A Genome-Wide Association Study of Intraocular Pressure in Latinos

BY

Drew Nannini
BS, University of Illinois at Urbana-Champaign, 2011
MA, Kansas City University of Medicine and Biosciences, 2012

THESIS

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Defense Committee:

Xiaoyi Gao, Cochair
Supriya Mehta, Cochair
Leslie Stayner

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALD	Adrenoleukodystrophy
CCT	Central Corneal Thickness
DNA	Deoxyribonucleic Acid
GWAS	Genome-Wide Association Study
HPFS	Health Professionals Follow-Up Study
IOP	Intraocular Pressure
LALES	Los Angeles Latino Eye Study
LD	Linkage Disequilibrium
MAGGS	Mexican American Glaucoma Genetic Study
Melbourne VIP	Melbourne Visual Impairment Project
miRNA	Micro Ribonucleic Acid
mmHg	Millimeter of Mercury
mRNA	Mature Ribonucleic Acid
µm	Micrometer
MYOC	Myocilin
NHS	Nurses' Health Study
NTG	Normal Tension Glaucoma
OPTN	Optineurin
PACG	Primary Angle Closure Glaucoma
PCA	Principal Component Analysis
POAG	Primary Open Angle Glaucoma
RNA	Ribonucleic Acid

LIST OF ABBREVIATIONS (continued)

SNP	Single Nucleotide Polymorphism

SUMMARY

A genome-wide association study of intraocular pressure (IOP) was conducted utilizing a sample of Latinos. Data were analyzed from 3,374 subjects from the Los Angeles Latino Eye Study (LALES) and the Mexican American Glaucoma Genetic Study (MAGGS). Subjects underwent a detailed ophthalmological examination. The average IOP measurement for both eyes was modeled using linear regression, controlling for age, gender, and principal components of genetic ancestry.

The overall mean (standard deviation) age for the sample was 56.95 (10.12) years. The sample consisted of unrelated individuals, 1,918 females and 1,372 males, 58.30% and 41.70%, respectively. The average IOP for both eyes was 14.56 (2.70) millimeters of mercury (mmHg), ranging from 6.92 mmHg to 26.00 mmHg, after excluding outliers. Males and females had an average IOP of 14.34 mmHg and 14.73 mmHg, respectively.

Results from the analysis yielded five suggestive single nucleotide polymorphisms (SNPs) that were associated with IOP. The top SNP associated with IOP was located at 21q21.2 (P=8.80×10^{-8}) on chromosome 21, between *TUBAP* and *VN2R20P*. This SNP was associated with a 1.33 mmHg increase in IOP for each effect allele. The second most significant SNP was rs12591689 (P=5.23×10^{-6}), located on chromosome 15 in *PCSK6*. This SNP was associated with a 0.31 mmHg increase in IOP per effect allele.

This study represents the first genome-wide association study (GWAS) of IOP in Latinos, and both replicated previously reported and identified new genetic variants associated with IOP. Further replication studies are needed, and implications for these genetic variants include screening and identifying individuals who harbor these polymorphisms that increase IOP.

I. INTRODUCTION

A. Background

Glaucoma is a prominent cause of irreversible blindness, and the second leading cause of overall blindness worldwide (H. A. Quigley, 1996). Glaucoma refers to disorders that lead to optic neuropathy, or damage to the optic nerve, and if left untreated, can result in vision loss or blindness. Of the several types of glaucoma, primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG) are the two most prevalent, with POAG being the most prevalent form. Both POAG and PACG currently affect approximately 70 million people worldwide (Pan & Varma, 2011). Estimates suggest that by 2020, a total of 79.6 million people will be affected by these types of glaucoma, with 58.6 million being affected by POAG (H. A. Quigley & Broman, 2006).

Given that the number of affected individuals is expected to increase over the next several years, as well as an increase in the aging population, glaucoma is becoming a significant public health issue. Commonly referred to as "the sneak thief of sight" (Glaucoma Research Foundation), glaucoma does not have any overt, early-onset symptoms during the course of the disease. By the time individuals experience visual field loss in either or both eyes, the disease may have progressed into its advanced stages, resulting in irreversible damage to the eye or eyes. Despite the possibility of irreversible vision loss or blindness, if glaucoma is diagnosed early in the disease process, through regular eye examinations, proper treatment may delay the disease progression. There is no known current cure for glaucoma; therefore, understanding the risk factors associated with glaucoma will help to identify those individuals who are predisposed to developing the

disease, will allow for targeted interventions to prevent the progression, and ultimately, may contribute toward the identification of a cure.

The purpose of this thesis is to identify genetic variants associated with IOP, a major risk factor of POAG, in a sample of Latinos from LALES and GWAS genotypes from both LALES and MAGGS. What follows is a brief history of early genetics, describing the methods employed to study the genetics of Mendelian diseases, as well as a discussion on modern genetics, including a brief overview of the advancements in genetics, and how they have affected the way we understand genetic mutations and disease development. The second chapter describes the basics of glaucoma, seminal POAG studies, and risk factors associated with POAG and IOP. A review of the genetic literature of POAG and IOP, a brief synopsis of the Latino population, and the effect of POAG on public health are additionally included in this chapter. The third chapter describes the main methodology of this thesis. The fourth chapter reports the descriptive and genotype analysis from the GWAS. The fifth chapter is a discussion of the study's findings, the strengths and weaknesses of the study, and future implications.

B. **A Brief History of Early Genetics**

Scientists have known for decades that many diseases have a genetic component. Gregor Mendel, who is considered to be the father of modern genetics, discovered the rules of heredity by studying generations of pea plants. Using such phenotypes as flower color, stalk height, and flower shape, he established the "Law of Segregation" and the "Law of Independent Assortment" (Griffiths, Wessler, Lewontin, & Carroll, 2007). These laws describe the nature of alleles, which are alternative forms of the same gene. The Law of

Segregation states that for any given trait, a pair of alleles split from each other during gamete formation, and there is a 50% chance of receiving either allele. Mendel's second law, the Law of Independent Assortment, asserts that alleles of different genes separate independently from each other during the formation of gametes. These basic laws provided the foundation for scientists to determine the patterns of heritance for a given trait, and allowed for the ability to identify which genotypes were associated with a given phenotype.

Initially, genetic research was conducted to determine the heredity of certain diseases in human populations utilizing study designs such as familial aggregation, segregation analysis, and linkage analysis. Familial aggregation studies, identifying an individual's risk of disease based upon whether other members of the family have the disease, have shown that Alzheimer's disease, schizophrenia, and Parkinson's disease are highly heritable within families where these diseases exist (Autere, Moilanen, Myllyla, & Majamaa, 2000; Devi et al., 2000; Lichtenstein et al., 2006). Confirmed familial aggregation studies are usually followed by segregation analysis identifying a Mendelian inheritance pattern; e.g., autosomal dominant or autosomal recessive. Through segregation analysis studies, cystic fibrosis and sickle cell disease have been classified as autosomal recessive disorders, and Huntington's disease as an autosomal dominant disorder (Ashley-Koch, Yang, & Olney, 2000; Romeo et al., 1985; Walker, 2007).

While familial aggregation and segregation studies have been successful in verifying a possible genetic component to a disorder and determining inheritance patterns for Mendelian-like diseases, these methods are mainly limited to single-gene disorders within pedigrees or families with phenotypic data for multiple generations. Even though the genetic variants associated with these diseases have large effect sizes, significantly

contributing to the pathogenesis of these diseases, most of the single-gene disorders studied with these two methods are considered to be rare, with disease-causing allele frequencies found in less than 1% in the population (Hirschhorn, 2005). Furthermore, these studies operate under the assumptions that genes are inherited independently of each other, and that a single gene is responsible for the development of the disease, which does not hold true for all diseases.

Mendel's laws, while correct, were simplistic and explained only a small portion of genetic inheritance. Scientists began to discover that there were multiple means in which genes were inherited. In addition to the classic Mendelian patterns, it was found that genes located on the same chromosome were often inherited together, whereas genes that are located on separate chromosomes are inherited independently (Shorvon, Perucca, & Jr., 2009). In addition, during meiosis, the process of recombination occurs to introduce genetic variability. Recombination is the exchanging of segments of DNA between two sister DNA strands, resulting in a new genomic sequence and a new combination of alleles. As the likelihood of recombination events within a region of a chromosome increases, the likelihood that the genetic linkage of two physically close genes will be disrupted also increases (Neiman & Linksvayer, 2006).

Familial aggregation and segregation analysis methods have offered particular insight into genetic disorders and their heritability, but they cannot pinpoint the location of the disease-causing variant. This can be accomplished through genetic linkage studies. Genetic linkage occurs when two genes are located close to each other on the same chromosome and the rate of recombination between the two loci is less than 50% (Khoury, Little, & Burke, 2004). Linkage analysis takes advantage of genetic linkage by using the

location of a known genetic marker to identify the genomic location of a disease-causing gene. For example, linkage analysis studies have identified a locus on chromosome 12 for Ehlers-Danlos syndrome, a locus on chromosome 18 for bipolar disorder, and a locus on chromosome 13 for breast cancer, notoriously known as BRCA2 (Rahman et al., 2003; Stine et al., 1995; Wooster et al., 1995).

Linkage analysis studies have been successful in mapping risk loci for Mendelian traits and disorders, but similar to familial aggregation and segregation studies, this methodology has its limitations. While previous studies have used linkage analysis to identify genes and genetic variants that affect rare phenotypes that follow Mendelian inheritance patterns, this methodology has had less success in mapping genetic variants that are associated with common diseases, such as type-2 diabetes mellitus, rheumatoid arthritis, and cardiovascular disease (Visscher, Brown, McCarthy, & Yang, 2012). The main reason linkage analysis studies have been unable to reliably map such variants is due to the variants' effect sizes in relation to disease status. Linkage analysis studies have proven to be successful at identifying variants with large effect sizes, but they are much less powerful in their ability to detect common variants, occurring in greater than 1% of the population, with modest-to-small effects sizes, an effect size of 1.5 (Hirschhorn & Daly, 2005). As seen in Figure 1, adapted from Manolio et al., Mendelian diseases have large effect sizes but are caused by rare variants, whereas common variants are smaller effect sizes but are more common (Manolio et al., 2009). With most interest in identifying genetic associations with diseases falling in between the dotted diagonal lines, segregation and linkage methods are used to assess variants with large effect sizes, but are unable to evaluate common variants; thus, another technique is required to measure these variants.

These previous methods of evaluating disease-causing genes and disease-predisposing variants have been applied to diseases with a single genomic cause with great success. Despite these achievements, these types of diseases only account for a small fraction of illnesses experienced by the human population. In the early 1900s, scientists began to witness that some traits did not follow patterns of inheritance in families according to Mendelian laws. Opponents of the strict Mendelian's laws, then called biometricians, believed that for some traits, individual differences were not attributable to a single locus of a chromosome. Rather, numerous different genes throughout the genome, with allelic variations, contribute to the overall occurrence and variation of a trait, with no single gene contributing a large effect (Risch, 2000).

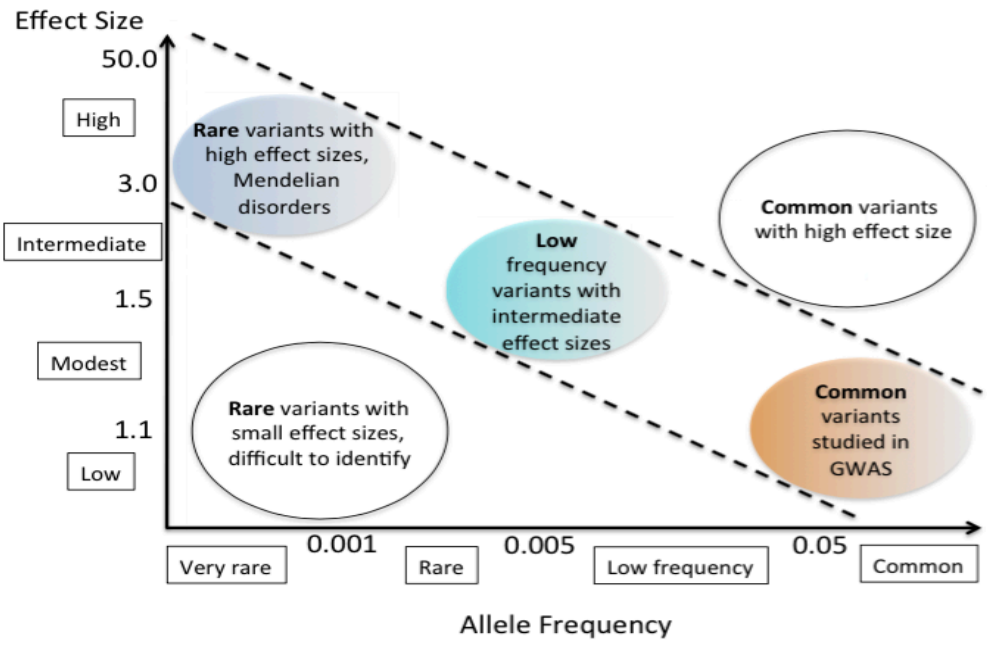

Figure 1. Frequency and effect size of genetic alleles.

The debate between the Mendelians and the biometricians continued until 1918, when Ronald A. Fisher published a paper attempting to settle the dispute. In the paper, Fisher argued that multiple genes, while following Mendel's laws, could work together to influence the expression of a phenotype (Fisher, 1919). That is to say that a particular phenotype of an individual is determined by the sum of numerous loci. While not absolute for all traits and diseases, this new concept did not devalue Mendel's theory, but rather sought to further explain disease occurrence in the absence of a single disease-causing gene. This concept of the additive effect of alleles contributing to phenotypes has since been the focus of research for diseases that are believed to be caused by a combination of alleles.

The landmark paper by Fisher founded the field now known in modern genetics as quantitative genetics. Instead of phenotypes that fall into discrete phenotypic classes, such as blood type, quantitative genetics studies phenotypes that vary continuously, such as height. This paradigm shift in the understanding of human phenotypes, one from single-gene traits with large effect sizes to one with polygenic characteristics with small effect sizes, engendered a new discipline of science. Guided by the understanding that several genes may contribute to certain phenotypes, researchers began to move away from simple Mendelian disorders and began to explore diseases that may have multiple genetic and environmental components.

Complex diseases are multifactorial in origin, caused by a combination of genetic, environmental, and lifestyle factors (Craig, 2008). These diseases usually have multiple, and often, an unknown number of genes contributing to the development of the disease. As such, these diseases do not follow the classic single-gene dominant or recessive Mendelian

inheritance patterns that familial aggregation and linkage analysis studies are typically used for. Compared to Mendelian diseases, where researchers studied these traits by examining the phenotype-to-genotype relationship, studying complex diseases in a similar matter proved to be difficult. These traditional methods of genetic research, when applied to complex diseases, were unable to identify the small effect sizes of genes. Rather than attempting to link the phenotype to the genotype, it was proposed that methods to link the genotype to the phenotype would elucidate the genetic architecture of complex diseases.

The concepts and knowledge put forth by Mendel and Fisher shifted the genetic landscape in terms of how genes are inherited and cause disease, and has shaped the way in which researchers view and study genes over the past century. At the time of Fisher's paper, researchers were limited in their ability to study diseases caused by several genes contributing small effect sizes due to the lack of knowledge and technology to assess the genetic architecture underlying these complex diseases.

C. **Modern Genetics**

The ability to study complex diseases required methods that were different from the methods used to study Mendelian diseases—researchers needed to be able to go beyond single-gene effect sizes. Over the past several decades, advancements in technology have enabled scientists to explore the human genome in new ways to determine the structure of the human deoxyribonucleic acid (DNA), and ultimately, the nucleotide sequence to identify and map genes throughout the genome. These steps have allowed scientists to discover genetic variation that has aided in the diagnosis, prevention, and management of complex diseases.

