As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Kenneth James Wysocki entitled Leukotriene Receptor Variation and Atopic Asthma and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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SIGNED: Kenneth James Wysocki
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James 1:5

Like the two wings of a bird, love and insight work cooperatively to bring about enlightenment - fundamental Buddhist teaching (Dalai Lama).
DEDICATION

This dissertation is dedicated those suffering with asthma, health care providers, scientists in pulmonary health, and to my partner, Randy, my source of joy, hope, and inspiration.

Genome Path by Kenneth Wysocki

DNA the spiral of life
3.3 billion bases lined up to tell a story
Genes in concert with each other
Expressing more than health and disease
Humans the recipients of order out of chaos across
Generations shaped by ancestors before us

Scientists connecting hypotheses and evidence
That we might dream of the possibilities
Humans emancipated from the chains of the unknown
Health care providers presenting the keys
Ours to open the doors to health

So goes the story as the pages are turned
Chapters revealing secrets and defining new paths
Individuals step forward
Communities become engaged
Countries broker these ideas
A world is awakened to see the new dawn

Will we sit and watch the world before us?
Will we pretend to not hear the call?
Will we instead engage in the dialogue?
Will we lend a hand to strengthen the cause?

DNA from our ancestors
Tell a story of our common thread
As genes are unfolded
So too our history is carved

It is out of chaos that we move forward
A beacon of light, the call of the wind
Molecular patterns of chemistry before us
Will we uproot what we have envisioned?
Or nourish the dream that winds before us?
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ABSTRACT

Atopic asthma is a complex disease process that has a significant social, personal and economic burden across all ages. Leukotriene-receptors are involved in the cascade of inflammation that may result in symptoms of atopy and asthma. Two leukotriene receptors have been identified in the lung. The cysteinyl leukotriene receptor 1 and cysteinyl leukotriene receptor 2 genes (i.e., CYSLTR1 and CYSLTR2) have been sequenced, and a number of single nucleotide polymorphisms (SNPs) within these genes have been identified.

The purpose of this study was to: (1) Determine the relationship between CYSLTR1 genotypes, CYSLTR2 genotype, atopy, elevated IgE level, and eosinophilia, (2) Determine the relationship between CYSLTR1 genotypes, CYSLTR2 genotype, asthma, and atopic asthma, and (3) Determine the degree of interaction between CYSLTR2 genetic variation and gender in atopic asthma.

Nested within two sub-studies of the Tucson Epidemiological Study of Airway Obstructive Disease (TESAOD) study, a prospective longitudinal cohort, 853 individuals were entered into this study. Study criteria included Non-Hispanic white adults, who consented to genetic testing in the two sub-studies. Tagging SNPs (i.e., rs320991, rs321006, rs321073, rs912278, and rs2407249) of the CYSLTR1 and CYSLTR2 genes were genotyped by Sequenom system. Serum IgE status and eosinophilia were obtained from existing dataset. Questionnaires collected in the parent study were used to obtain demographic and clinical data.

SNP rs321006 in the CYSLTR1 gene was associated with atopy among Non-Hispanic white women. SNP rs321073 in the CYSLTR1 gene was associated with atopic asthma among Non-Hispanic white women in a recessive genetic model of inheritance. Assuming a recessive
model, among female Non-Hispanic white adults, the odds of having rs321073 CC genotype was 5.82 times higher among those with atopic asthma than those without atopic asthma. No gene by gender interaction was found between SNP of interest in CYSLTR2 and atopic asthma. Genetic association of SNPs rs321006 with atopy and rs321073 with atopic asthma are novel findings to date.

Implications for nurses, clinicians, and scientists include better understanding of associations of these genetic variations with asthma, atopy, and atopic asthma that can generate further inquiry into other mechanisms of atopic asthma. These novel genetic associations with atopy and atopic asthma may have the potential for personalized medicine that might afford patients with appropriate treatment based on their genotype.
CHAPTER I: INTRODUCTION & THEORETICAL FRAMEWORK

Background

Asthma is an enormous economic, social, and quality of life burden in the United States. Evidence indicates that over 50% of asthma cases are attributed to atopy and this percentage is higher in men than women (Arbes, Gergen, Vaughn, & Zeldin, 2007). Asthma is a complex disease process involving many factors and pathways leading to wheezing, shortness of breath, chest tightness, and nighttime or early morning coughing (Centers for Disease Control and Prevention, 2011b). Atopy (or inherited predisposition to allergies) is the body’s hypersensitivity response to the environment triggering a complex inflammatory process that includes leukotrienes, leukotriene receptors, eosinophils, immunoglobulin E (IgE) and skin reactivity (Centers for Disease Control and Prevention, 2011a; Ronchetti, Macri, Ciofetta, Indinnimeo, Cutrera, et al., 1990; Stedman). Atopic asthma is a form of asthma that includes the observable traits of atopy (or atopic phenotype).

Leukotrienes are chemical mediators that have been implicated in the cascade of events that contribute to atopy, asthma, and chronic disease. Cysteiny1 leukotriene receptor 1 (CYSLTR1) and cysteiny1 leukotriene receptor 2 (CYSLTR2) are located in the lung and serve as the ligands for leukotrienes. Leukotriene - receptor complexes result in intracellular signaling, cellular responses, and subsequent clinical manifestations of atopy and asthma including mucus secretion, edema, and eosinophilia (Gould, & Sutton, 2008; Shelhamer, Levine, Wu, Jacoby, Kaliner, et al., 1995; Vercelli, 2008). Research has indicated that genetic polymorphisms and gender difference play a role in atopic asthma phenotypes and contribute to asthma severity, management, and progression (Gould, et al., 2008; Vercelli, 2008). Evidence suggests that
genetic variations in leukotriene receptors (CYSLTR1 and CYSLTR2) play a role in the expression of inflammation in atopic disease, airway dysfunction, and remodeling in asthma; however, these studies are limited to homogenous populations outside the United States (Evans, 2002; Hao, Sayers, Cakebread, Barton, Beghe, et al., 2006; Klotsman, York, Pillai, Vargas-Irwin, Sharma, et al., 2007).

Theoretical Framework

A physiologic framework guides this research (Figure 1). The framework contains physiologic concepts and associated empirical referents that can be measured. Central to the framework is the physiologic concept that leukotriene-receptor interactions lead to cellular responses that result in the atopic asthma phenotype. The ultimate cellular response is influenced by genetic variations in leukotriene receptors and gender (S. H. Kim, Oh, Kim, Palmer, Suh, et al., 2006). This research measured receptor polymorphisms and characteristics of the asthma phenotype including gender differences on cell signaling (e.g., IgE), and characteristics of atopic asthma, including cellular responses (e.g., elevated eosinophil count and skin prick test reactivity) and symptoms of asthma. In keeping with the intent of a theoretical framework, it was expected that this research would generate new information with practical use in society (Wright, & Subramanian, 2007), and specifically, in the care of those with atopic asthma.
A physiological framework guides this research. Leukotriene-leukotriene receptor interactions may be influenced by genetic variation of leukotriene receptors (i.e., cysteiny1 leukotriene receptors 1 and 2). These genetic variations may alter cell signaling (i.e., increased IgE) and cellular responses (i.e., eosinophilia) resulting in the atopic asthma phenotype (i.e., bronchoconstriction, mucous secretion, edema, and skin reactivity). This research focused on the concepts and measures in the shaded boxes.

Statement of Purpose

The purpose of this dissertation research is to determine whether CYSLTR1 and CYSLTR2 genetic variations are associated with inflammatory markers of atopy, with atopic asthma, and with gender. This study was conducted in an effort to better understand the development of atopic asthma from a genetic epidemiologic perspective; this dissertation research addressed three specific aims from a parent longitudinal study, Tucson Epidemiological Study of Airway Obstructive Disease (TESAOD), which included a homogenous Non-Hispanic white population.
Specific Aims

Specific Aim 1: Determine the relationship of the CYSLTR1 and CYSLTR2 genetic variations to inflammatory markers of atopy including IgE level, blood eosinophil count, and skin test reactivity.

Hypothesis 1a: There is a positive association between CYSLTR1 and CYSLTR2 genetic variations and IgE level.

Hypothesis 1b: There is a positive association between CYSLTR1 and CYSLTR2 genetic variations and blood eosinophil count.

Hypothesis 1c: There is a positive association between CYSLTR1 and CYSLTR2 genetic variations and skin test reactivity.

Specific Aim 2: Determine the relationship of CYSLTR1 and CYSLTR2 genetic variations to atopic asthma.

Hypothesis 2: There is a positive association between CYSLTR1 and CYSLTR2 genetic variations and atopic asthma.

Specific Aim 3: Determine gene by gender interactions in association with CYSLTR2 genetic variations in affecting the risk for atopic asthma.

Hypothesis 3: There is an interaction between gender and CYSLTR2 genetic variations in affecting the risk for atopic asthma.
Significance

Significance of the problem

Asthma has been identified as a serious public health problem in the United States with steady increases in prevalence since 1980. Mortality rates associated with asthma are relatively low; however, the lifetime prevalence is common (i.e., 1 in 15 Americans), affects all age groups, and contributes to a steady increase in social and economic burden (Akinbami, 2006). Symptoms of asthma are reversible for most individuals (i.e., mild intermittent or mild persistent asthma), however, some individuals do not have adequate control of their disease even with maintenance medications including long acting beta-agonists, steroids, and leukotriene receptor antagonists.

Significance of the study

With the focus on evidence-based medicine, national agencies have addressed the ongoing health care problem of asthma in the United States, identified a need for further research, and funded numerous research studies related to asthma. The United States Environmental Protection Agency (EPA) Office of Research and Development scientists established the Asthma Research Strategy focusing on research on environmental triggers and susceptibility factors contributing to asthma such as genetics, health status, socioeconomic status, residence and exposure history, and lifestyle and activity patterns (United States Environmental Protection Agency, 2002).

In relation to asthma, the Healthy People 2020 document calls for reductions in deaths, hospitalizations, emergency department visits, reductions in activity limitations, missed school or work days, and an increase in appropriate asthma care (United States Department of Health and
The National Institute of Health (NIH) produced Fact Sheets, available online at the Research Portfolio Online Reporting Tools webpage, to “tell the stories of research discovery, current treatment status, and future expectations for the prevention and treatment of diseases and conditions affecting the nation's health” (National Institute of Health Research Portfolio Online Reporting Tools, 2010). Genomic research in asthma falls within “NIH-supported clinical studies, initiatives and research endeavors” and “making progress in increasing our understanding of asthma and improving its treatment” (National Institute of Health Research Portfolio Online Reporting Tools, 2010).

In 2002, statistician Bert Sperling named Tucson the number one asthma hot-spot from a list of 25 United States cities with the highest prevalence of asthma (COPD International, 2002). The National Institute of Allergy and Infectious Diseases (NIAID) funded the Inner City Asthma Study (ICAS) naming the Arizona Respiratory Center in Tucson, Arizona, as one of seven centers nationwide (Morgan, Crain, Gruchalla, O'Connor, Kattan, et al., 2004; University of Wisconsin Madison, 2002). The Arizona Respiratory Center was also named one of five centers for the National Heart, Lung, and Blood Institute (NHLBI), Childhood Asthma Research and Education (CARE) Network to evaluate treatments for children who have asthma. Appropriately, this investigator chose Tucson, Arizona, for asthma research and included biomarkers of atopic asthma and gene variations among this population. NIH-National Institute of Nursing Research also followed by funding this dissertation research, Leukotriene Receptor Gene Variation and Atopic Asthma, in Tucson, Arizona. This dissertation research addresses the goals and objectives of the EPA, Healthy People 2010, NIH, and the NHLBI of increasing our
understanding of genetics associated with asthma that may lead to better clinical approaches, better health care outcomes, and reduced social and economic burden.

Significance to Nursing and Clinicians

Nurses, especially advanced practice nurses, and other health care clinicians assess patients and understand the importance of epidemiological research of asthma in their community. These health care providers are in a unique position to ensure appropriate interventions and patient/community awareness in treating this disease across the lifespan. Understanding the pathology and genetic etiology in the leukotriene pathway in asthma can improve preventive strategies, diagnostic tools, therapies and determining responsiveness to treatment. Advanced practice nurses are in an ideal position to contribute to the body of knowledge surrounding genetics and asthma, facilitate the translation of bench research to clinical practice, and influence appropriate interventions and positive health care outcomes for patients.

Introduction Summary

Atopic asthma is a complex disease process that has a huge societal and personal burden across all ages. Leukotriene-receptors are involved in the cascade of inflammation that may result in symptoms of atopy and asthma. Two leukotriene receptors have been identified in the lung and genes have been sequenced (i.e., CYSLTR1 and CYSLTR2) as well as variants in the sequence identified.

This study sets out to address goals and objectives that are consistent with those of the EPA, Healthy People 2010, NIH, and the NHLBI and that will increase our understanding of genetics associated with asthma. Achievement of the aims of this study may lead to better
clinical approaches, better health care outcomes, and reduced social, personal and economic burden.
CHAPTER II: BACKGROUND & LITERATURE REVIEW

Background and Significance

In 2001, it was estimated that over 50 million Americans suffered allergic disorders (American Academy of Allergy Asthma and Immunology; Kunjumoideen). In 2007, it was estimated that 22.9 million Americans had asthma (American Lung Association, 2009) and a greater number had allergic reaction to environmental exposure to allergens (Steinke, Rich, & Borish, 2008), which contributed to the atopic asthma phenotype. Approximately 70% of asthmatics also had allergies (World Health Organization, 2007). At the present time, it is estimated that the total number of Americans suffering from allergies and/or asthma is 60 million or one out of four individuals, representing a number that is greater than those with diabetes, cancer, coronary heart disease, stroke, Alzheimer’s, and Parkinson’s combined (Asthma and Allergy Foundation of America). (Figure 2)
Perhaps of greatest concern is the fact that asthma rates have continued to increase over the past three decades, an occurrence that has prompted large public health institutions to declare asthma as a serious public health problem in the United States (Clement, Jones, & Cole, 2008). During years between 1980 and 1994, the rate of asthma among children increased 160% and the overall prevalence in the population increased by 75% (Centers for Disease Control and Prevention, 1998). In the 1998-2009 National Health Interview Survey (NHIS), a continuous national survey of the civilian non-institutionalized population of the United States, the lifetime
asthma diagnosis among children (i.e., 10 to 17 years old) was over 16% and lifetime asthma diagnosis among adults (i.e., 18 to 84 years old) was over 10% (National Asthma Education and Prevention Program, 2007). In 2007, it was estimated that over 34 million Americans and 300 million worldwide had been diagnosed with asthma by a health care provider during their lifetime (American Lung Association. Epidemiology & Statistics Unit Research and Program Services, November 2007; World Health Organization, 2007). It is estimated that the number of people with asthma worldwide will increase by another 100 million by 2025 (World Health Organization, 2007).

Social and Economic Burden

Allergic disorders are considered the 5th leading cause of chronic disease, and the 3rd most common chronic disease among children under 18 (National Academy on an Aging Society, 1999). Health care cost estimate is $6 billion annually and loss of 4 million workdays per year for an additional loss of $700 million in lost productivity (National Academy on an Aging Society, 1999). Total indirect and direct costs of asthma in the United States is more than $30 billion annually (Kamble, & Bharmal, 2009). In 2001, the rate of mortality due to allergic reactions in the United States was over 700 per year and was primarily related to drug, food, insect, and latex anaphylaxis (Neugut, Ghatak, & Miller, 2001). 42.7 million Americans remain at risk of anaphylaxis during their lives (Matasar, & Neugut, 2003).

In 2007, asthma contributed to 14.7 million outpatient visits, approximately 1.7 million emergency room visits, and 456,000 asthma hospitalizations (Pitts, Niska, Xu, & Burt, 2008). Emergency department visits and hospitalizations for asthma were highest among children (Akinbami, 2006). In 2009, asthma contributed 10.5 million missed school days, 14.2 million
missed work days (Akinbami, Moorman, & Liu, 2011). Although mortality among children due to asthma is rare, in 2003, 195 children died from asthma or 0.3 deaths per 100,000 compared to 1.4 deaths per 100,000 adults (Akinbami, 2006). Over all mortality related to asthma is still high. Asthma is the cause of 11 deaths daily and over 4,000 deaths per year and listed as a contributing factor for approximately 7,000 other deaths per year (Akinbami, 2006; Centers for Disease Control and Prevention, 2001). In 2009, the Center for Disease Control (CDC) reported 8.2% of the U.S. population (24.6 million people) currently had asthma and rates decreased with age (Akinbami, et al., 2011; Centers for Disease Control and Prevention, 2011b).

The financial burden of asthma is estimated at 19.7 billion dollars in annual health care costs (American Lung Association, 2009). In retrospective analysis of 2004 data, the adjusted mean incremental total expenditure associated with asthma was estimated at $1,004.6 per person among children and $2,077.5 per person among adults. This estimate increased the annual direct medical expenditure attributable to asthma in 2007 dollars at $37.2 billion (Kamble, et al., 2009). The burden of atopic asthma goes beyond financial costs or missed workdays and lowers the quality of life when the affected individual suffers from excessive mucus production, feelings of chest heaviness, coughing, and shortness of breath. Differences in asthma prevalence were identified among particular populations in the United States (Centers for Disease Control and Prevention, 2011b) (Figure 3).
Figure 3: Asthma Prevalence in the United States, 2005

Reprinted from Center for Disease Control and Prevention website (Akinbami, 2006).

