for men, with non-significant negative associations for child onset asthma (POR=0.84, 95%CI: 0.31 to 2.30) and adult onset asthma (POR=0.58, 95%CI: 0.23 to 1.44).

Genetic Analysis

Estimated frequencies of HapA, HapB, and HapK haplotypes according to asthma and CAD status are shown in table 4.2. Haplotype K was significantly less prevalent among asthmatic compared to non-asthmatics (9.0% versus 14.6%, p-value=0.03). This corresponds to an odds ratio of 0.58 (95%CI: 0.35 to 0.96) Haplotype frequencies did not differ according to CAD status (table 4.2).

P-values for gene-asthma-sex and gene-asthma interactions on CAD prior to elimination from logistic models are shown in table 4.3. The association of HapA or HapB with CAD was significantly modified by asthma status and sex (p-value for Wald test of interaction of gene-asthma-sex= 0.027). Under this interaction, the presence of one copy of HapA or HapB haplotype was significantly associated with CAD among female asthmatics (POR=4.70, 95%CI:1.73 to 12.75) but not among male asthmatics, female non-asthmatics, or male non-asthmatics (figure 4.3). Interactions of the HapK haplotype with asthma and sex were not significant at alpha=0.05 (table 4.3). When we repeated the analysis using HapA and HapB separately and forced the three-way interactions for both haplotypes into the model,

we observed a similar trend to the combined HapA/HapB variable whereby HapA and HapB were both positively associated with CAD only among asthmatic women (figure 4.4).

Discussion

We found that the association of 5-lipoxygenase activating protein (ALOX5AP) genotype with CAD is significantly modified by female gender and asthma status. We also report for the first time that the HapK haplotype of LTA4H is significantly associated with asthma. Finally, we found that asthma was negatively associated with coronary artery disease among both men and women in this population of subjects undergoing coronary catheterization, contrasting previous studies reporting a positive association between asthma and atherosclerotic disease among women in free-living populations.

The negative association we found between asthma and CAD may be related to selection bias inherent in this population of patients referred for cardiac catheterization. Cardiac catheterization is an invasive procedure associated with risk of serious adverse events and is generally not performed unless subjects are suspected of experiencing an acute coronary syndrome or are determined to be at high risk of CAD through a combination of symptoms such as angina and shortness of breath, risk factor profile, and non-invasive tests. The shortness of breath and chest discomfort typically associated with asthma and seen among asthmatics in our

study may have increased the probability that asthmatics free of CAD were selected for catheterization. This notion is supported by the observation that the age-adjusted prevalence of asthma among CAD-free female catheterization patients was markedly higher (17.4% versus 11.9%) than the female prevalence of self-reported asthma in CDC's 2005 National Health Interview Survey (NHIS). However, prevalence of asthma among CAD-free male catheterization patients was similar to NHIS estimates for male asthma prevalence (7.3% versus 7.9%). The apparent gender difference in selection of CAD-free asthmatic patients for catheterization may be explained by increased symptom reporting that has been observed among women (Barsky, Peekna, and Borus 2001) and the stronger association of angina (Timmis, Feder, and Hemingway 2007) and shortness of breath (Abidov et al. 2005) with CAD among men. Interestingly, age-adjusted asthma prevalence among the CAD positive women in our study is greater than the NHIS estimate of asthma prevalence among U.S. women (14.3% versus 11.9%), resulting in an odds ratio of 1.25. The same comparison among men (5.4% versus 7.9%) results in an odds ratio of 0.67. These trends are consistent with our previous study of asthma and CAD (Onufrak S 2007).

To our knowledge this is the first study to assess the relationship of HapA, HapB, and HapK haplotypes with asthma although several studies have otherwise examined the ALOX5AP and LTA4H genes with inconsistent results (Choi et al. 2004; Kedda et al. 2005; Koshino et al. 1999; Lima et al. 2006; Sayers et al. 2003). Koshino reported a novel repeat polymorphism in the Poly-A promoter region of

ALOX5AP associated with asthma (Koshino et al. 1999). Kedda et al. found no evidence of an association between asthma and several polymorphisms of the ALOX5AP gene: IVS2+12C>A, intron II *Hind*III, IVS2+105T>C, and the repeat polymorphism of the Poly-A promoter region that Koshino had reported on previously (Kedda et al. 2005). Choi et al. reported no association of asthma or aspirin intolerance among asthmatics with a single SNP of ALOX5AP (ALOX5AP, 218A—>G) (Choi et al. 2004). Sayers et al. reported no associations between asthma and two polymorphisms of ALOXAP: a single SNP at -36G/A and a simple sequence length polymorphisms (A) involving two alleles (A23/A19) at positions −169 to −146 relative to the initiation codon (Sayers et al. 2003). Research on the LTA4H gene is more sparse: a 2000 study failed to find any polymorphisms in the LTA4H gene (Heinzmann et al. 2000) but a recent study by Lima et al. reported increased risk of asthma exacerbation associated with GG and GA genotypes at SNP rs2660845 among asthmatics taking Montelukast (Lima et al. 2006). This finding contrasts somewhat with our results in that a G substitution at rs2660845 is consistent with the HapK haplotype, which we found to be negatively associated with asthma prevalence. Further research on the association of HapK with asthma is necessary to clarify this relationship.

Our finding that HapA and HapB haplotypes were associated with CAD only among asthmatic women suggests that leukotrienes may be a contributing factor in previously reported associations of asthma with atherosclerotic disease in women. Leukotrienes have been implicated in the inflammation process of both

asthma and atherosclerosis. The HapA and HapB haplotypes have been previously associated with myocardial infarction (Helgadottir et al. 2004) and stroke (Helgadottir et al. Association between the gene encoding 5-lipoxygenase-activating protein and stroke replicated in a Scottish population 2005; Helgadottir et al. 2004) by the DeCode genetics study groups. Moreover, one of these studies reported increased production of Leukotriene B4 by ionomycin-stimulated neutrophils among subjects with history of MI, particularly among subjects with the HapA haplotype(Helgadottir et al. 2004). However, studies from other groups have failed to replicate these findings (Koch et al. 2007; Lohmussaar et al. 2005; Zee et al. 2006).

The primary weakness of this study is the fact that it is based on a population referred for cardiac catheterization, and thus the results may not be generalizable to the general population. This study population and the cross-sectional design make the study susceptible to selection bias. This selection bias can occur as a result of both physician selection of patients for catheterization as well as differential survival of patients prior to catheterization. For example, patients who die of CAD prior to catheterization may differ from those who survive and enter the study population. Furthermore, we relied on self-reporting of asthma history as well as other co-morbidities. Nonetheless, this is the first study to examine associations of HapA, HapB, and HapK haplotypes with asthma and to examine interactions of these genotypes with asthma and sex on prevalence of objectively documented coronary atherosclerosis.

In conclusion, asthma is negatively associated with CAD among both male and female patients referred for cardiac catheterization, which may be due to selection bias. The association of HapA and HapB haplotypes of ALOX5AP with CAD is significantly modified by gender and asthma status whereby CAD prevalence among asthmatic women is increased nearly five-fold by the presence of one copy of HapA or HapB. Further research is required to elucidate the relationship between asthma, leukotriene regulation, and atherosclerotic disease in the general population.

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Table 4.1: Baseline comparison of men and women according to self-reported asthma history.

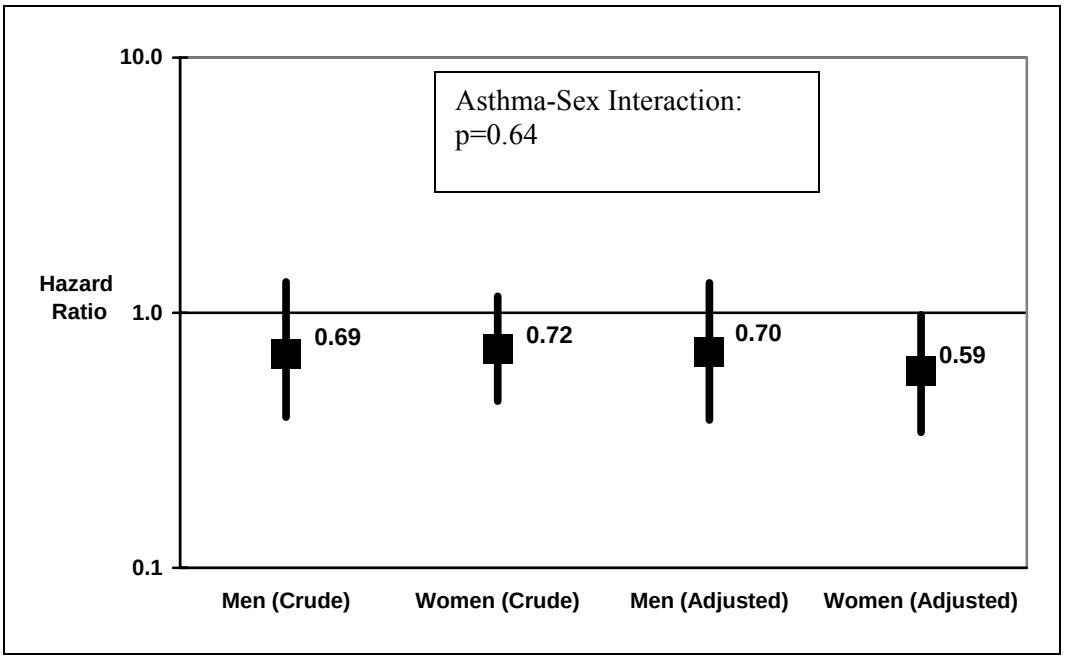
	Men		**Women**	
	Asthma n=63 (5.8%)	**No Asthma n=1015 (94.2%)**	**Asthma n=82 (15.9%)**	**No Asthma n=434 (84.1%)**
Demographics				
Age (y) (SD)	60.5 (9.7)	62.8 (11.3)	61.8 (11.4)	63.5 (11.5)
Obese (BMI ≥ 30) (%)	**57.6%***	**40.0%**	54.8%	46.8%
Race				
White (%)	84.1%	87.1%	82.9%	80.3%
Black (%)	11.1%	10.8%	15.9%	18.1%
Other (%)	4.8%	2.1%	1.2%	1.6%
High School Graduate (%)	84.1%	87.9%	80.0%	84.0%
Currently Employed (%)	53.9%	43.2%	33.8%	29.1%
Currently Married (%)	83.9%	82.9%	**0.0%***	**63.1%**
Comorbidities				
Heart Failure (%)	17.5%	13.5%	10.7%	13.0%
Stroke (%)	6.7%	7.1%	8.5%	8.7%
Diabetes (%)	**41.9%***	**28.7%**	**41.6%***	**28.2%**
Hypertension (%)	70.5%	64.4%	74.0%	65.9%
Hypercholesterolemia (%)	73.3%	66.9%	69.2%	60.9%
Current Depression (%)	18.3%	12.1%	20.3%	15.7%
Dyspnea				
Dyspnea listed as Reason for Catheterization (%)	39.7%	31.4%	43.2%	38.0%

Table 4.1 (continued): Baseline comparison of men and women according to self-reported asthma history.

	Men		Women	
	Asthma n=63 (5.8%)	**No Asthma n=1015 (94.2%)**	**Asthma n=82 (15.9%)**	**No Asthma n=434 (84.1%)**
Dyspnea Index (SD)	1.7 (1.7)	1.4 (1.5)	**2.4* (1.5)**	**1.9 (1.6)**
Seattle Angina Questionnaire				
Angina Frequency Index (SD)	77.4 (28.0)	79.8 (24.8)	77.0 (25.6)	78.0 (25.4)
Physical Limitation Index (SD)	85.8 (26.8)	87.9 (21.9)	81.9 (27.2)	82.8 (25.9)
Quality of Life Index (SD)	61.7 (26.5)	67.5 (26.4)	60.1 (29.0)	65.0 (27.1)
Behavioral				
Ever Smoker (%)	55.0%	65.2%	57.5%*	45.3%
Pack Years (SD)	**9.0* (14.4)**	**18.6 (26.5)**	**17.3* (25.7)**	**7.5 (16.4)**
Physically Active (>2Hours/Week) (%)	35.1%	29.9%	17.5%	21.3%
Current Drinking (%)	37.3%	38.8%	23.4%	21.2%

*$p<0.05$ comparing subjects with asthma to subjects reporting no history of asthma within each gender

Figure 4.1: Crude and Multivariate Adjusted* Prevalence Odds Ratios for Coronary Artery Disease According to Asthma Status and Sex Among Emory Cardiac Catheterization Patients



*Adjusted for age, catheterization lab facility, Black race, smoking status, diabetes, heart failure, current depression, hypertension, hyperlipidemia, shortness of breath, and physical activity

Figure 4.2: Prevalence of Self-Reported Asthma According to Age of Onset and Gender Among Subjects Undergoing Cardiac Catheterization