In 1953, two scientists working at the University of Cambridge put forth a new structure of DNA that would forever change the field of science. James Watson, an American research fellow, and Francis Crick, a British graduate student, proposed a new structure of DNA, consisting of two helical chains that coil around a single axis, contrary to an earlier proposed structure comprised of three intertwined chains (Pauling & Corey, 1953; Watson & Crick, 1953). The double-helix structure is formed by a sugar phosphate backbone made up of deoxyribose molecules and phosphate groups. Furthermore, four nitrogenous bases make up the center of the double helix: adenine (A), thymine (T), guanine (G), and cytosine (C). These four bases bond with one another—i.e., A bonds with T and G bonds with C—to join the two strands together. Consistent with Erwin Chargaff's rules, the ratio of pyrimidine (C and T) and purine (A and G) should be in a ratio of one to one. In other words, the number of A and T (and C and G) nucleotides should equal one another with each conjoined pair called a base pair (Chargaff, Lipshitz, & Green, 1952). Additionally, the combination of a single nitrogenous base, a deoxyribose molecule, and a phosphate group is called a nucleotide, which is the main building block of DNA.

When a long strand of DNA is generated and becomes coiled, a chromosome is formed. Humans have 23 pairs of chromosomes, or 46 individual chromosomes. Chromosomes are made up of segments of DNA that contains genes, a locus of DNA that codes for a specific function. Other areas of DNA consist of non-coding regions, segments of DNA that do not code for a function, and regulator regions, parts of the genome that either upregulate or downregulate the amount of gene expression. When a gene is expressed, a copy of the base pair sequence is transcribed into ribonucleic acid (RNA), which is exported out of the nucleus and into the cytoplasm. In the cytoplasm, this RNA is translated by a

ribosome, a particle that converts the genetic code into an amino acid sequence that forms proteins, which in turn expresses a phenotype. With the basic understanding of how DNA drives protein creation, scientists became increasingly fascinated with discovering the sequence of the human genome, with the focus on identifying new genes and furthering our understanding of the origin of complex diseases.

With the completion of the Human Genome Project in 2003, scientists had successfully decoded nature's blueprint for human life, with the ultimate goal of better understanding the molecular processes that contribute to diseases. The process included breaking each of the 23 pairs of chromosomes into smaller segments of DNA, placing these segments into bacterium to replicate, ordering the fragments, and then sequencing these segments. The results from sequencing revealed that the human genome is more than 99.9% identical between any two individuals (Lander et al., 2001; Venter et al., 2001). Despite this high percentage, the human genome contains 3.3 billion base pairs and estimates have reported that the amount of genetic variation between two individuals is 0.1%. Thus, for every 1,000 base pairs, 1 base pair will be different (Sachidanandam et al., 2001). Any difference in the frequency of base pairs that occurs less than 1% in the population is considered to be a mutation or rare variant. Any difference that occurs in greater than or equal to 1% in the population, compared to the wild type or the most common allele, is called a polymorphism or common variant (Schork, Murray, Frazer, & Topol, 2009).

Since the completion of the Human Genome Project, polymorphisms have been studied as genetic markers to identify genes that could be related to disease susceptibility. The most common form of a polymorphism is a single-base pair change or single nucleotide

polymorphism, also known as a SNP. For example, if a segment of DNA is coded as "ATGCTA" for the wild type, a smaller subset of the population may have the code "ATGATA." The exchange of a C for an A is an example of a SNP. When the Human Genome Project was completed, 1.42 million SNPs were identified throughout the genome (Sachidanandam et al., 2001). More recently, through continued research, the number of SNPs has grown over the past decade to 3.1 million SNPs in 2007 and about 14.4 million SNPs in 2010, with millions of SNPs waiting to be validated (Genomes Project et al., 2010; International HapMap et al., 2007). With the number of SNPs continually growing, the ability to identify genetic variation that may be related to disease susceptibility increases as well.

While SNPs are abundant in the genome, the effect of a SNP on a phenotype depends highly on where in the genome it is located. The SNPs in the coding region of a gene (exons) or in the regulatory region of a gene (segments of DNA that control the expression of a gene) most often alter the expression, structure, or function of the encoded protein. These SNPs are first to be analyzed to determine whether or not they are associated with a phenotype, because these SNPs are more likely to have a direct effect on the phenotype. Most SNPs, however, are located in non-coding regions of the genome and typically do not directly affect any phenotype (Syvanen, 2001). Additionally, the type of SNP plays a role in the severity of its effect. Thus, SNPs can either be "synonymous," in which the change in DNA code does not result in a change in the amino acid sequence, or "non-synonymous," in which the SNP does change the sequence of the amino acid. Non-synonymous SNPs are further broken down into "missense" and "nonsense." In missense SNPs, the sequence codes for a completely different amino acid that was previously called for. Nonsense SNPs

result in a premature stop codon that terminates the peptide sequence. Non-synonymous SNPs can cause different variants of genes, known as alleles, with each allele producing a unique protein. Premature termination often results in a nonfunctional protein, which can also alter its biological function.

The phenotypes investigated with the premodern genetics methods often involved either nonsense or missense genetic variation that resulted in severe outcomes. These genetic variations are subject to evolutionary forces such as natural selection. Negative selection is one of the most common forms of natural selection that affects a majority of genes and their role in evolution. Through negative selection, the frequency of genetic variation that puts a population of organisms at a disadvantage decreases due to the effects on the survival of the organism (Vasseur & Quintana-Murci, 2013). However, the negative selection of genetic variation depends on the extent of the effect. That is, if a genetic variation is highly detrimental to the survival of an organism or is lethal, these deleterious variations are removed from the population almost immediately. By comparison, genetic variations that have subtle effects, such as alleles underlying complex diseases, are less likely to be removed by negative selection and thus, remain in a population for many generations (Hirschhorn & Daly, 2005). Complex diseases tend to consist of many of these modest-effect genetic variants. Because no one SNP controls disease development, as compared to Mendelian diseases, negative selection is less effective at removing these variants. Thus, these individual genetic variants continue to persist in human populations and contribute to disease susceptibility.

D. **Genome-Wide Association Studies**

Through GWASs, SNPs have been useful in examining disease susceptibility, as well as locating genes that increase the risk of disease. Genome wide association studies involve collecting DNA samples from cases (individuals with a particular phenotype or disease), and controls, (phenotype or disease free individuals). Typically, GWASs are conducted by genotyping SNPs that are evenly spaced throughout the entire genome. Allele frequencies between the two groups are compared to determine if any SNP is associated with the phenotype. That is, if a SNP has a higher frequency in cases than controls, that SNP is said to be associated with the phenotype. Even though a SNP was determined to be associated with the phenotype, it may not be the actual cause of the disease due to it being linked with surrounding SNPs. Genome wide association studies can also be utilized to examine quantitative traits, and are unbiased in regards to the genomic location of the causal locus because there is no prior assumption of the location of the variant. Additionally, GWASs do not assume any prior knowledge of the biological meaning of associated SNPs (Pescatello & Roth, 2011). Due to the unbiased nature of GWASs, as well as no prior biological knowledge, GWASs offer opportunities to detect novel variants throughout the genome.

In population genetics, significant associations found between a genetic loci and a disease arise from three circumstances (Lander & Schork, 1994). The first situation occurs when the genetic variant is actually the cause of the trait. The second situation arises when a genetic variant that does not cause the trait is in linkage disequilibrium (LD) with the causal variant. The third situation occurs from an artifact due to the population admixture, instances in which populations have a recent ancestry from two or more continents (Seldin, Pasaniuc, & Price, 2011). This last scenario is a form of confounding.

Linkage disequilibrium is the nonrandom association between alleles at different genetic loci (Khoury et al., 2004). Generally, parts of a genome are inherited together, such as genes on the same chromosome, and therefore, are nonrandomly associated with each other. Genetic loci that are close in physical distance to each other typically exhibit a higher pattern of LD than loci that are further apart or on different chromosomes. Patterns of LD are generated over time through evolutionary factors, such as genetic drift, mutations, and migration and are broken by recombination of the genome (Hartl & Clark, 1997). If a genotyped SNP is found to be associated with a phenotype and this SNP is in high LD with another SNP, which is actually the true causal variant, the detected SNP acts as a surrogate marker for the true causal variant. Figure 2, adopted from Hirschhorn et al., depicts an illustration of such direct and indirect associations (Hirschhorn & Daly, 2005). While researchers do not actually know if the detected SNP is a surrogate marker or the causal SNP, follow-up studies employing fine mapping are needed to further elucidate the true causal variant. Fine mapping is a method for SNP validation. Even though surrogate SNPs are not the causal SNP, they still provide insight. Those SNPs that are in high LD with other SNPs may be advantageous; that is the SNPs are physically close to one another and will be inherited together over many generations (Jorde, 2000). In this sense, depending on the research question, the identification of a surrogate marker instead of the true causal SNP may be equivalent. For example, scientists who wish to understand the underlying biology in the pathogenesis of a disease to develop therapeutic drugs may want to identify the causal SNP, whereas if the aim of research is to detect an individual's disease susceptibility, the true identity of the causal SNP may not be as crucial (Attia et al., 2009).

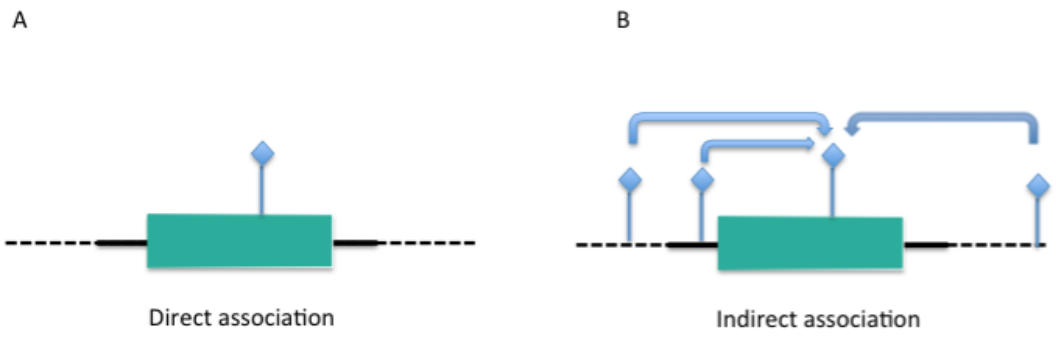

Figure 2. Indirect and direct SNP association.

Historically, populations of humans generally lived in small villages and remained isolated from other civilizations. Members from these societies often mated with individuals who were both physically close and genetically related to one another. Inbreeding reduces genetic diversity and increases the likelihood of being homozygous for deleterious recessive alleles, often resulting in higher risk of developing rare diseases (Charlesworth & Willis, 2009). Understanding the genetic structure of these social enclaves has allowed researchers to conclude that inbreeding increases detrimental rare variants in these populations while admixture and outbreeding reduces risk of such diseases (Rudan et al., 2006).

Admixed populations, such as African Americans, Mexican Americans, and Puerto Ricans, have recently been of genetic interest amongst researchers for several reasons. Within the past several centuries, the genetic composition of the Americas has drastically changed due to the colonization of North America by the Europeans, the succeeding African

slave trade, and the waves of migration from foreign countries before and after both world wars (Moreno-Estrada et al., 2013). This genetic reconstruction has brought together genomes from different continents, resulting in the creation of LD, different allele frequencies, and increased genetic diversity compared to the parental genomes.

Because GWASs often assume cases and controls are sampled from the same population, any differences in allele frequencies are related to the outcome rather than any background differences in the sample (L. Liu, Zhang, Liu, & Arendt, 2013). If this assumption does not hold true, false associations could be identified as a result of confounding. The genomes between individuals and populations are relatively similar with little genetic variation, but the prevalence of many diseases is significantly associated to the genetic ancestry of an individual (Freedman et al., 2006; Haffner, Hazuda, Mitchell, Patterson, & Stern, 1991). When cases and controls experience differences in allele frequencies and disease prevalence due to differing genetic ancestry, a phenomenon called population stratification has occurred. The systematic differences in allele frequencies and disease prevalence can lead to spurious associations that are due to population structure, rather than a true disease-associated locus. A classic study collected data on 4,920 Pima and Papago Indians to investigate type-2 diabetes and Gm genotypes (Knowler, Williams, Pettitt, & Steinberg, 1988). The researchers observed a negative association between the Gm genotypes and type-2 diabetes, suggesting that the absence of these genotypes is a risk factor for the disease. However, the Gm marker is less common and prevalence of diabetes is higher in individuals with higher American Indian ancestry compared to individuals with no American Indian ancestry. When stratified by American Indian ancestry, there was no longer an association between the Gm genotypes and type-2 diabetes. Because ancestry

was related to both allele frequency and the disease, ancestry introduced bias into the association.

The paper mentioned above illustrates that if population stratification is not properly corrected, bias and spurious associations will be introduced into the results. One method to correct for population stratification is to conduct principle component analysis (PCA), which aims to reduce the number of dimensions of the data while maximizing the amount of variability explained by the components (Price et al., 2006). Essentially, principal components are linear combinations of the SNPs that contain as much variation as possible between subjects. In the case of an admixed population, PCA reduces variation in ancestry to several principal components that explain most of the ancestral variability. By identifying the top principal components that explain most of the variation in a dataset, these components can be used as covariates in an association analysis to control for differences in allele frequencies across various ancestries. In addition to dimension reduction using PCA, cluster analysis can also control for population stratification by identifying homogenous subgroups that are similar to one another in a dataset.

Understanding the role of the human genome in disease risk and pathogenesis is critical for medical and public health officials. Through genetic research, primary prevention strategies for complex diseases include identifying individuals through screening for a genetic variant that is a known risk factor for a disease, so that behavioral modifications (e.g., exercise, diet, and smoking cessation) and preventive modifications (e.g., taking aspirin to reduce one's risk of a heart attack) may be recommended. Secondary prevention strategies include using a genetic biomarker to detect the disease early in the natural history of the disease, before any symptoms are experienced, so that treatment can

be provided earlier than normal. Tertiary prevention strategies include developing medications that target a specific gene or biological pathway in order to reduce complications of a disease, or to improve the likelihood of survival of an individual. One proposed method of tertiary prevention, while still in its infancy stage, is gene therapy, where mutated genes that cause a disease can be replaced with a wild-type gene. Researchers in Paris were successful in treating two 7-year-old boys with X-linked adrenoleukodystrophy (ALD), a severe brain demyelinating disease (Cartier et al., 2009). Briefly, ALD is caused by a deficiency in the ALD protein encoded by the *ABCD1* gene. Due to a lack of match for bone marrow donors, the researchers removed hematopoietic cells, genetically corrected ex-vivo the *ABCD1* gene, and reinserted the cells into the boys. Within a span of 30 months, the neural damage was halted. Even though the study was conducted in only two individuals, the success of this study illustrates the possibilities of gene therapy for multiple diseases in the future.

Where primary prevention attempts to raise awareness and educate individuals by identifying their genetic susceptibility to a disease in order to take appropriate behavioral and preventative modifications, secondary prevention aims to detect individuals who have the disease in order to receive targeted medical treatment. Tertiary prevention is in its beginning stage, but may lead to greater practice of gene therapy and personalized medication that will cater to an individual's genome, making the drug more efficient with fewer side effects.

While genetics is only part of an individual's risk for disease, attempting to elucidate the role of genetic risk factors on the occurrence of a disease is a critical part in understanding the mechanisms that drive disease manifestation. With the continuous

decryption of the architecture of the human genome, the information housed in the genetic code will provide insight into disease occurrence and the mechanisms in progression. The potential applications resulting from the understanding of population genetics provides medical and public health officials with knowledge to improve health and prevent disease throughout the world.