Atopy

Atopy is a hypersensitive immune system response to an allergen that leads to mast cell and basophil blood cell activation releasing inflammatory factors of histamine, leukotrienes, IgE, and other cytokine chemical messengers (Dorland, 2000; Kumar, Abbas, Fausto, Robbins, & Cotran, 2005). Inflammation accompanying atopy can involve numerous systems in the body, but is especially serious when this involves the respiratory system and triggers symptoms of asthma (Spergel, 2005, 2010).
Atopy or allergic disease commonly includes symptoms of sneezing, cough, nasal or respiratory congestion, runny nose, itchy skin, nose, eyes or throat, and watering eyes. Extreme allergic reaction can result in anaphylaxis including signs of hypotension and airway closure due to swelling. The best way to determine diagnoses is through skin tests to determine if a person has an IgE antibody reaction to a specific allergen. An extract of allergen applied is a scratch or puncture to the patient’s skin or is injected under the skin of the arm or back. Positive reactivity is a small, raised, reddened area (i.e., wheal or hive) of the skin with or without respiratory symptoms. Another method of testing for atopy is the radioallergosorbent test (RAST) blood test to determine level of IgE antibody in the presence of the allergen. This test may not be as sensitive and is more costly than the skin test method (Bousquet, Chatzi, Jarvis, & Burney, 2008).

Asthma

Asthma is common chronic complex disease process of reversible airflow obstruction in response to triggers and causing various combinations of cellular responses of airway mucosa edema, inflammation, increased secretions, and bronchial smooth muscle contraction (National Heart, 2007; Shelhamer, et al., 1995; Vercelli, 2008). Asthma response can range in severity from mild to life threatening and involve shortness of breath, chest tightness, wheezing, coughing during the early morning or late at night, and/or breathing problems worse after physical activity or after environmental exposure to airborne chemicals or allergens. Asthma is diagnosed through spirometer testing and/or bronchial challenge testing to determine the volume and ease of airflow on forced lung expiration. Other tests helpful in determining diagnosis of asthma include airflow tests during or after exercise using spirometer or peak flow meter.
Many factors can trigger an asthma attack including environmental exposure to inhaled irritants or allergens, infection, exercise, or changes in the weather (Akinbami, 2006). Beyond the initial trigger, asthma is a complex disease process involving many pathways including inflammation and bronchoconstriction. Asthma and associated intermediate phenotypes, such as atopy, are associated with genetic determinants (Asthma and Allergy Foundation of America; Palmer, & Cookson, 2000). Research has identified factors that have an impact on asthma including, but not limited to, β-agonists, Immunoglobulin E (IgE), Tumor Necrosis Factor, leukotrienes, and maternal effects. Many of the genetic variations associated with asthma have been identified in chromosomes 2, 3, 5, 6, 9, 11, 12, 13, 14, 17, and 19 (Gu, & Zhao, 2011; March, Sleiman, & Hakonarson, 2011).

**Atopic Asthma Phenotype**

The strongest identified predisposing factor for the development of asthma is atopy (Centers for Disease Control and Prevention, 2011b). Holgate et al. reported that, although 50% of the population had atopy, less than 10% had persistent asthma (Holgate, Bradding, & Sampson, 1996). Studies outside the United States indicate the rate for atopic asthma among children varies between countries with the lowest prevalence in Pichincha, Ecuador (0.2%) and highest prevalence in Hawkes Bay, New Zealand (13.4%) (Weinmayr, Weiland, Björkstén, Brunekreef, Buchele, et al., 2007). Atopy accounted for 56.3% of asthma cases in the United States and that percentage was greater in males than females (Arbes, et al., 2007).

Atopy is the tendency to mount IgE mediated inflammatory responses to environmental exposures (Ronchetti, et al., 1990; Stedman). Atopic asthma, also known as allergic asthma or extrinsic asthma, includes patients with asthma in response to environmental exposure to
airborne allergens (Akinbami, 2006; Stedman; Vercelli, 2008). Individuals with atopic asthma
test positive for atopic markers such as skin test reactivity, elevated IgE levels, and/or elevated
eosinophil count (Gould, et al., 2008; Stedman; Sunyer, Antó, Sabrià, Roca, Morell, et al., 1995).
This type of asthma usually begins in childhood but can present at any age (King, & Moores,
2008; Kumar, et al., 2005). Research in genetic linkage with atopic asthma has evolved into new
research findings with significant associations between gene variations and atopic asthma
(Arriba-Mendez, Sanz, Isidoro-Garcia, Davild, Laffond, et al., 2006; Cookson, & Moffatt, 2000).
Genetic studies in asthma have included gene mutations on all chromosomes with limited
research reported on the X-chromosome. This is rationale for Aim 1, to determine the
relationship of the CYSLTR1 and CYSLTR2 genetic variations (on the X-chromosome and
chromosome 13 respectively) to inflammatory markers of atopy. Further study is suggested to
elucidate influences in the inflammatory cascade of atopic asthma that can lead to new treatment
therapies (Gould, et al., 2008).

Gender Differences in Atopic Asthma

In 2005, the Center for Disease Control (CDC) estimated that adult women had about a
50% higher prevalence rate of asthma attacks than men (Akinbami, 2006). This pattern was
reversed when examining children ages 0-17 years. Asthma attack prevalence rate for boys was
30% higher than among girls (Akinbami, 2006). During childhood, boys have greater airway
hyper-responsiveness to methacholine airway challenge test for asthma, and girls exhibit an
increased prevalence in adolescence (Le Souef, Sears, & Sherrill, 1995). It is unclear why
prevalence of asthma is greater in boys up to puberty and reverses after puberty, and why severe
asthma is more predominant in females (Postma, 2007). Asthma mortality rate is higher in
females than males, accounting for nearly 65% of asthma related deaths (Centers for Disease Control and Prevention, 2001).

When comparisons were made with an asthma-atopic dermatitis and an asthma non-atopic dermatitis group, significant differences were observed in allele distribution within the CYSLTR1 gene variation (i.e., rs320995) in boys and there were no differences in allele distribution between these disease sub-groups in girls (Arriba-Mendez, et al., 2006). Among patients with asthma, prevalence of positive skin tests was greater in boys (Sears, Burrows, Flannery, Herbison, & Holdaway, 1993). At age 6 months, boys were associated with higher IgE concentrations (Klinnert, Nelson, Price, Adinoff, Leung, et al., 2001) and greater prevalence of any positive skin test than girls (Postma, 2007; Sears, et al., 1993). Boys nine years old had higher number of peripheral blood eosinophils and higher IgE levels when exposed to parental cigarette smoke in comparison to girls of similar age and exposure; this gender effect of eosinophil and IgE production contributed to greater numbers of atopy and asthma in boys than girls (Ronchetti, et al., 1990). Significant differences were observed in allele frequencies of three single nucleotide polymorphisms (SNPs) in the CYSLTR1 gene between Korean adult male subjects with and without asthma (i.e., SNPs -634C>T, -475A>C, and -336A>G) (S. H. Kim, Oh, et al., 2006). Although not clear, this research finding might suggest gender difference attributed to the CYSLTR1 gene being located on chromosome X.

Evidence suggests that hormones influence asthma phenotype as studies indicate risk of asthma and atopic conditions with early onset of menarche (Salam, Wenten, & Gilliland, 2006), reduced incidence of asthma after menopause (Lange, Parner, Prescott, Ulrik, & Vestbo, 2001; Troisi, Speizer, Willett, Trichopoulos, & Rosner, 1995), and increased incidence of asthma
symptoms with female hormone replacement therapy (Lange, et al., 2001; Postma, 2007; Troisi, et al., 1995). One factor is progesterone influences up-regulation of interleukin-4 (IL-4) to promote eosinophil recruitment (Piccinni, Giudizi, Biagiotti, Beloni, Giannarini, et al., 1995). Estrogen enhances eosinophil release, while testosterone inhibits it (Hamano, Terada, Maesako, Numata, & Konno, 1998). Estrogen augments mast cell degranulation while testosterone inhibits it (Vliagoftis, Dimitriadou, Boucher, Rozniecki, Correia, et al., 1992). Testosterone inhibits histamine (Zannolli, & Morgese, 1997), IgE and IL-4, mediators of inflammation, and increases Interleukin-10 (IL-10) gene expression, inhibiting pro-inflammatory cytokines (Canguven, & Albayrak, 2011). Research suggests that testosterone increases expression of cyclooxygenase 1 & 2 (COX-1 and COX-2), important in aspirin sensitive asthmatics (Cheuk, Leung, Lo, & Wong, 2000). Testosterone has been identified as an immunosuppressant and is likely to be protective, whereas, female sex steroids are pro-inflammatory and associated with atopy (Osman, 2003).

Although hormones may have an influence, it remains unclear why there are gender differences in prevalence of atopic asthma (Postma, 2007), differences in eosinophil count, IgE levels (Ronchetti, et al., 1990), and positive skin tests (Sears, et al., 1993). New evidence points to SNPs in CYSLTR1 and CYSLTR2 genes which play a role in atopic asthma susceptibility (Arriba-Mendez, et al., 2006; Hao, et al., 2006; Heise, O'Dowd, Figueroa, Sawyer, Nguyen, et al., 2000; Mellor, Frank, Soler, Hodge, Lora, et al., 2003; Thompson, Capra, Takasaki, Maresca, Rovati, et al., 2007); however these studies have small samples and/or limited information on gender association. This is rationale for Aim 3, to determine gene by gender interactions in association with CYSLTR2 genetic variations in affecting the risk for atopic asthma.
Leukotrienes

Cysteinyl leukotrienes (CysLTs) are lipid mediators generated in activated leukocytes from arachidonic acid (Evans, 2002; Hao, et al., 2006). Cysteinyl leukotrienes are up to 1,000 times more potent and prolonged bronchoconstrictors than histamine and 10,000 times more potent than methacholine which is typically utilized in bronchoconstrictive challenge tests (Adelroth, Morris, Hargreave, & O'Byrne, 1986; Barnes, Piper, & Costello, 1984; Holgate, et al., 1996). Leukotrienes have been implicated in the cascade of events contributing to asthma symptoms and disease chronicity. Inhalation of CysLTs can induce airway obstruction even in the non-asthmatic patient (Holgate, et al., 1996). CysLTs are involved in cell signaling by binding with cysteinyl leukotriene receptors 1 and 2 (CYSLTR1 and CYSLTR2, respectively) (Triggiani, Granata, Giannattasio, & Marone, 2005) (Figure 4).

Cysteinyl leukotrienes (leukotriene D₄ [LTD₄], leukotriene C₄ [LTC₄], and leukotriene E₄ [LTE₄] in order of potency of activation) are lipid mediators generated in activated leukocytes from arachidonic acid via the 5-lipoxygenase (5-LO)/LTC₄ synthase pathway (Evans, 2002; Hao, et al., 2006). The process begins when inflammatory stimuli triggers the translocation of phospholipase A₂ releasing arachidonic acid from cell membrane phospholipids and converts into an unstable intermediate leukotriene A₄ (LTA₄) with 5-LO activating protein as a cofactor. LTA₄ is converted to LTC₄ and then through removal of amino acids forms into LTE₄ and LTD₄ (Klotsman, et al., 2007). These cysteinyl leukotrienes then act through CYSLTR1 and CYSLTR2 on target cells including bronchial smooth muscle and inflammatory leukocytes (Klotsman, et al., 2007). At the cell level, CYSLTR1 and CYSLTR2 activation contributes to the pathophysiologic pathway of asthma including bronchoconstriction, plasma exudation,
mucus secretion and eosinophil recruitment (Evans, 2002; Hao et. al., 2006). It is suggested LTD₄ is able to augment growth factor-induced human airway smooth muscle cell proliferation through an ‘atypical’ CysLT receptor or through the classical CYSLTR1 of which montelukast has been shown to inhibit allergen-induced airway remodeling in a mouse model of asthma (Ravasi, Citro, Viviani, Capra, & Rovati, 2006) (Figure 4).
Figure 4: Leukotriene Cascade

Illustrated by Tantisira and Drazen, this leukotriene pathway is with cascade of events from arachidonic acid to leukotriene receptor leading to inflammatory process of edema, eosinophilia, mucus secretion, and bronchoconstriction associated in atopic asthma (Tantisira, & Drazen, 2009). Reprinted with permission (Elsevier Limited, #2654050546112).
Leukotriene inhibitors are the latest group of medications that have been used widely in management of chronic asthma unresolved by β-agonist and steroid use. Asthma and associated intermediate phenotypes are under a substantial degree of genetic control (Palmer, et al., 2000). Research has identified that genetic factors have an impact on asthma including, but not limited to, promoting inflammation (Fryer, Spiteri, Bianco, Hepple, Jones, et al., 2000), inability to regulate inflammation (Sandhu, & Casale, 2010), β2-adrenoreceptor activity (Guerra, Graves, Morgan, Sherrill, Holberg, et al., 2005), IgE serum level changes (Basehore, Howard, Lange, Moore, Hawkins, et al., 2004), Tumor Necrosis Factor alpha release (Yucesoy, Kurzius-Spencer, Johnson, Fluharty, Kashon, et al., 2008), leukotriene activity (Tantisira, et al., 2009), sex-hormone influence (Postma, 2007), and maternal effects (Cookson, et al., 2000).

Cysteiny1 Leukotriene Receptors 1 and 2

Cysteiny1 leukotriene receptors 1 and 2 are located on target cells including bronchial smooth muscle and inflammatory leukocytes (Klotsman, et al., 2007) contributing to the pathophysiology of asthma including bronchoconstriction, plasma exudation, mucus secretion and eosinophil recruitment (Evans, 2002; Hao, et al., 2006). Studies indicate CysLTs promote generation of T-helper type 2 cytokines, such as IL-4, IL-5, and IL-13, which further enhance production of CysLT by inducing the expression of CYSLTR1 identified in airway smooth muscle, alveolar macrophages, peripheral blood monocytes, eosinophils and endothelial cells. This up-regulation of CYSLTR1 expression promotes the pro-inflammatory effects of CysLTs (S. H. Kim, Oh, et al., 2006). Two human cysteiny1 leukotriene receptors found in the lung and are implicated in the inflammatory pathway have been clearly identified and cloned (Lynch, O’Neill, Liu, Im, Sawyer, et al., 1999; Online Mendelian Inheritance in Man OMIM (TM)).
CYSLTR1 and CYSLTR2 have a 38% amino acid identity and share a similar degree of identity with the purinergic class of G protein-coupled receptors (Heise, et al., 2000; Mellor, et al., 2003). However, each cysteinyl leukotriene receptor gene is quite unique. CYSLTR1 is expressed in bronchial smooth muscle cells, macrophages in normal human lung, eosinophils and peripheral blood monocytes, and may predispose some individuals to asthma susceptibility and severity. CYSLTR2 is expressed in the lung macrophages, mast cells, airway smooth muscle, lymph node, spleen, peripheral blood leukocytes, central nervous system and in the heart, and evidence indicates genetic polymorphism in this CYSLTR2 gene plays a role in asthma susceptibility (Hao, et al., 2006; Heise, et al., 2000; Mellor, et al., 2003; Thompson, et al., 2007).