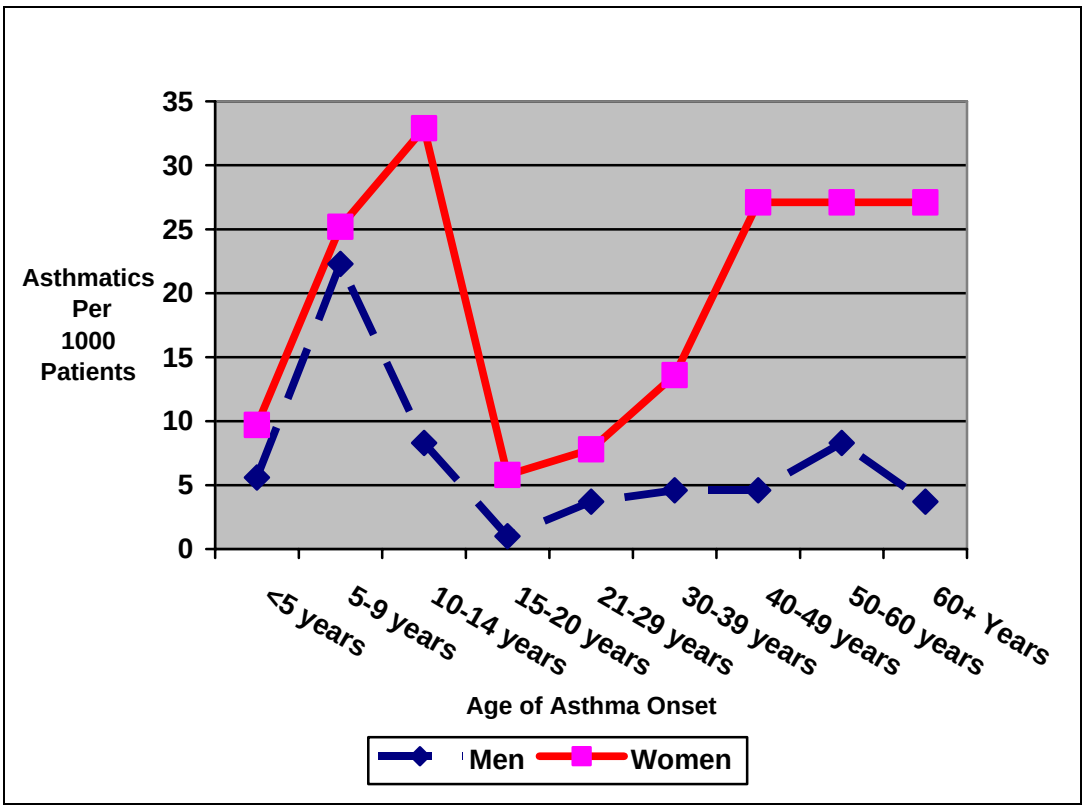


Table 4.2: Estimated haplotype frequencies among Emory cardiac catheterization patients according to asthma and coronary artery disease (CAD) status

	Asthma	No Asthma	Odds Ratio	CAD	No CAD	Odds Ratio
HapA	16.7%	14.9%	1.17 (0.78, 1.75)	14.9%	15.5%	0.97 (0.75, 1.27)
HapB	7.6%	7.4%	1.04 (0.53, 2.06)	7.2%	7.9%	0.89 (0.53, 1.36)
HapK	**9.0%**	**14.6%***	**0.58 (0.35, 0.96)**	13.7%	14.9%	0.90 (0.70, 1.17)

* p-value < 0.05

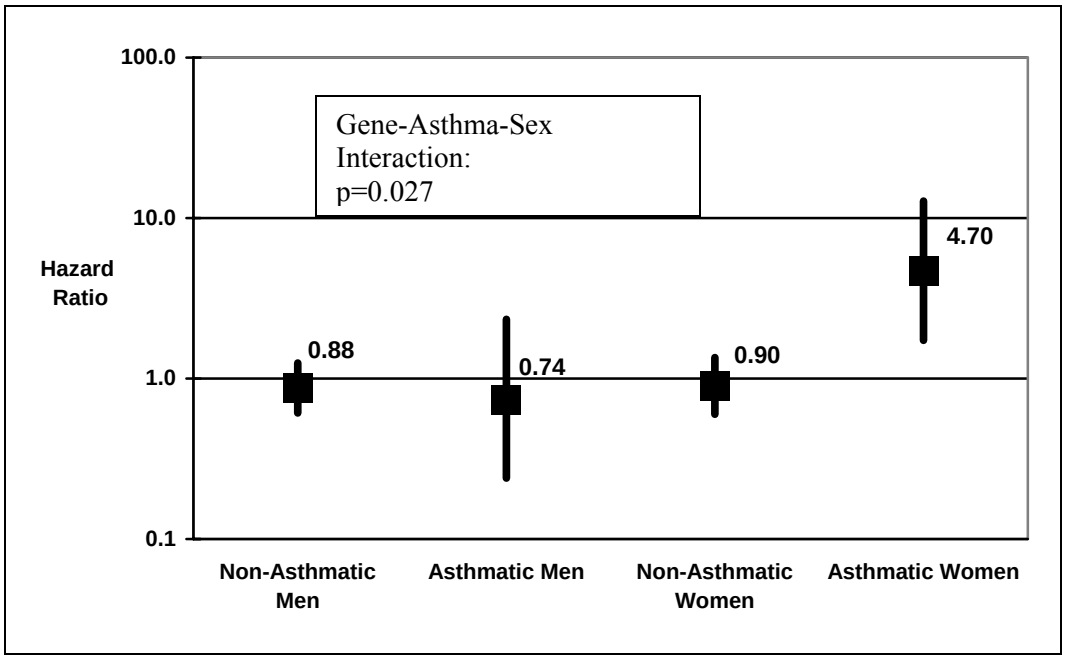
Table 4.3: Gene-Sex-Asthma and Gene-Asthma Interaction Tests*,** on Association of HapA or HapB, and HapK haplotypes with Coronary Artery Disease

Interaction	Coefficient	Standard Error	p-value
HapA/HapB*Asthma*Male	-1.818703	0.818532	**0.026**
HapK*Asthma*Male	2.230383	1.503061	0.14
HapA/HapB*Asthma	1.650073	0.545904	**0.0025**
HapK*Asthma	-0.195620	0.481979	0.68

* coefficients, standard errors, and p-values for interaction terms at time of removal or retention in model during backward elimination of non-significant interaction terms.

**Adjusted for age and race

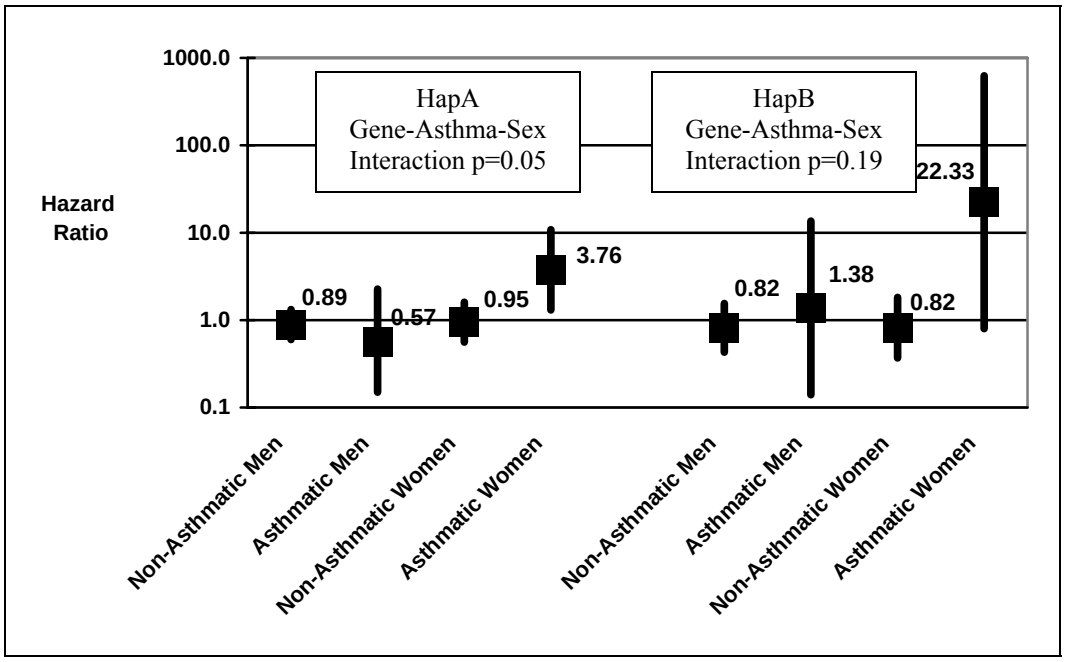
Figure 4.3: Adjusted* Association** of HapA or HapB Genotype with Coronary Artery Disease Among Emory Cardiac Catheterization Patients According to Asthma Status and Sex



*Adjusted for age and race

**Odds ratio describing the effect of one copy of either HapA or HapB on prevalence of CAD

Figure 4.4: Adjusted* Individual Associations** of HapA and HapB Genotypes with Coronary Heart Disease Among Emory Cardiac Catheterization Patients According to Asthma Status and Sex



*Adjusted for age and race

**Odds ratios describing the effect of one copy of either HapA or HapB on prevalence of CAD

CHAPTER 5

ADULT-ONSET ASTHMA IS ASSOCIATED WITH INCIDENT CORONARY HEART DISEASE AND STROKE AMONG WOMEN

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Abstract

Asthma has been associated with atherosclerotic disease in several studies with some evidence that this association may be limited to women. However, most previous studies have failed to account for the heterogeneity of asthma subtypes. We previously reported increased carotid intima medial thickness among women with adult onset asthma. In this study, we examine the association of adult and child onset asthma with incident coronary heart disease and stroke.

Subjects were classified according to self-report of physician diagnosed asthma and age of asthma onset. We used Cox proportional hazards models to test the association of adult and child onset asthma with incident CHD and stroke, testing for gender interaction. Subanalysis was also performed using only neversmokers.

Women with adult onset asthma experienced a two-fold increase in incident CHD and stroke which was independent of other risk factors including smoking, body mass index, and physical activity and persisted when the analysis was restricted to never-smokers. No significant association was found among women with child onset asthma or among men.

Adult onset asthma may be a significant risk factor for CHD and stroke among women but not men. Further research on cardiovascular disease among asthmatics should focus on asthma subtypes.

Introduction

Asthma has been associated with vascular disease, carotid atherosclerosis, coronary heart disease, or stroke in at least nine studies (Iribarren, Tolstykh, and Eisner 2004; Knoflach et al. 2005; Liss et al. 1999; Liss et al. 2000; Onufrak, Abramson, and Vaccarino 2006; Schanen et al. 2005; Soriano et al. 2005; Toren and Lindholm 1996; Zureik et al. 2004). Among studies that present results stratified by gender, there is a suggestion that the association may be stronger among or entirely limited to women (Iribarren, Tolstykh, and Eisner 2004; Onufrak, Abramson, and Vaccarino 2006; Schanen et al. 2005; Toren and Lindholm 1996). However, asthma is not a single disease but rather a collection of distinct underlying subtypes, with somewhat differing etiologies (Bel 2004; Wenzel 2006). Child and adult onset asthma differ in regards to asthma triggers (Wenzel 2006), gender distribution (Wenzel 2006), association with obesity (Chen, Dales, and Jiang 2006), and systemic inflammation (Olafsdottir et al. 2005). We previously reported an association between carotid intima medial thickness and adult onset asthma among women in the Atherosclerosis Risk in Communities (ARIC) study (Onufrak, Abramson, and Vaccarino 2006). This association was not observed among women with child onset asthma or among men with either adult or child onset asthma. In this study, we examined the association of asthma age of onset phenotypes with incidence of coronary heart disease and stroke according to gender within the ARIC cohort.

Methods

Study population

The Atherosclerosis Risk in Communities (ARIC) Study is a prospective study of the etiology of atherosclerotic, cardiovascular, and cerebrovascular disease in four US communities in North Carolina, Mississippi, Minnesota, and Maryland(The atherosclerosis risk in communities (ARIC) study: Design and objectives. The ARIC Investigators 1989). The study population of 15,792 men and women ages 45 to 64 years includes both black and white participants. Subjects completed a baseline clinic visit during 1987 to 1989 and were followed for incidence of CHD and stroke events. We used publicly available data with follow-up available through 2001 for 15,732 participants. We excluded subjects missing data for asthma status (n=28) or who reported ever having asthma but did not report physician diagnosis of asthma (n=131). We also excluded subjects with self reported history of stroke (n= 320) or prevalent coronary heart disease (n=692), defined as history of MI, silent MI, or revascularization surgery at baseline. This left 14,567 subjects for analysis.

Baseline assessment of asthma status and other covariates

Based on self report of physician diagnosed asthma and age of asthma onset, subjects were classified as having "adult-onset asthma" if age of onset was 21 years or above, or "childhood-onset asthma" if onset was before age 21. Smoking was measured by self-reported smoking status (former, current, or never). Classification of

diabetes was based on at least one of the following: a fasting plasma glucose greater than 126 mg/dL, a non-fasting plasma glucose greater than 200 mg/dL, self reported diabetes, or taking diabetes medications (ARIC Investigators *ARIC manual 1. General description and study management* 1987, *ARIC protocol 7. Blood collection and processing* 1987, *ARIC protocol 10. Clinical chemistry determinations. Version 1.0* 1987). Total cholesterol and high density lipoprotein (HDL) cholesterol were included in models as continuous variables. Hypertension was defined as a diastolic blood pressure greater than or equal to 90 mm Hg, a systolic blood pressure greater than 140 mm Hg, or self report of use of anti-hypertensive drugs(Jones and Hall 2004). Physical activity was assessed according to the Baecke scale based upon frequency, duration, and intensity of physical activity (Baecke, Burema, and Frijters 1982). Education level was classified according to the number of years of school completed (<12 years, 12-16 years, or >16 years). Asthma medication use (beta adrenergic and oral glucocorticoids) was classified according to use during the two week period prior to the baseline clinic visit and was ascertained by having subjects bring all prescription and non-prescription medications used during this period to the clinic during their baseline visit. Forced expiratory volume in one second (FEV1) and forced expiratory vital capacity (FVC) were assessed using spirometry according to the ARIC study protocol (ARIC Investigators *ARIC protocol 4. Pulmonary function assessment. Version 7.* 1987). FEV1 was categorized for use in multivariable analysis according to gender-specific quartiles. Chronic bronchitis and emphysema were classified based upon self report of physician diagnosis.

Laboratory Testing

Approximately 60 milliliters of blood were drawn by ARIC personnel from study subjects at the first clinic visit. Samples were either kept at room temperature, for hematology and clinical chemistry laboratories, or immediately stored on ice for hemostasis and lipid testing. Within 90 minutes of collection and after centrifugation, all samples were frozen at –70^{o}F for later processing. All laboratory tests were run at centralized chemical, hemostasis and lipid laboratories, and hematological tests such as complete blood count were run at local laboratories.