II. GLAUCOMA

A. Glaucoma Basics

Glaucoma is a group of eye disorders that are characterized by degenerative optic neuropathy of the retinal ganglion cells, resulting in visual field defects (Gemenetzi, Yang, & Lotery, 2012). The optic nerve is a bundle of nerve fibers located at the back of the eye, which transmits electrical impulses from the retina, through the optic disc, and to the brain to be processed into an image. At the center of the optic disc is a white depression called the optic cup. The amount of pressure inside the eye, or IOP, is a main risk factor for glaucoma. When the IOP becomes elevated, extra force is applied to the optic nerve, resulting in damage, and eventually the loss of function in the nerve fibers. As the optic cup deepens and widens, the nerve fibers within die, severing the transmission of electrical impulses from the retina to the brain, resulting in vision loss (Flammer, 2006).

The two most common forms of glaucoma are POAG and PACG, with the former occurring more frequently than the latter. Both forms are a result of inadequate drainage of the aqueous humor, a clear fluid that fills the area between the cornea and the iris. Briefly, the human eye is divided into three main fluid-filled compartments: the anterior chamber, located between the iris and the inner most layer of the cornea; the posterior chamber, situated behind the iris and in front of the lens; and the vitreous chamber, found between the lens and the retina (Hersh, Johnson, & Keating, 2008). The aqueous humor, produced by the ciliary body, flows through the pupil into the anterior chamber, where it is drained from the eye through the trabecular meshwork, located in the iridocorneal angle, eventually emptying into the bloodstream.

The aqueous humor is a critical component of the overall health of the eye, including providing nutrients to various ocular tissues, aiding ocular immune response to antigens, and exerting IOP to keep the shape of the eye spherical (To, Kong, Chan, Shahidullah, & Do, 2002). The trabecular meshwork provides some resistance in the outflow of aqueous humor in order to produce the necessary IOP for proper anatomical conditions to optimize ocular function. The balance of the continuous production of aqueous humor, along with the drainage from the eye via the trabecular meshwork, enables the IOP to remain at appropriate levels. An increase in the resistance or a complete blockage of this meshwork results in increased IOP, and may potentially lead to glaucoma. Typically, the IOP of a healthy eye with no form of glaucoma is between 10 mmHg and 21 mmHg.

B. **Primary Open Angle Glaucoma**

The most prevalent type of glaucoma in the United States, POAG affects 2% of adults over the age of 40 (Friedman et al., 2004; Harmon & Intrator, 2004). It is characterized as all of the following in at least one eye:

1. Evidence of glaucomatous optic nerve damage from either or both of the following:
    i. The appearance of the disc or retinal nerve fiber layers (e.g., thinning or notching of the disc rim, progressive change, nerve fiber layer defects)
    ii. The presence of characteristic abnormalities in the visual field (e.g., arcuate defect, nasal step, paracentral scotoma, generalized depression) in the absence of other causes or explanations
2. Adult onset

3. Open, normal-appearing anterior chamber angles
4. Absence of known other (e.g., secondary) causes of open angle glaucoma (Morrison & Pollack, 2003).

While IOP appears in many POAG causes, it is not considered to be a criterion for the clinical diagnosis because optic nerve damage can be present without elevated IOP. Furthermore, an IOP greater than 21 mmHg, can be observed without any optic nerve damage. This is a condition known as ocular hypertension. As such, IOP is one of the main risk factors associated with POAG, but does not have a role in defining the disease.

In order to diagnose individuals with POAG, ophthalmologists perform a comprehensive eye examination using multiple techniques (Becker, Shaffer, Stamper, Lieberman, & Drake, 2009). During a normal eye examination, applanation tonometry and ophthalmoscopy are procedures used to evaluate IOP and the optic nerve, respectively. After applying numbing drops to the eye, a tonometer is used to determine the force needed to flatten an area of the cornea 3.06 mm in diameter. The force needed to flatten the cornea is approximately equal in magnitude and opposite direction to the force inside of the eye, providing a measure of IOP. In addition, ophthalmologists examine the optic nerve head by dilating the pupil with eye drops and using an ophthalmoscope. By evaluating the optic nerve head, ophthalmologists are able to determine if there is damage to the nerve, and suggest other examinations to determine the possible cause of damage.

Ophthalmologists may also perform perimetry, gonioscopy, and pachymetry to further evaluate ocular health (Becker et al., 2009). In order to map the visual field, kinetic perimetry uses a variety of bright stimulus to test the visual boundaries of an individual. Abnormalities in the visual map detected by perimetry may suggest visual field loss due to

glaucoma. Gonioscopy is an examination of the anterior chamber angle of the eye, where the aqueous humor drains from the eye. After numbing the eye, mirrors are used to examine the angle to classify suspected glaucoma individuals into POAG or PACG. And lastly, pachymetry, or the measurement of the thickness of the cornea, central cornea thickness (CCT), may also be performed. Measurement of CCT plays a major role in the diagnosis of glaucoma for two reasons. The first is that IOP readings are affected by CCT; thinner CCTs underestimate the true IOP and thicker CCTs overestimate the true IOP. Secondly, thinner CCT is a significant risk factor in the development of glaucoma.

Because it takes years to develop, POAG is often asymptomatic until the late stages. The Early Manifest Glaucoma Trial aided to elucidate the natural history of POAG by randomizing individuals to treatment through the use of IOP-lowering eye drops and no treatment (Heijl, Bengtsson, Hyman, Leske, & Early Manifest Glaucoma Trial, 2009). After 48 months, 49% of controls had progressed into developing POAG, compared to only 30% of individuals who were receiving treatment (P=.004). In addition, this study illustrated differences in time to progression among the subjects. After six years of follow up, 68% of the control patients had shown glaucoma progression, with a median time to progression of 42.8 months (Heijl et al., 2009). Furthermore, individuals with high IOP, IOP ≥21 mmHg, had a median time to progression of 44.8 months, compared to 61.6 months for those individuals with normal IOP. These results demonstrate the variability in the time to progression for various types of glaucoma.

In addition to eye drops designed to lower IOP, different types of surgery are available to treat POAG, most notably laser trabeculoplasty and trabeculotomy (Becker et al., 2009; H. Quigley, 2011). Both surgical procedures are modification methods of the

trabecular meshwork to increase aqueous humor outflow. Laser trabeculoplasty uses laser beams to burn the meshwork tissue to increase the outflow through the trabecular meshwork. If laser trabeculoplasty is ineffective, trabeculotomy is recommended, in which part of the trabecular meshwork is removed. During the procedure, the conjunctiva and sclera are cut and folded back to reveal the trabecular meshwork. Tissue from the trabecular meshwork is removed, as well as tissue from the iris in order to prevent further blockage of the hole. The flaps are then sewn back and the newly formed hole allows the aqueous humor to bypass the trabecular meshwork and filter into the conjunctiva, where it is absorbed.

Even though routine eye examinations are recommended every two years, according to data collected from the National Health Interview Survey, only 35.2% of individuals have utilized eye care within the past year (D. J. Lee et al., 2009). Eye care utilization rate varied depending on the level of impairment, with individuals with no visual impairment having a utilization rate of 33.7%, 49.6% for some visual impairment, and 58.3% for severe visual impairment. The insidious nature of POAG makes screening for elevated IOP and damage to the optic nerve critical components in preventing the development of glaucoma. Furthermore, early diagnosis of visual field defects aids in preventing further blindness.

By increasing the overall eye care utilization rate, in combination with early treatment of elevated IOP, the progression of POAG can be slowed; in some cases, its development can be prevented. While both invasive and noninvasive treatments are available for those in the more advanced stages of POAG, early diagnosis and treatment are pivotal in preventing the pathogenesis of POAG that may result in irreversible blindness.

Understanding the risk factors of POAG, including identifying new risk factors, is of public health significance in order to reduce and prevent incident cases of POAG and to improve overall ocular health.

C. **Epidemiology of Primary Open Angle Glaucoma**

Several studies have examined the prevalence of POAG in different ethnic groups. Most notably, the seminal POAG studies are summarized in Table I. What follows is a brief description of each of the study's methodology sections, including the measurements taken, the means in which these measurements were evaluated, and the criteria used to diagnosis POAG.

The Baltimore Eye Study was a population-based prevalence survey conducted in Baltimore, Maryland, investigating glaucoma and other ocular disorders in individuals 40 years of age and older between January 1985 and November 1988 (Tielsch et al., 1991). A cluster sampling strategy was used to select 16 geographic clusters in Baltimore, with the clusters stratified by race. An initial interview was conducted for occupied residences within each cluster. Individuals who completed the enrollment interview were eligible for an ophthalmologic screening examination conducted at a neighborhood screening center. Subjects underwent several examinations, including visual acuity, applanation tonometry, stereoscopic fundus photography of the optic disc, and visual field testing using the Humphrey Field Analyzer. Subjects who had 17 or more visual field defects were defined as having potential field loss. These subjects were then evaluated using static and kinetic perimetry to further determine if the visual field loss was glaucomatous. The IOP was measured three times in each eye, and the median pressure reading was used to define

each eye, with the higher measurement of the two eyes as defining the IOP for an individual. Optic disc photographs were read by a single examiner to measure the vertical disc and cup size. Approximately 10% of all optic disc photographs were reviewed by another examiner and showed excellent agreement. In a small proportion of cases in which subjects were too ill or too insecure to leave their home, a modified screening examination was conducted, omitting gonioscopy and optic disc photography.

Subjects that met any predetermined criteria were referred for definitive ophthalmologic examination at an eye institute. The subjects received a comprehensive eye examination by one of four examiners, each of whom had a subspecialty in glaucoma, to arrive at a final diagnosis, based upon evidence of glaucomatous optic nerve damage and the presence of normal angles in the absence of other eye disorders. However, due to varying amounts of data available on different subjects, a second stage of final diagnosis included the principal investigator to review all data available for each of the potential cases deemed by the four examiners. The final classification, either as definite, probable, or uncertain-unknown was based upon glaucomatous optic nerve damage pursuant to the appearance of visual fields, optic disc and nerve fiber layer, whether the angle was open or closed, and whether the disease was primary or secondary. The IOP was not a criterion used for diagnosis.

The Barbados Eye Study was conducted in Bridgetown, Barbados, West Indies, between April 1988 and May 1992 (Leske, Connell, Schachat, & Hyman, 1994). A random sample of Barbadian-born citizens between the ages of 40 and 84 was selected based on national registration numbers by the Barbados Statistical Services Department.

Table I

DESCRIPTION OF SEMINAL POAG STUDIES

	Study						
	Baltimore Eye Study		Barbados Eye Study	Blue Mountains Eye Study	Visual Impairment Project	Proyecto VER	LALES
Study Type	Prevalence		Prevalence	Prevalence	Prevalence	Prevalence	Prevalence
Location	Baltimore, MD		West Indies	Australia	Australia	Arizona	Los Angeles, CA
Sample Size	5,308		4,631	3,654	3,265	4,774	6,142
Publishing Year	1991		1994	1996	1997	2001	2004
Gender, %							
Women	60.3		57.0	56.7	53.8	61.2	58
Men	39.7		43.0	43.3	46.2	38.8	42
Race	AA (45.1%)	NHW (54.9%)	Blacks (97.1%)	NHW	NHW	Latinos	Latinos
Prevalence	4.74	1.29	6.8[b]	2.4	1.7	1.96	4.74
Age-Specific Prevalence							
40–49	1.23	0.92	1.4[b]		0.1	0.50	1.32
50–59	4.05	0.41	4.1[b]	0.3[a]	0.6	0.59	2.92
60–69	5.51	0.88	6.7[b]	1.1	1.9	1.73	7.36
70–79	9.15	2.89	14.8[b]	4.2	5.2	5.66	14.72
≥ 80	11.26	2.16	23.2[b]	8.2	6.2	12.63	21.76
IOP (mmHg) mean ± SD							
Cases	21.48±6.46	24.15±5.23	27 ± 9	17.6±2.8[d]	17.2±6.5	18.5±8.7	17.1±4.7[e]
Controls	16.00±4.18	17.17±3.35	17 ± 2[c]	15.9±2.6[d]	14.2±2.7	15.6±3.2	14.5±3.1[e]

LALES = Los Angeles Latino Eye Study; AA = African American; NHW = Non-Hispanic White; IOP = Intraocular Pressure

[a] Blue Mountains Eye Study combined individuals with ages 40–59 into one group.

[b] Results were calculated using blacks (93.1%) and mixed race (4%) individuals.

[c] Excluded 34 persons with other types of glaucoma and 577 with IOP > 21 mmHg of the overall sample.

[d] Means calculated from incidence data from same study (Burdon et al., 2014).

[e] Estimates used n=5,927 (participants who completed clinical examination in clinic) (Kuzin et al., 2010).

Participants were contacted and asked to attend an eye clinic for an extensive standardized examination, including visual acuity, automated perimetry by a Humphrey Field Analyzer, three IOP measurements of each eye, anterior chamber depth measurement, and fundus photographs of the optic disc. Subjects with IOP greater than 21 mmHg in either eye, abnormal visual fields, poor visual acuity, family history of eye diseases, or inability to have fundus photographs taken were referred for a comprehensive ophthalmologic examination with repeated tonometry, perimetry, and gonioscopy. The same ophthalmologist examined all referred participants at the clinic. Two independent graders at the clinic classified the optic disc photographs and a third grader reviewed any discrepancies.

All data from the data collection center and the fundus photography reading center were sent to a coordinating center for independent glaucoma classification. Classification of definite POAG required at least two abnormal visual field tests by the automated perimetry on two or more occasions, and at least two signs of optic disc damage based on the fundus photographs, excluding those with narrow angles and irrelevant for IOP.

The Blue Mountains Eye Study was a population-based survey investigating prevalent eye diseases in a community outside of Sydney, Australia, between January 1992 and January 1994 (Mitchell, Smith, Attebo, & Healey, 1996). Using census data, all permanent noninstitutionalized residents born before January 1, 1943 were invited for a detailed eye examination at a local clinic. At the clinic, a detailed questionnaire and eye examination was conducted. The eye examination was conducted in two phases. In the first phase, visual field was tested using the Humphrey Visual Field Analyzer. Applanation tonometry and stereo disc photography were also conducted for both eyes. The second phase consisted of a subset of participants returning for additional visual field test,

gonioscopy, and tonometry. A single measurement was taken for tonometry, but was repeated for measurements that were unreliable. Vertical cup-disc ratios were measured, and damage to the optic nerve was assessed using the stereo optic photographs. One or two graders graded all photographs, with discrepancies being reviewed by the principle investigator.

Glaucoma suspects were asked to return for a second exam if they had a history of glaucoma, if the visual field had defects, or if the optic disc had signs suggesting glaucoma. A diagnosis of POAG was assigned if glaucomatous visual field loss was present, in combination with optic disc damage, and if there were no signs of a closed angle or secondary glaucoma. Two glaucoma specialists and two ophthalmologists conducting other surveys in Australia assessed the visual field printouts and disc photographs of cases where POAG was suspected. In order for a diagnosis of POAG, the consensus by both glaucoma specialists was needed.

The Melbourne Visual Impairment Project (Melbourne VIP) was a population-based prevalence study on ocular diseases (Wensor, McCarty, Stanislavsky, Livingston, & Taylor, 1998). Eligible participants were residents 40 years of age and older and were randomly selected from the 1986 census. Each participant was contacted for an initial interview and invited for an eye examination at a local screening center. The eye examination consisted mainly of visual acuity testing, visual field testing, IOP measurement, and a standardized clinical assessment.

Visual fields were measured using the Humphrey Field Analyzer. If the ophthalmologist suspected the results to contain a visual field defect, the test was repeated. Tonometry was used to measure IOP, and if the reading was greater than 21 mmHg, the

test was repeated. If the IOP was still greater than 21 mmHg, the last reading was the final measurement for those individuals. In addition, fundus and stereo photographs of the optic disc were taken and measurements of the vertical cup disc ratio were conducted.

Participants who had a past history of glaucoma, an IOP greater than 21 mmHg, who experienced visual field defects, or had an enlarged vertical cup disc ratio were glaucoma suspects. The records of these individuals were compiled and presented at a meeting with a panel of six experienced ophthalmologists that included two glaucoma subspecialists. The panel graded the visual fields and photographs of the optic disc separately and in a masked fashion. Each panel member used his or her own clinical judgment to classify each participant into definite, probable, possible, or no categories for POAG. No specific criteria were used. Cases that had discrepancies between experts resulted in an open discussion.