A PubMed and OMIM search identified candidate genes and gene associations with asthma on all human chromosomes (Online Mendelian Inheritance in Man OMIM (TM)). The CYSLTR1 gene has been mapped to chromosome Xq13-q21 (Lynch, et al., 1999; Online Mendelian Inheritance in Man OMIM - TM) at position 77,341,174 to 77,396,130 (The International HapMap Consortium, 2003). The CYSLTR2 gene has been mapped to chromosome 13q14.12-q21.1, within in an one million base pair region linked to atopic asthma (Daniels, Bhattacharrya, James, Leaves, Young, et al., 1996; Heise, et al., 2000; Kimura, Noguchi, Shibasaki, Arinami, Yokouchi, et al., 1999; Online Mendelian Inheritance in Man OMIM - TM; Takasaki, Kamohara, Matsumoto, Saito, Sugimoto, et al., 2000) at position 48,178,952 – 48,181,499 (The International HapMap Consortium, 2003). Most of the current reports of association of these genes to atopic asthma are limited to small samples under 400 in Korea (S. H. Kim, Oh, et al., 2006), and Tristan da Cunha, an island in the South Atlantic Ocean between Africa and South America (Thompson, Storm van's Gravesande, Galczenski, Burnham,
Siminovitch, et al., 2003), Japan (Zhang, Migita, Koga, Shibasaki, Arinami, et al., 2006), Spain (Sanz, Isidro-Garcia, Davila, Moreno, Laffond, et al., 2006), intermarried Hutterite families in the USA originating from Germany (Ober, Cox, Abney, Di Rienzo, Lander, et al., 1998), and China (Hong, Zhou, Tsai, Wang, Liu, et al., 2009). (Appendix A)

Reports of CYSLTR1 genetic variations associated with atopic asthma include 927T>C and 899G>A (Arriba-Mendez, et al., 2006; Thompson, et al., 2007). Earlier studies reported no CYSLTR1 genetic variations associated with atopic asthma (Hao, et al., 2006; Zhang, et al., 2006). This difference in findings could be attributed to small sample size and different ethnic populations. Other studies reported CYSLTR1 genetic variations associated with asthma but did not specifically study associations with atopic asthma, IgE levels, eosinophilia, or skin reactivity (S. H. Kim, Jeong, Cho, Kim, Lee, et al., 2008; S. H. Kim, Oh, et al., 2006; S. H. Kim, Yang, Park, Ye, Lee, et al., 2007; S. H. Kim, Ye, Hur, Lee, Sampson, et al., 2007; S. H. Kim, Ye, Lee, & Park, 2006; Lee, Kim, Kim, Kim, Kang, et al., 2007; Sanz, et al., 2006). CYSLTR2 genetic variation, 604 A/G, was associated with atopy (Thompson, et al., 2003) and CYSLTR2 genetic variations, -1220A > C and 601A>G, were associated with asthma (Fukai, Ogasawara, Migita, Koga, Ichikawa, et al., 2004; Pillai, Cousens, Barnes, Buckley, Chiano, et al., 2004). In the first stage of screening Hutterite families in the USA, analysis found association between asthma and chromosome 13 (i.e., 13q14) but not identified in the second stage of the screening (Ober, et al., 1998). Although there are reports of studies of CYSLTR2 genetic variations with atopy or asthma separately, there is no study to investigate the association of CYSLTR2 genetic variations with atopic asthma. Due to limits in the available research of association of CYSLTR1 and CYSLTR2 genetic variations to atopic asthma in the United States, this is the rationale for Aim
2, to determine the relationship of CYSLTR1 and CYSLTR2 genetic variations to atopic asthma in a large homogeneous population in the United States (i.e., TESAOD). Appendix A illustrates the studies to date related to CYSLTR1 and CYSLTR2 genetic variations to atopy, asthma, and atopic asthma from around the world. This served as framework to identifying potential SNPs and significance of this study in Tucson, Arizona, USA.

IgE level, Blood Eosinophil Count, and Skin Test Reactivity

IgE level, blood eosinophil count and skin test reactivity are standard biomarkers of atopy (Daniels, et al., 1996). Leukotrienes involved in the inflammatory process of atopy and asthma have affinity for and activate cysteinyl leukotriene receptors and activate IgE and eosinophil production (Krawiec, & Wenzel, 2001; Sunyer, et al., 1995). Although symptoms of asthma and increased airway responsiveness correlate with increased IgE levels, many persons with increased IgE levels are not asthmatic (Sunyer, et al., 1995). The relationship between IgE and leukotriene receptor gene have not been fully elucidated in their correlation with atopic asthma. Blood eosinophilia and skin reactivity to common allergens were related to bronchial responsiveness but to a lesser extent than IgE (Sunyer, et al., 1995). Cysteinyl leukotrienes are mediators in the transport of eosinophils and leukocytes to airway sites of allergic inflammation and promote eosinophil survival in vitro (National Center for Biotechnology Information, 2011b). The prevalence of asthma has been shown to increase with increasing numbers of positive skin tests (Postma, 2007).

Although IgE level, eosinophilia, and skin reactivity have been utilized as a standard in defining atopic asthma, and are typically measured in studies involving CYSLTR1 and CYSLTR2 genetic variations in atopic asthma, only one study reported no significant association
between total IgE and CYSLTR1 SNP rs320995 (Hao, et al., 2006). Genetic studies of CYSLTR1 and atopic asthma included IgE level and/or eosinophil counts, and did not report associations (Arriba-Mendez, et al., 2006; Lee, et al., 2007). Other studies reported CYSLTR1 genetic variations associated with asthma but, did not specifically study associations with atopic asthma, IgE levels, eosinophilia, or skin reactivity (S. H. Kim, et al., 2008; S. H. Kim, Oh, et al., 2006; S. H. Kim, E. M. Yang, et al., 2007; S. H. Kim, Y. M. Ye, et al., 2007; S. H. Kim, Ye, et al., 2006; Lee, et al., 2007; Sanz, et al., 2006). Hao and colleagues studied the association of the CYSLTR1 genetic variations of 927T/C with IgE level; however, authors reported no significant association (Hao, et al., 2006). Although CYSLTR2 genetic variations have been studied in atopy and asthma cited above, these did not include IgE levels, eosinophilia, or skin reactivity. This is the rationale for Aim 1, which is to determine the relationship of the CYSLTR1 and CYSLTR2 genetic variations with inflammatory markers of atopy including IgE level, blood eosinophil count, and skin test reactivity. Thus there is a gap in knowledge on markers of atopy and CYSLTR1 and CYSLTR2 genetic variations. By analyzing the associations of all of these inflammatory markers with CYSLTR1 and CYSLTR2 genetic variations and atopic asthma within a large population in the United States, we might gain new evidence on the influence these genetic variations may have on inflammatory markers and atopic asthma.

Background Summary

Atopic asthma is an expensive health care problem as well as a social and quality of life burden. Evidence indicates that over 50% of asthma cases are attributed to atopy (Arbes, et al., 2007) and this percentage is higher in males than females (Sears, et al., 1993). Evidence also
suggests that genetic variations in leukotriene receptors CYSLTR1 and CYSLTR2 play a role in the expression of inflammation in atopic disease and airway dysfunction and remodeling in asthma. Studies involving CYSLTR1 and CYSLTR2 genetic variations, atopic asthma, and IgE level, eosinophilia, and skin reactivity are limited.

Current knowledge on atopic asthma associated with CYSLTR1 and CYSLTR2 genes is limited to small homogeneous populations outside the United States. The current gap in knowledge includes large association studies of CYSLTR1 and CYSLTR2 genes with IgE levels, eosinophilia, and skin reactivity in the United States. Although there is evidence of gender difference in CYSLTR1 genetic variations and atopic asthma, there is no report of CYSLTR2 genetic variations, gender, and atopic asthma. Current treatment modalities are unable to adequately block the inflammation cascade in all patients with atopic asthma. If therapies could be developed to prevent or reverse atopy or block the effect on asthma, a large percent of asthma cases could be prevented. This research helps to fill the gap in knowledge regarding associations of CYSLTR1 and CYSLTR2 genetic variations, gender, and other inflammatory markers of atopy such as IgE, eosinophil count, and skin sensitivity testing. This research may lead to innovative tailored preventive strategies and may have important pharmacogenetic implications in the treatment of the disease itself. An example of a preventative strategy is performing genetic testing of a panel of genes including CYSTLR gene(s) if a patient has a history of atopy to help determine risk of developing atopic asthma. Prevention of allergen triggers and early treatment might reduce risk of triggering atopic asthma. Finally, if atopic asthma was diagnosed, results of genetic testing might indicate which treatment the patient might best respond to.
CHAPTER III: METHODS

Design

The genetic association questions in this dissertation research were answered using data from a descriptive longitudinal cohort study, TESAOD. This dissertation study includes demographic and clinical data related to atopic asthma from questionnaires collected in two TESAOD sub studies, “The Relation of Innate Immune Genes to COPD” (HSC #05-0628-01) and “The Relation of LPS Receptor Complex Polymorphisms to COPD” (HSC #03-0789-01). To address the specific aims of this dissertation study, five SNPs related to the two leukotriene genes of interest were added to the batch DNA genotyping from the two TESAOD sub studies. Here-after “TESAOD study” will refer to the collection of studies performed over the nearly 40 year history of the longitudinal TESAOD cohort. Here-after, the two recent TEASOD sub studies will be referred to as the “parent studies.” Here-after, this dissertation research will be referred to as “this study.” In order to understand the methodologic basis of this study, the sample population, protocols and methods of the parent studies will be described first, followed by the power calculation, then the sample population, protocols and methods of this study.

Sample Population—Parent Studies

TESAOD is a descriptive, longitudinal, prospective cohort study over 30 years that includes a stratified cluster sample of 1,655 Tucson, Arizona households initially enrolled between March 1972 and April 1973 based on 1970 Tucson census block statistics to study general airway obstructive disease in this population (Lebowitz, Knudson, & Burrows, 1975). This population of 1,655 households represented a total initial enrollment of 3,805 individuals (Lebowitz, et al., 1975). Exclusion criteria of TESAOD and the parent studies were Non-English
speaking individuals (B. Boyer, personal communication, 2010). During the follow up period (1972-1996), the TESAOD population of 3,805 grew to 5,377 through marriages and births (Guerra, Sherrill, Kurzius-Spencer, Venker, Halonen, et al., 2008; Morse, Lebowitz, Knudson, & Burrows, 1977).

In 2005, two parent studies described above were initiated with subjects from the reactivated large TESAOD population. Approximately 1872 individuals were identified to participate and attempted to contact in the parent studies based on vital status and contact information. Of those, 420 were lost to follow-up, 1452 were randomly contacted, 467 did not return the invitation packet, and 56 refused, and 909 initially enrolled; however, only 897 were active (i.e., completed the consent and provided blood or buccal DNA sample) and became the total sample for these parent studies (C. Venker, personal communication, 2009).

Protocols—Parent Studies

During the first 24 years of follow-up, TESAOD studies were comprised of 13 questionnaires. All participants were eligible to take part in the first 12 questionnaires, whereas questionnaire 13 was conducted on selected respiratory / residence status subgroups (Guerra, Sherrill, et al., 2008). Questionnaires were administered approximately every two years and included demographic data, disease status, medication use, up to 12 spirometric lung function tests, four allergy skin prick tests, three measurements of IgE levels, and seven measurements of blood eosinophil counts. During the period of the parent studies, 2005 to 2008, DNAs were extracted from the parent study participants by Arizona Respiratory Center technicians, whole genome amplified for each participant, and banked at the Arizona Respiratory Center labs located at the University of Arizona, Tucson, Arizona for future genotyping. De-identified
specimens were shipped to the Facility for Mutation and Methylation Analysis, Nebraska Medical Center, Omaha, Nebraska for Sequenom genotyping. Sequenom is an advanced genotyping technology using primers to amplify a gene region of interest, then a single extension primers to the SNP of interest before determining genotype using mass spectrometry.

Methods—Parent Studies

Skin Reactivity Testing

Skin reactivity tests were carried out according to the prick techniques up to three times for each study subject during various TESAOD study periods and included the most common allergens in the Tucson area, including house dust, Bermuda grass, tree mix (olive, mulberry, mesquite, and cottonwood), weed mix (rabbit bush, saltbush, careless weed, Russian thistle, desert ragweed, and slender ragweed), Dematiaccae mold mix (Alternaria, Helminthosporium, Cladosporium, Curvularia, Spondylocladium, and Stemphylium species) (Appendix B). Participants were considered atopic if a reaction of at least 2mm greater than the size of the glycerin control test was recorded for at least one of these tests (Guerra, Sherrill, Martinez, & Barbee, 2002). Skin reactivity tests were performed during questionnaires #1, 6-13.

Serum IgE and Eosinophil Tests

Blood samples were obtained from parent study participants, were frozen within two hours of collection and stored at -70ºC (P. Graves, personal communication, 2008). Serum IgE levels were measured within two weeks of obtaining the samples, utilizing the Paper Radioimmunosorbent Test (PRIST) method in international units/ml, up to three times for each subject at least six years old while enrolled in the study (Guerra, et al., 2002). IgE levels were tested during questionnaires #1, 6, and 11. Percentages of eosinophils were obtained from a
differential count of white blood cells drawn from parent study participants within two weeks of questionnaires #1, 6-13.

DNA Extraction

In the parent studies, blood was obtained from participants living in the Tucson area, and buccal swabs were obtained from participants living out of the Tucson area starting in 2005. Specimens were de-identified and processed by Arizona Respiratory Center staff. Blood was frozen within two hours of collection, DNA isolated within a month, and stored at -70°C (P. Graves, personal communication, 2008). DNA from buccal swabs was refrigerated when received, isolated within two weeks, and frozen at -70°C (P. Graves, personal communication, 2008). DNA specimens isolated from buccal swabs were utilized when blood samples were not available. DNA isolation from blood was performed utilizing QiaAmp DNA Blood Mini Kit, catalog #51106 (250x) or #51104 (50x) (Qiagen, 2009). DNA isolation from buccal swabs was performed utilizing QiaAmp DNA Mini Kit #51104 (50x) (Qiagen, 2009). Whole genome amplification from purified genomic DNA was performed utilizing the Qiagen Repli-g Mini Kit in a PCR (Qiagen, 2009). The process includes a Multiple Displacement Amplification (MDA) approach where exonuclease-resistant random primers attach to template DNA for uniform amplification across the entire genome with high fidelity (Qiagen, 2010).

Genotyping

In 2010, DNA specimens at Arizona Respiratory Center labs were thawed at room temperature for preparation to be sent out of state for Sequenom genotype analysis. A robot pipette at least 2 µl of DNA aliquot with approximate concentration of 100 ng/µl into respective plate wells (approximately 200 ng of DNA). Due to limited amounts of buccal DNAs, there may
have been less than 200 ng sent for these subjects (P. Graves, personal communication, 2011). DNA plates were dried down, packed in dry ice, and shipped to the Facility for Mutation and Methylation Analysis, Nebraska Medical Center, Omaha, Nebraska for Sequenom genotype analysis using Sequenom manufacture protocols. The specific method used for Sequenom genotype analysis is described under “Methods-This Study.”

Power Estimation of Sample Size—This Study

The intended sample size of the parent studies was 1,000 participants (S. Guerra, personal communication, 2007). Preliminary power estimation was performed a priori to determine the appropriate sample size needed to obtain 80% statistical power for this study using available data including TESAOD population proportion of atopic asthma and minor allele frequencies of CYSLTR1 and CYSLTR2 SNPs of interest. Power was computed utilizing Quanto power computation program (Gauderman, & Morrison, 2009). For power computations alpha level was set at two-sided 0.05. With a total intended population of 1,000 subjects for Aims 1 and 2 and utilizing an additive model, there was sufficient power to detect an odds ration between 1.8 and 2.4 among males (i.e., moderate to large genetic effect) and between 1.5 to 1.8 among females (i.e., moderate genetic effect). For Aim 3, the analyses in this study would have power ≥ 80% to detect odds ratio of 1.3 and 1.9 between CYSLTR2 and gender (i.e., moderate genetic effect).

Sample Population—This Study

A priori inclusion criteria for this study included: (1) written consent in either of the parent studies, (2) banked DNA, (3) 18 years of age by January 1, 2005, and (4) restriction to the largest ethnic group within the parent TESAOD studies, Non-Hispanic white. From the 897 active participants in the parent studies, 28 did not meet the criteria for age and ethnicity for this
study. An additional 16 individuals were not included in this study due to genotyping failure, including those without a reported genotype call (i.e., computer generated determination of genotype) or heterozygote call for males for a gene located on an X-chromosome. Thus, the total number of Non-Hispanic white individuals at least 18 years of age with written consent and genotype data in this study was 853, and here-after referred to as “eligible participants”.
(Figure 5)

Figure 5: Study Population

This diagram illustrates how enrollment for this study relates to the parent studies and the TESAOD study.

To further compare if this study group was representative of the larger TESAOD population, two other group classifications were identified: eligible non-participating group and deceased group. An eligible non-participating group was defined as living Non-Hispanic white individuals at least 18 years of age as of January 1, 2005, who had participated in any of the previous TESAOD study and sub studies over a 24-year follow-up, but was lost to follow-up, did not consent to the recent parent studies and/or did not provide DNA samples. A deceased group
was defined as any Non-Hispanic white individual that previously participated in a TESAOD study but died before the conclusion of the parent studies in 2008, and did not provide blood or buccal sample.

Protocols—This Study

Longitudinal data from TESAOD study and DNAs collected from the parent studies provided the framework for this study. Protocols for this study were similar to the parent studies with two exceptions: (1) only questionnaires and clinical data specifically related to atopy, asthma, atopic asthma, elevated IgE, and eosinophilia were used, and (2) genotypes for the two genes of interest (CSTYLR1 and CSTYLR2) were performed. The principal investigator of this study only had access to questionnaire, clinical, and genotype data germane to this study.

Methods—This Study

Questionnaires

For this study, a secondary analysis of questionnaires from the parent studies was performed. Questionnaires from the parent studies included demographic information, medication use, smoking status, asthma status, and personal health information. As mentioned in the methods for parent studies above, previously collected, processed, and recorded clinical data included IgE level, skin prick test results, and blood eosinophil count were used.