Ascertainment of incident CHD and stroke events

For our primary analysis, incident coronary heart disease (CHD) was defined as definite or probable myocardial infarction or fatal coronary heart disease. We also performed subanalyses in which incident CHD events included revascularization procedures and silent MI detected through electrocardiogram. Incident strokes included both ischemic and hemorrhagic strokes. Potential coronary heart disease and stroke events were identified in cohort members through annual follow-up, survey of area hospital discharge lists, and state vital statistics. Where discharge summaries indicated diagnosis codes for cardiovascular disease, diabetes, stroke, or included stroke related keywords, hospital records were abstracted by trained study personnel. Out-of-hospital deaths were investigated by means of death certificates, interview with one or more next of kin, and a physician questionnaire, coroner reports or autopsy reports. Myocardial

infarction events were classified based upon chest pain, cardiac enzyme levels, and ECG results (ARIC Investigators *Atherosclerosis Risk in Communities (ARIC) study surveillance component procedures protocol 3, version 4.* 1987). Fatal CHD classification was based on chest pain symptoms, cause of death from the death certificate, and available hospital information and medical history, including ARIC clinic visits. For stroke events, records were reviewed in detail by a member of the ARIC study Stroke-Mortality and Morbidity Classification Committee and the patient was classified according to the type of stroke that occurred (ischemic or hemorrhagic). This process has been described in further detail by ARIC Investigators (ARIC Investigators *Atherosclerosis Risk in Communities (ARIC) study surveillance component procedures protocol 3, version 4.* 1987).

Statistical analysis

Analysis was completed using SAS version 9. Baseline covariates were compared by asthma history among men and women separately using chi-square tests, Fisher exact tests, and pooled or unpooled t-tests. Missing values for covariates were imputed using multiple imputation methods(Barnard and Meng 1999). Crude incidence density rates of CHD and stroke were calculated among men and women for non-asthmatics, childhood onset asthmatics, and adult-onset asthmatics. Crude and multivariate hazard ratios comparing each asthma subtype to non-asthmatics were computed using Cox proportional hazards models. Multivariate models were adjusted for age, body mass index (BMI), black race, smoking status, diabetes, hypertension, education level, low and

high density lipoprotein levels, and leisure physical activity. We tested for interaction between asthma and gender in crude and multivariate models using Wald chi-square tests. In subanalyses, we used an expanded definition of CHD to include revascularization and silent MI..

We also performed additional analyses to investigate the impact of asthma medications, lung function, and the respiratory comorbidities chronic bronchitis and emphysema on the association of asthma with cardiovascular outcomes. To further examine the possible confounding effect of smoking and the possible misclassification of chronic obstructive pulmonary disease (COPD) as asthma on our results, we repeated the analysis in the subgroup of individuals who never smoked in the past. In these additional analyses we used a combined outcome of incident CHD or stroke to maximize the number of events in the model.

Results

The distribution of asthma age of onset among men and women is shown in figure 5.1. The prevalence of child onset asthma was higher among men (3.3%) than women (2.5%), while adult onset asthma was more common among women (3.4%) compared to men (2.0%). Compared to their non-asthmatic counterparts, men and women with adult onset asthma were older, had a higher prevalence of diabetes and hypertension, more pack-years of smoking, higher fibrinogen levels, and a higher prevalence of beta adrenergic and glucocorticoid steroid asthma medication use at baseline (table 5.1). Women with adult onset asthma also had significantly higher body mass index (BMI),

lower physical activity, and were more often post-menopausal than women without a history of asthma. Compared to nonasthmatics, FEV1, percent expected FEV1, and FEV1/FVC were lower among men and women with either asthma subtype but were lowest among those with adult onset asthma. Likewise, chronic bronchitis, emphysema, and use of asthma medications were more prevalent among all asthmatics but were most prevalent among adult onset asthmatics (table 5.1).

In all, 979 subjects experienced myocardial infarction or fatal CHD during 181,145 person-years of follow-up for an overall CHD rate of 5.40 per 1000 person-years. Women, but not men, with adult onset asthma experienced a two-fold increase in rate of CHD (hazard ratio (HR): 2.10, 95% Confidence Interval (CI) : 1.40 to 3.16) compared to their non-asthmatic counterparts (table 5.2). This association was attenuated but remained significant (HR: 1.78, 95% CI: 1.18 to 2.67) after adjustment for age, BMI, black race, smoking status, diabetes, hypertension, education level, low and high density lipoprotein cholesterol levels, and physical activity (table 5.2). Child onset asthma was not significantly associated with incident coronary heart disease among women or men. Tests of interaction between gender and adult onset asthma were significant ($p<0.05$) in all CHD models, while interaction tests of child onset asthma with gender were not. Results were similar in subanalyses where incident CHD also included revascularization procedures and silent myocardial infarctions, with an adjusted hazard ratio of 1.86 (95%CI: 1.31 to 2.63) for women with adult onset asthma and nonsignificant associations observed among all other asthma-gender subgroups.

There were 531 incident strokes during 183,181 person-years of stroke follow-up for an overall stroke rate 2.90 per1000 person-years. Similar to the results for CHD, adult onset asthma was associated with incident stroke among women (crude HR: 2.36, 95% CI: 1.48 to 3.76) but not men (crude HR: 0.35, 95% CI: 0.09 to 1.41) with a significant gender interaction ($p<0.05$) (table 5.3). The association of adult onset asthma with stroke in women remained significant (HR: 2.08, 95% CI: 1.30 to 3.32) after adjustment for demographic variables and established CHD risk factors (table 5.3). The small numbers of stroke events precluded multivariate analysis among men. Child onset asthma was not significantly associated with incident stroke among men or women and the interaction of child onset asthma and gender was nonsignificant (table 5.3).

Because of the similarity of results for the CHD and the stroke outcomes, we performed additional analyses using a combined endpoint of incident cardiovascular disease, including CHD or stroke (figure 5.2). In the fully adjusted model including covariates for asthma medications, FEV1, chronic bronchitis, and emphysema, the HR for the association of adult-onset asthma with this combined outcome in women was 1.68 (95% CI: 1.21 to 2.35), while none of the other gender and asthma type subgroups showed an elevated incidence of cardiovascular disease compared to non asthmatics (figure 5.2). Results remained robust in analyses restricted to never-smokers, which again confirmed a significant association of adult onset asthma among women (adjusted HR: 2.05, 95% CI:1.28 to 3.31) but not among men (adjusted HR: 1.04, 95% CI: 0.43 to 2.53) or among women or men with child onset asthma (table 5.4).

Discussion

In this large, community-based follow-up study, women with adult onset asthma experienced a nearly two-fold increase in the rate of coronary heart disease and stroke, which was independent of other risk factors including smoking, BMI, and physical activity and persisted when the analysis was restricted to never-smokers. This result is consistent with our previous finding that women, but not men, with adult onset asthma have increased carotid intima medial thickness compared to their nonasthmatic counterparts (Onufrak, Abramson, and Vaccarino 2006) and with other literature suggesting a role of asthma in atherosclerotic disease among women but not men (Iribarren, Tolstykh, and Eisner 2004; Onufrak, Abramson, and Vaccarino 2006; Schanen et al. 2005; Toren and Lindholm 1996).

This is the first study to test the association of asthma age of onset subtypes with cardiovascular outcomes. It is recognized that "asthma" is not a uniform disease, but rather a constellation of distinct conditions (Bel 2004; Miranda et al. 2004; Wenzel 2006). Adult onset asthma differs from child onset asthma in several aspects, including its distribution among men and women (Wenzel 2006), and its immunological and inflammatory pathophysiology (Bel 2004; Miranda et al. 2004). Nevertheless, previous studies of asthma and atherosclerotic outcomes have generally ignored asthma subtypes. Three previous studies have presented gender specific results for the association between asthma and CHD. Toren et al. reported an age-adjusted standardized mortality ratio for ischemic heart disease of 1.4 (95%CI: 0.8 to 2.0) among asthmatic men and 2.5 (95%CI:

1.7 to 3.3) among asthmatic women(Toren and Lindholm 1996). In a retrospective cohort study of a large insurance cohort, Iribarren et al. reported multivariate adjusted hazard ratios of 1.22 (95% CI: 1.14 to 1.31) among asthmatic women and 0.99 (95% CI: 0.93 to 1.05) among asthmatic men (Iribarren, Tolstykh, and Eisner 2004). Similarly, an earlier report from the ARIC study found an elevated risk of stroke in asthmatic women, but not men, compared to non-asthmatic subjects, although no association was found with CHD outcomes in either women or men (Schanen et al. 2005). None of these previous reports distinguish among asthma subtypes. By doing so, we have uncovered an important risk associated with adult onset asthma among women.

The precise mechanisms underlying the association between adult-onset asthma and atherosclerotic vascular disease in women are unclear. Asthma may predispose to atherosclerosis through specific pathophysiologic pathways perhaps linked to the chronic inflammatory response of this disorder. Alternatively, the association between asthma and atherosclerosis may be due to an inherent joint susceptibility to both diseases through shared inflammatory pathways. For example, cysteinyl leukotrienes, potent inflammatory mediators, are implicated in the pathogenesis of both asthma (Bisgaard 2001) and atherosclerosis(Zhao and Funk 2004). Furthermore, genes that regulate the production of cysteinyl leukotrienes have been associated with both asthma (Kalayci et al. 2006; Kim et al. 2005; Kim et al. 2006; Lima et al. 2006; Moissidis et al. 2005; Thompson et al. 2006) and atherosclerosis (Dwyer et al. 2004; Helgadottir et al. Association between the gene encoding 5-lipoxygenase-activating protein and stroke replicated in a scottish population 2005; Helgadottir et al. A variant of the gene encoding leukotriene a4 hydrolase confers

ethnicity-specific risk of myocardial infarction 2005; Helgadottir et al. 2004), suggesting that both conditions have a common pathophysiological substrate of enhanced inflammation which may be, in part, genetically determined. Thus, asthma may be a marker of genetic susceptibility to inflammation and thus, susceptibility to atherosclerotic disease. This hypothesis may also help explain the apparent gender specificity of the association of asthma and atherosclerosis: in a recent study, a promoter polymorphism of Leukotriene C4 Synthase Gene, the rate-limiting enzyme in cysteinyl leukotriene production, was associated with carotid intima medial thickness and coronary calcium among women but not among men(Iovannisci et al. 2007).

Why is the association between asthma and cardiovascular disease only observed in women with adult-onset asthma? Estrogen levels, which increase at puberty, modulates the release of proinflammatory cytokines from activated monocytes, macrophages (Kramer, Kramer, and Guan 2004), and vascular cells (Miller et al. 2004; Xing et al. 2007) and also regulates the production of leukotrienes from mast cells (Zaitsu et al. 2007). The incidence rate of asthma among women is temporally associated with changes in estrogen levels, with incidence increasing after puberty (de Marco et al. 2000) and peaking during the onset of menopause (Balzano et al. 2001) (figure 5.1). Thus, the increase in asthma susceptibility that occurs during these times may be a consequence of estrogen-modulated alterations in inflammatory cytokine and leukotriene regulation. Because leukotrienes and many cytokines are common to the etiology of both asthma and atherosclerosis, alterations in their regulation that promote the development of asthma may also have an impact on the development of atherosclerosis. Thus, asthma onset

among adult women may indicate an underlying estrogen-modulated proinflammatory state that also increases the risk of atherosclerotic disease.

Our study has several strengths. Foremost, the ARIC cohort is large, multiracial and prospective, and includes rich and high quality subject data. We were able to control for potentially important confounding variables such as smoking, physical activity, and asthma medication use and we further addressed confounding by smoking by performing sub-analyses restricted to never smoking subjects. The major weakness of our study is the fact that asthma status was based upon self-report of physician diagnosis. Although there is some evidence to suggest that self-reported asthma yields high specificity (Toren, Brisman, and Jarvholm 1993), there is also literature to suggest that misdiagnosis of COPD as asthma occurs frequently, and more so among women (Chapman, Tashkin, and Pye 2001). Because approximately 85% of COPD cases have a history of smoking, the persistence of the association of adult onset asthma with CHD among never smoking women suggests that the observed association is not likely to be due to misdiagnosed COPD cases. Nonetheless, there remains the need for further research in which asthma classification is objectively determined through established clinical guidelines. Furthermore, because child-onset asthma is more often of allergic etiology, while the adult-onset type is more often nonallergic, it is possible that age of onset is a proxy for allergic and nonallergic asthma subtypes. Unfortunately, we did not have information on IgE levels, asthma triggers, or presence of allergies to differentiate asthma according to allergic status. Other limitations include small numbers of events, particularly stroke,

among men with adult onset asthma and the fact that our study is observational and thus we cannot exclude the influence of unmeasured confounding factors.

In conclusion, women with adult-onset asthma have nearly double the risk of CHD and stroke, independent of other risk factors compared to women without asthma. Our results indicate that adult-onset asthma is a robust marker of cardiovascular risk uniquely for women. While the mechanisms underlying this gender-specific susceptibility need exploration in future studies, our findings are of significant public health importance given the approximately five to seven million women in the United States that are affected by adult onset asthma.

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Figure 5.1: Self-Reported Asthma Age of Onset Among Men and Women.