Proyecto VER was a population-based survey of ocular disease prevalence among Hispanic persons 40 years and older in the Pima and Santa Cruz counties of southern Arizona between April 1997 and September 1999 (H. A. Quigley et al., 2001). The 1990 census data were used to randomly select groups of persons living in sections of the counties, with most of the population living in Nogales and Tucson. If participants were eligible, an extensive home interview was administered and an eye examination was conducted at a nearby clinic. The ocular examination consisted of visual acuity measurement, visual field testing by Humphrey Field Analyzer II, applanation tonometry, slit-lamp examination to estimate the anterior chamber, and dilated fundus examination. Additionally, all subjects underwent stereophotography of the optic disc and nerve fiber layer. A glaucoma specialist reviewed all the data on persons with IOP greater than 22 mmHg in either eye, a cup-disc ratio of 0.7 or greater, visual field defects in either eye, a

shallow anterior chamber, or vision loss attributed to glaucoma. Additionally, a single glaucoma specialist evaluated all the optic disc stereophotographs.

A diagnosis of POAG was made for an individual if the angle was open by gonioscopy and if one of the following conditions was met: (1) if one eye had optic disc damage and a visual field defect in the same eye; (2) if at least one eye did not satisfactorily complete the visual field testing had a cup-disc ratio that is equal to or greater than the 99.5 percentile value for the population; or (3) if a subject did not complete visual field testing in whom the optic disc was not visible, the visual acuity test results indicated the individual was considered to be legally blind, and the IOP was greater than the 99.5 percentile value for the population.

The LALES consisted of self-identified Latinos in La Puente California who were 40 years of age or older (Varma et al., 2004). Residences within six census tracts were identified, and all eligible individuals were invited to participate in both a home interview and a clinic examination. Eye examinations were conducted at a local eye examination center.

Participants' peripheral vision was tested using the Humphrey Automated Field Analyzer II. If the results were normal, no further testing was done; but if the results were abnormal, the test was run again. For participants that required two or more tests, two glaucoma specialists evaluated the field-loss patterns. The optic nerve was evaluated using stereoscopic optic disc photographs and was classified as consistent with characteristics of glaucoma, abnormal but non-glaucomatous, normal, or unsure.

Based on fundus photographs and clinical examination data, the specialists determined if vision field loss was due to glaucoma. The diagnosis of glaucoma was

determined via a three-step process. Initially, two glaucoma specialists evaluated all clinical history gathered during the baseline interview and examination data. Second, the specialists determined POAG status using the optic disc photographs and visual field results independently of each other. And thirdly, if there was disagreement between the two specialists, a third specialist assessed the data. The principal investigator also reviewed the data of all the POAG cases.

Primary open angle glaucoma cases were identified as having an open angle, visual field abnormality, and optic disc damage due to glaucoma in at least one eye. Cases of POAG were also identified as having an open angle, and having one of four criteria: (1) end-stage disease with poor visual acuity and a vertical cup-disc ratio of 1.0; (2) at least one abnormal visual field test with visual field defects and optic disc damage; (3) optic disc damage with no visual field irregularity; and (4) various groupings of visual field tests and optic disc abnormalities that are both compatible with glaucoma. The IOP was not included as part of the definition of POAG.

While these studies were innovative in quantifying the prevalence of POAG among various populations, there was substantial variation in the methodologies, including the eye examination and the criteria to diagnose cases. Although all studies evaluated the optic disc of all eligible study participants, there was variation in defining glaucomatous nerve damage. For example, the LALES classified the optic nerve as compatible with glaucoma if the vertical cup-disc ratio was equal to or greater than 0.80, as compared to Proyecto VER, Melbourne VIP, and the Barbados Eye Study that considered a vertical cup-disc ratio of equal to or greater than 0.70. This difference in diagnosis criteria may introduce misclassification bias of the outcome, potentially resulting in lower prevalence rates for

LALES due to stricter criteria compared to the other studies. Additionally, the criteria used to diagnose POAG cases varied between studies. The Proyecto VER and LALES used the conventional criteria of defining POAG, but also included additional criteria, such as identifying cases as having various components of either visual field defects or optic disc abnormalities, instead of the criteria from previous studies where components of both conditions must be present. While the authors defend that there could not be an alternative explanation for the disc finding or the visual field defect other than glaucoma, this relaxing of the traditional criteria for POAG cases may introduce misclassification bias of the outcome, and may increase prevalence rates, due to the inclusion of more subjects. Additionally, the Melbourne VIP diagnosed POAG cases with no specific criteria. Cases were determined based on each panel member's clinical experience, making comparability of the final diagnosis criteria between this study and other studies difficult.

Other sources of variation between the studies include inter-grader reliability when evaluating the stereo fundus photographs, and the effect of technology on data quality. Accurate and consistent optic disc grading in the overall evaluation of POAG criteria is significant in order to avoid misdiagnosis and potential burden on individuals. In order to ensure reliable diagnosis of glaucomatous optic disc damage, investigators may have two graders evaluate photographs independently of each other and then compare their findings. All the original POAG studies made use of two stereograph and fundus graders except for Proyecto VER. While having only one grader does not render the study of poor quality, having at least two graders allows for the measurement of agreeableness between these individuals and thus, serves as a proxy for a more reliable diagnosis. And lastly, the time between the first and last study spans 13 years, in which the scientific community's

understanding of the disease and approaches of studying it has changed. The technology used to examine the eye has also advanced, potentially affecting the quality of the data when comparing studies that use different instruments for measurement. For example, Proyecto VER and LALES both used the Humphrey Field Analyzer II for visual field testing, whereas earlier studies used the Humphrey Field Analyzer. The newer version of the equipment may be better able to grade the visual field of an individual, thus better distinguishing them as either a potential case or not, compared to the older version. As a result, this may lower misclassification based on outcome.

While these studies are slightly different in some aspects of their methodology, they are comparable on other facets. Eligible participants for all the studies, regardless of the initial baseline interview, underwent eye examinations, compared to examining only subjects with a family history of glaucoma or other ocular diseases. By examining all participants, the investigators avoid potential selection bias by having a representative sample of the overall distribution of POAG cases in the entire target population. Secondly, none of the studies used IOP as a criterion for the diagnosis of POAG. Because cases can have POAG with or without elevated IOP, inclusion of this criterion would neglect those with normal IOP and attenuate the true prevalence. And lastly, all the studies had a relatively large sample, leading to more reliable results, thus increasing the likelihood that a statistically significant finding is actually true.

1. **Risk Factors**

a. **Race**

The Baltimore Eye Survey was the first study to investigate the prevalence of POAG in African Americans and non-Hispanic Whites using the same examination criteria (Tielsch et al., 1991). Previous studies had been conducted by investigators using alternative procedures in different populations. By conducting a prevalence study in different populations, but with the same procedures and diagnosis criteria, the results are more comparable and reliable in regard to reducing systematic error and increasing both internal and external validity across different racial groups. As such, the Baltimore Eye Survey found African Americans have a higher prevalence of POAG compared to non-Hispanic Whites after adjusting for age, 4.74% and 1.29%, respectively. The referral rate for eye examinations was higher among African Americans than Whites, 39.1% and 28.6% (P<.0001), respectively. While there were no differences between basic demographic data between respondents and nonrespondents, response to screening was inversely related to age, with the highest response rate among those 40 to 44 years of age (84.8%), and the lowest among those 85 years and older (69.2%) (P<.001). In addition, the overall response rate to definitive examination was slightly higher among African Americans compared to Whites, 82.9% and 79.0% (P=.02), respectively. Nonresponse bias may have been introduced into the study due to both younger participants and African Americans participating, and thus, the true prevalence for older individuals and White individuals may be higher than what was reported. Possible reasons for the lower participation rate may be that older individuals are uncomfortable to participate in a study, or not as willing to travel to an eye clinic, and White individuals may be more proactive

about maintaining a healthy lifestyle, and perhaps feel as though they do not need to concern themselves about ocular health.

The prevalence of POAG in the predominately Black study population of the Barbados Eye Study showed a slightly higher prevalence of 6.8% and higher referral and response rates to definitive examination, 64% and 93%, respectively, compared to the African Americans in the Baltimore Eye Survey (Leske et al., 1994). Participation was higher among individuals 40 to 44 years of age (89.6%) compared to individuals 80 years or older (72.6%) (P<.02). Similar to the Baltimore Eye Survey, the prevalence for older individuals may be underestimated due to the lack of response from this age group.

The Blue Mountains Eye Study and the Melbourne VIP, both conducted in non-Hispanic White populations in Australia, showed similar prevalences of 2.4% and 1.7%, respectively (Mitchell et al., 1996; Wensor et al., 1998). The Melbourne VIP had a slightly lower response rate compared to the Blue Mountains Eye Study, 83% and 87.9%, respectively. In analyzing participants and nonparticipants in the Melbourne VIP, these individuals were similar in all aspects except that non-English speaking persons were slightly less likely to participate. These individuals may not have fully understood the study, or felt uncomfortable enrolling in a study that did not use their native language, potentially introducing nonresponse bias and a lower prevalence for this subgroup.

The Proyecto VER study and LALES examined the prevalence of POAG in Hispanics in Arizona and California and found the prevalence to be higher among those in LALES, 2.0 and 4.7, respectively (H. A. Quigley et al., 2001; Varma et al., 2004). Nonparticipants in the Proyecto VER study tended to be younger and had a higher proportion of males as well as reporting better health and fewer vision problems compared to participants. In LALES,

those who completed the initial baseline interview were on average younger and more likely to be females compared to those who did not complete the interview. Compared to participants who completed the eye examination, those who only completed the interview were more likely to be older, born in the United States, more acculturated, more educated, and had higher incomes. There were, however, no differences in gender, employment, or marital status. Nonparticipation in the eye examination may be due to the higher levels of education and income, where these individuals may be more proactive about ocular health, or may be able to afford health insurance, and thus, may not feel as though they need to participate.

A meta-analysis was performed evaluating the prevalence of POAG in various races and determined that the prevalence for POAG for Black, White, and Asian populations was 4.2%, 2.1%, and 1.4%, respectively (Rudnicka, Mt-Isa, Owen, Cook, & Ashby, 2006). While the meta-analysis summarized results from previous studies on POAG among various racial groups, there was substantial variation in the eye examination methods, instruments, and case definition of POAG. Despite the heterogeneity in examination methods, instruments, and case definition, the authors did not exclude any studies solely on these reasons. Additionally, the meta-analysis contained several studies involving Hispanic populations; however, these Hispanic studies were combined with the non-Hispanic White studies in the analysis as a result of no significant difference in POAG prevalence between Hispanics and non-Hispanic whites. With the systematic differences in methodology and case definition among the studies, as well as the combining of different racial groups, this study may not reflect the true prevalence of POAG among these populations. Future studies should attempt to use similar definitions for the diagnosis of POAG in order to increase

comparability between studies. Although this meta-analysis combines studies with varying study characteristics, the individual studies show significant racial differences in the prevalence of POAG. In order to further understand these differences, it is important to investigate the genetic architecture of these racial groups to identify any differences that may contribute to the pathogenesis of POAG.

b. **Gender**

By comparison to ethnicity, the role of gender in the risk of POAG is debatable. Women in the Blue Mountains Eye Study had a significantly higher prevalence of POAG compared to men with an age-adjusted odds ratio of 1.55 (95% CI: 1.03–2.32) (Mitchell et al., 1996). The prevalence of elevated IOP among males and females was 3.6% and 3.8%, respectively, and was not significant when using logistic regression (odds ratio, 0.95; 95% CI: 0.67–1.35). In contrast, the Barbados study determined that men had a higher prevalence of POAG than women, 8.3% compared to 5.7%, respectively (Leske et al., 1994). Additionally, there was no difference in IOP between males and females, 18.8±5.7 and 18.7±4.7, respectively. Other studies, including the Baltimore Eye Study, (2.70% for males and 2.35% for females with *P*=.39 after adjusting for age and race), and Melbourne, (*P*=.15), studies found no significant difference between males and females concerning the prevalence of POAG (Tielsch et al., 1991; Wensor et al., 1998). Additionally, neither the LALES, (5.44% for males and 4.35% for females after adjusting for age), nor the Proyecto VER (1.09 [95% CI: 0.71–1.69]) studies found significant differences in gender in Hispanic populations (H. A. Quigley et al., 2001; Varma et al., 2004). The prevalence of elevated IOP was not statistically significantly different between males and females in LALES, 3.00% and

3.96%. As a result, gender is not normally considered to be a risk factor for POAG (Morrison & Pollack, 2003).

c. **Age**

Although gender has not been consistently identified to influence POAG or IOP, age has been implicated in several of the previously mentioned studies. The age-specific prevalence rates in Table I illustrate an exponential relationship between age and POAG occurrence. In the Baltimore study, the prevalence of POAG in Whites was 3.15 times higher in individuals in their 70s compared to individuals in their 40s, 2.89% and 0.92% respectively. The pattern in Blacks was even more pronounced using the same age groups with a 7.4 times higher prevalence, 9.15% for individuals in their 70s and 1.23% for individuals in their 40s (Tielsch et al., 1991). The study participants in the Barbados study who were in their 70s had an 10.6 times higher prevalence of POAG compared to participants in their 40s, 14.8% and 1.4% respectively (Leske et al., 1994). Furthermore, IOP increased with age, with an increase in 1 mmHg per decade. That is, 50–59-year-old subjects had a mean IOP of 18.2±4.2, 60–69 mean IOP of 19.4±5.4, and 70–79 mean IOP of 20.4±6.6. Latinos who were in their 70s had an 11.2 times higher prevalence of POAG than Latinos in their 40s in the LALES (Varma et al., 2004). Similar to the Barbados study, the prevalence of elevated IOP increased with each additional decade of age with a prevalence of 4.26% for 50–59-year-old subjects, to 4.76% and 5.48% for 60–69, and 70–79-year-old subjects, respectively. In all of the original POAG studies, the prevalence of POAG and elevated IOP increased as age increased.

While the previously mentioned variables are unable to be changed, there are only a few studies that have been conducted to investigate the relationship between various

modifiable lifestyle factors and the risk of developing POAG. Identifying modifiable risk factors is attractive in that the discovery of such factors could serve as primary prevention as well as inexpensive methods to combat POAG development. Additionally, detecting such lifestyle factors may aid in our understanding of the pathogenesis of the disease. Several studies have investigated the association between POAG and modifiable lifestyle factors, including coffee consumption, smoking, and alcohol consumption. These studies are briefly summarized below.

d. **Coffee consumption**

A recent study reported the association between coffee consumption and risk of POAG using data from the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS) (Kang, Willett, Rosner, Hankinson, & Pasquale, 2008). Both studies are cohort studies that have followed participants through the years answering questionnaires about lifestyle habits and the occurrence of newly diagnosed illnesses. In both cohorts, dietary intake data were collected every four years in which respondents had to respond with the frequency intake of each item. Respondents could answer from one of nine responses that included "never or less than once per month" to "6 or more time per day." The average intake of caffeinated beverages per year was converted to daily intakes using the US Department of Agriculture food-composition sources. Cases of POAG were identified through the questionnaires and confirmed with medical records. During the follow-up of this study, 1,011 incident POAG cases were diagnosed. Compared to individuals who drank no caffeinated coffee per day, those who drank less than 1 per day, 1 cup, 2 cups, 3–4 cups, or 5 or more cups per day had a 1.07 (95% CI: 0.87–1.32), 1.07 (95% CI: 0.86–1.34), 1.17 (95% CI: 0.94–1.46), 1.20 (95% CI: 0.93–1.54), and 1.62 (95% CI:

1.00–2.59) times increased risk of developing POAG, respectively. Additionally, the *P* for trend was 0.02, suggesting that increasing intake of caffeinated coffee is associated with risk of developing POAG. However, the cell size for the highest coffee consumption category was small and was borderline significant with the 95% CI including the null. While this study identified a borderline significant association between caffeinated coffee consumption and increased risk of POAG, this was the first study to investigate this relationship and thus, additional studies are needed to elucidate this relationship.