Specifically, data were analyzed from limited access and password protected existing information obtained from parent studies. Aligned with the definition of asthma in previous parent studies, asthma in this study was defined as a positive response to a standardized self-completed questionnaire (e.g., “Have you ever had asthma?”) and considered physician-confirmed if the subject reported ever having had asthma and been seen, diagnosed, or treated for
asthma by a doctor (Guerra, Sherrill, et al., 2008) (Table 1). Aligned with the definition of atopy in the parent studies, atopy in this study was defined through skin reactivity at least 2mm greater than the size of the glycerin control recorded for at least one of the tested allergens (Guerra, et al., 2002; Guerra, Sherrill, Venker, Ceccato, Halonen, et al., 2010) (Appendix B). Atopic asthma was defined as a patient diagnosed with both asthma and atopy.
Table 1: Asthma Status Questions

This table presents the specific questions related to asthma in each of the parent study questionnaire and that were used in this study. The full questionnaires have been reported previously (Barbee, Halonen, Lebowitz, & Burrows, 1981; Burrows, Martinez, Halonen, Barbee, & Cline, 1989; Guerra, Sherrill, et al., 2008; Guerra, et al., 2010; Lebowitz, et al., 1975).

<table>
<thead>
<tr>
<th>Questionnaire number</th>
<th>Asthma questions</th>
</tr>
</thead>
</table>
| Survey 1             | Have you ever had asthma?  
                       | Have you ever seen a doctor about your asthma? |
| Survey 2             | During the past year have you developed asthma?  
                       | In the past year, have you seen a doctor about your asthma? |
| Survey 3             | During the past year, have you seen a doctor for asthma? |
| Survey 4             | During the past year, have you seen a doctor for asthma? |
| Survey 5             | During the past year, have you seen a doctor for asthma? |
| Survey 6             | Have you ever had asthma?  
                       | Did a doctor ever tell you that you had asthma? |
| Survey 7             | Have you ever had asthma?  
                       | Did a doctor ever tell you that you had asthma? |
| Survey 8             | Have you ever had asthma?  
                       | Did a doctor ever tell you that you had asthma? |
| Survey 9             | Since the last questionnaire, have you had or been treated for asthma? |
| Survey 10            | Since the last questionnaire, have you had or been treated for asthma? |
| Survey 11            | Have you ever had asthma?  
                       | Have you ever seen a doctor about your asthma? |
| Survey 12            | Have you ever had asthma?  
                       | Have you ever seen a doctor about your asthma? |
| Survey 13            | Have you ever had asthma?  
                       | Have you ever seen a doctor about your asthma? |

With respect to IgE levels, previous parent study investigators reported that sampled serum IgE levels had a logarithmic distribution and varied according to age and sex, and thus reported them as age-sex-standardized z-scores, defined as the number of standard deviations by
which an individual’s log IgE level differed from the mean log IgE for his or her age-sex-specific group (Barbee, et al., 1981; Burrows, et al., 1989; Dodge, Burrows, Lebowitz, & Cline, 1993). In another reported TESAOD study, serum IgE levels were also converted into log10 for calculations (Barbee, Lebowitz, Thompson, & Burrows, 1976). Serum IgE was considered elevated if at least one standard deviation higher than the mean of the subject's sex-age-specific category in any of the measurements performed during the study (Crestani, Lohman, Guerra, Wright, & Halonen, 2007; Guerra, Sherrill, et al., 2008; Guerra, et al., 2010). Thus, aligned with previous TESAOD studies, elevated serum IgE levels in this study was defined as mean log10 age-sex-standardized z-score transformed IgE level ≥ 1. In a previous TESAOD study, eosinophils were considered elevated if > 4% or z-scores at least one standard deviation higher than the age-sex-specific mean in any of the measurements performed during the study (Crestani, et al., 2007; Guerra, Sherrill, et al., 2008). Thus, aligned with previous TESAOD studies, elevated eosinophil count in this study was defined as an eosinophil level > 4% or z-score ≥ 1.

DNA Processing

DNA was processed as described in the parent studies at the Arizona Respiratory Center labs in Tucson, Arizona. SNPs of interest for this study were identified (see below) and extension primers for SNPs of interest were purchased (Integrated DNA Technologies) prior to sending DNA as a batch for individual Sequenom genotyping in Omaha, Nebraska (Table 3).

Single Nucleotide Polymorphism Identification—This Study

This study utilized a candidate gene association approach for single nucleotide polymorphism identification of CYSLTR1 and CYSLTR2 genes. When SNPs are in linkage disequilibrium, haplotype tagging SNPs can be chosen that cover the diversity within a region. It
is possible to identify genetic variations without genotyping every SNP in a chromosomal region by identifying non-random association of alleles at two or more loci (i.e., linkage disequilibrium) (Ziegler, & König, 2010). SNPs in a region of the chromosome in high linkage disequilibrium are considered tag SNPs. Choosing a tag SNP in a particular chromosome region means that it is highly correlated or represents other SNPs in linkage disequilibrium of that region. Thus, it is possible to choose tag SNPs that likely represent other SNPs across respective regions of the gene of interest (Nussbaum, McInnes, Willard, Thompson, & Hamosh, 2007; Ziegler, et al., 2010). Tag SNPs were identified for the CYSLTR1 and CYSLTR2 genes in this study. Tag SNPs were chosen based on the web based open source Genome Variation Server utilizing the HapMap-CEU population [i.e., Utah residents with Northern and Western European ancestry from the Centre d'Etudes du Polymorphisme Humain (CEPH) human genome collection] which represented a Non-Hispanic white population like this study population (SeattleSNPs, 2011). CEU is one of the 11 populations in HapMap phase 3, a sample collection comprised of 1,301 samples from 11 populations and including the original 270 samples used in Phase I and II of the International HapMap Project (National Center for Biotechnology Information, 2011b).

Parameters chosen for tag selection with Genome Variation Server (GVS) included minimum value for variations to belong to the same cluster ($r^2$ threshold) = 0.8, minor allele frequency (MAF) > 5%, 5,000 bases upstream of transcription start, and 2,000 bases downstream (National Center for Biotechnology Information, 2011a; SeattleSNPs, 2011). Data coverage (%) for tag SNPs, the minimal data coverage in percent for a variation to be considered as a potential tag SNP, was chosen at 85%. Data coverage (%) for clustering, the minimal data coverage in percent for a variation to be clustered potentially with other variations, was chosen at 70%.
Thus, one tag SNP from each of the three blocks in the CYSLTR1 gene loci (rs320991, rs321006, and rs321073) and one tag SNP for each of the two blocks in the CYSLTR2 gene loci (rs912278F and rs2407249) were utilized in this research (Table 2). Extension primers used for each respective SNP are illustrated in Table 3.
Table 2: CYSLTR1 and CYSLTR2 tag SNPs

CYSLTR1 and CYSLTR2 tag SNPs for this research (based on population: HapMap-CEU).

SNP type: intron (transcribed but non-coding region), coding-synonymous (within an exon and translated, no protein amino acid change), utr-3 (within an exon following a coding region, but not translated), and 3' near gene (intergenic, but within 2000 bases of a transcribed region) (SeattleSNPs, 2011).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Bin</th>
<th>Minor allele</th>
<th>Average minor allele frequency</th>
<th>rs number</th>
<th>SNP type</th>
<th>Transcribed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYSTLR1</td>
<td>1</td>
<td>C</td>
<td>0.24</td>
<td>rs321073</td>
<td>Intron</td>
<td>Reverse</td>
</tr>
<tr>
<td>CYSTLR1</td>
<td>2</td>
<td>C</td>
<td>0.33</td>
<td>rs320991</td>
<td>Intron</td>
<td>Reverse</td>
</tr>
<tr>
<td>CYSTLR1</td>
<td>3</td>
<td>A</td>
<td>0.14</td>
<td>rs321006</td>
<td>Intron</td>
<td>Reverse</td>
</tr>
<tr>
<td>CYSTLR2</td>
<td>1</td>
<td>G</td>
<td>0.41</td>
<td>rs912278</td>
<td>utr-3</td>
<td>Forward</td>
</tr>
<tr>
<td>CYSTLR2</td>
<td>2</td>
<td>G</td>
<td>0.13</td>
<td>rs2407249</td>
<td>3' near gene</td>
<td>Forward</td>
</tr>
</tbody>
</table>
Table 3: PCR Extension Primers

This table illustrates PCR extension primer sequences for CYSLTR1 and CYSLTR2 tag SNPs of interest.

<table>
<thead>
<tr>
<th>SNP rs#</th>
<th>Extension primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs321073</td>
<td>TGTTAAAAGTAGAAACCTGAA</td>
</tr>
<tr>
<td>rs320991</td>
<td>gcgGGCGATCCTTGGGTATGTGTAACC</td>
</tr>
<tr>
<td>rs321006</td>
<td>ATTATGAGTAAATTCTCCTATGT</td>
</tr>
<tr>
<td>rs912278</td>
<td>GAGGGACTAAGTCAGTC</td>
</tr>
<tr>
<td>rs2407249</td>
<td>agggGCCTTTTCATGTTACGGCCTTCCC</td>
</tr>
</tbody>
</table>

Single Nucleotide Polymorphism Detection—This Study

Sequenom is a new generation of high-throughput genotyping. This technology allows multiple SNPs to be analyzed within a sample plate well (i.e., multiplexing) incorporating a PCR process amplifying a short DNA sequence surrounding the SNP of interest and single primer extension process at the location of the SNP of interest. PCR can amplify large quantities of DNA within a few hours by mixing DNA with primers on either side of the DNA (forward and reverse). The MassExtend reaction in Sequenom is a primer extension process using the iPLEX Gold assay, a single termination mix and universal reaction condition for SNPs of interest (Seqenom, 2011). Primers utilized were oligonucleotides with less than 20 bases. This PCR process was used to detect sequence differences in DNA at the SNP level.

Unlike TaqMan PCR, which utilizes fluorescent probes to detect sequence differences, Sequenom PCR results in allele-specific differences in mass between extension products. The
Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) analyzer uses mass spectrometry technology to detect these differences in mass between alleles at specific loci in real time. Allele detection was performed using matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry. The SpectroCHIP® had 384 positions or wells. Specific data analysis software was used to differentiate between SNP alleles resulting in genotype call. Detailed information and interaction with processed data was stored in the TYPER database.

Sequenom project explorer software allowed selection of single or multiple chips to obtain an overview of the genotypes. Four colors displayed the percent of genotype calls per well on the computer screen and actual percent of successful genotype call rates were provided.

Computer screen graphics included analyte signals, genotypes and mass range with annotations for all peaks. Rough judgment of intensities, resolution, and signal-to noise ratios were provided. The screen display for well data computer allowed a view of one particular well at a time for genotype call(s) and confidence calculations of the call(s) (i.e., user call). The screen display of plate data provided information for the entire plate such as area, resolution, and primer peak scores. Computer screen histogram illustrated no call, low mass homozygous, heterozygous, and high mass homozygous and allows the user to quickly identify assay stability (Sequenom, 2011). Cluster plots provided illustration of low mass allele vs. high mass allele for chosen assay. Hardy-Weinberg values, an analysis to determine genotyping data quality, were calculated for each genotyped population per assay. Up to 36 SNPs could be multiplexed in a single well and processed up to 384 samples in parallel (Seqenom, 2011); however, 20 SNPs or less were multiplexed in a single well for this study and the parent studies in parallel.

Sequenom allelic discrimination assay as a biomethod to determine genotype has been
proven effective, quick, reliable (Gabriel, Ziaugra, & Tabbaa, 2009) and was utilized in this research. Polymorphisms were genotyped using the Sequenom mass spectrometry allelic discrimination at the Facility for Mutation and Methylation Analysis, Nebraska Medical Center, Omaha, Nebraska. Prior to genotyping DNA for this study, stock DNA samples (i.e., Coriell DNA plates with DNA from HapMap CEPH population) were genotyped utilizing Sequenom at this Omaha facility and compared to known genotype data of these samples to determine concordance (i.e., reliability). After concordance was verified, sent DNA was genotyped. DNA was placed in 96 well plates and 384 specimens were run in parallel. Samples from this study were run on the shared DNA plates with the parent study. Coriell DNA plate with trios were utilized for DNA assays and inheritance patterns (i.e., Hardy Weinberg Equilibrium) and were checked by Dr. Tricia Levan (Director, Facility for Mutation and Methylation Analysis). Primers and extension assays were obtained from Integrated DNA Technologies (IDT). PCRplexes for CYSLTR1 and CYSLTR2 were performed utilizing Assay Design 3.1 and the Sequenom system. The genotype analysis was completed using SpectroTYPER 4.0 software.

Statistical Analysis

Statistical analysis was completed utilizing SPSS and STATA statistical software packages. Descriptive statistics were utilized to summarize the demographic and clinical characteristics of the eligible participant group, eligible non-participant group, and deceased group. Inferential statistics were employed to describe correlation between eligible participant group and eligible non-participant group (i.e., ANOVA and Chi-square). Hardy Weinberg Equilibrium analysis was utilized. Inferential statistical analysis was utilized to draw conclusions about associations between SNPs of interest and atopy, asthma, atopic asthma, elevated IgE level, and eosinophilia
based on contingency tables. Contingency tables were analyzed using Chi-square and Fisher’s Exact Test when the number within a contingency table cell was less than five. False Discovery Rate (FDR) adjustment was performed to reduce risk of spurious association or Type I error. Inferential statistical analysis (Chi-square or Fisher’s Exact Test) was performed to determine genetic effect (i.e., mode of phenotypic inheritance). Detailed statistical analyses for the Hardy Weinberg Equilibrium, population sample, genetic association, False Discovery Rate, and genetic model analysis are described in detail below.

**Hardy Weinberg Equilibrium**

Hardy Weinberg Law is principle in population genetics that describes the relationship between allele and genotype frequencies in a large, randomly mating, population remains constant over generations and Hardy Weinberg Equilibrium (HWE) is an analysis to determine if there is the fixed relationship between allele and genotype frequencies remains constant. HWE assumes that in the population there is no random mating, no selection or migration, no mutation, no population stratification, and infinite population size. HWE is useful in determining genotyping data quality in genetic epidemiology studies (Ziegler, et al., 2010). HWE tests for deviations using contingency table analyses. Tests for Hardy-Weinberg equilibrium in controls and allelic or genotypic association in cases versus controls were evaluated by Chi-square ($\chi^2$) test. HWE analysis was performed for all 5 genotypes in this study utilizing an open source online HWE calculator (Domingues, 2010). A p-value of less than 0.05 was regarded as significant.

**Population Sample Analysis**

Descriptive statistical analysis was performed to obtain frequency of asthma, atopy,
atopic asthma, smoking status, elevated IgE status, and eosinophilia. Mean values with standard deviations were calculated for age of participants on January 1, 2005, pack year history among smokers, and number of questionnaires completed.

Demographic analysis was performed for three groups in order to describe this study population (i.e., eligible participating, a sub population of the total TESAOD population) with the rest of the TESAOD population not included in this study (i.e., eligible non-participating and deceased). In order to compare this study population (i.e., eligible participating) with other living members of TESAOD (i.e., eligible non-participating), ANOVA and Chi-square inferential statistical analysis were used to describe similarity or difference between this study sample population (i.e., eligible participants) and the parent study sample population (i.e., eligible non-participants). A p-value of less than 0.05 was regarded as significant.

Genetic Association Analysis

Each genetic variation has three possible genotypes (i.e., CC, CT, or TT) and was treated as categorical data. However, since CYSLTR1 gene is within chromosome X, data for males was dichotomous based on having minor allele or not. Atopy status (i.e. positive skin test reactivity), asthma status, atopic asthma status, elevated IgE (based on at least one IgE level log10 z-score \( \geq 1 \)) and eosinophilia (based on at least one eosinophil/white blood cell ratio > 4% or eosinophil count z-score \( \geq 1 \)) are dichotomous data. Inferential statistical analysis of genetic association with asthma, atopy, atopic asthma, elevated IgE status, and eosinophilia, was carried out using contingency table analyses (i.e., Chi-square analysis or Fisher exact test when needed because of small contingency square sample size less than 5). Logistic regression modeling was used to analyze the interaction of CYSLTR2 genetic variation and gender in atopic asthma.
Since CYSLTR1 gene is located on the X-chromosome, males would only have one allele versus two alleles for females. Thus, analysis of SNPs of interest in the CYSLTR1 gene was stratified for sex. A p-value of less than 0.05 was regarded as significant.

Analysis of interaction was performed through Chi-square test of homogeneity with p-value of less than 0.10 regarded as significant. Confounding was analyzed through multivariate logistic regression; with confounding considered if difference between adjusted and unadjusted odds ratio > 10%.

False Discovery Rate

In studies with multiple analyses, adjustments are performed to reduce risk of familywise error, also known as alpha inflation or cumulative Type I error. Bonferroni is a method that calculates a new pairwise alpha to keep the familywise alpha value at 0.05 or less and may overcorrect for Type I error (Newsom, 2006). Benjamini and Hochberg False Discovery Rate is a sequential method with more power than the Bonferroni procedure taking into consideration the alpha level ranked p-values, then correcting for the previous number of tests rather than all the tests in the group (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001; Newsom, 2006). The Benjamini and Hochberg False Discovery Rate post hoc adjustment was utilized. A p-value of less than 0.05 was regarded as significant.