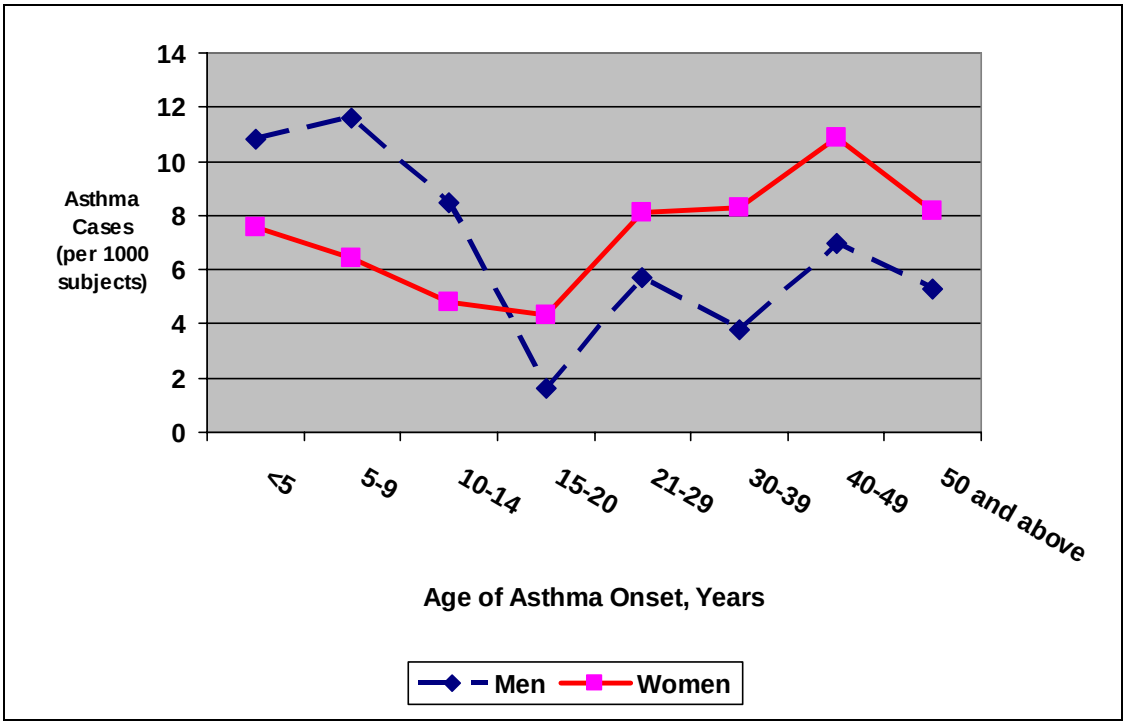


Table 5.1: Baseline comparison of men and women according to self-reported asthma history

	Men			Women		
	No Asthma	**Child Onset* Asthma**	**Adult Onset* Asthma**	**No Asthma**	**Child Onset* Asthma**	**Adult Onset* Asthma**
	n=5931	**n=210**	**n=131**	**n=7809**	**n=203**	**n=283**
Demographics						
Age (y)	54.3	53.3†	55.4†	53.7	52.9†	54.3
Black Race (%)	23.3	19.1	15.3†	29.6	30.1	33.2
High School Graduate (%)	77.5	82.3	65.7†	77.2	76.4	69.3†
Body Mass Index	27.4	27.2	27.3	27.8	28.3	28.9†
Comorbidities						
Diabetes (%)	10.5	10.6	14.5	10.8	17.2†	17.8†
Hypertension (%)	32.4	29.2	37.4	34.1	33.0	43.6†
Chronic Bronchitis (%)	4.0	15.1†	27.9†	9.2	39.6†	43.1†
Emphysema (%)	1.9	4.3†	8.4†	0.9	3.9†	5.3†
Behavioral						
Current Smoking (%)	27.6	22.4	21.4	24.6	22.8	29.0
Pack Years	21.7	20.1	24.3	10.0	11.2	13.8†
Leisure Physical Activity Index	2.34	2.35	2.35	2.38	2.33	2.29†
Spirometry						
FEV_1 (liters)	3.37	3.11†	2.74†	2.44	2.20†	2.05†
FEV_1 (% predicted)	91.4	83.2†	74.6†	97.1	86.5†	83.3†
FEV_1 / FVC	73.5	68.3†	63.5†	75.9	71.1†	70.3†
Lipids						
LDL Cholesterol (mg/dL)	138.7	134.6	139.1	136.0	132.9	134.0

Table 5.1 (continued): Baseline comparison of men and women according to self-reported asthma history.

	Men			Women		
	No Asthma	**Child Onset† Asthma**	**Adult Onset† Asthma**	**No Asthma**	**Child Onset† Asthma**	**Adult Onset† Asthma**
	n=5931	**n=210**	**n=131**	**n=7809**	**n=203**	**n=283**
HDL Cholesterol (mg/dL)	44.8	44.8	47.2†	57.7	58.2	57.9
Inflammatory Markers						
Albumin (mg/dL)	3.92	3.96	3.91	3.83	3.78†	3.81
Fibrinogen (mg/dL)	295.4	293.9	305.7	306.6	315.2	315.5†
Hormonal Variables						
Current Hormone Replacement Therapy (%)	-	-	-	19.2	22.6	15.7
Post-Menopausal or Hysterectomy (%)	-	-	-	67.1	58.9†	75.9†
Asthma Medication Use						
Beta Adrenergics (%)	0.6	6.2†	29.0†	0.4	13.3†	22.6†
Glucocorticoids (%)	0.5	2.9†	12.2†	1.0	3.5†	9.2†

* Child onset = age < 21 years; adult onset = age ≥ 21 years;

† $p<0.05$ comparing subjects within asthma subtype to subjects reporting no history of asthma within each gender

Table 5.2: Incident Coronary Heart Disease Rates and Rate Ratios Among Men and Women According to Asthma History

	Males			Females		
	No History of Asthma	**Childhood Onset Asthma**	**Adult-Onset Asthma**	**No History of Asthma**	**Childhood Onset Asthma**	**Adult-Onset Asthma**
Crude Rate*	**7.85**	**8.52**	**6.22**	**3.52**	**3.49**	**7.34**
(Cases / Person-Years)	**(565 / 72006)**	**(22 / 2582)**	**(10 / 1607)**	**(348 / 98963)**	**(9 / 2580)**	**(25 / 3407)**
Crude Hazard Ratio (95% CI)	**1.0 (Ref)**	**1.08 (0.71, 1.66)**	**0.80 (0.43, 1.49)**	**1.0 (Ref)**	**0.99 (0.51, 1.93)**	**2.10 (1.40, 3.16)**
Multivariate Adjusted† HR (95% CI)	**1.0 (Ref)**	**1.25 (0.82, 1.92)**	**0.71 (0.38, 1.32)**	**1.0 (Ref)**	**0.95 (0.49, 1.83)**	**1.78 (1.18, 2.67)**

* Per 1000 person years

† Adjusted for age, BMI, black race, smoking status, diabetes, hypertension, education level, low and high density lipoprotein levels, and physical activity

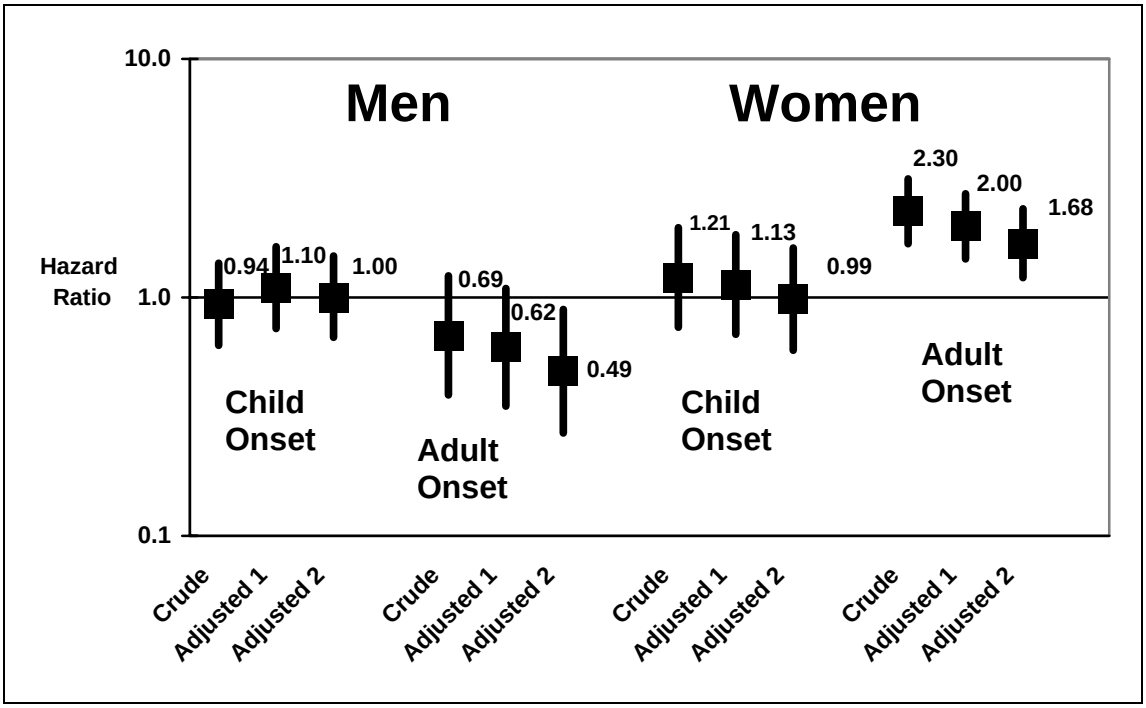
Table 5.3: Incident Stroke Rates and Rate Ratios Among Men and Women According to Asthma History

	Males			Females		
	No History of Asthma	**Childhood Onset Asthma**	**Adult-Onset Asthma**	**No History of Asthma**	**Childhood Onset Asthma**	**Adult-Onset Asthma**
Crude Rate*	**3.50**	**2.27**	**1.22**	**2.39**	**3.51**	**5.57**
(Cases / Person-Years)	**(257 / 73489)**	**(6 / 2639)**	**(2 / 1634)**	**(238 / 99443)**	**(9 / 2565)**	**(19 / 3411)**
Crude Hazard Ratio (95% CI)	**1.0 (Ref)**	**0.65 (0.29, 1.45)**	**0.35 (0.09, 1.41)**	**1.0 (Ref)**	**1.47 (0.76, 2.87)**	**2.36 (1.48, 3.76)**
Multivariate Adjusted† HR (95% CI)	**1.0 (Ref)**	**-**	**-**	**1.0 (Ref)**	**1.25 (0.64, 2.44)**	**2.08 (1.30, 3.32)**

* Per 1000 person years

† Adjusted for age, BMI, black race, smoking status, diabetes, hypertension, education level, low and high density lipoprotein levels, and physical activity

Figure 5.2: Hazard Ratios for Incident CHD or Stroke According to Asthma Age of Onset and Gender



Adjusted 1: Age, BMI, black race, smoking status, diabetes, hypertension, education level, low and high density lipoprotein levels, and physical activity

Adjusted 2: Adjusted for above plus FEV1, Chronic Bronchitis, Emphysema, and use of glucocorticoid or beta adrenergic medicines

Table 5.4: Never-smokers Only - Incident Combined CHD or Stroke Event Rates and Rate Ratios Among Men and Women According to Asthma History

	Males			Females		
	No History of Asthma	**Childhood Onset Asthma**	**Adult-Onset Asthma**	**No History of Asthma**	**Childhood Onset Asthma**	**Adult-Onset Asthma**
Crude Rate*	**8.31**	**5.49**	**10.69**	**4.75**	**5.40**	**10.59**
(Cases / Person-Years)	**(181 / 21757)**	**(5 / 910)**	**(5 / 468)**	**(251 / 52868)**	**(7 / 1296)**	**(18 / 1699)**
Crude Rate Ratio (95% CI)	**1.0 (Ref)**	**0.65 (0.27, 1.58)**	**1.31 (0.54, 3.18)**	**1.0 (Ref)**	**1.14 (0.51, 2.43)**	**2.24 (1.39, 3.62)**
Multivariate Adjusted HR† (95% CI)	**1.0 (Ref)**	**0.74 (0.30, 1.80)**	**1.04 (0.43, 2.53)**	**1.0 (Ref)**	**1.10 (0.52, 2.33)**	**2.05 (1.28, 3.31)**

* Per 1000 person years

† Adjusted for age, BMI, black race, diabetes, hypertension, education level, low and high density lipoprotein levels, and physical activity

CHAPTER 6

ADULT-ONSET ASTHMA IS ASSOCIATED WITH INCREASED CAROTID ATHEROSCLEROSIS AMONG WOMEN IN THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY

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Abstract

Some studies have suggested that asthma may be a risk factor for coronary heart disease and stroke, particularly in women. Child and adult-onset asthma differ according to inflammatory characteristics and gender distribution. We examined whether childhood-onset and adult-onset asthma were associated with carotid artery intima-media thickness (IMT) in men and women in the Atherosclerosis Risk in Communities (ARIC) study. In unadjusted analyses, the weighted mean far wall IMT thickness for women with history of adult-onset asthma was significantly greater than that of women without history of asthma (0.731mm vs. 0.681mm; $p<0.0001$) while IMT for women with history of childhood-onset asthma (IMT=0.684mm) did not differ substantially from non-asthmatic women. Mean IMT did not differ significantly according to asthma history among men. When the data were fitted to a linear model, the interaction between asthma status and gender was significant ($p=0.006$). After adjusting for age, race, BMI, smoking status, smoking pack years, diabetes, hypertension, physical activity, education level, and high and low density lipoprotein levels, the mean IMT difference between women with adult-onset asthma and no history of asthma was attenuated but remained significant (0.713mm vs. 0.687mm, $p=0.008$). In conclusion, adult-onset asthma but not child-onset asthma is associated with increased carotid atherosclerosis among women but not among men.

Introduction

Previous studies have linked potent inflammatory mediators known as leukotrienes, and the genes that regulate them, to both asthma and atherosclerosis (Dwyer et al. 2004; Jala and Haribabu 2004; Votava 1984). Other studies have suggested that asthma itself may be a risk factor for coronary heart disease and stroke, particularly in women (Iribarren, Tolstykh, and Eisner 2004; Schanen et al. 2005; Toren and Lindholm 1996). Despite this evidence, the importance of asthma as a risk factor for atherosclerotic vascular disease is not well-established. In addition, asthma is a heterogeneous disease with many recognized phenotypes (Bel 2004), which may have differential effects on atherosclerosis risk and therefore should be examined separately. One phenotypic distinction involves age of onset with two asthma subtypes, child-onset or adult-onset asthma. These asthma subtypes are distinct entities both for inflammatory pathophysiology (Hsu et al. 2004; Miranda et al. 2004) and patient susceptibility, with adult-onset asthma being more common in women and the child-onset subtype more common in men (Osman 2003).

In the present study we sought to examine the relationship between asthma and its subtypes (child-onset and adult-onset) with carotid artery intima-media thickness (IMT) in men and women who were participants in the Atherosclerosis Risk in Communities (ARIC) study. We hypothesized that IMT would be greater in subjects with history of either child- or adult-onset asthma, particularly in women.

Methods

The Atherosclerosis Risk in Communities (ARIC) study is a prospective study of the etiology of atherosclerotic, cardiovascular, and cerebrovascular disease in four communities in North Carolina, Mississippi, Minnesota, and Maryland. The study population of 15,792 men and women ages 45 to 64 years includes both black and white participants. Carotid IMT was measured in subjects during a baseline clinic visit during 1987 to 1989.