In the Blue Mountain Eye Study, IOP did not statistically differ between regular coffee drinkers and non-coffee drinkers among subjects without POAG or elevated IOP, and was also was not significantly different among subjects with elevated IOP, *P*=.42 and *P*=.91, respectively (Chandrasekaran, Rochtchina, & Mitchell, 2005). The IOP, however, did differ among subjects with POAG, with non-coffee drinkers having a mean IOP of 16.84±0.97 and a mean IOP for regular coffee drinkers of 19.63±0.76 (*P*=.03), after adjusting for age, sex, systolic blood pressure, myopia, current smoking, and diabetes. While the results from this study indicate that coffee consumption is associated with elevated IOP among POAG cases, the number of cases in both regular and non-coffee drinkers was small, 19 and 30, compared to subjects with neither POAG nor elevated IOP, 861 and 2,115, respectively.

e. **Cigarette smoking**

Cigarette smoking is a widely known modifiable risk factor for many human diseases. The association between smoking and POAG occurrence has not been well studied with some studies yielding nonsignificant results. For example, results from the Proyecto VER study were nonsignificant with individuals who were ex-smokers or current smokers having a 0.96 (95% CI: 0.61–1.51) and 0.71 (95% CI: 0.35–1.48) times lower odds

of POAG compared to individuals who never smoked, after adjusting for age. One study used two large ongoing cohorts, the NHS and the HPFU, to investigate the relationship between POAG and smoking (Kang et al., 2003). Following these individuals from 1980 to 1996, and from 1986 to 1996 respectively, questionnaires on risk factors and newly diagnosed illnesses were mailed out to participants biennial. In the NHS, smoking exposure was ascertained for current and past smokers with regard to at what age they started smoking, the average number of cigarettes smoked per day, and if they stopped, when they quit smoking. In the HPFS, participants were asked whether they had smoked more than 20 packs in their lifetime, and if they answered yes, participants were asked the mean number of cigarettes smoked per day at various age categories and if they quit smoking, how long ago did they stop. A total of 450 cases of POAG were identified through questionnaires and then verified through medical records. Among current smokers, compared to those who never smoked, those who smoked 1–14, 15–24, and 25 or more cigarettes per day had a 0.96 (95% CI: 0.60–1.53), 0.72 (95% CI: 0.42–1.23), and 1.00 (95% CI: 0.52–1.84) times greater risk of developing POAG, respectively, with a *P* for trend=.70. Additionally, pack-years of smoking had a more significant trend of 0.06, with 1–9, 10–19, 20–29, and 30 or more pack years had a 1.12 (95% CI: 0.66–1.92), 0.72 (95% CI: 0.53–0.98), 0.85 (95% CI: 0.59–1.23), and 0.78 (95% CI: 0.55–1.11) times increased risk of POAG compared to individuals who never smoked, respectively. In both pack years and cigarettes per day, heavy smoking did not have a significant relationship with risk of POAG.

While there appears to be a modest inverse relationship between risk of POAG and pack years of smoking, data from the Blue Mountain Eye Study suggest smoking increases IOP (A. J. Lee, Rochtchina, Wang, Healey, & Mitchell, 2003). After adjusting for age and sex,

the mean IOP of subjects who never smoked was 16.03±0.07, 16.06±0.09 for ex-smokers, and 16.34±0.13 for current smokers. Compared to never-smokers, IOP did not significantly differ for ex-smokers (*P*=.78) but IOP was significantly higher among current smokers (*P*=.04). The current study included cigarettes, cigars, and pipes, which may, individually, have different effects on IOP due to various tobacco types and amount of smoke inhaled. Despite a significant difference, the authors concluded that the reduction in IOP gained from cessation of smoking alone may not delay or prevent the onset of POAG.

f. **Alcohol consumption**

Consumption of alcohol has both positive and negative effects on health. For example, consuming moderate levels of alcohol has been reported to lower risk of a stroke, but drinking high levels of alcohol increases risk of liver cancer. The role of alcohol on the incidence of POAG has been poorly studied. A recent study used females and males from the NHS and the HPFU study to investigate the relationship between alcohol consumption and risk of POAG (Kang, Willett, Rosner, Hankinson, & Pasquale, 2007). Following females from 1980 to 2002 and males from 1986 to 2002, participants filled out biennial questionnaires about numerous lifestyle habits including alcohol consumption and newly diagnosed illnesses, such as POAG. Similar to that of coffee consumption, participants were asked to estimate the average frequency of consuming alcohol. Cases of POAG were identified via questionnaires and verified through medical records. Compared to individuals who do not drink any alcohol, those who consumed 1–9 grams, 10–19 grams, 20–29 grams, or 30 or more grams of alcohol per day had a 0.99 (95% CI: 0.83–1.19), 0.96 (95% CI: 0.76–1.22), 0.95 (95% CI: 0.68–1.33), 0.71 (95% CI: 0.49–1.04) increased risk of POAG, with a *P* for trend=.09. For reference, one beer contains 13.2 grams of alcohol, a

four-ounce glass of wine has 10.8 grams of alcohol, and one shot of liquor has 15.1 grams of alcohol. While none of these are significant, the *P* for trend suggests a modest inverse relationship between alcohol consumption and risk of developing POAG.

Similar to the effect of alcohol on risk of POAG, studies investigating the effect of alcohol and IOP have led to insignificant findings. Results from recent studies have yielded no significant increase in IOP due to alcohol. A prospective population-based cohort study of residents of Rotterdam, the Netherlands, who were 55 years and older (n=3,939) found that when dividing alcohol into groups (beer, wine, liquor, and sherry), consumption of any group did not significantly increase or decrease IOP among men or women (Ramdas et al., 2011). Additionally, the Beijing Eye Study, a population-based cohort study conducted in Northern China for subjects 40 years of age and older (n=4,439), found no significant association between a binary dependent variable of alcohol consumption with IOP (L. Xu, You, & Jonas, 2009). Subjects who were considered consumers of alcohol had a mean IOP of 16.1±2.9, and nonconsumers of alcohol had a mean IOP of 15.9±3.0 (*P*=.19). While consumption of moderate levels of alcohol may be beneficial for various aspects of an individual's health, alcohol consumption does not act in a beneficent or maleficent manner on ocular health, in regards to IOP.

g. **Central corneal thickness**

Central cornea thickness has increasingly become an important determinant of ocular health. Several epidemiological eye studies have investigated the association of CCT with POAG and have shown it as a risk factor. Participants in the Ocular Hypertension Treatment Study who had a CCT of 555 μm or less had a three times greater risk of developing POAG compared to those with a thickness of 588 μm or greater, with a

hazard ratio of 1.71 per 40 μm decrease (Gordon et al., 2002). Similarly, the Early Manifest Glaucoma Trial and the LALES found a hazard ratio of 1.25 and an odds ratio of 1.30 per 40 μm decrease in CCT, respectively (Jiang et al., 2012; Leske et al., 2007). These studies show that the odds of POAG are higher among individuals with thinner CCT compared to those with thicker CCT. Furthermore, CCT at baseline independently predicted the occurrence of POAG.

h. **Intraocular pressure**

The association between IOP and POAG has been a subject of controversy in terms of using IOP as a criterion to diagnose POAG. As previously stated, normal IOP ranges between 10 mmHg and 21 mmHg, and IOP greater than 21 mmHg is considered elevated. Intraocular pressure is not considered to be part of the definition of POAG due to the occurrence of elevated IOP in the absence of POAG as well as the converse. The latter type, where there is an occurrence of glaucoma in the absence of elevated IOP, is a form of POAG known as normal tension glaucoma (NTG). As such, elevated IOP is considered to be a risk factor for POAG with more elevated IOP resulting in a greater risk for POAG. The Baltimore Eye study revealed that compared to individuals with IOP less than 15 mmHg, an IOP of 16–18 mmHg had a relative risk of 2.0, 19–21 mmHg had a relative risk of 2.8, 22–29 mmHg had a relative risk of 12.8, 30–34 mmHg had a relative risk of 39.0, and greater than or equal to 35 mmHg had a relative risk of 40.1 for developing POAG (Sommer et al., 1991). While these relative risks imply that the probability of developing POAG is 40 times higher for extremely high IOP, the number of individuals in these categories is small, making the estimates unstable and unreliable relative risks.

Several cohort studies investigated the role of IOP as a risk factor for the incidence of POAG. Surviving members from the Barbados Eye Study were reexamined four (n=3,427) and nine (n=2,795) years after the initial study (Nemesure et al., 2007). Compared to the baseline population, those included in the follow-up were younger and more likely to be females. Additionally, baseline nonparticipants had higher IOP compared to participants in the follow-up study, 19.5±5.7 mmHg and 18.1±5.6 mmHg, respectively. Similar to the eye examinations for the original study, POAG cases required visual field defects, as well as optic disc damage, with no other possible causes. Over the nine-year follow-up, of the 4,008 eligible individuals, there were 3,222 individuals (80.4%) at risk with an overall incidence of 4.4%. After adjusting for age, gender, hypertension, and IOP-lowering treatment, compared to individuals with baseline IOP equal to or less than 17 mmHg, those with IOP greater than 17 and equal to or less than 19, greater than 19 and equal to or less than 21, greater than 21 and equal to or less than 23, greater than 23 and equal to or less than 25, and greater than 25 had a relative risk of 1.3 (95% CI: 0.6–2.4), 3.1 (95% CI: 1.8–5.5), 5.4 (95% CI: 2.7–10.5), 7.9 (95% CI: 3.8–16.2), and 13.1 (95% CI: 7.1–24.1), respectively. While the relative risks illustrate a linear dose response relationship between IOP and incidence of POAG, similar to the Barbados study, the small cell sizes for the higher IOP levels result in unstable estimates and wide confidence intervals.

Members of the LALES were also followed up four years after the initial examination (Jiang et al., 2012). Among the 4,538 participants with eye examinations at both the baseline and follow-up time periods, 3,939 participants had reliable data. Participants tended to be older, more likely to be married, and have health insurance, compared to nonparticipants, with no differences in gender, education level, or income level. Over the

four-year time period, the overall incidence rate was 2.3%. Among participants that developed POAG, their mean IOP at baseline was 17 mmHg, compared to a mean of 14 mmHg for participants who did not develop POAG. A nonlinear dose-response relationship was observed between baseline IOP and incidence of POAG after four years. After adjusting for age, axial length, lack of vision insurance, waist-to-hip ratio, and CCT at baseline, compared to individuals with an IOP at baseline less than 10 mmHg, participants with an IOP between 10 and less than 13, 13 and less than 16, 16 and less than 19, and 19 and less than 22 had relative risks of 2.35 (95% CI: 0.31–18.12), 2.06 (95% CI: 0.27–15.64), 3.72 (95% CI: 0.39–28.32), and 5.79 (95% CI: 0.71–47.22), respectively. Additionally, participants with an IOP between 22 and less than 25, or an IOP greater than 25 had relative risks of 19.82 (95% CI: 2.21–177.71) and 30.85 (95% CI: 2.26–421.51), respectively, compared to participants with an IOP less than 10 mmHg. Although the latter two categories of IOP were the only two statistically significant due to the confidence interval not containing the null, the small number of cases in each of these categories results in wide confidence intervals and unstable estimates. As such, the relative risks for lower baseline IOP are more reliable than the relative risks for higher baseline IOP.

The IOP is the only currently known modifiable risk factor for POAG. Several randomized clinical trials have found that reducing IOP through medical means has been effective in delaying and preventing POAG. The Ocular Hypertension Treatment Study was a randomized clinical trial to assess the efficacy of topical ocular hypotensive medication in delaying and preventing POAG (Kass et al., 2002). Conducted at 22 clinical centers, participants ages 40 to 80 years, with an IOP ranging from 25 mmHg to 32 mmHg in one eye and 21 mmHg to 32 mmHg in the opposite eye, open angles, and normal visual field and

optic discs were equally randomized to either the observational or medication group. Participants were randomized to the treatment group to decrease their IOP to 24 mmHg or less, and reduce their IOP by at least 20% from the average qualifying and baseline IOP. If needed, medications were changed to achieve these aims. The topical medications included all topical ocular hypotensive medications that were commercially available in the United States. Additionally, the primary outcome was the development of POAG in one or both eyes. Of the 3,328 individuals who enrolled in the study, only 1,636 were eligible for the study. The treatment group had 817 individuals; 819 were assigned into the control group; with no statistically significant differences between the two groups. After 60 months, the IOP goal was met in 87% of the medicated participants in both eyes and 7% of participants achieved this goal in one eye. In the treatment group, the mean and standard deviation reduction in IOP was 22.5% ± 9.9% compared to the reduction in IOP in the control group of 4.0% ± 11.6%. Furthermore, the probability of developing POAG was lower in participants who received medication compared to those who did not receive the medication, with a hazard ratio of 0.40 (95% CI: 0.27–0.59). This study showed that topical ocular hypotensive medications are effective in reducing IOP and potentially preventing POAG from occurring in individuals with ocular hypertension.

D. **Genetics of Primary Open Angle Glaucoma**

Genetics appear to play a significant role in both the prevalence and the pathogenesis of POAG. Several studies have investigated the heritability of POAG in related individuals. The Baltimore Eye Study found an association between family history and POAG. Study participants who reported a first-degree relative who had POAG had a 2.85

(95% CI: 1.82–4.46) times greater odds of having POAG compared to individuals who did not have a first-degree relative with POAG, after adjusting for age and race. Among first-degree relatives, full siblings of the study participants had the strongest association, with an odds ratio of 3.69 (95% CI: 2.10–6.48) compared to siblings who did not have POAG, while the association was weakest in the children of the study subjects with an odds ratio of 1.12 (95% CI: 0.26–4.86) compared to the children of study subjects without POAG (Tielsch, Katz, Sommer, Quigley, & Javitt, 1994). The lower odds of POAG among children is likely due to a small number of cases in this group. Additionally, the children of study subjects are younger than the subjects themselves, and with the risk of POAG increasing with age, the occurrence of POAG would be lower in children than in adults. Similarly, siblings in the Barbados study had a 4.5 greater odds of glaucoma if one or more siblings had a history of glaucoma, compared to siblings who do not have a history of glaucoma (Nemesure, Leske, He, & Mendell, 1996). Lastly, a population-based study conducted in the Netherlands found individuals with a first-degree relative who had POAG had a 22% lifetime risk compared to 2.3% for individuals who did not have a first-degree relative with POAG, yielding a risk ratio of 9.2 (Wolfs et al., 1998).

These segregation studies aided in understanding the genetic architecture of POAG and enabled scientists to identify and map several genes that are associated with POAG. The first gene to be identified with POAG by linkage analysis was myocilin (*MYOC*), which is located on chromosome 1 (Stone et al., 1997). The *MYOC* encodes the myocilin protein, previously known as trabecular meshwork inducible-glucocorticoid response protein, which is produced by the ciliary body and trabecular meshwork, both of which regulate IOP (Ortego, Escribano, & Coca-Prados, 1997; Polansky et al., 1997). A mutation in this gene

results in elevated IOP and usually requires surgical interventions to control the disease. The prevalence of *MYOC* mutations varies in POAG cases between 2% and 4%. The mutation is considered to be an autosomal dominant trait with carriers of the mutation developing POAG 90% of the time (Alward et al., 1998; Fingert et al., 1999). In normal ocular tissues, myocilin protein is present in the aqueous humor but is absent in patients with mutations in *MYOC* (Jacobson et al., 2001). While the biological process of how the absence of this protein elevates IOP and results in POAG is unknown, the identification of a potentially causal gene is beneficial for screening purposes.