Genetic Model

In classical genetics model of inheritance is described as co-dominant, recessive, dominant, or additive. A co-dominant model refers to all genotypes contributing to and visible in the trait (i.e., phenotype). A phenotype visible among those with a homozygote genotype for the minor allele is considered recessive. A phenotype visible among those with either
homozygote genotype for the minor allele or heterozygote genotype is considered dominant. A phenotype visible as a combined effect of each allele in the genotype is considered additive or dose dependent (Nussbaum, et al., 2007; Ziegler, et al., 2010). The initial genetic association analysis of this study assumed a co-dominant model. After significant association was determined for SNPs of interest, further analysis was performed to determine the best genetic model for the genotype. Since CYSLTR1 gene is located on the X-chromosome, genetic model analysis was performed utilizing the female eligible participant population. Tests for recessive and dominant models of inheritance included contingency table analysis of grouped genotypes for SNPs of interest (i.e., Chi-square analysis). Tests for additive model of inheritance included logistic regression analysis of individual genotypes for SNPs of interest. A p-value of less than 0.05 was regarded as significant.

Protection of Human Subjects

Human Subject Sample

Subjects enrolled in the longitudinal parent studies had been informed of ongoing analysis of DNA, biomarkers, and other data. Written consents were obtained in subjects enrolled in the parent studies and included permission for use of genomic samples for studies related to respiratory diseases (Guerra, 2005a, 2005b). Internal Review Board (IRB) approval for ethical review of activities involving human biological/genetic materials was obtained from the University of Arizona prior to starting data collection (Appendix C). Confidentiality, security of data, anonymity, and ability to withdraw from the study at any time without penalty was understood by all parent study participants and was carried over in this dissertation study. All data and genetic DNA samples were coded.
Protection of Human Subjects

Safeguards to protect subject rights and welfare in this study were outlined in the consent forms in the two parent studies detailed above. Subjects enrolled in the two parent studies were informed of analysis of DNA, biomarkers, and physiologic data. Written informed consents were obtained from participants in these two studies. Confidentiality, security of data, anonymity, and ability to withdraw from the study at any time without penalty was explained to all parent study participants during the informed consent process. Under the authorization section of the previous parent studies’ consent forms, it was stated: “I may ask questions at any time and I am free to withdraw from the project at any time without causing bad feelings or affecting my medical care” (Guerra, 2005a, 2005b). All data and genetic DNA samples were identified by numeric study IDs.

Access to the parent study data was permitted to authorized personnel only. Understanding that handling and storage of genetic and molecular data has potential risks for breach of confidentiality, discrimination, and effects on emotional well-being, this author had access to parent study data limited to just study participants and only information outlined in the original parent studies.

Potential Benefits of this Study to the Subjects and Others

Data obtained from this study did not have any immediate benefit for participants included in this study. However, knowledge gained from this proposed study has the potential to affect prevention and management of asthma in the future.

Inclusion of Women Children, and Minorities

Women represented approximately 52% of the subjects enrolled in parent study cohorts.
Since 52% of the parent study population was female, the intent of this study was to have at 52% female participation. The study did not involve vulnerable populations such as children, pregnant women, prisoners, or cognitively impaired subjects. Although asthma and atopic disease phenotype is present in children, most children initially enrolled in the parent studies were adults as of January 2005. Only subjects ≥ 18 years old, enrolled in parent studies, were included in this study. The parent studies started in 1972 and a large majority of those enrolled were Non-Hispanic whites. This is an unavoidable limitation.

Methods Summary

This study was nested within two parent studies, which employed a 30-year, prospective longitudinal cohort design. Previously stored buccal and blood specimens were processed for DNA and Sequenom system was utilized for genotyping CYSLTR1 and CYSLTR2 tag SNPs of interest to this study. Previously collected blood specimen data were used to evaluate IgE status, and eosinophilia. Questionnaires collected in the parent studies were used to describe demographic variables. Eligible participants in this study included Non-Hispanic white individuals at least 18 years of age, who consented to genetic testing in the parent studies and genotype call was determined for the CYSLTR1 and CYSLTR2 SNPs.

Power computations were performed with Quanto computation software. Hardy Weinberg Equilibrium analysis was performed. Stata and SPSS statistical software packages were used for statistical analysis. Descriptive statistics were employed to analyze the demographic variables. Chi-square analysis and logistic regression were employed to answer questions derived from the specific aims of this study. In the interest of reducing Type I error, Benjamini and Hochberg False Discovery Rate post hoc adjustment was utilized. Genotypes
were tested for co-dominant, recessive, dominant, and additive genetic effects.
CHAPTER IV: RESULTS

Overview

The Aims of this study were to: (1) Determine the relationship between CYSLTR1 genotypes, CYSLTR2 genotype, atopy, elevated IgE level, and eosinophilia, (2) Determine the relationship between CYSLTR1 genotypes, CYSLTR2 genotype, asthma, and atopic asthma, and (3) Determine the degree of interaction of CYSLTR2 genetic variation and gender in atopic asthma. This chapter includes demographic data, HWE, genotypic data, FDR, and genetic model analysis.

Demographic Data Analysis

Demographic data are presented in Table 4. Total eligible participants in this study were 853. As of January 2005, the mean age for eligible participant population was 55.3 years, significantly older than the eligible non-participant population (p < 0.001). Females totaled 56.9% of the eligible population. Over half of the eligible participant population and eligible non-participant population smoked at some point in their lives. Smokers among eligible participants had an average pack year history of 20.32 years and were statistically different from the eligible non-participant population (p = 0.003). Prevalence of asthma based on physician diagnosis was 20.3% and significantly higher than the eligible non-participant group (p = 0.001). Prevalence of atopy based on ever having a recorded skin prick test ≥ 2mm was 72.8% and significantly higher than the non-participant group (p < 0.001). Prevalence of atopic asthma in the eligible participant group was 17.1% and significantly higher than the eligible non-participant group (p<0.001). This prevalence of atopic asthma in this Tucson, Arizona, population is higher than most other areas reported around the world (Weinmayr, et al., 2007).
Prevalence of ever having an elevated IgE level was 20.5% in the eligible participant group, higher than the eligible non-participant group, however not statistically significantly different (p = 0.416). Prevalence of eosinophilia was 25.5% among eligible participants, significantly higher than the eligible non-participant group (p = 0.001). Not including the latest questionnaire, the number of surveys completed by the eligible participant group was considerably higher than the eligible non-participant group (p < 0.001). Between the eligible participants and non-participant groups, there were several significant differences in age, gender, ever having smoked, asthma status, atopy status, atopic asthma status, rhinitis, eosinophilia, and number of questionnaires completed.
Table 4: Characteristics of Subjects

This table of subject characteristics includes asthma status, atopy status, atopic asthma, elevated IgE, eosinophilia, and number of recorded questionnaires completed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Eligible participating</th>
<th>Eligible non-participating</th>
<th>Deceased</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample population</td>
<td>853</td>
<td>2682</td>
<td>1664</td>
<td></td>
</tr>
<tr>
<td>Age as of January 2005: Mean (standard deviation, SD)</td>
<td>55.34 (15.40)</td>
<td>46.72 (18.61)</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male % (total number, N)</td>
<td>43.1 (368)</td>
<td>51.9 (1392)</td>
<td>45.3 (753)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ever smoker % (N)</td>
<td>50.9 (405)</td>
<td>55.3 (1040)</td>
<td>63.0 (1030)</td>
<td>0.035</td>
</tr>
<tr>
<td>Among smokers, pack years as reported on last completed questionnaire: mean (SD)</td>
<td>20.32 (0.97)</td>
<td>16.47 (0.54)</td>
<td>42.24 (0.95)</td>
<td>0.003</td>
</tr>
<tr>
<td>Asthma status % based on physician diagnosis (N)</td>
<td>20.3 (173)</td>
<td>15.3 (399)</td>
<td>16.1 (264)</td>
<td>0.001</td>
</tr>
<tr>
<td>Atopy status % based on SPT ≥ 2mm (N)</td>
<td>72.8 (562)</td>
<td>56.5 (999)</td>
<td>39.3 (597)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atopic Asthma % (N)</td>
<td>17.1 (144)</td>
<td>8.9 (223)</td>
<td>8.4 (136)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elevated IgE % based on at least one log10 z-score ≥ 1 (N)</td>
<td>20.5 (138)</td>
<td>18.9 (213)</td>
<td>12.9 (180)</td>
<td>0.416</td>
</tr>
<tr>
<td>Eosinophilia % based on at least one eos/wbc &gt; 4% or z-score ≥ 1 (N)</td>
<td>25.2 (171)</td>
<td>18.8 (225)</td>
<td>15.8 (209)</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of questionnaires completed: Mean (SD)</td>
<td>10.35 (3.17)</td>
<td>6.4 (3.91)</td>
<td>7.04 (3.93)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Genetic Data Analysis

Conservative genotype calls were made on strong signals easily detected by the Sequenom system. There were eleven heterozygous genotype calls for SNPs in the CYSLTR1 gene located on the X-chromosome. Since it is impossible for males to be heterozygotic for genes located on the X-chromosome, these were considered genotype call failures, and thus were not included in genetic association analysis.

Hardy Weinberg Equilibrium

HWE analysis was performed for all 5 genotypes in this study (Domingues, 2010). HWE in this study population was met for rs320991, rs321006, rs321073, and rs912278; and thus, these genotypes were entered into further analysis. HWE for rs2407249 was not met; and thus, this genotype was not entered into further analysis. (Table 5)

Table 5: Hardy Weinberg Equilibrium

This table includes calculations for HWE among females for CYSLTR1 SNPs of interest and among males and females for CYSLTR2 SNPs of interest.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chi square (1 df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs320991 Females</td>
<td>0.162</td>
<td>0.6873</td>
</tr>
<tr>
<td>rs321006 Females</td>
<td>0.138</td>
<td>0.7107</td>
</tr>
<tr>
<td>rs321073 Females</td>
<td>1.829</td>
<td>0.1762</td>
</tr>
<tr>
<td>rs912278 Combined</td>
<td>0.369</td>
<td>0.5434</td>
</tr>
<tr>
<td>rs2407249 Combined</td>
<td>13.890</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Specific Aim 1 Analysis

Analysis included determining the relationship of the CYSLTR1 and CYSLTR2 genetic variations to markers of atopy including IgE level, blood eosinophil count, and skin test reactivity. The hypotheses of an association between CYSLTR1 and CYSLTR2 genetic variations and atopy, elevated IgE level and eosinophilia were tested (Table 6). There was a statistically significant association between SNP rs321006 and atopy among males (p = 0.029) and females (p = 0.003). However, only the latter remained significant after False Discovery Rate (FDR) adjustment. There were no statistically significant associations between these SNPs of interest, elevated IgE level, and eosinophilia (Table 6).

Specific Aim 2 Analysis

Analysis included determining the relationship of CYSLTR1 and CYSLTR2 genetic variations to asthma and atopic asthma. There was a statistically significant association between SNP rs321073 and asthma and atopic asthma among females (p = 0.014 and p = 0.007, respectively). Only the association with atopic asthma remained significant after FDR adjustment. There were no statistically significant associations between any of the SNPs of interest, asthma, and atopic asthma among males (Table 6). The proportion of females with a specific genotype associated with asthma, atopy, and atopic asthma are listed in Table 7.
Table 6: P-values for Genotype Association

This table illustrates the association between genotypes of interest and health outcomes using a co-dominant model (significant association reported as p-value < 0.05). Benjamini and Hochberg False Discovery Rate post hoc corrected p-values are in parenthesis.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Asthma</th>
<th>Atopy</th>
<th>Atopic Asthma</th>
<th>Elevated IgE</th>
<th>Eosinophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs320991</td>
<td>0.797</td>
<td>0.104</td>
<td>0.564</td>
<td>0.274</td>
<td>0.522</td>
</tr>
<tr>
<td>Males</td>
<td>(0.932)</td>
<td>(0.239)</td>
<td>(0.987)</td>
<td>(1.918)</td>
<td>(0.731)</td>
</tr>
<tr>
<td></td>
<td>0.129</td>
<td>0.431</td>
<td>0.229</td>
<td>0.978</td>
<td>0.581</td>
</tr>
<tr>
<td></td>
<td>(0.452)</td>
<td>(0.431)</td>
<td>(0.801)</td>
<td>(0.978)</td>
<td>(0.680)</td>
</tr>
<tr>
<td>rs321006</td>
<td>0.296</td>
<td>0.029</td>
<td>0.535</td>
<td>0.331</td>
<td>0.400</td>
</tr>
<tr>
<td>Males</td>
<td>(0.690)</td>
<td>(0.101)</td>
<td>(1.246)</td>
<td>(1.158)</td>
<td>(0.932)</td>
</tr>
<tr>
<td></td>
<td>0.415</td>
<td>0.003</td>
<td>0.939</td>
<td>0.505</td>
<td>0.335</td>
</tr>
<tr>
<td></td>
<td>(0.726)</td>
<td>(0.021)</td>
<td>(0.939)</td>
<td>(1.177)</td>
<td>(1.178)</td>
</tr>
<tr>
<td>rs321073</td>
<td>0.831</td>
<td>0.257</td>
<td>0.814</td>
<td>0.536</td>
<td>0.242</td>
</tr>
<tr>
<td>Males</td>
<td>(0.831)</td>
<td>(0.359)</td>
<td>(0.952)</td>
<td>(0.938)</td>
<td>(1.694)</td>
</tr>
<tr>
<td></td>
<td>0.014</td>
<td>0.271</td>
<td>0.007</td>
<td>0.887</td>
<td>0.551</td>
</tr>
<tr>
<td></td>
<td>(0.098)</td>
<td>(0.317)</td>
<td>(0.049)</td>
<td>(1.038)</td>
<td>(0.964)</td>
</tr>
<tr>
<td>rs912278</td>
<td>0.600</td>
<td>0.191</td>
<td>0.574</td>
<td>0.679</td>
<td>0.696</td>
</tr>
<tr>
<td>Combined</td>
<td>(0.840)</td>
<td>(0.334)</td>
<td>(0.804)</td>
<td>(0.950)</td>
<td>(0.696)</td>
</tr>
</tbody>
</table>
Table 7: Genotype Frequencies

Focusing on the disease-gene associations with statistical significance among females, this table illustrates the proportions of individuals with respective genotypes and listed outcomes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Asthma</th>
<th>Atopy</th>
<th>Atopic Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>105/476 (22%)</td>
<td>324/436 (74%)</td>
<td>88/470 (19%)</td>
</tr>
<tr>
<td>rs321006 Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>80/342 (23%)</td>
<td>222/310 (72%)</td>
<td>64/337 (18%)</td>
</tr>
<tr>
<td>CT</td>
<td>22/123 (18%)</td>
<td>96/114 (84%)</td>
<td>21/122 (17%)</td>
</tr>
<tr>
<td>TT</td>
<td>2/13 (15%)</td>
<td>6/12 (50%)</td>
<td>2/13 (15%)</td>
</tr>
<tr>
<td>rs321073 Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>8/16 (50%)</td>
<td>13/14 (93%)</td>
<td>8/16 (50%)</td>
</tr>
<tr>
<td>CT</td>
<td>29/164 (18%)</td>
<td>111/148 (75%)</td>
<td>25/161 (16%)</td>
</tr>
<tr>
<td>TT</td>
<td>68/296 (23%)</td>
<td>199/122 (73%)</td>
<td>55/273 (19%)</td>
</tr>
</tbody>
</table>

False Discovery Rate

After FDR adjustment, among females, only SNPs rs321006 and rs321073 met level of statistical significance (p-value < 0.05) for association with atopy and atopic asthma, respectively. Thus, further genetic model analysis focused on these two SNPs associated with atopy and atopic asthma.

Specific Aim 3 Analysis

Analysis included determining gene by gender interactions in association with CYSLTR2 genetic variation in affecting the risk for atopic asthma. Genetic variations, gender and diagnosis of atopic asthma are categorical data. The independent variables in this analysis are CYSLTR2 SNP genotype and gender; the dependent variable is atopic asthma. Logistic regression was utilized to analyze the interaction of CYSLTR2 genetic variation and gender in atopic asthma, testing the hypothesis that there was an interaction between gender and CYSLTR2 genetic
variation in affecting the risk for atopic asthma. In analysis of a model that included sex, genotype, and an interaction term of sex by genotype to predict atopic asthma among Non-Hispanic adults, there was no statistically significant interaction (p-value for the interaction term = 0.4463). (Table 8)

Table 8: Genotype Frequency Stratified for Sex

This table illustrates the association between SNP rs912278 genotypes and atopic asthma stratified by sex.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Atopic Asthma N = 143/838</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs912278 Females</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>14/88 (16%)</td>
</tr>
<tr>
<td>CT</td>
<td>48/229 (21%)</td>
</tr>
<tr>
<td>TT</td>
<td>27/159 (17%)</td>
</tr>
<tr>
<td>rs912278 Males</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>9/73 (12%)</td>
</tr>
<tr>
<td>CT</td>
<td>24/173 (14%)</td>
</tr>
<tr>
<td>TT</td>
<td>54/362 (15%)</td>
</tr>
</tbody>
</table>

Genetic Models

Testing the recessive model of inheritance (i.e., the association of disease state with homozygotic minor alleles) there was a borderline statistical significant association between SNP rs321006 and atopy among females (p = 0.051), and a statistically significant association between SNP rs321073 and atopic asthma among females (p = 0.004) (Table 9). Testing the dominant model of inheritance there was a statistically significant association between SNP rs321006 and atopy among females (p = 0.043) There was no statistical significance in analysis
of an additive model of inheritance. The dominant model appeared to best fit the data for association between atopic asthma and rs321006 among female Non-Hispanic whites (Table 9); however, considering the proportions of atopy with the genotypes of SNP rs321006 (Table 7), a dominant model is ambiguous and is not supported.