For the present study we examined whether far wall IMT measurements of subjects at baseline differed according to self-reported asthma history. The far wall thickness was chosen because it has been shown to be measured more accurately than the near wall (Wikstrand and Wendelhag 1994). We excluded 167 subjects because of missing asthma history data, 1154 subjects because of missing carotid IMT data, and 81 subjects due to history of endarectomy because IMT thickness may have been altered in these subjects. Carotid angioplasty and stenting were not yet being performed at the time IMT was measured in these subjects. An additional 763 subjects were excluded because of missing data for smoking, hypertension, diabetes, body mass index (bmi), physical activity, and lipid profile. This left 13,627 subjects available for analysis.

Measurement of Asthma and Covariates

Asthma history of subjects was classified according to whether they answered yes or no to the questions "Ever had asthma?" and "Was it confirmed by a doctor?". Subjects who reported having asthma but whose diagnosis was not physician confirmed (9.4% of those reporting asthma) were excluded from the study population. We further classified asthmatic subjects as having "adult-onset asthma" if they reported age of onset of 21 years of age or above or as having "childhood-onset asthma" if onset age was before age 21.

Self reported pack-years of smoking and current smoking status were used as measures of smoking behavior. Diabetes was defined as having an 8 or more hour fasting plasma glucose level greater than 126, a non-fasting plasma glucose greater than 200, or self report of diabetes or taking medications for treatment of diabetes (ARIC Investigators *ARIC manual 1. General description and study management* 1987, *ARIC protocol 7. Blood collection and processing* 1987, *ARIC protocol 10. Clinical chemistry determinations. Version 1.0* 1987). Hypertension was defined as diastolic blood pressure greater than or equal to 90 mm Hg, systolic pressure greater than 140 mm Hg, or self report of taking medication for hypertension in the two weeks prior to interview(Jones and Hall 2004). Physical activity was assessed according to self reported frequency, duration, and intensity of physical activity using a questionnaire and scale developed by Baecke et al. This scale features three index variables for physical activity according to work, sports, and leisure (non-sport)

(Baecke, Burema, and Frijters 1982). Education was measured as a three-level ordinal variable according to the number of years of school completed (<12 years, 12-16 years, or >16 years). Use of beta adrenergic and oral glucocorticoid asthma medications, which have been associated with cardiovascular disease (Au et al. 2003; Wei, MacDonald, and Walker 2004), was assessed for the period of two weeks prior to the baseline clinic visit. Subjects were asked to bring all prescription and non-prescription medications used during this period to the clinic visit and medications were coded according to drug category.

Pulmonary function was assessed by ARIC personnel using a Collins Survey II Volume Displacement spirometer according to established protocol (ARIC Investigators *ARIC protocol 4. Pulmonary function assessment. Version 7.* 1987). Because we hypothesized that increased atherosclerosis among asthmatics would be a result of systemic inflammation and not diminished lung function, which is independently associated with CHD (Schroeder et al. 2003), we examined whether the asthma-IMT association persisted after controlling for lung function measured as force expiratory volume in one second (FEV_1). The latter was categorized according to gender-specific quartiles.

Measurement of Carotid IMT

Carotid IMT was measured using B-mode ultrasound by trained technicians. Measurements were adjusted for reader differences and temporal trends and missing

measurements were imputed using maximum likelihood procedures. The overall mean far wall IMT was calculated by averaging the mean far wall measurements for up to six different far wall sites for each subject: the left and right common bifurcation, internal carotid, and common carotid (optimal angle). Further details on IMT measurement procedures in the ARIC study have been published previously (ARIC Investigators *High-resolution b-mode ultrasound reading methods in the Atherosclerosis Risk in Communities (ARIC) cohort*. 1991; ARIC Investigators *High-resolution b-mode ultrasound scanning methods in the Atherosclerosis Risk in Communities study (ARIC).* 1991).

Analysis

Baseline covariates were compared among subjects reporting no history of asthma, history of childhood-onset asthma, and history of adult-onset asthma separately among men and women using chi-square tests or pooled or unpooled t-tests.

Within each gender, t-tests were used to compare the weighted mean IMT of subjects with adult-onset asthma or childhood-onset asthma to the mean IMT of subjects with no history of asthma. The weight given to the overall mean far wall IMT for specific subjects was determined by dividing the number of far wall sites used to calculate the overall mean for each subject by six (the maximum number of sites). In this way, subjects whose mean IMT was based on more sites were given

more weight than those whose mean IMT was determined by fewer measurements. Using a class variable representing asthma status (none, adult-onset, or childhood-onset), we first tested for the asthma-gender interaction and then fit separate multivariable linear regression models for each gender that included age, race, education, body mass index, smoking status, smoking pack years, diabetes, hypertension, physical activity, and high and low density lipoprotein cholesterol levels. In these models, the multivariable adjusted mean IMT for each subtype of asthma was compared to the adjusted mean IMT of subjects with no history of asthma within each gender. We also tested for the interaction of asthma with smoking status in each gender-specific model. We also compared means when this model was refit excluding subjects with diabetes and hypertension. We then compared the means generated from additional multivariable models in which prevalent coronary heart disease, lung function and asthma medication use were also included.

IMT was also evaluated as a dichotomous outcome variable using logistic regression. For this analysis, significant atherosclerosis was defined as having a mean far wall IMT ≥1mm. This cutpoint is based upon previous research in this cohort suggesting that it is clinically significant in the prediction of incident coronary heart disease and stroke in both men and women (Chambless et al. 2000; Chambless et al. 1997)[7]. In our study population, this cutoff corresponds to the upper 10.4% and 4.4% of far wall IMT measurements for men and women, respectively. Multivariate analysis was performed using the same control variables as in the regression analysis.

We also performed additional analyses to investigate whether lung function was associated with IMT differently among men and women. Within each gender, we compared crude and multivariable adjusted mean weighted IMT according to gender-specific quartile of FEV_1. We performed trend tests within each gender using an ordinal variable for FEV_1 quartile to determine if decreasing lung function was associated with increasing mean IMT. Additionally, we also examined IMT according to current or former asthma status as well as asthma duration.

Results

The prevalence of adult-onset asthma was higher in women than in men (3.4% verses 2.2%) while childhood-onset asthma was more common among men than in women (3.1% verses 2.4%) (Chi-Square $p<0.0001$). The weighted mean far wall carotid IMT was 0.683 mm for women and 0.779 mm for men.

Both men and women with history of adult-onset asthma were older, had less education, lower FEV_1, more pack years of smoking, and were more likely to have diabetes and hypertension than their non-asthmatic counterparts (table 6.1). Women, but not men, with adult-onset asthma also had elevated BMI, were more likely to be African American, and reported lower leisure physical activity. Women with adult-onset asthma included a higher proportion of current and former smokers compared to non-asthmatic women, while men with adult-onset asthma had a higher proportion of former smokers and less current smokers. In both men and women, childhood-onset

asthma was associated with lower FEV_1, but to a lesser degree than adult-onset asthma. Childhood-onset asthma was also associated with a greater prevalence of diabetes among women but not among men. Use of beta-adrenergic and glucocorticosteriod drugs was elevated among all asthmatics but was greatest among those with adult-onset asthma. Work and sport physical activity did not differ according to asthma history among either men or women.

The weighted mean far wall IMT thickness for women with history of adult-onset asthma was significantly greater than that of women without history of asthma (0.731mm vs. 0.681mm; $p<0.0001$) while IMT for women with history of childhood-onset asthma (IMT=0.684mm) did not differ substantially from non-asthmatic women (table 6.2). Among women who reported never having smoked, the crude mean IMT was 0.667mm for non-asthmatics, 0.663mm for child-onset asthmatics, and 0.684 for adult-onset asthmatics. Men with either asthma phenotype had lower mean IMT compared to men with no history of asthma but these differences were not statistically significant. Among men who had never smoked, mean IMT was 0.727 for non-asthmatics, 0.724 for child-onset asthmatics, and 0.709 for adult-onset asthmatics. When the data were fitted to a crude linear model, the interaction between asthma status and gender was significant ($p=0.006$).

We fit separate multivariable models for each gender. After adjusting for age, race, BMI, smoking status, smoking pack years, diabetes, hypertension, physical activity, education level, and high and low density lipoprotein levels, the mean IMT

difference between women with adult-onset asthma and no history of asthma was somewhat attenuated but remained significant (0.713mm vs. 0.687mm, p=0.007) (table 6.2). The adjusted mean IMT for women with childhood-onset asthma (0.703mm) was also greater than that of non-asthmatic women but the difference was non-significant (p=0.21). Mean IMT for men with either type of asthma remained less than that of non-asthmatic men after adjustment. The interaction between smoking status and asthma was not significant within the male model (p=0.055) or female model (p=0.28). Addition of prevalent coronary heart disease, lung function and asthma medication use to the models did not generally change the results (table 6.2). The results were also similar when we excluded patients with hypertension and diabetes. Among women, adult onset asthmatics had significantly higher mean IMT (IMT=0.688mm; p=0.0096) compared to non-asthmatics (IMT=0.656mm) while child onset asthmatics did not (IMT=0.669mm; p=0.28). Among men, IMT was 0.754mm for nonasthmatics, 0.714 for adult onset asthmatics, and 0.757 for child onset asthmatics.

In analyses of the dichotomized outcome, women with history of adult-onset asthma were approximately twice as likely (odds ratio [OR]: 2.31, 95% CI, 1.34 to 3.96) to have mean far wall IMT thickness of 1mm or greater compared to women without history of asthma (table 6.3) while no significant association was observed among women with childhood-onset asthma. After multivariate adjustment, the odds ratios for women with adult-onset asthma were somewhat reduced but the association remained significant (Odds Ratio [OR]: 1.79, 95% CI, 1.00 to 3.22). Addition of

prevalent coronary heart disease, lung function and asthma medications to the model did not substantially change these estimates (table 6.3). No significant associations were observed among men with either type of asthma.

Decreasing lung function was significantly associated with greater carotid intima medial thickness in both men and women (p-values for trend tests<0.0001). Women in the lowest quartile of FEV_1 had mean IMT of 0.747mm compared to 0.634mm among women in the highest quartile (figure 6.1). Among men, those in the lowest quartile of lung function had IMT of 0.855mm compared to 0.718mm for men in the highest quartile. After adjustment for age, smoking, asthma, and other variables, these differences were reduced and IMT remained elevated only among men and women in lowest quartile of FEV1.

In supplemental analyses, asthma duration was not related to IMT among men or women of any asthma type and did not suggest a dose-response relationship (figure 6.2). In analyses comparing current and former asthma status with nonasthmatic status (figure 6.3), crude IMT was significantly greater in women who reported either current (IMT=0.707mm) or former (IMT=0.777mm) adult onset asthma compared with nonasthmatic women. Differences among women with child onset asthma or men with any asthma type were smaller and not significant.

Discussion

We found that adult-onset asthma was associated with carotid atherosclerosis among women. However, asthma was not associated with carotid atherosclerosis among women with childhood-onset asthma or men with either asthma phenotype. Women with adult-onset asthma had crude mean far wall IMT approximately 0.050 mm thicker than their non-asthmatic counterparts, which is comparable in magnitude to the difference between currently smoking women and non-smoking women in this cohort. This difference was reduced by half, but not eliminated, after adjustment for smoking, physical activity, lung function, asthma medication use, and other potential confounders.

The results of our study lend evidence to the notion that asthma is associated with atherosclerosis among women but not among men and further suggest that this relationship is limited to women with adult-onset asthma. Our results are consistent with several previous studies which have suggested that asthma is associated with coronary heart disease and stroke and that these associations are either limited to or more pronounced among women (Iribarren, Tolstykh, and Eisner 2004; Schanen et al. 2005; Toren and Lindholm 1996). Toren et al reported stronger associations between asthma and cardiovascular disease among women (All Vascular Disease SMR = 1.9, 95%CI 1.3 to 2.4; Ischemic Heart Disease SMR = 2.5, 95% CI: 1.7 to 3.3) than among men (All Vascular Disease SMR = 1.6; 95% CI: 1.1, 2.1; Ischemic Heart Disease SMR = 1.4; 95%CI: 0.8 to 2.0) (Toren and Lindholm 1996). Iribarren et al reported multivariate adjusted relative rates of CHD of 1.22 (95% CI: 1.14 to 1.31)

among asthmatic women and 0.99 (95% CI: 0.93 to 1.05) among asthmatic men in a large managed care population (Iribarren, Tolstykh, and Eisner 2004). In another study using the ARIC cohort, the rate ratios for stroke differed substantially among men (HR = 0.72, 95% CI: 0.26 to 1.95) and women (HR = 2.20, 95% CI: 1.25 to 3.90) although the authors reported that effect modification on the asthma/stroke association by gender was statistically non-significant (Schanen et al. 2005).

To our knowledge, our study is the first to examine asthma age of onset subtypes with respect to atherosclerotic risk. The fact that the association was limited to adult-onset asthma was an unexpected finding. The lack of association between child onset asthma could potentially be due to the fact that many such participants were no longer affected by asthma and were primarily affected only as children. However this seems unlikely as supplemental analysis revealed that women with current and former child onset asthma did not differ in regards to IMT. Another possibility is that child-onset asthmatics did not experience increased atherosclerosis due to different duration of corticosteroid use. However, literature suggests that use of corticosteroids may have either beneficial or detrimental cardiovascular effects depending on the dosage and administration route (Wei, MacDonald, and Walker 2004) (Huiart et al. 2005), Thus, it is difficult to predict what impact these drugs might have on the observed association.

Although the mechanism behind the observed differential association of IMT and asthma based on age of onset is unknown, distinct inflammatory mechanisms in

these two types of asthma may explain our findings (Hsu et al. 2004; Miranda et al. 2004). Adult-onset asthmatics have higher bronchial tissue eosinophil counts (Hsu et al. 2004)and twice as high urinary leukotriene E4 levels compared to childhood-onset asthmatics (Miranda et al.), despite the fact that the prevalence of allergen specific immunoglobulin E (IgE) decreases with increasing age of asthma onset (Hsu et al. 2004). The number of polymorphonuclear (PMN) cells in bronchial secretions, which produce leukotrienes, is also higher among adult-onset asthmatics while childhood-onset asthmatics have a greater number of CD3(+) lymphocytes (Miranda et al. 2004). Taken together, these data indicate a larger role of leukotriene-mediated inflammatory response in adult-onset asthma than the childhood-onset subtype, which in turn may lead to greater atherosclerotic risk.