While *MYOC* is associated with elevated IOP, a second and third gene have been associated with both NTG and POAG. Optineurin (*OPTN*) is the second gene to be associated with POAG and is located on chromosome 10 (Rezaie et al., 2002). The first study to designate *OPTN* examined 54 families with autosomal dominant POAG. It was found that genetic variants in *OPTN* occurred in 16.7% of the affected families. Furthermore, the researchers used segregation analysis to identify E50K, a missense mutation that changed the normal amino acid of glutamic acid at position 50 to a lysine. This mutation was found in 124 family members, of whom 38 were affected with POAG, of which 7 had elevated IOP, and the remaining with normal IOP. The mutation appears to be the most strongly associated variant of POAG, especially among NTG (Allingham, Liu, & Rhee, 2009). This study, however, was conducted in related individuals and thus, are more likely to be similar than unrelated individuals, resulting in clustering and within-family correlation. A study conducted in 315 unrelated British study participants with POAG, 132 with NTG and 183 with high tension glaucoma, found 2 (1.5%) and 0 normal and higher tension glaucoma with the E50K mutation (Aung et al., 2003). Additionally, a gene located on chromosome 5,

WDR36, was found by mapping of sequence variants. Four non-synonymous mutations were identified in 17 POAG individuals, 11 of whom had elevated IOP and 6 had normal IOP (Monemi et al., 2005). Specifically, the missense mutation D658G, resulting in an amino acid change from an aspartic acid to a glycine, was the only significant mutational variant in POAG cases compared to controls and was found to segregate in all affected members of one family. The mutation D658G has been reported to be rare with a prevalence of approximately 1.6%, while other studies have reported no association of this variant with POAG (Fingert et al., 2007; Hewitt, Dimasi, Mackey, & Craig, 2006; Pasutto et al., 2008).

These segregation and linkage analysis studies were successful in identifying several genes that are strongly associated with POAG. These single genes, most notably *MYOC* and *OPTN*, are responsible for 5% of POAG cases (Fingert, 2011). In addition to the above genes discovered, numerous genetic loci have been associated with the etiology of POAG in the literature. More than twenty genetic variants have been associated with POAG across many chromosomes, including chromosomes 1, 2, 3, 4, 6,7, 9, 11, 16, 17, and 19 (Allingham et al., 2009). Even though most of these genetic variants have not been verified by other independent studies, these variants offer locations in the genome for researcher to further investigate.

E. **Genetics of Intraocular Pressure**

Due to the clinical heterogeneity of POAG, investigating the genetic architecture of disease-related quantitative traits or endophenotypes may aid in elucidating true disease genes. As the only modifiable risk factor of POAG, understanding genes associated with IOP may shed light on the pathogenesis of this disease. Several GWASs have been conducted to

identify common variants associated with IOP. The results from these studies are summarized in Table II. The first GWAS on IOP included a meta-analysis of four independent European samples for a total of 11,972 participants. Of the four studies, the smallest study (n=2,035) tended to be younger (48.8 ±14.4) and have a smaller percentage of samples with IOP greater than 22 mmHg (1.2), compared to the largest study, n=5,794, 68.8 ± 8.9, and 3.3, respectively (van Koolwijk et al., 2012). Using the mean IOP, measured with a Goldmann applanation tonometer from both eyes or the IOP from one eye if data for the other eye were not available, associations between IOP and SNPs were assessed using linear regression adjusting for age and sex, while assuming an additive model for the effect of the risk allele. Results from the individual cohorts were combined into a meta-analysis and SNPs were determined to be genome-wide significant with a *P* value less than 5×10^{-8} and suggestive of an association at a *P* value less than 1×10^{-5}, after adjustment for multiple testing. One SNP, rs11656696 in *GAS7*, reached genome-wide significance and was associated with a 0.26 mmHg decrease in IOP for each copy of the A allele. Six other SNPs had *P* values that were suggestive of an association. When these results were combined in a joint analysis with four other replication cohorts, results not shown in Table II, each copy of the A allele from the previous SNP was associated with a 0.19 mmHg reduction in IOP, and rs7555523 in *TMCO1* was associated with a 0.28 mmHg increase in IOP with each copy of the minor allele C. Additionally, mature ribonucleic acid (mRNA) expression analysis using ocular tissues revealed expression of *GAS7* in the trabecular meshwork and optic nerve and expression of *TMCO1* in the trabecular meshwork and the retina.

A second GWAS study was performed on 2,175 subjects from Sydney, Australia, and identified a suggestive SNP on chromosome 7, rs59072263, in the discovery set that was

associated with a 0.61 increase in IOP for each copy of the G allele (Blue Mountains Eye & Wellcome Trust Case Control, 2013). Additionally, this SNP is in between two genes, *ICA1* and *GLCCI1*, the former encoding a protein that aids in secretory vesicle trafficking and the latter playing a role in glucocorticoid sensitivity in tissues. Similar to the first GWAS, the second study measured IOP with a Goldmann applanation tonometer and adjusted for age and sex during linear regression.

A third study performed on 2,774 subjects yielded a genome-wide significant SNP, rs2286885, located within an intron of *FAM125B*, and was associated with a 0.56 mmHg increase in IOP for each copy of the A allele (Nag et al., 2014). This study, however, was predominately female, ~95%, and measured IOP with a noncontact air-puff tonometer, potentially making comparability between studies difficult due to different demographic and measurement features.

The fourth study performed a meta-analysis of GWASs results of three smaller studies, all of European ancestry (Ozel et al., 2014). Of the three studies included in the GWAS, the smallest study had more females compared to the other two studies and was older, 62.1%, 53.4%, 52.8% and with mean ages of 76.6 years, 68.4 years, and 67.8 years, respectively. Additionally, the first two studies adjusted for age, gender, and the first five principal components for population stratification compared to the last study, which adjusted for diabetes medication, spherical equivalent, age-macular degeneration type, and the third principal component. While no SNPs reached genome-wide significance in the individual studies, the meta-analysis yielded one significant SNP, rs7518099, in an intron of *TMCO1*. This SNP was associated with a 0.76 mmHg increase in IOP for each copy of the G allele. While the heterogeneity between studies in the meta-analysis was not significant,

IOP was measured using either Goldmann applanation or Tonopen telemetry, potentially introducing differences in data quality. The fifth and last published GWAS on IOP conducted a meta-analysis including 35,296 subjects, 27,558 of European descent and 7,738 of Asian descent, from 18 studies (Hysi et al., 2014). After adjusting for age and sex and assuming an additive model, seven regions of the genome were associated with IOP, two of which were already reported, *TMCO1* and *GAS7*. In addition, a third locus associated with IOP has previously been associated with POAG, *CAV1* (Thorleifsson et al., 2010). Of the seven associated IOP loci, two loci were not statistically homogeneous between studies, suggesting there are differences in the effect at these loci among the studies and a random effects model should be used. In order to assess the clinical effect of these associated SNPs on POAG, the researchers performed a second meta-analysis using 4,284 POAG cases and 95,560 controls. Results from this meta-analysis confirmed previous associations at *TMCO1*, *CAV1-CAV2*, and *GAS7*, as well as new associations at regions near *ABCA1*, *FNDC3B*, and on chromosome 11.

Primary open angle glaucoma is a complex disease that is clinically heterogeneous. As such, dissecting the genetic basis of POAG through endophenotypes, such as IOP, may aid in further elucidating the biological mechanisms underscoring this disease. Intraocular pressure is one of the major risk factors of POAG and currently the only modifiable risk factor. Previous GWASs have identified loci that are associated with IOP, with similar loci associated with POAG. Furthermore, mRNA expression analysis showed some of these genes are expressed in ocular tissues. While these results identified genetic determinants associated with IOP, a majority of these GWASs were conducted in individuals of European ancestry. With differences in disease prevalence and allele frequencies among different

ethnic backgrounds, conducting such studies in different ethnic groups is critical in understanding the genetic etiology of this disease in different populations. To date, there has been no GWAS on IOP in Latinos.

Table II

RESULTS FROM PREVIOUS GWAS ON IOP

Chr	Gene	SNP	Position	Risk Allele	Delta IOP	*P* value	Ethnic Population
1	*TMCO1*	rs7555523	165718979	C	0.3	5.7×10^{-6}	European [a]
1	*TMCO1*	rs7555523	165718979	C	0.235	2.19×10^{-9}	European/Asian [e]
1	*TMCO1*	rs7518099	165736880	G	0.76	8.8×10^{-8}	European [d]
3	*FNDC3B*	rs6445055	171992387	A	-0.177	4.19×10^{-8}	European/Asian [e]
3	*FOXP1*	rs2117760	70850461	A	0.22	4.1×10^{-6}	European [a]
3	*SATB1*	rs9841621	18409077	G	-0.81	8.9×10^{-6}	European [a]
5	*CSF1R*	rs216146	149445921	T	0.22	1.4×10^{-6}	European [a]
7	*ICA1-GLCCI1*	rs59072263	8152067	G	0.61	9.02×10^{-6}	European [b]
7	*CAV1*	rs10258482	116150095	A	0.196	1.87×10^{-11}	European/Asian [e]
9	*ABCA1*	rs2472493	107695848	G	0.159	2.80×10^{-11}	European/Asian [e]
9	*ABO*	rs8176743	136131415	T	0.261	3.08×10^{-11}	European/Asian [e]
9	*FAM125B*	rs2286885	129246487	A	0.56	3.48×10^{-8}	European [c]
10	*BMPR1A*	rs7894966	88618624	G	0.67	1.6×10^{-7}	European [a]
11	*NUP160-PTPRJ*	rs747782	47940925	C	0.203	1.04×10^{-11}	European/Asian [e]
16	*ADAMTS18-NUDT7*	rs1826598	77572955	A	0.32	6.06×10^{-6}	European [a]
17	*GAS7*	rs9913911	10031183	G	-0.179	1.03×10^{-11}	European/Asian [e]
17	*GAS7*	rs11656696	10033679	A	-0.26	9.8×10^{-9}	European [a]

Chr, chromosome;

[a] van Koolwijk, L. M., et al., *Common genetic determinants of intraocular pressure and primary open-angle glaucoma.* PLoS Genet, 2012.

[b] Blue Mountains Eye, S. and C. Wellcome Trust Case Control, *Genome-wide association study of intraocular pressure identifies the GLCCI1/ICA1 region as a glaucoma susceptibility locus.* Hum Mol Genet, 2013.

[c] Nag, A., et al., *A genome-wide association study of intra-ocular pressure suggests a novel association in the gene FAM125B in the TwinsUK cohort.* Hum Mol Genet, 2014.

[d] Ozel, A. B., et al., *Genome-wide association study and meta-analysis of intraocular pressure.* Hum Genet, 2014.

[e] Hysi, P. G., et al., *Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma.* Nat Genet, 2014.

F. **Latinos**

According to the 2010 United States Census, 308.7 million people live in the United States, with 50.5 million, approximately 16% of the total population, of Hispanic origin (Ennis, 2011). In 2000, the Hispanic population was 35.3 million, representing 13% of the 281.4 million residents (Guzman, 2001). Between 2000 and 2010, more than half of the total growth was due to the increase in the Hispanic population. Among the increase of 15.2 million Hispanics, Mexican individuals represent the largest subpopulation of growth, with an 11.2 million rise in this subpopulation. This population experienced the largest growth from 20.6 million in 2000 to 31.8 million in 2010, a 54% increase. Furthermore, Mexicans represent approximately three-fourths of the overall increase in the Hispanic population, and 64% of the total Hispanic population in 2010.

The US Census defines Hispanics as "a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin regardless of race" (Ennis, 2011). While this term is excessively broad, often used to describe a single group sharing similar heritage, the biogeographical ancestry of Hispanics is rather heterogeneous. Several studies investigating self-identified Hispanics have found varying genetic proportions of European, African, and Native American ancestries. Individuals classified as Dominicans have tended to have a higher proportion of West African ancestry compared to Puerto Ricans and Mexicans, 41.8%, 23.6%, and 5.6%, respectively (Bryc et al., 2010). Similarly, Mexicans have higher proportions of Native American ancestry compared to Cubans, Dominicans, and Puerto Ricans (Manichaikul et al., 2012). The effect of genetic ancestry has been associated with various biological differences. For example, African ancestry was found to be inversely related with lung function, such that for every increase in percent of

African ancestry, there was an associated decrease in the forced expiratory volume in the lungs (Kumar et al., 2010). The study of complex diseases may be confounded by genetic admixture due to differences in allele frequencies and disease prevalence from various ancestries. Therefore, proper adjustment is needed to control for population stratification (Kittles et al., 2002).

G. **Primary Open Angle Glaucoma and Public Health**

Glaucoma is a significant public health issue affecting 70 million people worldwide, and is the second leading cause of blindness worldwide, causing blindness in approximately 8.4 million individuals. In the United States, POAG affects 2.2 million people and is expected to increase with the aging population and increase in ethnic minority populations. The Centers for Disease Control and Prevention estimated that the economic burden of POAG is $2.9 billion in direct medical costs (Rein et al., 2006). Currently, detection and diagnosis of POAG is achieved through eye examinations, with most new cases being detected once observable symptoms have occurred. Developing convenient and affordable screening methods that can be utilized in developed regions, as well as underserved regions, is crucial in confronting this public health issue. In order to reduce the number of new cases, as well as alleviate the economic burden associated with POAG, identifying novel risk factors and implementing these findings into public health screening programs will lead to a vast improvement in the well-being of individuals across the world.

As the largest minority group in the United States, identifying critical differences in the genetic architecture of Mexican Americans, or Latinos, and other ethnic groups will aid in understanding the genetic basis of complex diseases. Studies investigating the genetic

influence on the development of POAG in Latinos are lacking. Furthermore, studying the genetic makeup of Latinos may elucidate the role of ancestry and the effect of different proportions of ancestry on the pathogenesis of the disease.

Glaucoma is a complex disease that is clinically heterogeneous and a leading cause of blindness worldwide. Understanding the role of genetic factors on IOP in Latinos, one of the largest ethnic populations in the United States, is of public health significance in order to reduce the prevalence, and associated cost, of POAG. In order to identify genetic determinations of IOP among Latinos, a genome-wide association study for IOP was conducted in this population. Findings from this study will provide insight into the pathogenesis of POAG and hope to suggest new approaches to screening, diagnosis, and medical interventions for this disease.

III. MATERIALS AND METHODS

A. Ethics Statement

The research that was conducted was approved by the University of Southern California Health Sciences Campus, the University of Illinois at Chicago, and Los Angeles Biomedical Research Institute at Harbor-University of California, Los Angeles. All clinical investigation was conducted according to the Declaration of Helsinki principles.

B. Study Sample

The study sample for this thesis consisted of 3,374 unrelated Latinos that were originally recruited for LALES, a population-based study conducted in La Puenta, California, with the specific aim of documenting the prevalence of ocular diseases in Latinos. The phenotype data were collected from LALES and the genotyping of these individuals was performed by both LALES and MAGGS. Written, informed consent was obtained for all subjects in the study and all subjects were 40 years of age or older.

C. Intraocular Pressure Measurements

Three IOP measurements for each eye were obtained during a detailed ophthalmologic examination for all subjects by Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland). The average of these three measurements was taken to yield a single IOP measurement for each eye. The average IOP measurement for the right and left eye was taken and resulted in the final IOP measurement for each subject. If IOP measurements were available for only one eye, the average of these values determined the final IOP measurement for the subject.

D. **Genotyping and Quality Control**

Samples were genotyped from LALES and MAGGS using Illumina HumanOmniExpress Beadchip (~730K markers; Illumina, Inc., San Diego, California). The genotyping of the samples was performed by the Genotyping Laboratory of the Institute for Translational Genomics and Population Sciences at the LA-Biomed Research Institute at Harbor-University of California, Los Angeles. Duplicates were introduced into the genotyping sample to assess the genotyping lab's reproducibility. In order to call SNPs, a commercial software (Illumina GenomeStudio, v2011.1; Illumina, Inc.) was used. If a subject had a call rate less than 97%, the subject was excluded from further analysis. The program PLINK was used to perform quality control for both subjects and genotypes (Purcell et al., 2007). Based on genotypic data, subjects were removed from the analysis if there was inconsistency between reported and genetic gender or if there were unexpected duplicates. After the removal of the intentional duplicates, the unexpected duplicates, and inconsistent gender, 3,374 unrelated samples remained.