Table 9: Genetic Models

This table illustrates the association between genotypes of interest and health outcomes using recessive, dominant, and additive models of inheritance among Non-Hispanic white female adults (association reported as p-value < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Recessive model</th>
<th>Dominant model</th>
<th>Additive model</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs321006 for atopy</td>
<td>0.051</td>
<td>0.043</td>
<td>0.252</td>
</tr>
<tr>
<td>rs321073 for atopic asthma</td>
<td>0.004</td>
<td>0.973</td>
<td>0.302</td>
</tr>
</tbody>
</table>

Since a recessive model appeared to best fit the data for association between atopic asthma and rs321073 among female Non-Hispanic whites (Table 9), a recessive model of inheritance is supported in further analysis of this SNP and atopic asthma (Table 10). Genetic model analysis for rs321006 appears to be inconclusive. However when looking at the proportions of individuals with respective genotypes and listed outcomes in Table 7, the rs321006 TT genotype appears to be protective for asthma, atopy, and atopic asthma.
Table 10: Atopic Asthma and rs321073 Recessive Odds Ratio

Assuming the recessive model, this table illustrates the unadjusted calculation of odds ratio (OR) of having the rs321073 CC (vs. TT & CT genotypes combined) in atopic asthma among female Non-Hispanic white adults (OR = 5.82). Observations are filtered for missing data for variables of age, smoking status, and number of completed questionnaires for appropriate comparison between crude and adjusted odds ratio.

<table>
<thead>
<tr>
<th></th>
<th>TT or CT</th>
<th>CC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No atopic asthma</td>
<td>345</td>
<td>6</td>
<td>351</td>
</tr>
<tr>
<td>Atopic asthma</td>
<td>79</td>
<td>8</td>
<td>87</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>424</td>
<td>14</td>
<td>438</td>
</tr>
</tbody>
</table>

Separate analysis of atopic asthma and rs321073 CC genotype includes adjustment for age, smoking status, and number of completed questionnaires among Non-Hispanic white females. Number of completed questionnaires was included in the analysis since frequency of engagement in TESAOD studies might confound an association in this model. The odds ratio for the association between atopic asthma and rs321073 after adjustment for age, smoking status, and number of completed questionnaires (adjusted OR = 6.24, p = 0.001, 95% CI 2.07 – 18.81), did not change relevant to the unadjusted model of atopic asthma and rs321073 alone (crude OR = 5.82, p = 0.001, 95% CI 1.96 – 17.26). The difference between these two models (6.7%) did not meet the a priori parameter to be able to consider confounding (>10%), thus, the unadjusted
model reported here. Assuming a recessive model, among female Non-Hispanic white adults with atopic asthma, the odds of having rs321073 CC genotype was 5.82 times higher than female Non-Hispanic white adults without atopic asthma (p = 0.001, 95% CI 1.96 – 17.26).

Assuming a recessive genetic model for rs321006 genotype, further analysis was performed. The crude odds ratio for the association between atopy and rs321006 TT genotype was 0.61 (p = 0.052, 95% CI 0.37 – 1.005). The odds ratio for the association between atopy and rs321006 after adjustment for age, smoking status, and number of completed questionnaires (adjusted OR = 0.62, p = 0.067, 95% CI 0.37 – 1.03), did change relevant to the unadjusted model and remained not statistically significant.

Results Summary

Of the 897 individuals initially enrolled in the two parent studies, 853 individuals met criteria for inclusion in this dissertation study. There were statistically significant differences between the eligible participant group and the eligible non-participant groups for demographic and clinical data except elevated IgE. Having met criteria for HWE in this study population for rs320991, rs321006, rs321073, and rs912278, these genotypes were entered into further analysis. HWE in this study for rs2407249 was not met and subsequently, this genotype was not entered into further analysis.

For Aim 1 in this study, there was a statistically significant association between SNP rs321006 and atopy among Non-Hispanic white males and females. For Aim 2 in this study, there was a statistically significant association between SNP rs321073 and asthma status and atopic asthma status among Non-Hispanic white females. For Aim 3 in this study, there was no statistically significant interaction between gender and CYSLTR2 genotype in atopic asthma.
among Non-Hispanic white participants. After FDR adjustment, statistical significance was reached only between rs321006 and atopy, and between SNP rs321073 and atopic asthma among Non-Hispanic white female adults.

Analysis of recessive model of gene expression for SNP rs321073 located on the X-chromosome was supported for atopic asthma among female Non-Hispanic white adults. Utilizing the recessive model, among female Non-Hispanic white adults with atopic asthma, the odds of having rs321073 CC genotype was 5.82 times higher than female Non-Hispanic white adults without atopic asthma (p = 0.001, 95% CI 1.96 – 17.26). It appears that rs321006 TT genotype may have a protective effect for atopy among Non-Hispanic white females.
CHAPTER V: DISCUSSION

Introduction

This descriptive longitudinal study design allows estimating relative risks for atopy, asthma, atopic asthma, elevated IgE level, and eosinophilia (Khoury, Little, & Burke, 2004; Merrill, & Timmreck, 2006). In this chapter, HWE, the major findings relative to each study Aim, and limitations are discussed including sample population selection, study protocol, study design, and study methods. Table 11 illustrates the identifiable positions of SNPs in CYSLTR1 and CYSLTR2 as reported in this study (i.e., first five rows) and as identified in the literature and detailed in Appendix A (i.e., remaining five rows of that table). Further discussion includes implications of the study findings for pharmacogenetics, implications for clinical practice, implications for asthma related genomic research, and suggested future research.

Study Aims Outcomes

Specific Aim 1 Outcomes

Specific Aim 1 was designed to determine the relationship of the CYSLTR1 and CYSLTR2 genetic variations to markers of atopy including IgE level, blood eosinophil count, and skin test reactivity. Among the SNPs of interest in these cysteinyi leukotriene receptor genes, there was only one SNP (i.e., rs321006), located on the CYSLTR1 gene, associated with atopy (i.e., positive skin test reactivity) among males and females. However, after FDR adjustment, only the association between rs32106 and atopy showed statistical significance among female Non-Hispanic white adults. Tagging SNP rs321006 represents one of the three regions covering the CYSLTR1 gene and it appears that rs321006 has not been previously reported in association with atopy among males or females (Appendix A and Table 11).
disequilibrium refers to the correlation between two alleles or two genotypes and indicates that these two alleles are physically close on the DNA strand (Ziegler, et al., 2010). Analysis of linkage disequilibrium, reveals SNP rs321006 in moderate linkage disequilibrium with rs321029 and rs321073 (i.e., correlation coefficient $r^2$ values of 0.060 and 0.055, respectively). Although SNP rs321029 was reported in the literature as a leukotriene pathway candidate gene in asthma, this study finding provides a novel association between SNP 321006 and atopy in this region of the CYSLTR1 gene.

SNP rs321006 is located in an intron region of the CYSLTR1 gene. Changes in the intron region of a gene can influence gene expression through transcription, polyadenylation, mRNA export, translation efficacy and the rate of mRNA decay (Nott, Meislin, & Moore, 2003). It is possible that SNP rs321006 might be linked with an unidentified, functional polymorphism and thus pre-dispose females to atopy. Expression analysis research of this gene is lacking.

Epigenetics refers to changes in gene expression or phenotype due to mechanisms other than changes in DNA sequence and includes methylation or other epigenetic factor tagging the DNA sequence and influencing activation or repression of a gene in that chromosome region and is a growing area of genetic research as another possible association with disease outcomes (Nussbaum, et al., 2007; Ziegler, et al., 2010). Methylation typically occurs in regions rich in GC sequence (i.e., CpG islands) and might be involved in methylation of DNA (Jirtle, & Skinner, 2007; Nussbaum, et al., 2007). SNPs of interest on the CYSLTR1 gene (i.e., rs321006 and rs321073) and CYSLTR2 gene (i.e., rs912278 or rs2407249) were not located in regions rich in GC sequence and thus epigenetics most likely not an influence at these SNP locations (National Center for Biotechnology Information, 2011c, 2011d; United States Department of Health and
SNP rs320991 located in the CYSLTR1 gene is located in a region with GC sequence and could be reason for possible change in gene expression; however, there was no identified association with elevated IgE level, blood eosinophil count, or skin test reactivity among this study population (National Center for Biotechnology Information, 2011c, 2011d; United States Department of Health and Human Services, 2011). Thus, associations of SNPs rs321006 and rs321073 and atopy and atopic asthma respectively are most likely a result of changes in transcription without epigenetic influence of these respective CYSLTR1 gene intron regions.

Although SNP rs321006 appeared to affect atopy status according to a dominant genetic model (i.e., CT and TT genotypes) as illustrated in Table 9, results for the genetic model border on statistical significance (i.e., dominant model $p = 0.043$ and recessive model $p = 0.051$). In reviewing Table 7, the proportions of individuals with rs321006 CC, CT, and TT genotypes in association with atopic status does not support a dominant genetic model, but perhaps a recessive genetic model where TT genotype contributes to protection from atopy among Non-Hispanic white females.

Although previous genetic association studies have included CYSLTR1 gene variation, IgE level and eosinophilia, there were no reports of significant association. There was no significant genetic association between CYSLTR1 SNPs of interest in this study, IgE level, and eosinophilia, which remains consistent with current literature. There was no significant genetic association between CYSLTR2 SNP of interest in this study, IgE level, and eosinophilia. This is the first published report of association analysis results between these variables.
Specific Aim 2 Outcomes

Specific Aim 2 was designed to determine the relationship of CYSLTR1 and CYSLTR2 genetic variations to asthma and atopic asthma. Among the SNPs of interest in these cysteinyl leukotriene receptor genes, only one SNP located on the CYSLTR1 gene (i.e., rs321073) was associated with asthma and atopic asthma among females, but not among males. However, after FDR adjustment, only the association between SNP rs321073 and atopic asthma showed statistical significance among female Non-Hispanic white adults. A recessive model best fit the data for association between atopic asthma and rs321073 among female Non-Hispanic whites. Assuming the recessive mode of gene expression among female Non-Hispanic white adults, the odds ratio of having the rs321073 CC genotype in atopic asthma (OR = 5.82) reflects a large genetic effect in this population.

Tagging SNP rs321073 represents another one of the three regions covering the CYSLTR1 gene in this study and it has not been previously reported in association with asthma or atopic asthma in females (Appendix A and Table 11). However, SNP rs321073 is in complete linkage disequilibrium with SNP rs320995 (i.e., correlation coefficient $r^2 = 1.00$) previously reported in the literature with significant association with atopic asthma (Arriba-Mendez, et al., 2006; SeattleSNPs, 2011). This association was noted when choosing tag SNPs in the beginning stages of this research study however primers could not be obtained for rs320995. Although the report of SNP rs321073 associated with atopic asthma is a novel finding and is in complete linkage disequilibrium, this research also represents a novel association between SNP rs321073 and atopic asthma in a Non-Hispanic white female population.
SNP rs321073 is located in an intron region of the CYSLTR1 gene. Changes in this intron region could influence gene expression (Nott, et al., 2003). There are 3 other nucleotide polymorphisms within 100 bases of SNP rs321073 that are insertion polymorphisms (i.e., rs72500752, 68 bases upstream from SNP rs321073, rs61463264, 69 bases upstream from SNP rs321073, and rs66483565, 84 bases upstream from SNP rs321073). An insertion polymorphism in an intron region can directly affect gene expression as mentioned above. It is possible that SNP rs320173 might be linked with an unidentified, functional polymorphism and thus predispose females to atopic asthma.

For the CYSLTR2 gene, SNP rs912278 was not identified in association with asthma or atopic asthma and rs2407249 did not meet HWE to be entered into genetic analysis.

Specific Aim 3 Outcomes

Specific Aim 3 was designed to determine gene by gender interactions in association with CYSLTR2 genetic variation in affecting the risk for atopic asthma. HWE was not met for SNP rs2407249 and not entered into this analysis. There was no interaction between gender and SNP rs912278 among eligible participants with atopic asthma. SNP rs912278 is a tag SNP representing the second of two genomic regions in CYSLTR2 gene as reported by the open source Genome Variation Server (GVS) for the representative CEU population (SeattleSNPs, 2011).
Table 11: SNP Sequence

This table illustrates the identifiable position of SNPs in CYSLTR1 and CYSLTR2 (National Center for Biotechnology Information, 2011c, 2011d; United States Department of Health and Human Services, 2011).

<table>
<thead>
<tr>
<th>rs number</th>
<th>Gene</th>
<th>Human Genome Variation Society (HGVS) name (Assessment: NT or NM)</th>
<th>Sequence position (chromosome location)</th>
<th>Sequence with SNP in red (NM sequence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs321073</td>
<td>CYSLTR1</td>
<td>NT_011651.17:g.836649C&gt;T.17</td>
<td>836649 C&gt;T (77540341)</td>
<td>GAAACCTGAA C CTACAGCTGC</td>
</tr>
<tr>
<td>rs321006</td>
<td>CYSLTR1</td>
<td>NT_011651.17:g.865917</td>
<td>865917 G&gt;A (77569609)</td>
<td>AGAGGTTTAG G ACATAGGAGA</td>
</tr>
<tr>
<td>rs320991</td>
<td>CYSLTR1</td>
<td>NT_011651.17:g.828933</td>
<td>828933 G&gt;A (77532625)</td>
<td>AGCATGGCCA C GGTTACACAT</td>
</tr>
<tr>
<td>rs912278</td>
<td>CYSLTR2</td>
<td>NT_024524.14:g.30263487</td>
<td>30263487 A&gt;G (49283487)</td>
<td>TAAGTCAGTC A TCATACTAAA</td>
</tr>
<tr>
<td>rs2407249</td>
<td>CYSLTR2</td>
<td>NT_024524.14:g.30263795</td>
<td>30263795 A&gt;G (49283795)</td>
<td>AATACATTTG A GGGAAAAGGCC</td>
</tr>
<tr>
<td>rs2412222</td>
<td>CYSLTR1</td>
<td>NT_024524.14:g.30263487</td>
<td>846813 A&gt;G (77550505)</td>
<td>ACAAGTCCCA A TGAGATGAAA</td>
</tr>
<tr>
<td>rs320995</td>
<td>CYSLTR1</td>
<td>NT_011651.17:g.824625 or NM_006639.2:c.927</td>
<td>824625 A&gt;G or (77528317)     927T&gt;C</td>
<td>AATGCTTTCT G AATGTAGACA or (TGTCTACATT C AGAAAGCATT)</td>
</tr>
<tr>
<td>rs321029</td>
<td>CYSLTR1</td>
<td>NT_011651.17:g.880054</td>
<td>880054 A&gt;G (77583746)</td>
<td>TCTGGAGAAA A GGGACATAGC</td>
</tr>
<tr>
<td>rs28365142</td>
<td>CYSLTR1</td>
<td>NT_011651.17:g.824935 or NM_006639.2:c.617</td>
<td>824935 or 617T&gt;G (77528627)</td>
<td>GTAACAGACA A TTATAATAAC or (GTTATTATAA T TGTCGTTAC)</td>
</tr>
<tr>
<td>rs321092</td>
<td>CYSLTR1</td>
<td>NT_011651.17:g.858000</td>
<td>858000 A&gt;G (77561692)</td>
<td>AGGAGGTTCG CTCTGTCACA</td>
</tr>
</tbody>
</table>
DNA sequence reported using NM accession number was identified as the reverse DNA sequence reported using NT accession number; however, there were research studies reporting SNPs on the CYSLTR1 gene that rs numbers could not be identified (i.e., 136 G/A, 275C/A, 336A>G, 434T/C, 475A>C, 634C>T, 898G/A, and 899G/A). Although the rs numbers could not be identified for these, it appears that none are a pseudo label for those listed in Table 11 above and in this study. It appears that the two SNPs of interest (i.e., rs321006 and rs321073) represent novel significant associations between atopy and atopic asthma respectfully.