The mechanism of the gender specificity reported in this study and in others is also unknown. Our analysis suggests that lower lung function does not appear to be associated with greater increases in IMT among women than among men, therefore lung function should not be the underlying mechanism for the stronger association of asthma history in women than in men. There is literature to suggest a role of female hormones. The human uterus is capable of synthesizing leukotrienes and uterine leukotriene release peaks during menstruation, suggesting hormone-dependent leukotriene production (Rees et al. 1987). It is possible that this hormonal effect on leukotriene production in the uterus may also affect inflammation elsewhere in the body and therefore predispose asthmatic women to atherosclerosis. Indeed, 30-40% of asthmatic women seen at allergy clinics report worsening of asthma during the

premenstrual and menstrual phase of their menstrual cycles (Vrieze, Postma, and Kerstjens 2003). Women's potentially greater vulnerability to the inflammatory consequences of asthma may parallel their distinct susceptibility to other inflammatory and immunologic diseases. For example, rheumatoid arthritis and lupus disproportionately affect women and are associated with substantially higher risk of coronary heart disease (Pasceri and Yeh 1999; Petri 2001; Van Doornum, McColl, and Wicks 2002).

A major limitation of this study is self reporting of asthma status. Asthma self-reporting, especially self-reporting of physician diagnosed asthma, has low sensitivity but high specificity (Toren, Brisman, and Jarvholm 1993). Because of the number of nonasthmatics in this study is very large compared to the number of asthmatics, misclassification with poor sensitivity but high specificity should have little or no effect on the validity of the point estimates. Nonetheless, although we only included asthmatic subjects who reported that their asthma diagnosis was confirmed by a physician, some of these patients may actually have chronic obstructive pulmonary disease (COPD) rather than asthma. COPD is associated with systemic inflammation and atherosclerosis (Sin and Man 2005). Even the gender specificity of the association we observed could potentially be explained by such misclassification as physicians may be more likely to misdiagnosis COPD as asthma in women than in men (Chapman, Tashkin, and Pye 2001). To investigate the impact of such misdiagnosis, we examined whether the association differed significantly according to smoking status because COPD is rare among non-smokers. We found that the

interaction with smoking was not statistically significant among women. Nonetheless, it is still possible that some of the association we detected among women with adult-onset asthma is attributable to the mixing of misdiagnosed COPD cases with adult-onset asthmatics.

Self reporting of asthma may also result in differential reporting rates according to the perceived health status of participants. We included subjects with symptomatic coronary heart disease in our study population, which is valid if it is assumed that the asthma-IMT association does not change substantially following diagnosis of CHD, regardless of treatment regimen. However, it is possible that subjects who perceive themselves to be in poor cardiovascular health would be more likely to recall other comorbidities such as asthma. To account for this, we controlled for prevalent coronary heart disease in the analysis. Furthermore, the gender specificity of the results would also seem to contradict a large role of such reporting differences as an explanation to the results, since there is no reason to suspect that recall bias occurs differently by gender.

Because the study is observational, confounding is also potential source of bias. Women with adult onset asthma had significantly higher prevalences of hypertension and diabetes compared to other women. However, the association of adult onset asthma with IMT in women persisted after controlling for these factors in multivariable models. This association remained even when we excluded subjects with hypertension and diabetes from analysis. One might also expect smoking,

physical activity, lung function, and asthma medication use to be potentially strong confounders of the association between asthma and atherosclerosis. While physical activity did not differ meaningfully according to asthma history, men and women in our study with adult-onset asthma did report greater pack years of smoking, had lower FEV1 measurements, and had a greater prevalence of asthma medication use than either non-asthmatics or those with childhood-onset asthma. In particular, women with adult-onset asthma reported almost 50% greater pack years of smoking compared to non-asthmatic women. Although we were able to control for these variables, we cannot discount the possibility of residual confounding as a potential source of bias.

In conclusion, adult-onset asthma is associated with carotid atherosclerosis among women but not among men. In contrast, childhood-onset asthma is not significantly associated with atherosclerosis among either women or men. This association, which should be confirmed in other cohorts, may have substantial public health importance because adult-onset asthma affects 3-4% of women and atherosclerotic disease is the leading cause of death among women. If confirmed, this association would help to identify women who could benefit from more attention to cardiovascular disease risk.

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Table 6.1: Baseline comparison of men and women according to self-reported asthma history.

	Men			Women		
Variable	No History of Asthma n=5766	Asthma Onset Age<21 Years n=189	Asthma Onset Age≥21 Years n=132	No History of Asthma n=7102	Asthma Onset Age<21 Years n=180	Asthma Onset Age≥21 Years n=258
Age	54.6	54.1	56.0*	53.8	53.0	54.6*
Education Level						
<12 Years	23.5%	21.9%	34.1%**	22.2%	20.6%	30.6%**
12-16 Years	36.7%	35.8%	27.3%**	45.2%	50.6%	38.4%**
>16 Years	39.8%	42.3%	38.6%**	32.6%	28.9%	31.0%**
Body Mass Index	27.3	27.2	27.2	27.5	27.6	28.5**
Black Race	21.4%	16.4%	13.6%*	27.0%	25.6%	33.7%*
Diabetes	10.9%	9.0%	15.2%	9.9%	13.9%	17.5%**
Hypertension	33.4%	28.6%	36.4%	32.8%	32.2 %	43.4%**
Prevalent Coronary Heart Disease	8.0%	7.7%	7.6%	1.9%	2.3%	3.2%
Smoking Status						
Current	27.6%	23.8%	19.7%*	24.8%	22.2%	28.7%
Former	43.8%	44.4%	53.8%*	21.9%	27.2%	24.0%
Never	28.6%	31.8%	26.5%*	53.4%	50.6%	47.3%
Smoking Pack Years	23.0	22.2	26.3	10.3	11.6	14.7**
Physical Activity						
Sports Index	2.58	2.59	2.66	2.34	2.34	2.28
Leisure Index	2.34	2.35	2.36	2.39	2.33	2.28**
Work Index	2.35	2.31	2.21	2.05	2.05	2.12

Table 6.1 (continued): Baseline comparison of men and women according to self-reported asthma history.

	Men			Women		
Variable	No History of Asthma n=5766	Asthma Onset Age<21 Years n=189	Asthma Onset Age≥21 Years n=132	No History of Asthma n=7102	Asthma Onset Age<21 Years n=180	Asthma Onset Age≥21 Years n=258
FEV_1 (liters)	3.34	3.10**	2.73**	2.45	2.21**	2.05**
Asthma Medication Use						
Beta Adrenergics	0.6%	5.3%**	30.3%**	0.5%	13.9%**	21.3%**
Glucocorticoids	0.5%	2.1%*	13.6%**	1.0%	3.3%**	8.9%**

*p<0.05; **p<0.01 comparing subjects within asthma subtype to subjects reporting no history of asthma within each gender

Table 6.2: Crude and Adjusted Mean Far Wall Carotid Intima Medial Thickness (IMT) Measurement According to Gender and Self-Reported Asthma History

	Men			Women		
	No History of Asthma n=5766	Asthma w/ Age of Onset<21 n=189	Asthma w/ Age of Onset≥21 n=132	No History of Asthma n=7102	Asthma w/ Age of Onset<21 n=180	Asthma w/ Age of Onset≥21 n=258
Crude Weighted* Mean Far Wall IMT (SE) [p-value] ‡	0.780 (0.003)	0.772 (0.018) p=0.62	0.757 (0.015) p=0.12	0.681 (0.002)	0.684 (0.013) p=0.81	0.731 (0.013) **p<0.0001**
Model 1** Multivariate Adjusted Least Square Mean Far Wall IMT (SE) [p-value] ‡	0.785 (0.003)	0.776 (0.014) p=0.51	0.760 (0.016) p=0.13	0.687 (0.002)	0.701 (0.011) p=0.21	0.713 (0.010) **p=0.007**
Model 2† Multivariate Adjusted Least Square Mean Far Wall IMT (SE) [p-value] ‡	0.785 (0.003)	0.775 (0.014) p=0.49	0.758 (0.017) p=0.13	0.686 (0.002)	0.705 (0.012) p=0.12	0.716 (0.010) **p=0.005**

*Weighted according to number of outer wall sites measured (to a maximum of six) to calculate mean outer wall thickness for a given individual. Weight for the i^{th} individual (W_i)=(number of sites)$_i$ / 6

** Adjusted for age, BMI, black race, smoking status and pack years, diabetes, hypertension, education level, low and high density lipoprotein levels, and physical activity

† Adjusted for Model 1 covariates plus prevalent coronary heart disease, lung function (FEV1) and use of Glucocorticoid and Beta-Agonist Asthma Medications

‡ p-values for t-tests comparing asthma subtype to non-asthmatics within each gender

Table 6.3: Frequencies and Odds Ratios* Comparing Mean Outer Wall Carotid Intima Media Thickness (IMT) ≥ 1.0mm According to Gender and Self-Reported Asthma History

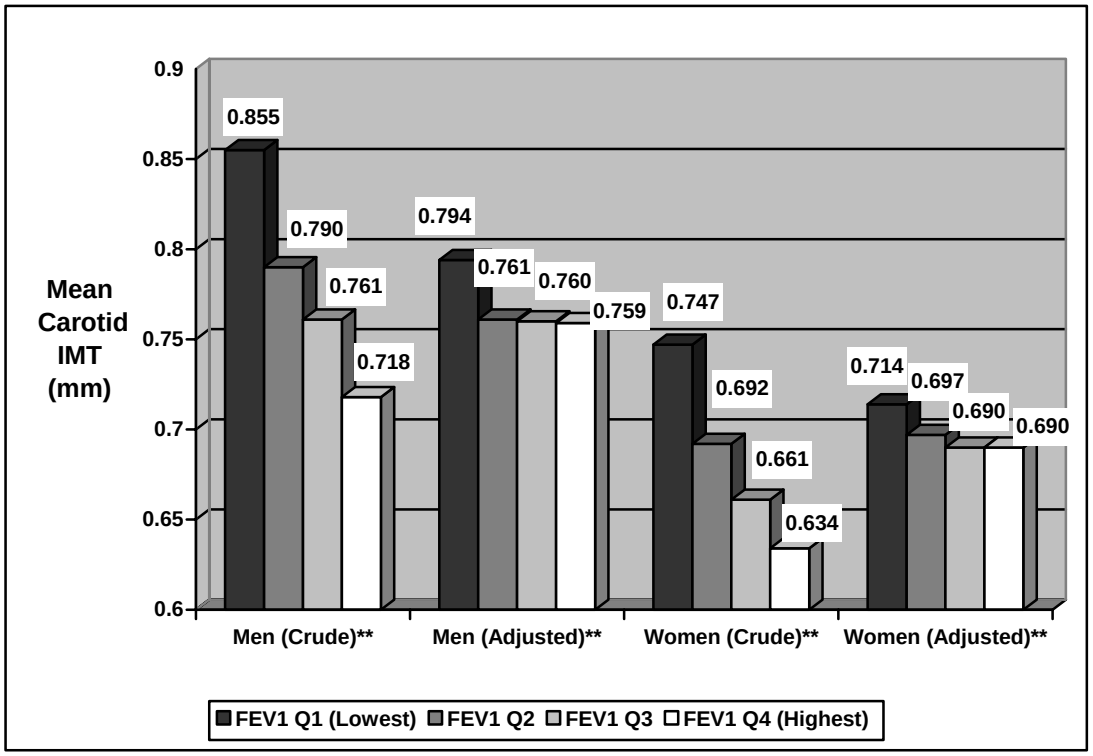
	Men			Women		
	No History of Asthma n=5766	Asthma w/ Age of Onset<21 n=189	Asthma w/ Age of Onset≥21 n=132	No History of Asthma n=7102	Asthma w/ Age of Onset<21 n=180	Asthma w/ Age of Onset≥21 n=258
Percentage of subjects with mean outer wall IMT≥1.0mm	10.4%	10.1%	12.9%	4.3%	3.3%	8.5%
Crude Odds Ratio (95% CI)	1.00 (Ref)	**0.83 (0.45, 1.52)**	**1.12 (0.59, 2.13)**	1.00 (Ref)	**0.68 (0.23, 1.99)**	**2.31 (1.34, 3.96)**
Model 1** Multivariate Adjusted Odds Ratio (95% CI)	1.00 (Ref)	**0.89 (0.46, 1.71)**	**0.93 (0.47, 1.84)**	1.00 (Ref)	**0.78 (0.26, 2.35)**	**1.79 (1.00, 3.22)**
Model 2† Multivariate Adjusted Odds Ratio (95% CI)	1.00 (Ref)	**0.83 (0.43, 1.63)**	**0.79 (0.38, 1.66)**	1.00 (Ref)	**0.84 (0.27, 2.56)**	**1.87 (1.01, 3.46)**

*****Odds ratios weighted according to number of outer wall sites measured (to a maximum of six) to calculate mean outer wall thickness for a given individual. Weight for the i^{th} individual (Wi)=(number of sites)i / 6

** Adjusted for age, BMI, black race, smoking status and pack years, diabetes, hypertension, education level, low and high density lipoprotein levels, and physical activity

† Adjusted for Model 1 covariates plus prevalent coronary heart disease, lung function (FEV1) and use of Glucocorticoid and Beta-Agonist Asthma Medication

Figure 6.1: Mean Crude and Adjusted* Carotid Intima Medial Thickness According to Gender-Specific Quartile of FEV1



* Adjusted for age, race, asthma status, BMI, smoking status, smoking pack years, diabetes, hypertension, physical activity, education level, and high and low density lipoprotein levels

**P-Values for all trend tests<0.0001

Figure 6.2: Crude Mean Carotid Intima Medial Thickness According to Asthma Type and Duration