Additionally, subjects were removed based on phenotypic data if their measurements were extreme values (n=7), any values greater than three times the interquartile range, if IOP measurements were missing (n=55), or if there were remaining duplicates in the sample (n=22). After these quality control criteria were applied, 3,290 samples remained for further analysis. Genotype quality control was also performed to ensure only high-quality SNPs were included in the analysis. The SNPs were excluded if SNP call rates were less than 95%, the minor allele frequency was less than 1%, or if Hardy-Weinberg equilibrium *P* value for a SNP was less than 10^{-6}. These SNP quality control criteria resulted in 619, 712 SNPs for the final analysis.

E. **Statistical Analysis**

To control for population stratification, principal components of ancestry were calculated using the software EIGENSOFT (Patterson, Price, & Reich, 2006). The top four principle components were used as covariates in the final analysis. To visually examine the distribution of test statistics generated from the analysis, a quantile-quantile plot was generated. Under the null hypothesis, the test statistics should lie on a diagonal with any deviations indicating confounding, except at the extreme right tail. Additionally, a genomic control inflation factor, a ratio of the median of the observed distribution of test statistics to the expected median, was calculated to further quantify the influence that population stratification and relatedness has on the test statistics (Devlin & Roeder, 1999). Linear regression was conducted to investigate the relationship between IOP as the dependent variable and SNPs as the main explanatory variables. Age, gender, and the first four principle components were included in the analysis as covariates. Additionally, an additive genetic model was assumed. Those SNPs with values of $P<5 \times 10^{-8}$ or $P<1 \times 10^{-5}$ were declared genome-wide significant and suggestive, respectively.

IV. RESULTS

A. Descriptive Analysis

After strict quality control of both the genotypic and phenotypic data, 3,290 unrelated Latinos remained for further analysis. Basic descriptive results from the study population are summarized in Table III. The overall mean (standard deviation) age for the sample was 56.95 (10.12) with the minimum age of 40 years of age and the maximum age of 92 years of age. The sample consisted of 1,918 females and 1,372 males, 58.30% and 41.70%, respectively. The average IOP for both eyes was 14.56 (2.70) mmHg, with the minimum reading of 6.92 mmHg and the maximum reading of 26.00 mmHg, after removal of outliers. Furthermore, 2.12% or 70 of the 3290 study subjects had IOP that was greater than 21 mmHg.

Table III

STUDY SAMPLE CHARACTERISTICS

Sample Size	Females, %	Age (y), Mean (SD)	IOP (mmHg), Mean (SD)	IOP (mmHg), Range
3,290	58.30%	56.95 (10.12)	14.56 (2.70)	6.92–26.00

y, years; SD, standard deviation

A boxplot and histogram were generated to visualize the distribution of IOP measurements for the study participants, including those individuals considered to be outliers (Figure 4). In viewing the histogram, the overall distribution of IOP appears to be slightly right skewed with several IOP measurements greater than 25 mmHg. The boxplot further distinguishes four extreme IOP measurements that are greater than 30 mmHg. Using the third quartile plus three times the interquartile range as the cutoff for extreme

values, individuals with an IOP greater than 26.67 mmHg were removed from the analysis (Fielding & Gilbert, 2006). This cutoff resulted in seven individuals being removed from further analysis. Of these seven individuals, five were female, the average age for all seven subjects was 62.57 (12.59) years of age with a minimum and maximum age of 45 and 86, respectively. The average IOP was 31.25 (3.53) with a minimum and maximum IOP of 27.75 mmHg and 36 mmHg, respectively.

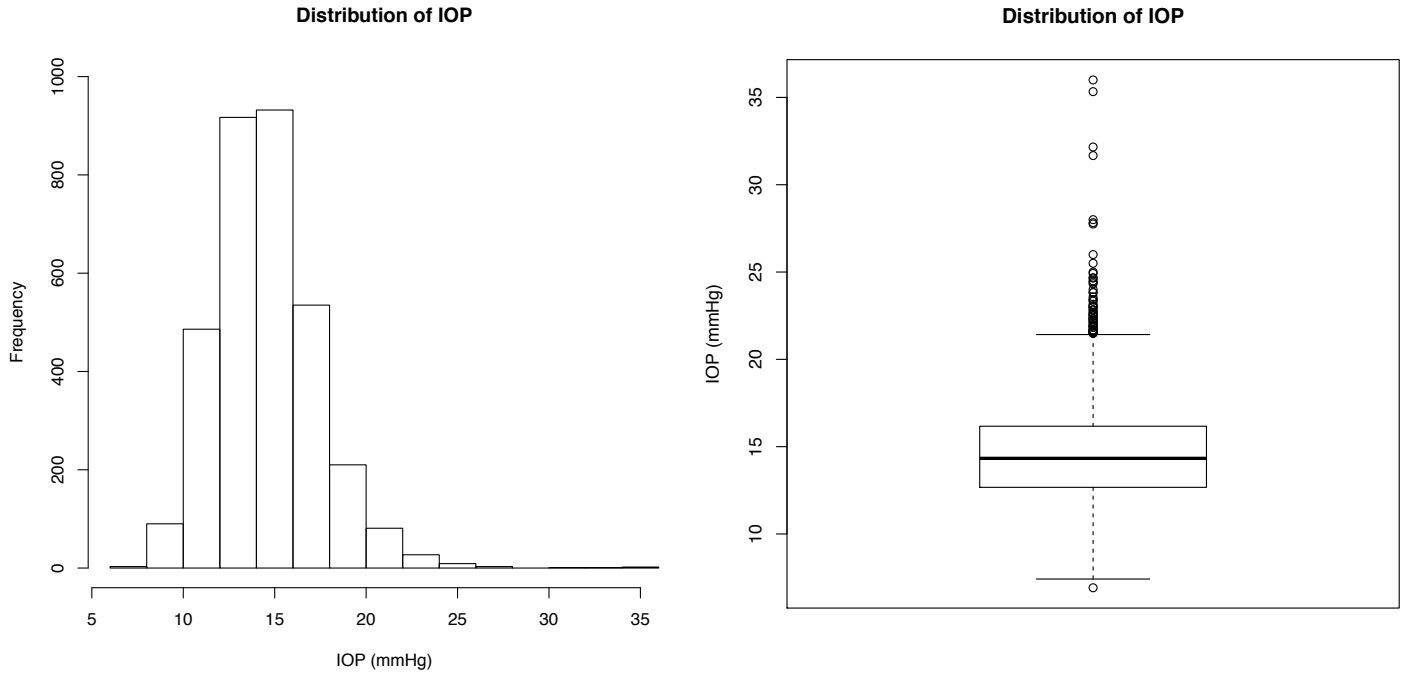

Figure 3. Histogram and boxplot of intraocular pressure measurements.*
*Plots contain all samples with complete phenotypic and genotypic data (n=3,297)

Additionally, age- and gender -specific distributions of IOP are summarized in Table IV. For both genders, compared to the youngest age group, the average IOP for all of the following age groups, stratified by decades, increased. Individuals between the ages of 70 and 79 had the highest IOP, followed by individuals between the ages of 60 and 69. When stratified by gender, females had a higher overall IOP compared to males (P<.001). Furthermore, females had higher IOP for all the age groups, except between the ages of 70 and 79. Unlike individuals included in the study sample, males who were removed from the analysis had a higher mean IOP compared to females, 33.5 (2.59) and 30.35 (3.67), respectively. Overall, the mean IOP increased from 14.08 (2.47) mmHg at the age of 40 to 49 years of age to 14.81 (2.91) mmHg at 80 years of age or older (P<.0001).

Table IV

AGE AND GENDER SPECIFIC DISTRIBUTION OF IOP

	IOP (mmHg), Mean (SD) (n)		
Age, y	Males	Females	Both Genders
40–49	13.91(2.54), 371	14.20(2.41), 539	14.08(2.47), 910
50–59	14.31(2.61), 470	14.74(2.69), 689	14.56(2.67), 1159
60–69	14.44(2.69), 338	15.22(2.88), 469	14.89(2.83), 807
70–79	15.09(2.74), 158	14.95(3.06), 182	15.01(2.91), 340
80+	14.78(3.35), 35	14.82(2.48), 39	14.81(2.91), 74
All ages	14.34(2.66), 1372	14.73(2.72), 1918	14.56(2.70), 3290

B. **Genotypic Analysis**

In order to evaluate the amount of control for population stratification, the genomic control inflation factor and a quantile-quantile plot were generated, shown in Figure 5. The

x-axis represents the expected $-\log_{10}(P \text{ value})$ and the y-axis represents the observed $-\log_{10}(P \text{ value})$. After adjusting for age, gender, and the first four principal components of ancestry, the genomic control was 1.02, suggesting that the inflation of test statistics due to population stratification was well controlled. The observed test statistics do not deviate from the line, except at the extreme right tail, further verifying proper control of population stratification and that the SNPs at the tail represent true associations.

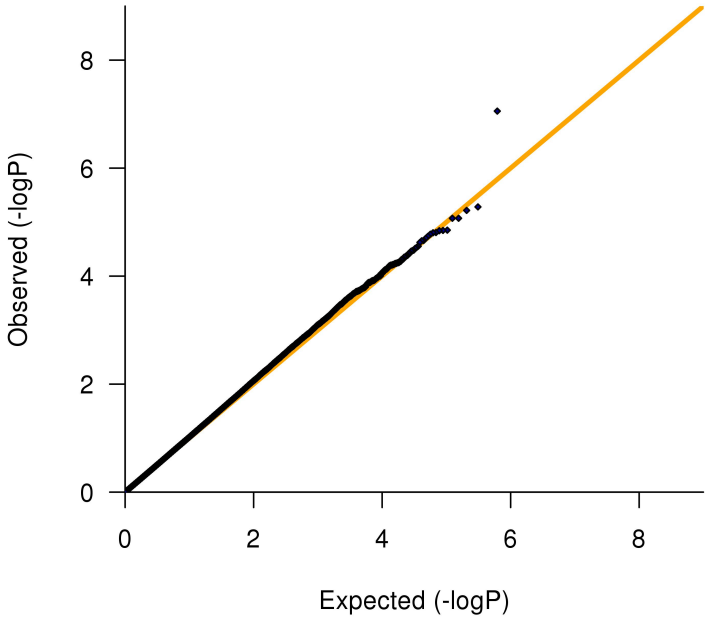

Figure 4. A Quantile-Quantile plot of the –log (*P* values).

A Manhattan plot of the genome-wide *P* values is presented in Figure 5. The x-axis shows the chromosome numbers and y-axis shows the $-\log_{10}(P \text{ value})$. In this analysis, no

SNP reached the traditional genome-wide significance cutoff of 5×10^{-8}. There were 5 SNPs that were suggestive of an association at the 1×10^{-5} cutoff. The top SNPs associated with IOP are summarized in Table V.

Table V

TOP SNPS ASSOCIATED WITH INTRAOCULAR PRESSURE

Chr	SNP	Position	Effect Allele	Beta	*P* value
6	rs276550	137401777	C	-0.30	6.07×10^{-6}
8	rs4738128	72245087	C	-0.41	8.49×10^{-6}
15	rs12591689	101981375	T	0.31	5.23×10^{-6}
16	rs2303218	66972968	C	0.53	8.49×10^{-6}
21	21q21.2	-	T	1.33	8.80×10^{-8}

Chr, Chromosome

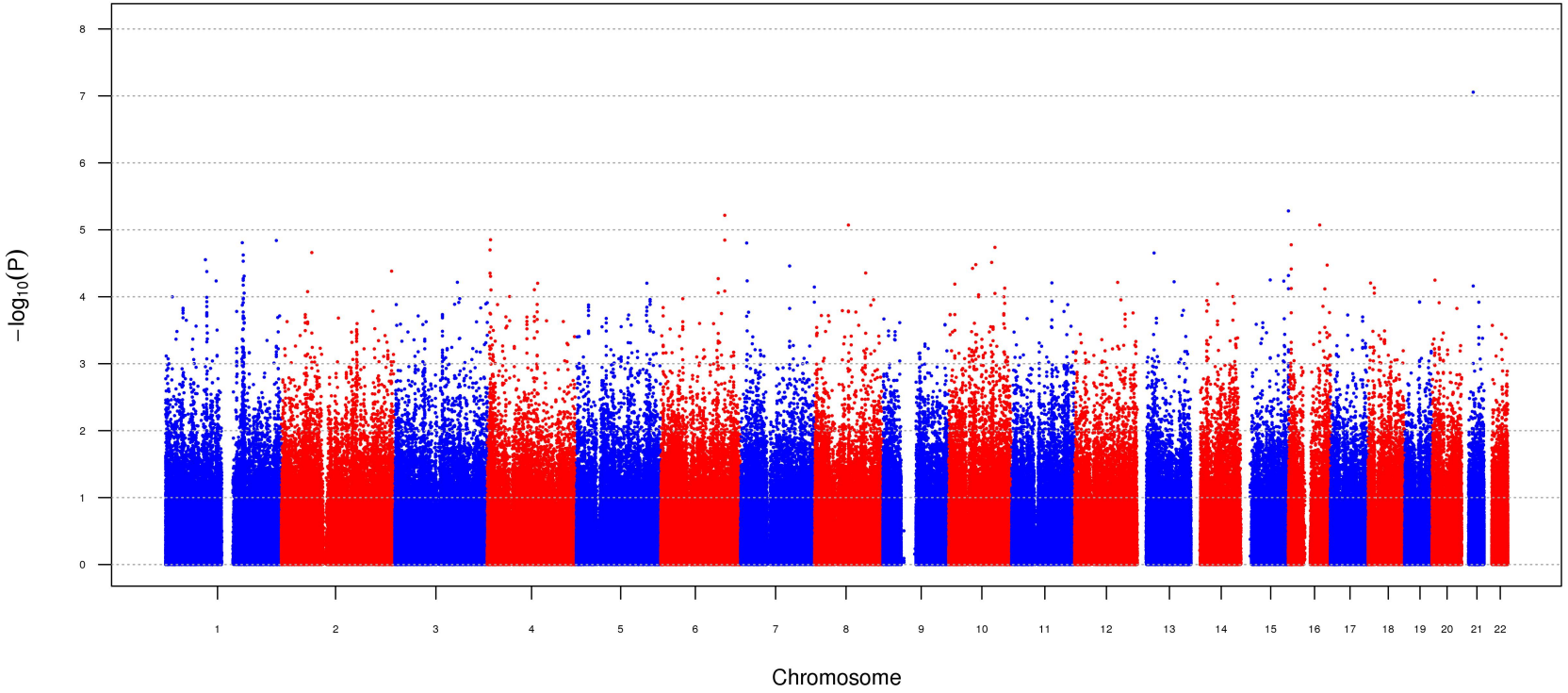

Figure 5. A Manhattan plot of the 619,712 genotyped SNPs from 3,290 individuals.