These study results are consistent with previous reports of CYSLTR1 genetic variation associated with atopy or asthma among adult females: CYSLTR1 SNP rs320995 associated with atopy among females in the UK (Hao, et al., 2006), CYSLTR1 SNP rs320995 associated with asthma among a Chinese population (Hong, et al., 2009), and CYSLTR1 SNP 899G>A associated with atopy and asthma among a Tristan da Cunha population (Thompson, et al., 2007). However, these study results are inconsistent with specific reports of CYSLTR1 genetic variation associated with atopy, asthma, and atopic asthma among adult females: CYSLTR1 SNP rs320995 associated with atopic asthma among males in Spain (Arriba-Mendez, et al., 2006; Sanz, et al., 2006), and CYSLTR1 SNPs rs320995, 434T/C, 275C/A, and 136G/A not associated with atopy or asthma among a Japanese population (Zhang, et al., 2006). Although there is linkage disequilibrium between SNPs rs321006 and rs321073 in this study and SNP rs320995 supporting consistencies in the literature, there are most likely other factors (to explain inconsistencies with the literature), like environment, gene-gene interactions and epigenetics, that may play an important role in the expression of atopy and atopic asthma and need to be explored further.
Limitations

Among previously reported research in the literature, incomplete reporting of research sample selection, study design, and statistical analysis contributes to the difficulty in the interpretation, synthesis of study results, and reliability when designing a new study such as this presented study (Kottner, Audige, Brorson, Donner, Gajewski, et al., 2011). This literature bias contributed to difficulty in selecting tag SNPs for this study and difficulty in comparing and contrasting with previous genetic association studies. A type I error is a spurious or erroneous conclusion that the outcome of interest is associated with the independent variable when it is not (i.e., false positive), in other words, detecting an association when the association does not actually exist. There are a number of ways to control for a possible type I errors in the study design and study statistical analysis. Limitations will be discussed from the perspective of sample selection, protocol, design, methods, and analysis.

Sample Selection—Parent Studies

The parent TESAOD studies represent a cross section of the Tucson population as it appeared 40 years ago. The recruitment approach was based on a stratified cluster sample of primarily “Anglo-white blocks” (Lebowitz, et al., 1975). Thus, the parent TESAOD study populations represent English speaking individuals and primarily Non-Hispanic white. Unlike the bias when enrolling in a research study within a clinic population or enrollment based on ability to contact individuals by phone call, there is no reason to suspect a selection bias of opt in or opt out of the study would have been contributed by a random door to door enrollment approach as in the parent TESAOD studies. Thus, the population framework of the parent studies represents a good canvas of the Tucson population 40 years ago.
Sample Selection—This Study

Not all individuals in this study currently reside in Tucson, Arizona. This study sample population did not represent the population in the parent studies. There were significant differences in the demographic make up of the eligible participant and eligible non-participant groups. Increased age among eligible participants versus eligible non-participants might have been related to personal health care practices engaging with health care system and thus surviving longer than non-participants. There is no plausible reason at this time to explain greater smoking habits among eligible participants vs. eligible non-participants. Increased rates of asthma, atopic status, and atopic asthma among the eligible participants than eligible non-participants could be related to increased rates of health care utilization, perceived direct benefit with respiratory study participation when having diagnosis, or over all poor respiratory status; and could contribute to selection bias into this study. However, higher prevalence in the eligible participant group may have also been contributed by increased engagement in testing (i.e., questionnaires and biometric measurements) over the 30-year history of these parent studies as evident in the greater number of questionnaires completed (eligible participant group number of completed questionnaires mean of 10.25 versus eligible non-participant group mean of 6.4, p < 0.001).

Power estimation a priori was based on enrollment of 1,000 participants into this study. Although 1,000 participants were anticipated, 897 individuals consented, and only 853 qualified for this study based on a priori enrollment criteria of Non-Hispanic white adults with genotypic data. This difference of 147 reflects approximately a 15% decrease in anticipated sample size for this study. The eligible participant population for this study was still larger than most genetic
epidemiological studies of CYSLTR1 and CYSLTR2 gene variation and asthma reported in the literature to date. Although large effects were identified among adult Non-Hispanic white females, the sample in the analysis contingency tables was small. Larger sample size may have strengthened the association between rs321006 and atopy among males even after FDR adjustment.

Hardy Weinberg Equilibrium

HWE for rs2407249 was not maintained in analysis of genotypic data. Reason why this SNP did not reach HWE might be related to poor SNP primers, failure of the genotyping assay, or problems with detection of the genotype signal to be able to determine correct genotype call. Conservative genotype calls by the Sequenom computer program for SNP rs2407249 totaled 83.6%; approximately 2% were considered genotype failures. Extension primers are different than the product and typically used up and thus do not overlap in the genotype spikes, but not always. Since there were up 20 genotype assays per DNA plate well and 4 extension primers per genotype, it was possible that the extension primer might have somehow interfered with the genotypic call in this SNP of interest. With more precision, pyrosequencing as a method of genotyping, could be considered in the future if current methods are not able to genotype this gene of interest accurately. However, at this time pyrosequencing is a slower process and method is more expensive than Sequenom (Sequenom, 2011; Zhou, Poe, Limor, Grady, Goldman, et al., 2006).

Study Protocol

Following strict protocols, blood and buccal specimens were collected, DNA was processed and banked at the Arizona Respiratory Labs in Tucson, Arizona, before being placed
on a 384 well DNA plate, dried down, and shipped on dry ice to the Facility for Mutation and Methylation Analysis, Omaha, Nebraska, for Sequenom genotyping. Although machines did most of the processing of the specimens, like any other genetic study, it is still possible that human error could enter into collecting poor blood or buccal samples, extracting the DNA from blood and buccal swabs, handling and storage of DNA material, or contaminating DNA samples (Khoury, 2010).

Study Design

Study design can affect the internal validity or the inference of causality of the research. Control of type I error includes large enough sample size based on a power of 80% to ensure being able to detect at least a moderate main genetic effect. Power computations were performed a priori to be able to detect a moderate effect in atopic asthma. Participation rate and loss to follow up were the major limitations of this cohort study design. Of the 1450 initially targeted participants, many did not return the invitation packet leaving a total of 897 participants. Of them, 853 were eligible participants for this dissertation study. This was lower than the a priori intended population size of 1,000.

Although a design strength, the long timeframe and resources required for a cohort study may be a potential limitation for generalizability and future studies. Misclassification bias, or inability to determine cases vs. controls appropriately (i.e., asthma vs. non-asthma), could be a potential problem in research studies. Fortunately, TESAOD studies have been active for over 30 years, and a large amount of data has already been collected. Misclassification bias was controlled by confirming self report of asthma with physician confirmed diagnosis. Misclassification bias for asthma status was controlled by comparing questionnaire results from
multiple TESAOD study questionnaires to determine if ever diagnosed with asthma. Misclassification bias for atopy status was controlled by not basing on self report of allergies, but rather by analyzing multiple allergy skin prick tests results and determining atopy status based on a positive skin prick test result. Elevated IgE status and eosinophilia were also determined from multiple tests of subjects over a 30-year parent study research history. It is possible that misclassification by self report of ethnicity may have occurred; however, genotyping for ethnic status was not employed in this study. Although associations were identified in this study between SNPs of interest and disease status among this homogeneous population, the outcomes of this research cannot be generalized to other ethnic populations.

Survivor bias can affect the overall prevalence of a genotype and phenotype. With treatments available over the past couple of decades more individuals have been able to control their asthma and reduce mortality. Although mortality rates related to asthma appear to be leveling off, the rates remain high. It is possible that the prevalence rates of genotypes and asthma, atopy, and atopic phenotypes could change over a period of 30 years of cohort analysis related to survival. It is also possible that the genotype or allele frequencies might be statistically different from the general Non-Hispanic white population of European decent if individuals migrated to the desert environment due to respiratory health issues, thus bringing their rare genotype variations into the Tucson hereditary mix.

Most gene-disease associations are not consistently replicated. In viewing results of this study in comparison to other studies of these genes of interest in atopic asthma, publication bias should also be discussed. It is known that published positive findings may skew the actual known body of knowledge away from negative or no association in genetic association studies.
(Szklo, 1991). Despite over 1,000 papers published with candidate gene studies related to asthma, identifying over 100 candidate genes, few are consistently replicated (March, et al., 2011). In addition to previous studies, this study contributes to the knowledge of CYSLTR1 and CYSLTR2 genetic variations associated with asthma. Asthma is a multifactorial disease. The outcomes of this study provide more puzzle pieces in the quest to understand the polygenetic and complex traits of atopic asthma.

Study Methods

Methodological errors can affect the construct validity or effective measurement of the research variables. Separate benches, protective lab gear, and other precautions were taken to reduce possible contamination of specimens during DNA extraction. Potential limitations of Sequenom included inaccurate results due to amplification of contaminated specimens and inappropriate assay design. Accurate results were ensured by choosing primers that were 15-30 bases in length and amplicon length less than 150 bases (PREMIER Biosoft International, 2009). Cycling conditions were followed based on assay protocol and with less lab technician hands-on manipulation due to the self contained process within Sequenom. Even though the Sequenom genotyping system was able to achieve high percentage of conservative genotype calls, eleven heterozygous genotype calls for SNPs in the CYSLTR1 gene located on the X-chromosome were dropped from the final genetic association analysis. This represents system a minor limitation in genotyping.

It is possible that there were problems with DNA quantity from buccal swabs and thus less DNA may have been plated for those individuals. A source of genotyping error relates to low template DNA concentrations which could result in an allele failing to amplify appropriately
This reasoning is a bit perplexing in the example of rs2407249 genotype assay failure since the same DNA was plated across all DNA plates and the plates with the first four SNPs of interest all met HWE. If for some unknown reason the DNA concentration of the plate with the rs2407249 genotype assay was lower, this would also affect genotype assays of other SNPs in the parent studies, and at this initial stage of analysis, there has been no report of HWE not being met in the parent studies (C. Venker, personal communication, 2011).

With combined SNPs of interest for this study and the parent studies, up to 20 SNPs were run in each DNA plate well at one time. This means that 4 extension primers are added for each genotype per DNA plate well. It is possible that primers can hybridize at inappropriate locations on the DNA and some PCR amplifications can be more robust than others (P. Graves, personal communication, 2011). SNP rs2407249 was placed on a separate plate from the other 4 SNPs of interest in this study and was most likely genotyped with a different assay from the other SNPs. These issues of primer interaction, PCR assay problems, and separate plate assays may have contributed to the rs2407249 genotype assay failure and thus, unable to meet HWE in this study.

It should also be noted that genotyping for this study utilized Sequenom technology unlike previous genetic association studies for CYSLTR1 and CYSLTR2 where TaqMan genotyping was utilized. Due to the high reliability of Sequenom, this genotyping platform was expected to be relatively robust to genotyping error (Huang, Nelson, Zimmermann, Dudarewicz, Wenzel, et al., 2006).

Analysis

A way to control for type I error is to set parameters in the statistical analysis of the research finding; this includes selecting a p-value cut off, probability of chance, low enough to
ensure not selecting a false positive result (p-value < 0.05 in most cases). To ensure low
probability of a type I error in this study, a p-value cut off was set at less than 0.05 (p < 0.05).
Statistical significant associations remained between rs321006 and atopy among female Non-
Hispanic white adults and between rs321073 and atopic asthma among female Non-Hispanic
white adults after adjusting for multiple comparisons using FDR.

A confounding variable is an extraneous variable that has a positive or negative
relationship between the independent variable and dependent variable. Confounding variables
need to be controlled to reduce the risk of reporting a spurious relationship between the
independent variable and dependent variable. Genotype is considered time-independent since
disease or health outcomes are typically not expressed until much later. In genetic association
studies, confounding variables of race and sex can be controlled in study design or reported by
stratifying the statistical analysis. This study design included a strict adult Non-Hispanic white
population and statistical analysis included stratification by sex for CYSLTR1 SNPs of interest.

Other factors to be considered in comparing results from this study and previously
reported studies include complex biological networks (i.e., gene by gene interactions and gene by
environment interactions) that were not analyzed in the scope of this study. It was originally
thought that there might be an association between the SNPs of interest and elevated IgE status
and/or eosinophila as intermediate phenotypes, intermediate markers in the pathway from genetic
variation to fully expressed disease, or as surrogate markers in the atopic asthma phenotype.
Consistent with previous studies of CYSLTR1 and CYSLTR2 genetic variation, there was no
discovery of association between SNPs of interest and elevated IgE status or eosinophila in this
study.
Implications

Genes are transcribed one base pair at a time. Knowledge is built one brick at a time. Atopic asthma has been identified as a chronic disease process with huge economic, social, and quality of life burden. This inflammatory respiratory disease process, response to treatment, and outcome has been associated with genetic variation. The research health care community is just beginning to unravel the mysteries behind gene translation, gene-gene interactions, gene-gender interactions, and gene-environmental interactions in atopic asthma. There is great potential for novel interventions to free those affected from this disease burden.

The philosophic view of this principal investigator incorporates critical social theory/emancipation and postmodern discourse, emphasizing the quest for truth and the evolving construction of knowledge, grounded in self-reflection, and a cognitive interest in emancipation, as suggested by Habermas (Crotty, 1998; Rodgers, 2005). Critical social theory functions in the criticism of searching for quality instruction, advancing the emancipatory function of knowledge, and liberating human beings from the circumstances that enslave them (Horkheimer, & Adorno, 1972). Nursing is in a unique position to be a primary provider of education about genomics, disease process, and interventions as well as facilitating the patient’s informed decision making. Nurses and other health care providers can help patients suffering from atopic asthma to move beyond disease burden towards efficient health management.

There is a call for standardization of known pulmonary biomarkers of local inflammation and identification of new ones (Dodig, Richter, & Zrinski-Topic, 2011). There is also a call for intervention strategies for asthma that incorporate appropriate assessment, controller therapy, education, and promotes patient engagement (Clement, et al., 2008). Understanding the
pathology and genetic etiology of atopic asthma, may improve preventative strategies, diagnostic tools, therapies and aid in determining responsiveness to treatment. This genetic epidemiological approach to research, focusing on influences of CYSLTR1 and CYSLTR2 genetic variations, gender, and markers of atopy, such as IgE, eosinophil count, and skin sensitivity testing in atopic asthma, may lead to innovative tailored preventive strategies by identifying persons with higher risk of atopic asthma. This research may have important pharmacogenetic implications in the treatment of atopic asthma since rs321006 and rs321073 represent SNPs in an intron region that may have a functional impact on CYSLTR1 and where current medications have been targeted to help those affected by atopic asthma (S. H. Kim, Y. M. Ye, et al., 2007; S. M. Kim, Bowers, Pal, Strong, Terwilliger, et al., 2007). New approaches to assessment and treatment of atopic asthma in different populations may reduce health disparity and increase quality of life for millions around the world. Other implications include: innovative methods to identify persons with higher risk of atopic asthma, improved methods in determining responsiveness to treatment of atopic asthma, better choices for patients in the management of their asthma, potential development of pharmacogenetic treatments for atopic asthma, and reduced health disparity and increased quality of life for millions with atopic asthma.

Future Research

At the time of the analysis for this study, there were no data available to determine if current residence in Tucson or outside Tucson might have influenced individual’s choice in enrolling in the parent studies and thus sample population for this study. This may be an interesting analysis as future studies are planned for the TESAOD parent population.

Haplotype is a combination of alleles on the chromosome that are transmitted together,
and when grouped together in genetic association analysis provide a more powerful representation of a combination of gene variations contributing in a particular outcome (Ziegler, et al., 2010) such as atopic asthma. Future study might include analysis of haplotype to determine the level of significance of more than one genotype in the genetic association model of atopic asthma.

Not all asthma patients respond to leukotriene inhibitors when taken to control chronic asthma and this is not indicated as a first line of treatment for asthma or to be utilized independently (Global Initiative for Chronic Obstructive Lung Disease, 2008). Ongoing research with leukotriene inhibitors has indicated differences in efficacy, adverse effect profile among the current available leukotriene inhibitors on the market (Dahlen, 2006; Morden, St Anna, & Slotkick, 2004). Although SNPs have been identified within the CYSLTR1 gene, studies do not indicate patient response to leukotriene medication (Lee, et al., 2007). In previously reported CYSLTR1 studies comparing an asthma-atopic dermatitis and an asthma non-atopic dermatitis group, significant differences were observed in the male allele distribution and there were no differences in allele distribution between these disease sub-groups in females (Arriba-Mendez, et al., 2006). These research findings might help build a framework for further pharmacogenetics research related to asthma.

Consistent with previous reports of the complex nature of asthma genetics (Guerra, & Martinez, 2008), future research might employ genetic associations of other candidate genes with asthma, atopy, and atopic asthma, gene by gene interactions, gene by environment interactions, and epigenetic variants associated with these genes of interest and asthma, atopy, and atopic asthma. Future research might employ these findings with other biomarker studies to explore
better use of biomarkers in determining disease phenotype, disease progression, and disease control or remission. As technology increases and pricing drops, pyrosequencing might be considered in the future to capture many more SNPs across these genes of interest in respiratory association studies with more precision.

Findings from this dissertation study may contribute to innovative approaches to pharmacotherapy, whereby blood assay tests could determine a tailored treatment plan for the individual based on genetic variations including those found in the leukotriene receptor. There is evidence that genetic variation also contributes to an individual’s ability to process information and even contribute to behavior. In the future, knowing more about human genomics may help the health care provider to not only tailor the treatment plan but also know the best way to present this information based on genetic information about the individual. Knowing this information would assist the patient or caregiver in making informed decisions and utilize a tailored treatment plan that maximizes healthy outcomes. Nurse scientists and practitioners are in a unique position as health care providers, health educators, and researchers to contribute to the vast arena of genomics and health.