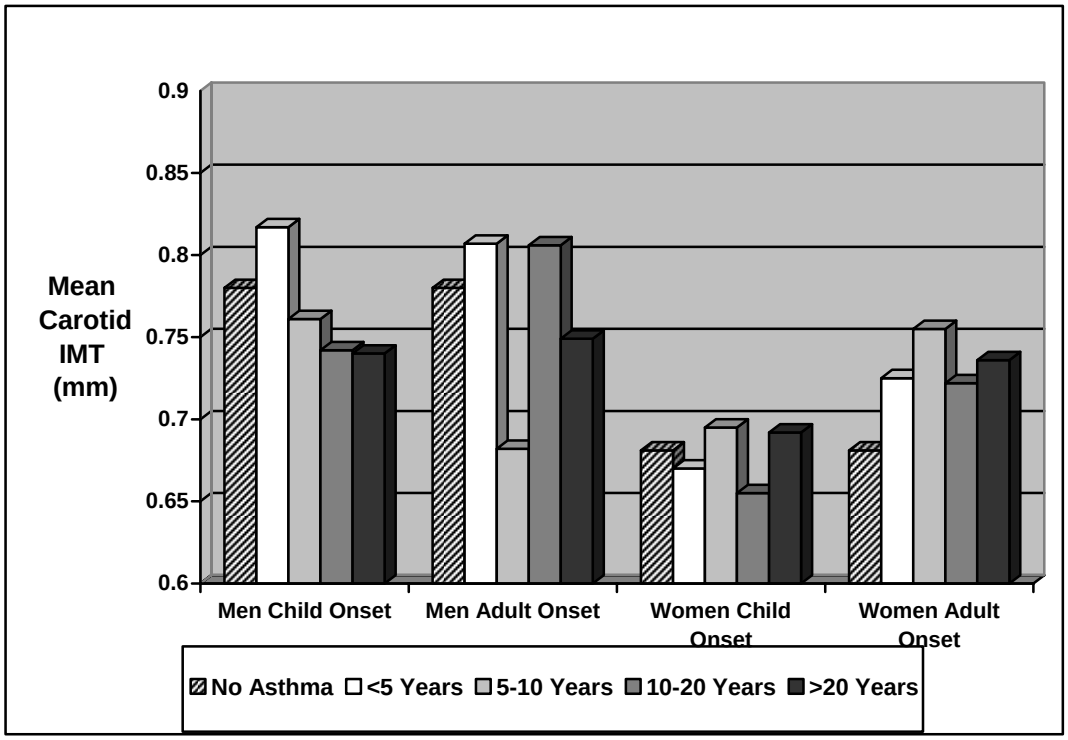
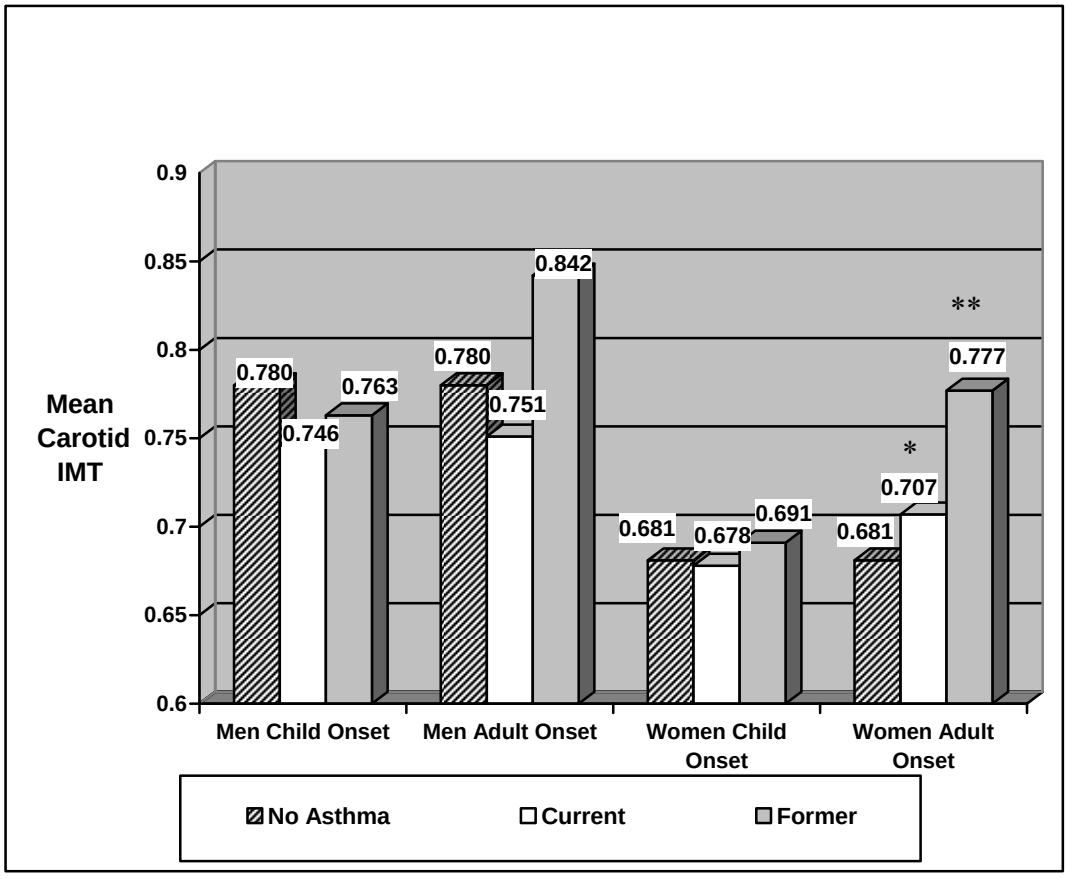


Figure 6.3: Crude Mean Carotid Intima Medial Thickness According to Asthma Type and Current Asthma Status



* p<0.05; ** p<0.0001; Comparing Mean IMT of Former or Current Asthma to No Asthma within Gender

CHAPTER 7: CONCLUSIONS

Atherosclerotic disease, which includes coronary heart disease and stroke, represents the leading cause of death among Americans (Smith 2005). Previous research suggests that asthma may be a risk factor for atherosclerotic disease (Iribarren, Tolstykh, and Eisner 2004; Knoflach et al. 2005; Liss et al. 1999; Liss et al. 2000; Schanen et al. 2005; Soriano et al. 2005; Toren and Lindholm 1996; Zureik et al. 2004) and some studies suggest that asthma may be a risk factor only among women (Iribarren, Tolstykh, and Eisner 2004; Schanen et al. 2005; Toren and Lindholm 1996). Nonetheless, there is insufficient evidence for this notion to be widely accepted and the underlying mechanism behind the observed asthma-atherosclerosis association remains unknown. Furthermore, literature indicates that asthma is not a single disease but rather a constellation of distinct subtypes and it is unknown whether specific asthma subtypes are differentially associated with atherosclerotic disease.

The primary goals of this dissertation were to confirm previous studies suggesting that asthma may be a risk factor for atherosclerotic disease, to determine whether the asthma-atherosclerosis association differed according to gender and asthma subtype, and to investigate the possibility that leukotriene-mediated inflammation is a biologic mechanism behind this association. This was done in three separate studies. In the first study, we investigated whether asthma was associated with coronary artery disease (CAD) among patients undergoing cardiac

catheterization at Emory University Hospital and further examined whether the asthma-atherosclerosis association differed by asthma age of onset subtype and gender. In the same study we also determined whether haplotypes of two genes involved in the production of leukotrienes interacted with asthma and gender in their association with CAD. In the second study of the dissertation, we examined the association of adult and child onset asthma subtypes with incident CAD and stroke and whether these associations differed by gender using data from the Atherosclerosis Risk in Communities (ARIC) study. The final study of the dissertation also utilized ARIC data and examined the gender-specific associations of adult and child onset subtypes with carotid intima-media thickness (cIMT), a marker of systemic atherosclerosis.

The results of both studies utilizing ARIC data suggest that atherosclerotic risk differs importantly according to asthma age of onset and gender. Women with a history of adult onset asthma experienced an approximately two-fold higher rate of CAD and stroke and had greater cIMT compared to nonasthmatic women. These associations were not observed among women with child onset asthma and neither child onset nor adult onset asthma was associated with atherosclerotic disease among men. Furthermore, the associations observed among women with adult onset asthma were independent of smoking status and spirometry-assessed pulmonary function.

In contrast to the ARIC studies, adult onset asthma and child onset asthma were negatively associated with CAD among both male and female patients referred

for cardiac catheterization. These results should be interpreted with caution because the catheterization population is not representative of the general population and the results are therefore susceptible to selection bias. Specifically, referral for cardiac catheterization is more likely among patients presenting with shortness of breath and chest discomfort, symptoms of both CAD and asthma. When these symptoms are reported by patients with asthma, they may be mistaken for symptoms of CAD rather than asthma, leading to over-selection of CAD-free asthmatics into the catheterization population. Indeed the age-adjusted prevalence of asthma among CAD-free female catheterization patients was almost 150% greater than that of the general population (NCHS 2005). Importantly, this study also found that the HapA and HapB haplotypes of the ALOX5AP gene were associated with CAD only among women with asthma. This finding supports the notion that inflammation is a mechanism behind the association of asthma with atherosclerotic disease. Furthermore, we also report for the first time that the HapK haplotype of the LTA4H gene is negatively associated with asthma.

Taken together, the results of this dissertation suggest that adult onset asthma is a risk factor for atherosclerotic disease among women but not among men in the general population and that leukotriene-mediated inflammation may be an underlying mechanism for this increased risk. The results also suggest that asthmatics may be referred for cardiac catheterization more often than necessary, perhaps due to the overlapping symptom profiles of asthma and CAD.

Recommendations for Further Research

The limitation common to all three studies of this dissertation is the use of self-reported asthma status. Furthermore, asthma subtype classification was limited to age of onset subtypes because laboratory and clinical asthma data were not available. As such, further research on the relationship between asthma and atherosclerosis should aim to measure asthma objectively using uniform clinical criteria, and further explore the role of asthma subtypes defined using laboratory and clinical indicators. Measurements of inflammatory markers such as C-reactive protein, interleukins, tumor necrosis factor, and alpha and urinary leukotrienes would also be beneficial to further investigate the role of inflammation as a potential mechanism in this relationship.

The interaction of ALOX5AP genotype, asthma status, and female gender on CAD which we observed among catheterization patients should be confirmed in a cohort more representative of the general population. Further studies should also explore the role of other genes in the arachidonic acid cascade as well as genes involved in other inflammatory pathways.

CHAPTER 8

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APPENDIX A

EMORY INTERNAL REVIEW BOARD (IRB) APPROVAL LETTER FOR CATHETERIZATION STUDY

EMORY UNIVERSITY

Institutional Review Board

Stephen Onufrak
SOM: Med: Cardiology
1518 Clifto RD NE 4th Flr
Atlanta, GA 30322

RE: **NOTIFICATION OF PROTOCOL APPROVAL**
PI: Stephen Onufrak
IRB ID: **731-2006**
TITLE: Asthma as a risk factor for coronary heart disease among patients undergoing cardiac catheterization
DATE: July 14, 2006

The research proposal referenced above was reviewed and APPROVED under the Expedited review process. This approval is valid from 7/5/2006 until 7/4/2007. Thereafter, continued approval is contingent upon the submission of a renewal form that must be reviewed and approved by the IRB prior to the expiration date of this study.

A waiver of authorization has been granted by the Emory University IRB for the purpose of determining eligibility and conducting this study. This waiver was reviewed and approved under the review procedure note above. The approval is granted based on this board's determination that all criteria for waiver of authorization have been met. The PHI that may be used or disclosed for this use is limited to: data previously collected for research purposes.

Any serious adverse events or issues resulting from this study should be reported immediately to the IRB and to any sponsoring agency (if any). Amendments to protocols and/or revisions to informed consent forms/process must have approval of the IRB before implemented.

All inquires and correspondence concerning this protocol must include the IRB number and the name of the Principal Investigator.

If you have any questions or concerns, please contact the IRB office at 404-712-0720 or at email address irb@emory.edu. Our web address is http://www.emory.edu/IRB. Thank you.

Sincerely,

Dayle Geroski, PhD
Vice Chair, Institutional Review Board

CC: Laura Viola Vaccarino MD, PhD

Emory University
1256 Briarcliff Road
4th Floor, South Wing
Atlanta, Georgia 30306

Tel 404.727.5646
Fax 404.727.1358
IRB@emory.edu

PAGE 2 of 2 - PROTOCOL APPROVAL

This approval is valid from 7/5/2006 until 7/4/2007.

IRB ID: **731-2006** DATE: July 14, 2006

The above referenced protocol was approved including the information below. Please review this information for accuracy. If there are any discrepancies, please notify the IRB office.

This study has no subjects.

Personnel		**Human Subjects Education Certification Information**
Onufrak, Stephen	Main Investigator	CITI - Refresher Course (06-Jun-2006)
Vaccarino, Laura Viola	Co-Investigator	CITI - MED Refresher course (13-Jul-2006)

Number of Approved Emory Subjects 1600 **(This number indicates the number of subjects you can consent.)**

Sites

Briarcliff Campus (Emory West)
Rollins Building
School of Public Health

APPENDIX B

EMORY INTERNAL REVIEW BOARD (IRB) APPROVAL LETTER FOR ARIC STUDY

EMORY UNIVERSITY | Institutional Review Board

Stephen Onufrak
RSPH: Epidemiology, 4th Floor
1518 Clifton RD NE
INTEROFFICE MAIL

RE: **NOTIFICATION OF PROTOCOL APPROVAL**
PI: Stephen Onufrak
IRB ID: **707-2004**
TITLE: Asthma as a Risk Factor for CHD in the ARIC Study
DATE: August 04, 2004*

The research proposal referenced above was reviewed and APPROVED under the Expedited review process. This approval is valid from 7/1/2004 until 6/30/2005. Thereafter, continued approval is contingent upon the submission of a renewal form that must be reviewed and approved by the IRB prior to the expiration date of this study.

Any serious adverse events or issues resulting from this study should be reported immediately to the IRB and to any sponsoring agency (if any). Amendments to protocols and/or revisions to informed consent forms/process must have approval of the IRB before implemented.

Page 2 of this letter contains a list of study personnel. If it indicates that the Principal Investigator (PI) does not have human subjects certification, the study will be suspended in thirty (30) days unless proof of training is provided to the IRB Office.

During this suspension period, all enrollment and other research activities related to this protocol must cease and it could affect the use of research funds for this project. If other study personnel are not certified, they may not participate in any part of the study until the IRB Office has been provided with proof of certification.

All inquires and correspondence concerning this protocol must include the IRB number and the name of the Principal Investigator.

If you have any questions or concerns, please contact the IRB office at 404-727-5646 or at email address: irb@emory.edu. Our web address is http://www.emory.edu/IRB. Thank you.

Sincerely,

J. W. Keller

James W. Keller, MD
Chairman, Institutional Review Board
JWK/mc

*** Regenerated Approval– Status was changed to 'Expedited'.**

Emory University
1256 Briarcliff Road
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An equal opportunity, affirmative action university

Tel 404.727.5646
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Email IRB@emory.edu

PAGE 2 of 2 - PROTOCOL APPROVAL

This approval is valid from 7/1/2004 until 6/30/2005.

IRB ID: **707-2004** DATE: August 04, 2004

The above referenced protocol was approved including the information below. Please review this information for accuracy. If there are any discrepancies, please notify the IRB office.

This study has no subjects.

Personnel		**Human Subjects Education Certification Information**
Onufrak, Stephen	Main Investigator	CITI - MED 1, 2, 3, 7, 12, 14, 17 (14-Jun-2004)
Vaccarino, Laura Viola	Co-Investigator	CITI - MED 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 (18-Nov-2003) SHB 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 (18-Nov-2003)

Number of Approved Emory Subjects 0 **(This number indicates the number of subjects you can consent.)**

Sites
Briarcliff Campus (Emory West)
School of Public Health

Funding Agencies
NOT FUNDED

APPENDIX C

SUBJECT QUESTIONNAIRE FOR CATHETERIZATION STUDY

Cardiology Genebank—Patient Questionnaire

PATIENT IDENTIFICATION

Today's Date _ _ / _ _ / _ _ _ _

Last Name _____

First Name _____

Middle Initial ____

Date of Birth _ _ / _ _ / _ _ _ _

SSN _ _ _ - _ _ - _ _ _ _ ☐ N/A ☐ Unknown

Hospital Medical Record_____

Hospital _____

BASELINE INTERVIEW STATUS

For cath patients: Was interview completed before or after cath. ? ○ Before ○ After

If interview not conducted, check primary reason:

○ Refused interview	○ Died prior to interview attempt
○ Too ill to interview	○ Discharged alive prior to interview
○ Hard of hearing	○ Non-English speaking
○ Hospice care	○ Other _____

HEIGHT AND WEIGHT

Subject's Height (as recorded from chart): ____feet ____inches

Subject's Weight (as recorded from chart): _____ pounds

PATIENT CONTACT INFORMATION

Patient information:

Address (line 1) _____
Address (line 2) _____
City _____
State _ _
ZIP code _ _ _ _ _
Home phone (_ _ _) _ _ _ - _ _ _ _
Work phone (_ _ _) _ _ _ - _ _ _ _
Cell phone (_ _ _) _ _ _ - _ _ _ _
e-mail _____

RELATIVE CONTACT INFORMATION

Name of relative or other contact:

Last Name _____
First Name _____
City _____
State _ _
Phone (_ _ _) _ _ _ - _ _ _ _
Relationship: _____

Name of an additional relative/contact:

Last Name _____
First Name _____
City _____
State _ _
Phone (_ _ _) _ _ _ - _ _ _ _
Relationship: _____

PHYSICIAN CONTACT INFORMATION

Primary Care Physician Information:

Name of PCP _____
City _____
State _____
Phone (if known) _____

If Don't Know Information, Check Here _____

If Don't Have Primary Care Physician, Check Here _____

Cardiologist Information:

Name of Cardiologist _____
City _____
State _____
Phone (if known) _____

If Don't Know Information, Check Here _____

If Don't Have Cardiologist, Check Here _____

REASONS FOR CATHETERIZATION

How did it come about that you were referred for catheterization? (check all that apply)

__ Heart attack
__ Angina
__ Shortness of breath
__ Fatigue
__ Test results
__ Doctor recommended—Don't Know reason
__ Other (specify_____)

PATIENT DEMOGRAPHICS

What is your age?
_____ years.

What is your gender?
__ Male
__ Female

How would you describe your race?
__Caucasian (White)
__African American (Black)
__Hispanic
__Asian
__Native American
__Other

What is your marital status?
__Married
__Widowed
__Divorced
__Separated
__Never Married

What is your employment status?
__Employed Full Time (more than 35 hrs./week)
__Employed Part Time (less than 35 hrs./week)
__Unemployed
__Unable to work due to disability
__Retired

What is your highest level of education?
__Elementary or Middle School
__Some High School
__High School Graduate
__Some College
__College Graduate
__Graduate Education or Degree

PATIENT HEALTH HISTORY (must be answered by patient alone, or by patient with assistance from interviewer)

Please indicate whether a **doctor** has ever diagnosed you with one of the following diseases/conditions:

Heart Attack __Yes → how many heart attacks?__ → age at first diagnosis? ___→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Angina (Chest Pain) __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Blocked or Clogged Arteries in the Heart
(Coronary Heart Disease) __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Heart Failure __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Stroke __Yes → how many strokes?___ → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Blockage or Hardening
of the Arteries in the Neck __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Blood Clot (thrombosis)
in the Legs, Arms or Lungs __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) __
__No
__Don't Know

Blocked arteries in your legs
(Periph. Vascular Disease) __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

PATIENT HEALTH HISTORY (CONTINUED)

Please indicate whether a **doctor** has ever diagnosed you with one of the following diseases/conditions:

Arrhythmia
(irregular heart beat) __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

High Blood Pressure __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

High Cholesterol __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Diabetes __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Cancer __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Depression __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Sleep Apnea __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

Asthma __Yes → age at first diagnosis? ____→ disease/condition now treated by a doctor? (y/n) ___
__No
__Don't Know

HISTORY OF CABG, PTCA, OR, HEART TRANSPLANT

Have you ever had coronary bypass surgery (CABG)?

___Yes → How many surgeries?____ → Year of most recent surgery _____
___No

Have you ever had angioplasty (Stent/Baloon/PTCA/PCI)?

___Yes → How many angioplasties?____ → Year of most recent angioplasty _____
___No

Have you had a heart transplant?

___Yes → Year of Transplant _____
___No

ANGINA (must be answered by patient alone, or by patient with assistance from interviewer)

Over the past 4 weeks, on average, how many times have you had chest pain, chest tightness or angina?

4 or more times per day	1-3 times per day	3 or more times per week but not every day	1-2 times per week	Less than once a week	None over the past 4 weeks**
☐	☐	☐	☐	☐	☐

**If answered "None over the past 4 weeks" to the question above, please skip to page 12

The following is a list of activities that people often do during the week. Although for some people with several medical problems it is difficult to determine what it is that limits them, please go over the activities listed below and indicate how much limitation you have had due to chest pain, chest tightness or angina over the past 4 weeks.

	Extremely Limited	Quite a bit Limited	Moderately Limited	Slightly Limited	Not at all Limited	Limited for other reasons or did not do the activity
Dressing yourself	☐	☐	☐	☐	☐	☐
Walking indoors on level ground	☐	☐	☐	☐	☐	☐
Showering	☐	☐	☐	☐	☐	☐
Climbing a hill or a flight of stairs without stopping	☐	☐	☐	☐	☐	☐
Gardening, vacuuming or carrying groceries	☐	☐	☐	☐	☐	☐
Walking more than a block at a brisk pace	☐	☐	☐	☐	☐	☐
Running or jogging	☐	☐	☐	☐	☐	☐
Lifting or moving heavy objects (e.g. furniture, children)	☐	☐	☐	☐	☐	☐
Participating in strenuous sports (e.g. swimming, tennis)	☐	☐	☐	☐	☐	☐

ANGINA (CONTINUED)

Over the past 4 weeks, on average, how many times have you had to take nitroglycerin (nitroglycerin tablets or spray) for your chest pain, chest tightness or angina?

4 or more times per day	1-3 times per day	3 or more times per week but not every day	1-2 times per week	Less than once a week	None over the past 4 weeks
☐	☐	☐	☐	☐	☐

Over the past 4 weeks, how much has your chest pain, chest tightness or angina limited your enjoyment of life?

It has extremely limited my enjoyment of life	It has limited my enjoyment of life quite a bit	It has moderately limited my enjoyment of life	It has slightly limited my enjoyment of life	It has not limited my enjoyment of life at all
☐	☐	☐	☐	☐

If you had to spend the rest of your life with your chest pain, chest tightness or angina the way it is right now, how would you feel about this?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
☐	☐	☐	☐	☐

SHORTNESS OF BREATH/SRH (must be answered by patient alone, or by patient with assistance from interviewer)

Over the past 4 weeks, have you experienced **shortness of breath**…

	Yes	No
…hurrying on level ground or walking up a slight hill?	☐	☐
…walking with people your own age on level ground?	☐	☐
…walking at your own pace on level ground?	☐	☐
…washing or dressing?	☐	☐

How often do you think or worry that you may have a heart attack or die suddenly?

I can't stop thinking or worrying about it	I often think or worry about it	I occasionally think or worry about it	I rarely think or worry about it	I never think or worry about it
☐	☐	☐	☐	☐

In general, would you say your health is:

Excellent	Very Good	Good	Fair	Poor
☐	☐	☐	☐	☐

FAMILY HISTORY (must be answered by patient alone, or by patient with assistance from interviewer)

Please fill out the table below about the health history of your parents and brothers/sisters. If you are unsure of an answer, please leave blank.

Relative	Blood Relative (y/n/dk)	Living or Dead (L/D)	Age now or age at death	Heart Attack? (y/n/dk) If yes, what was the age at first heart attack?	Angioplasty (PTCA) or Coronary Bypass Surgery(y/n/dk) If yes, at what was age at first CABG/PTCA?	Congestive heart failure? (y/n/dk)	Stroke (y/n/dk)	Diabetes/High Blood Sugar (y/n/dk)	Cancer (y/n/dk)
Father									
Mother									

BEHAVIORAL (must be answered by patient alone, or by patient with assistance from interviewer)

(1) Do you currently smoke cigarettes?
__Yes
__No (skip to question 5)

(2) On average, how many cigarettes do you smoke per day?
___ Less than 1 cigarette per day
___ 1 to 5 cigarettes per day
___ 6 to 10 cigarettes per day
___ 11 to 15 cigarettes per day
___ 16 to 20 cigarettes per day
___ 21 to 40 cigarettes per day
___ More than 40 cigarettes per day

(3) For how many years have you been smoking this number of cigarettes per day? ____

(4) Before that time, how many cigarettes did you smoke per day? (check all that apply)
___ Did not smoke (skip to question 9)
___ Less than 1 cigarette per day for ___ years
___ 1 to 5 cigarettes per day for ___ years
___ 6 to 10 cigarettes per day for ___ years
___ 11 to 15 cigarettes per day for ___ years
___ 16 to 20 cigarettes per day for ___ years
___ 21 to 40 cigarettes per day for ___ years
___ More than 40 cigarettes per day for ___ years

(skip to question 8)

(5) Did you smoke cigarettes in the past?:
__Yes
__No (skip to question 9)

(6) how many years has it been since you stopped smoking cigarettes?__

(7) When you were smoking cigarettes, on average, how many cigarettes did you smoke per day? (check all that apply)

___ Did not smoke (skip to question 9)
___ Less than 1 cigarette per day for ___ years
___ 1 to 5 cigarettes per day for ___ years
___ 6 to 10 cigarettes per day for ___ years
___ 11 to 15 cigarettes per day for ___ years
___ 16 to 20 cigarettes per day for ___ years
___ 21 to 40 cigarettes per day for ___ years
___ More than 40 cigarettes per for day ___ years

(8) Are you currently smoking cigars or pipes?
__Yes
__No

BEHAVIORAL (CONTINUED)

(9) Do you currently consume any alcohol?
__Yes
__No (skip to question 14)

(10) During an average **week**, how many cans or bottles of beer do you consume?

___ Less than 1 can/bottle per week
___ 1 to 3 cans/bottles per week
___ 4 to 7 cans/bottles per week
___ 8 to 14 cans/bottles per week
___ More than 14 cans/bottles per week

(11) During an average week, how many glasses of wine, sangria, or champagne do you consume?

___ Less than 1 glass per week
___ 1 to 3 glasses per week
___ 4 to 7 glasses per week
___ 8 to 14 glasses per week
___ More than 14 glasses per week

(12) During an average week, how many drinks of hard liquor of liquor such as tequila, gin, vodka, scotch, rum whiskey, liquors do you drink (either as shots or as mixed drinks)?

___ Less than 1 drink per week
___ 1 to 3 drinks per week
___ 4 to 7 drinks per week
___ 8 to 14 drinks per week
___ More than 14 drinks per week

(13) Considering all types of alcoholic beverages,, how many times during an average 30 day period do you drink 5 or more drinks on a single occasion? _____

(14) Was there a time in the past that you drank significantly more alcohol than you drink now?
__Yes
__No

(15) Prior to the onset of your symptoms, how many hours in a typical week did you regularly engage in vigorous physical activity (for example, running/jogging, rapid walking, aerobics, tennis, basketball, weight lifting etc.).
____ hours.

MOOD (must be answered by patient alone, or by patient with assistance from interviewer)

Over the last 2 weeks, how often have you been bothered by any of the following problems?

	Not at all	Several Days	More than half the days	Nearly every day
Little interest or pleasure in doing things	☐	☐	☐	☐
Feeling down, depressed, or hopeless	☐	☐	☐	☐
Trouble falling or staying asleep, or sleeping too much	☐	☐	☐	☐
Feeling tired or having little energy	☐	☐	☐	☐
Poor appetite or overeating	☐	☐	☐	☐
(Feeling bad about yourself, or that you are a failure or have let yourself or your family down	☐	☐	☐	☐
Trouble concentrating on things, such as reading the newspaper or watching television	☐	☐	☐	☐
Moving or speaking so slowly that other people could have noticed. Or the opposite—being so fidgety or restless that you have been moving around a lot more than usual	☐	☐	☐	☐
Thoughts that you would be better off dead or of hurting yourself in some way	☐	☐	☐	☐

Are you currently taking medications or receiving counseling for depression?

__Yes
__No