Conclusion

Outcomes of this study identified population differences between the eligible participant and eligible non-participant groups most likely related to the number of questionnaires completed over the history of the TESAOD studies and increased likelihood for participants to engage in research to keep abreast of their health or disease status. Statistically significant associations were found between the SNP rs321006 and atopy status among female Non-Hispanic white adults and further investigation is needed to confirm the genetic model.
Statistically significant associations were found between the SNP rs321073 and atopic asthma status among female Non-Hispanic white adults and appears to support a recessive genetic model. Large genetic effect was identified in the rs321073 recessive model (i.e., greater odds those with atopic asthma have the CC genotype). There appears to be a protective effect against atopy with the rs321006 TT genotype among Non-Hispanic white females.

Considerations for interpretation of results included controls for power / sample size, misclassification, and selection bias; however, it is possible there may be a small survivor bias in this study. Methodological errors were controlled by strict adherence to specimen processing, DNA amplification, and genotyping. Sequenom genotyping reduced the potential for error due to human handling of specimens and call in the genotyping process. In data analysis, type 1 error was controlled by FDR.

Implications for nurses, clinicians, and scientists include a better understanding of associations of these genetic variations with asthma, atopy, and atopic asthma that might generate further inquiry into other mechanisms in the pathway toward atopic asthma. Personalized medicine might afford patients with appropriate treatment based on their genotype. This study adds to the body of knowledge of genes associated with disease and helps build a platform based on which we might explore genetic assays that can be performed to identify asthma phenotype and perhaps one day help with appropriate and tailored treatment options. Future research might explore these genes of interest with current and future leukotriene inhibitors used to treat atopy and atopic asthma. Suggested future research for atopic asthma includes epigenetics, gene by gene interactions, and gene by environment interactions.
APPENDIX A

ANNOTATED BIBLIOGRAPHY OF STUDIES DESCRIBING GENETIC POLYMORPHISMS ASSOCIATED WITH ATOPY AND ASTHMA
<table>
<thead>
<tr>
<th>First author (year)</th>
<th>Sample ethnicity and race</th>
<th>Sample size</th>
<th>Rs used or snp name</th>
<th>Brief finding (positive or negative association)</th>
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<tbody>
<tr>
<td>Arriba-Mendez, et al. (2006)</td>
<td>Spanish</td>
<td>166 participants (79 adult controls, 87 children with asthma)</td>
<td>rs320995 (CYSLTR1)</td>
<td>C allele more common among males with allergic asthma (AA) and atopic dermatitis (AD). When comparing AA-AD vs. AA- no AD, significant differences observed (47% vs. 15%). No differences in this allele distribution were observed between these disease sub-groups and females. Although IgE levels were higher in asthmatics than controls there was no report of association of IgE level and genetic variation.</td>
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<tr>
<td>Lima, et al. (2006)</td>
<td>69% Caucasian, 26% African American</td>
<td>252 participants</td>
<td>rs2412222 (G&gt;A) rs320995F_F (A&gt;G) rs321029 (G&gt;A) rs321092 (A&gt;G)</td>
<td>Leukotriene pathway candidate genes in asthma were typed successfully (CYSLTR1). Minor allele frequency for Whites vs. African Americans respectively: rs2412222 (G&gt;A) 0.249 vs. 0.401 rs320995F_F (A&gt;G) 0.261 vs. 0.422 rs321029 (G&gt;A) 0.280 vs. 0.013 rs321092 (A&gt;G) 0.284 vs. 0.065 There was a table and discussion of the other genes (ALOX5, LTA4H, and LTC4S) in the article related to associations of polymorphisms of these and asthma exacerbation but no mention of the CYSLTR1 gene. Suspect that there was no significant finding to report.</td>
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<tr>
<td>First author (year)</td>
<td>Sample ethnicity and race</td>
<td>Sample size</td>
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<td>Hong, et al. (2009)</td>
<td>Chinese</td>
<td>Two independent samples, including 170 asthmatic cases and 347 controls in the initial sample, and 202 asthmatic cases and 332 controls in the confirmation sample, were recruited from the same region of China.</td>
<td>rs320995 (CYSLTR1)</td>
<td>Subjects without the thymidine allele in SNP rs320995 had a 3.1 times higher risk of asthma, which remained significant after accounting for multiple testing. This association was replicated in the confirmation sample and validated by meta-analysis. Further, sex-specific analysis was performed, but no sex difference was found. The present study provided coherent evidence that CYSLTR1 gene variation is associated with risk of asthma.</td>
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<tr>
<td>Thompson, et al. (2007)</td>
<td>Tristan da Cunha</td>
<td>112 participants (54 asthmatics and 58 nonasthmatics)</td>
<td>899G&gt;A (CYSLTR1)</td>
<td>The CysLT1 300S variant was more common in the atopics and asthmatics from Tristan da Cunha. However, women in this population were statistically more likely to carry the 300S variant. The odds ratio for atopy in a 300S carrier was more than six-fold higher than in participants with only the wild-type B300 allele.</td>
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<td>Hao, et al. (2006)</td>
<td>UK (affected sib-pair families)</td>
<td>341 sib-pair families</td>
<td>rs320995, 617T/G, 898G/A (CYSLTR1)</td>
<td>Family-based association tests showed that the 927 T allele (in the coding region) was associated with atopy severity, especially in female subjects, but not with asthma diagnosis or severity, atopic status, bronchial hyper-responsiveness to methacholine or forced expiratory volume in 1second. The frequency of polymorphism of 617T/G and 898G/A were shown to be negligible in this UK Caucasian population. SNP 927T/C not significantly associated with total IgE.</td>
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<td>Choi, et al. (2004)</td>
<td>Korean</td>
<td>93 aspirin-intolerant asthma (AIA) patients, 181 patients with aspirin-tolerant asthma (ATA), and 123 normal healthy controls (NC).</td>
<td>rs320995 (CYSLTR1)</td>
<td>No significant association between 927T/C polymorphism and AIA development in this Korean population. (In this study, individuals were not distributed by sex.)</td>
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<td>First author (year)</td>
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<td>Sample size</td>
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<tr>
<td>Kim, et al. (2008)</td>
<td>South Korean</td>
<td>94 with aspirin induced asthma and 152 with aspirin tolerant asthma</td>
<td>-634C&gt;T (CYSLTR1)</td>
<td>-634C&gt;T polymorphism has been indentified as significant genetic risk factor for developing aspirin induced asthma</td>
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<tr>
<td>Kim &amp; Ye, et al. (2007)</td>
<td>Korean</td>
<td>89 patients with aspirin intolerant asthma.</td>
<td>-634C&gt;T (CYSLTR1)</td>
<td>Patients with the -634C&gt;T (CT or TT) polymorphism were more likely associated with leukotriene receptor antagonist requirements for asthma control than the common genotype (CC).</td>
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<tr>
<td>Lee, et al. (2007)</td>
<td>Korean</td>
<td>100 children with exercise-induced bronchoconstriction (EIB) (68 were classified as responders to montelukast and 32 were classified as non-responders.)</td>
<td>rs320995 (CYSLTR1)</td>
<td>No significant association was observed between montelukast responsiveness and LTC4S [A(-444)C] or CysLTR1 [T(+927)C] genotype, either alone or in combination. However, bronchial hyper responsiveness and total IgE appear to predict the degree of montelukast responsiveness in Korean asthmatic children with EIB. No report of association of CysLTR1 [T(+927)C] SNP and IgE levels or eosinophils in this study even though measured. Authors point out the poor power values (power &lt;0.8) in this study and suggest more than 1000 study subjects because the difference in allele frequency is not prominent. Authors suggest further studies in larger cohorts of EIB patients with montelukast and other linked polymorphisms with these SNPs.</td>
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<tr>
<td>Kim &amp; Ye, et al. (2006)</td>
<td>Korean</td>
<td>95 aspirin-intolerant asthma (AIA) patients and 101 aspirin-induced urticaria/angioedema (AIU)</td>
<td>-634C&gt;T (CYSLTR1)</td>
<td>The frequency of the -634C allele was higher in the aspirin-induced asthma population.</td>
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<tr>
<td>First author (year)</td>
<td>Sample ethnicity and race</td>
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<tr>
<td>Kim &amp; Yang, et al. (2007)</td>
<td>Korean</td>
<td>159 aspirin-intolerant asthma (AIA) patients and 116 aspirin-induced chronic urticaria/angioedema (AICU)</td>
<td>-634C&gt;T (CYSLTR1)</td>
<td>Significant association of -634C&gt;T polymorphism with aspirin-induced asthma. The frequency of the CT or TT genotype was significantly higher in the AIA group than in the AICU group. Results indicate that the CysLTR1 -634C&gt;T polymorphism aggravates asthmatic symptoms after aspirin/NSAID exposure by increasing the expression level of CYSLTR1 in the asthmatic airways of AIA patients, whereas this SNP does not appear to be involved in AICU.</td>
</tr>
<tr>
<td>Kim &amp; Oh, et al. (2006)</td>
<td>Korean</td>
<td>105 aspirin-intolerant asthma (AIA) patients, 110 patients with aspirin-tolerant asthma (ATA), and 125 normal healthy controls (NC).</td>
<td>-634C&gt;T, -475A&gt;C, -336A&gt;G (CYSLTR1)</td>
<td>Male AIA patients had significantly higher frequencies of the minor alleles (T,C,G) of the CysLTR1 promoter SNPs than male control participants. There was no significant differences in allele and genotype frequencies among these three groups within female participants. These three promoter SNPs in an aspirin-intolerance asthma population was also associated with increased CysLT1 expression in vitro.</td>
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<td>First author (year)</td>
<td>Sample ethnicity and race</td>
<td>Sample size</td>
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<tr>
<td>Zhang, et al. (2006)</td>
<td>Tsukuba City, Japan for probands of families with asthma and Matsukawa, Japan, for probands of families with allergic rhinitis.</td>
<td>137 families (466 members) with mite sensitive asthma and 48 families (188 individuals) with allergic rhinitis.</td>
<td>434T/C, 275C/A, 136G/A, rs320995 (CYSLTR1)</td>
<td>Identified four polymorphisms (c.-618-434T/C, c.-618-275C/A, c.-618-136G/A, and 927C/T), and transmission disequilibrium tests revealed that none of these polymorphisms were associated with the development of asthma/rhinitis. However, the TCG and CAA haplotypes in the promoter region caused different transcriptional activity. Authors suggest that CYSLTR1 polymorphisms are not likely to be involved in the development of asthma/rhinitis, but it is possible that these polymorphisms could influence drug responses in individuals with atopic diseases.</td>
</tr>
<tr>
<td>Sanz, et al. (2006)</td>
<td>Spanish population (Spain)</td>
<td>208 individuals (130 asthmatic subjects and 78 controls)</td>
<td>rs320995 (CYSLTR1)</td>
<td>In the group of male patients, the C allele of 927T&gt;C CYSLTR1 was more common among patients with asthma than controls. The combination of 927T CYSLTR1 and -444A LTC4S was less common in male patients with asthma than in controls (Fisher's p-value = 0.04; Monte Carlo p-value (after 104 simulations) = 0.04 and the combination of 927C CYSLTR1 and -444A LTC4S was slightly more frequent in patients with asthma. No differences were observed in the female group. 5’-AAATCATGTTTTGGTTGCG-3’ and 5’-ATTTTCATGTTTTGACTG-3’ (for 927T&gt;C CYSLTR1)</td>
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<tr>
<td>First author (year)</td>
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<td>Thompson, et al. (2003)</td>
<td>Tristan da Cunha</td>
<td>604 A/G (CYSLTR2)</td>
<td>CysLT2 receptor variant was associated with atopy (21%) among a Tristan da Cunha population compared with those who were non-atopic (7%) (Fisher's exact test, p = 0.0016) in a manner that was independent of asthma (two-way ANOVA, p = 0.0015). This variant was activated with four-fold less potency by leukotriene D4 (LTD4) in a calcium flux assay. The CysLT2 receptor partial agonist, BAY 9773, also showed four-fold lower potency with this variant.</td>
<td></td>
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<tr>
<td>Fukai, et al. (2004)</td>
<td>Japanese</td>
<td>137 families</td>
<td>-1220A &gt; C (CYSLTR2)</td>
<td>A transmission disequilibrium test revealed that the -1220A &gt; C polymorphism is associated with the development of asthma (p = 0.0066).</td>
</tr>
<tr>
<td>Pillai, et al. (2004)</td>
<td>Mixed</td>
<td>Primary study set included 268 families from Denmark and 91 families from Minnesota (1336 samples total). Replication set included 384 families (total 1632 samples) from 10 collection centers (Families included: Aberdeen, UK, 77; North Carolina, US, 30; Leichester, UK, 35; Oslo, Norway, 65; Perth, Australia, 36; Sheffield, UK, 37; Stoke-on-Trent, UK, 51; and Thessaloniki, Greece, 53)</td>
<td>601A&gt;G, rs3803187, rs912278 (CYSLTR2)</td>
<td>A significant association of the coding polymorphism, 601A&gt;G, with asthma was observed (p = 0.003). Haplotype analysis using 2 marker and 3 marker combinations (rs3803187, rs912278) did not reveal lower p-values compared to the 601A&gt;G single marker analysis. The G allele was significantly under transmitted to asthmatics suggesting that this allele is protective against asthma.</td>
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<tr>
<td>First author (year)</td>
<td>Sample ethnicity and race</td>
<td>Sample size</td>
<td>rs used or snp name</td>
<td>Brief finding (positive or negative association)</td>
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<tr>
<td>Kim, et al. (2008)</td>
<td>South Korean</td>
<td>94 patients with aspirin induced asthma and 152 with aspirin tolerant asthma</td>
<td>2079 C&gt;T and 2534 A&gt;G (CYSLTR2)</td>
<td>No significant association with these polymorphism and risk factor for developing aspirin induced asthma.</td>
</tr>
<tr>
<td>Klotsman, et al. (2007)</td>
<td>Mixed race</td>
<td>174 participants (81 male, 93 female) however some were missing data and only 166 patients were analyzed</td>
<td>rs912277, rs912278 (CYSLTR2)</td>
<td>No significant association for baseline predicted FEV1 and baseline morning peak expiratory flow. Results suggest that variant CYSLTR2 polymorphisms may enhance response to montelukast (leukotriene receptor antagonist) indicated with increase in peak expiratory flow.</td>
</tr>
<tr>
<td>Park, et al. (2005)</td>
<td>Korean</td>
<td>642 participants (490 asthmatics)</td>
<td>c.-819T&gt;G, c.2078C&gt;T, c.2534A&gt;G, c.c.2545+297A&gt;G (CYSLTR2)</td>
<td>Patients with rare alleles c.-819T&gt;G, c.2078C&gt;T, c.2534A&gt;G, were higher in those with AIA than in those with ATA.</td>
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APPENDIX B

SKIN PRICK TEST
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APPENDIX C

IRB APPROVAL
**HiSPP Correspondence Form**

**Date:** 10/23/09  
**Investigator:** Kea Wysocki, MS  
**Advisor:** Leslie Ritter, PhD, RN and Stefano Guerra, PhD, MD  
**Project No/Title:** 09-0566-01 Leukotriene receptor Gene Variation and Atopic Asthma  
**Current Period of Approval:** 10/13/09 - 10/12/10

---

### IRB Committee Information

- **IRB1** – IRB0000291  
- **FWA Number:** FWA00064218  
- **Full Committee Review**  
  1st review – 10/13/09

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### Nature of Submission

- **New Project**

| Documents Reviewed Concurrently | Approved
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<td>VOIF (received 06/05/09)</td>
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<tr>
<td>Notice of Research Fellowship Award from the National Institute of Nursing Research</td>
<td>Ack</td>
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<tr>
<td>Other (define): Background Materials</td>
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<tr>
<td>Copy of Letter from Dr. Guerra authorizing the use of data</td>
<td>Appr</td>
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<tr>
<td>Copy of Dr. Guerra Continuing Review showing current approval of referenced project</td>
<td>Ack</td>
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<tr>
<td>Copy of Dr. Guerra Project Approval Letter</td>
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<td>Copy of Dr. Guerra Project Approval Form</td>
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<tr>
<td>Copies of Dr. Guerra Consents showing subject notification of the possibility of this research</td>
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<tr>
<td>Copy of Dr. Guerra Questionnaire showing data collection points</td>
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---

### Committee/Chair Determination

- **Approved** as submitted effective 10/13/09

---

### Additional Determination(s)

**Expeditied Continuing Review (45 CFR 46.110 Category 9):** Continuing review of research, not conducted under an investigational new drug application or investigational device exemption where categories (2) through (8) do not apply but the IRB1 Committee has determined and documented at the 10/13/09 convened meeting that the research involves no greater than minimal risk and no additional risks have been identified.

---

**David G. Johnson, MD**  
Chair, IRB1 Committee  
UA Institutional Review Board  
DG3/88J

**10/23/09**

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**Reminders:** Continuing Review materials should be submitted 30-45 days prior to the expiration date to obtain project re-approval.  
- Projects may be concluded or withdrawn at any time using the forms available at [www.irb.arizona.edu](http://www.irb.arizona.edu).  
- No changes to a project may be made prior to IRB approval except to eliminate apparent immediate hazard to subjects.  
- Original signed consent forms must be stored in the designated departmental location determined by the Department Head.
REFERENCES


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