The top SNP is located at 21q21.2 (P=8.80×10^{-8}) on chromosome 21. The effect allele T of this SNP was associated with 1.33 mmHg increase in IOP for each allele. The SNP located at 21q21.2 is an intergenic SNP that is flanked by two genes. The *TUBAP* gene is located 1.66 million base pairs upstream of 21q21.2 and *VN2R20P* is located 1.16 million base pairs downstream. The second most significant SNP was rs12591689 (P=5.23×10^{-6}, hg19 position 101981375), located on chromosome 15. The effect allele T of rs12591689 was associated with a 0.31 mmHg increase in IOP per allele. rs12591689 is located in the intron region of *PCSK6*. The third most significant SNP was rs276550 (P=6.07×10^{-6}, hg19 position 137401777) on chromosome 6. For each copy of the C allele at this position, there was an associated 0.30 mmHg reduction in IOP. This SNP is located in an intergenic region, bordered by two genes. The SNP rs276550 is located 367kb downstream of *IL20RA* and 258kb upstream of *IL22RA2*. The fourth most significant SNP associated with IOP was rs2303218 (P=8.485×10^{-6}, hg19 position 66972968), located on chromosome 16. The effect allele C was associated with 0.53 mmHg increase in IOP per effect allele. The SNP rs2303218 is located in the intron region of *CES2*. And the last top SNP was rs4738128 (P=8.494×10^{-6}, hg19 position 72245087) on chromosome 8. For each of the C allele at this position, there was an associated 0.41 mmHg reduction in IOP. The SNP rs2303218 is also located in the intron region of *EYA1*.

While the previously reported SNPs were neither genome-wide significant nor suggestive SNPs for the association with IOP, two of the previously reported SNPs were able to be replicated, using a P value cutoff of 0.0083. That is, the traditional significance cutoff, 0.05, divided by the number of previously reported SNPs in the current GWAS, 6. The beta coefficients and P values were extracted from the dataset and summarized in

Table VI. Of the 16 previously reported SNPs, 6 SNPs were directly genotyped in this sample. The most significant SNP (P=3.09 ×10^{-4}), rs7518099 on chromosome 1, was associated with a 0.32 mmHg increase in IOP for each copy of the C allele. Additionally, the second most significant SNP on chromosome 9, rs8176743 (P=4.62 ×10^{-3}), was associated with a 0.39 mmHg increase in IOP per copy of the T allele. Furthermore, the effects of these SNPs are in the same direction of the effects as previously reported, providing further support of true associations.

Table VI

REPLICATED SNPS FROM PREVIOUS STUDIES

Chr	SNP	Position	Effect Allele	Beta	*P* value
1	rs7518099	165736880	C	0.33	3.09×10^{-4}
3	rs9841621	18409077	G	-0.083	0.49
5	rs216146	149445921	C	-0.033	0.61
9	rs8176743	136131415	T	0.39	4.62×10^{-3}
11	rs747782	47940925	C	0.054	0.55
16	rs1826598	77572955	A	0.20	0.01

Chr, Chromosome

V. DISCUSSION

A genome-wide association study was conducted on 3,290 Latinos from the LALES on IOP. To date, this is the first GWAS performed to investigate an association between IOP and SNPs in a Latino population. Glaucoma is a leading cause of blindness, affecting 70 million individuals worldwide. Glaucoma is a group of heterogeneous diseases that are characterized by glaucomatous optic nerve damage and visual field defects and can cause irreversible blindness if left untreated. Primary open angle glaucoma represents a type of glaucoma in which the angle inside the anterior chamber is open, yet there is a reduction in drainage. Considered to be the "sneak thief of sight," POAG does not present any obvious symptoms early in the course of the disease. By the time an individual becomes cognizant of any visual impairments, the disease may already be in the advanced stages and irreversible damage to the optic nerve may have occurred.

Studies have predicted that the prevalence of POAG is expected to increase in the future. Given these speculations, in combination with a large aging population, POAG represents a significant public health issue. Because there is no known cure for POAG, routine eye examinations and identification of risk factors associated with POAG will enable early detection of this disease and potentially the avoidance of blindness. Epidemiological studies have identified IOP as one of the main risk factors for POAG. Most notably, elevated IOP increases an individual's risk of developing POAG. While not all individuals with elevated IOP develop POAG, IOP is the only modifiable risk factor and medications used to treat POAG are targeted to lower IOP. Due to the interconnectedness between POAG and IOP, exploring the genetic architecture of IOP may help to shed light on the genetic determinants of POAG.

The identification of genetic factors associated with certain phenotypes has been a source of scientific interest for centuries. Beginning with Gregor Mendel, the basic understanding of heredity using pea plants slowly evolved into our understanding of the human genome. Segregation analysis enabled researchers to determine the pattern of inheritance, that is, whether a disease was autosomal dominant or autosomal recessive. These analyses led to the understanding that some diseases have genetic components. Linkage analysis then allowed scientists to determine the chromosomal location of a disease causing gene. Despite these achievements, these analyses were only successful at mapping genes that follow a Mendelian inheritance pattern, that is, single-gene disorders. Segregation and linkage analyses were less successful in identifying genetic loci that are associated with complex diseases due to the small-effect sizes of the individual genetic determinants. With the discovery of DNA and subsequently the completion of the Human Genome Project, researchers were able to test for associations between a disease and individual genetic markers. Genome-wide association studies can now allow researchers to investigate the entire genome for possible associations of a phenotype with individual nucleotide polymorphisms.

Departing from chromosomal regions as the main genetic variant unit, the modern unit is a SNP. These may have a biological role in disease pathogenesis by affecting coding regions of genes, transcriptional bindings sites, and mRNA regulation. Compared to the genetic variants that are associated with Mendelian traits, SNPs are considered to be common variants of the human genome. In order to study complex diseases, diseases that are influenced by multiple genes with small-effect sizes, researchers use SNPs to uncover genetic associations with these diseases or other quantitative traits.

Early studies have identified genetic components in the pathogenesis of POAG. The prevalence of POAG was higher among siblings of individuals with the disease compared to siblings without the disease, 10.4% and 0.7%, respectively (Wolfs et al., 1998). Additionally, several genes have been identified to increase an individual's risk of POAG, including *MYOC*, *OPTN*, and *WDR36*. These genes, however, only account for 5% of POAG cases, suggesting additional genetic determinants may contribute to the occurrence of the disease. To further elucidate the genetic architecture of POAG, known endophenotypes of this disease, including IOP, have been studied to evaluate genetic variants that are associated with these quantitative traits that may increase an individual's risk of POAG.

In the present study, five SNPs were determined to be suggestive of an association with IOP at a cutoff of $P<1\times10^{-5}$. The most significant SNP, located at 21q21.2, is in an intergenic region of chromosome 21. Both of the genes flanking the SNP at 21q21.2, *TUBAP* (tubulin alpha pseudogene) and *VN2R20P* (vomeronasal 2 receptor 20 pseudogene), are pseudogenes. A pseudogene is a defunct genomic locus that is similar to functional genes in regard to sequencing but has had a mutation, such as a frame shift or a premature stop codon, that results in a nonfunctional gene (Pei et al., 2012). Pseudogenes can be classified into three categories: processed, duplicated, and unitary. Processed pseudogenes are created by retrotransposition, where mRNA is reverse transcribed and inserted into the genome, and lacks introns. Duplicated pseudogenes are genes that are duplicates of functional genes and unitary pseudogenes are unprocessed, functional genes that accumulate spontaneous mutations.

While pseudogenes are nonfunctional coding sequences of former genes, recent evidence suggests that some pseudogenes are functionally active and may play a role in the

pathogenesis of many diseases. One study investigated *PTEN*, a tumor suppressor where a monoallelic mutation is prevalent at cancer presentation and dialleleic mutation is associated with more advanced cancers (Poliseno et al., 2010). The pseudogene *PTEN1*, a processed pseudogene of *PTEN*, is highly homologous with *PTEN*. A set of micro-RNAs (miRNAs), were used to assess the effect of these miRNAs on both *PTEN* and *PTEN1*. Those miRNAs targeted at *PTEN* suppressed both *PTEN* and *PTEN1* and in both normal and prostate cancer tissues, both were directly correlated, suggesting that both may be co-regulated. Additionally, overexpression of *PTEN1* resulted in a derepression of *PTEN* transcript and protein. Consequently, the pseudogene *PTEN1* acted as a "decoy" for *PTEN* by binding to miRNAs and therefore, avoiding repression of *PTEN*. Although pseudogenes do not directly code for a protein that is related to a disease, these reported genes may have a biological effect on protein coding regions that share identical sequences. With regard to IOP, *TUBAP* and *VN2R20P* may not directly regulate IOP but further research is needed to determine whether these pseudogenes affect other genes. As such, caution must be given when investigating the role of pseudogenes on disease, and with sequencing becoming more affordable, elucidating the function of these pseudogenes may help further shed light on the genetic architecture of complex human diseases (Pink et al., 2011).

The second most significant SNP, rs12591689, is located in an intron of *PCSK6*, proprotein convertase subtilisin/kexin type 6. A recent GWAS reported an association between *PCSK6,* also known as *PACE4*, and handedness in individuals with dyslexia (Scerri et al., 2011). Additionally, *PCSK6* plays a regulatory role in the formation of the anterior central nervous system of right and left, as well as left and right axis formation, with mutations in these resulting in situs ambiguous, left pulmonary isomerism, or complex

craniofacial malformations (Constam & Robertson, 2000). Though the direct biological relevance between *PCSK6* and IOP remains unclear, further research is needed to determine if proteins coded by *PCSK6* are present in or have an effect on ocular tissue and whether or not *PCSK6* directly regulates IOP.

The third most significant SNP, rs276550, is located between *IL20RA* and *IL22RA2* on chromosome 6. The *IL20RA* gene, interleukin 20 receptor alpha and also referred to as *IL-20R1*, encodes a protein that is a subunit for the cytokine receptor of interleukin 20, a main component of epidermal function. The role the immune system has on the pathogenesis of POAG, most notably inflammatory factors, has been investigated by several recent studies. Specifically, the functions the aqueous humor has on maintaining a normal homeostatic environment has been of interest in measuring various cytokine concentration levels. Most notably, IL-8 levels in the aqueous humor were significantly higher in patients with POAG compared to patients with cataracts (Kuchtey, Rezaei, Jaru-Ampornpan, Sternberg, & Kuchtey, 2010; Takai, Tanito, & Ohira, 2012). Furthermore, one study found that IL-8 concentrations were significantly higher among individuals with severe visual field defect compared to individuals with mild visual field defect (Kuchtey et al., 2010). Additionally, IL-8 concentrations in the aqueous humor were positively correlated with IOP (Takai et al., 2012). Even though the exact mechanism between IOP and cytokines is not well understood, the correlation and concentrations of cytokines in ocular tissue suggest that inflammation may play a role in IOP and the development of POAG. One explanation proposes that elevated IOP may disrupt the blood-aqueous barrier, permitting inflammatory cells to enter and results in increased concentrations of these cytokines in the aqueous humor (Huang et al., 2014).

While not as studied in regards to concentration levels in ocular tissue, the involvement of *IL20RA* in other chronic inflammatory diseases has been examined. The SNPs in this gene were analyzed in 254 individuals diagnosed with plaque psoriasis and 224 healthy controls (Kingo et al., 2008). A haplotype block, a group of alleles that are clustered and are often inherited together, within *IL20RA* was associated with psoriasis with an odds ratio of 3.14 (95% CI: 1.61–6.14). Additionally, another study was conducted to examine the expression of IL-20 receptors in trabecular meshwork cells (Keller et al., 2014). Western immunoblotting revealed that IL-20 receptors, both IL-20R1 and IL-20R2 were detected in trabecular meshwork and were up-regulated when cytokine treatment with IL-20 was applied for 24 hours. Furthermore, immunolocalization localized IL-20R1 and IL-20R2 to the cell membrane and cytoplasm of trabecular meshwork cells. The identification of receptors embedded in the trabecular meshwork, as well as a previous association with another chronic inflammatory disease, may enable researchers to develop treatments that target these receptors to potentially effect IOP and the pathogenesis of POAG.

The second gene that rs276550 is flanked by is *IL22RA2*, interleukin 22 receptor alpha 2. This gene codes for a soluble protein that binds to and inhibits interleukin 22, IL-22, activity (W. Xu et al., 2001). Similar to IL-20, IL-22 is a cytokine that plays a significant role in tissue responses during inflammation. Additionally, IL-22 is highly expressed in other chronic inflammatory diseases, such as psoriasis, inflammatory bowel disease, and rheumatoid arthritis (Zenewicz & Flavell, 2011). Ulcerative colitis, a type of inflammatory bowel disease, is a chronic inflammation in the lining of the large intestine. A recent GWAS performed in 1,052 individuals with ulcerative colitis and 2,571 controls, all of European

ancestry, discovered an association between a SNP near *IL-22* and ulcerative colitis (P=2.5×10^{-12}) (Silverberg et al., 2009). The association of *IL-22* with several chronic inflammatory diseases in various tissues in the body illustrates the role *IL-22* has in inflammatory response and is suggestive that *IL-22* may be associated with other inflammatory diseases. Furthermore, the biological interaction between *IL-22* and *IL-22RA* suggests that *IL-22RA* may play a direct role in the pathogenesis of these inflammatory diseases and potentially other diseases, including POAG.

The fourth most significant SNP, rs2303218, is located on chromosome 16, in an intron of *CES2*, carboxylesterase 2. Carboxylesterase is a family of enzymes that are responsible for the metabolism of lipids and drugs. This gene encodes for a protein that is a major enzyme in the metabolism of drugs in the intestine, including aspirin, cocaine, heroin, irinotecan, and 6-acetylmophine (Williams, James, & Roberts, 2014). The degree to which medication and drugs impact POAG and IOP has been investigated by several studies. A double-blinded randomized control trial, with a crossover phase, was conducted to determine if IOP is decreased in patients with ocular hypertension and glaucoma (Linden & Alm, 2000). A total of 28 patients with either glaucoma or hypertension were included in the study and had their IOP taken every 20 minutes for two hours over two succeeding days. On the second day, the participants were given either 500 mg of aspirin or a placebo 15 hours before the second session of measurements. This two-day procedure was repeated again after a minimum of 13 days. After the second round, there were no significant differences in IOP between the placebo-treated and the aspirin-treated groups. Despite the conclusion of the researchers that aspirin does not affect IOP, this study

provided only a single dose of aspirin and thus, does not study the cumulative effect of long-term use of aspirin.

Apropos to the previous study, a case-control study of 7,334 individuals was conducted to determine whether the use of statin and other anti-hyperlipidemic medications were associated with POAG (McGwin et al., 2004). The researchers discovered that longer duration of statin use was associated with a lower POAG risk (*P*=.04) with individuals who took statins for 24 months or more having a 0.60 (95% CI: 0.39–0.92) times reduced odds of POAG compared to those who did not use statins. A third study, conducted using 7,093 participants from the European Prospective Investigation into Cancer-Norfolk Eye Study, investigated whether the use of medication was associated with IOP (Khawaja et al., 2014). Initially, the researchers determined that participants taking beta blockers, nitrates, statins, or aspirin had a lower IOP average than participants not taking any of these medications. Additionally, after adjusting for possible confounders, including age, gender, BMI, and blood glycosylated hemoglobin level, all of these medications remained significant. When these four medications were included into the same model, only beta blockers remained significantly associated with IOP. Further investigation enabled researchers to determine that lower IOP associated with aspirin and statins was explained by the concurrent use of beta blockers. While these studies provide evidence to support that aspirin is not associated with POAG or IOP, *CES2* may still play a role in the metabolism of other drugs and lipids that could affect either IOP or POAG. Additionally, the previously reported studies focused on one of several drugs that *CES2* processes and further studies are needed to determine whether or not this gene may play a

role in IOP homeostasis or the development of POAG through the process of drug and lipid metabolism.

The fifth, and last, most associated SNP was rs4738128, located on chromosome 8 in an intron of *EYA1*. The *EYA1* gene, eyes absent homolog 1, is a human homolog of Drosophila eyes absent gene and has been reported to be the causal gene of branchio-oto-renal syndrome, a genetic condition that results in malformations of the ears, kidney, and neck (Abdelhak et al., 1997). Mutations in *EYA1* have also been detected in individuals with congenital cataracts and ocular anterior segment anomalies (Azuma, Hirakiyama, Inoue, Asaka, & Yamada, 2000). Tissues that maybe affected due to anterior segment anomalies include the cornea, iris, lens, ciliary body, and trabecular meshwork (Gould & John, 2002). Abnormalities in these tissues may affect the production of aqueous humor or drainage structures. Depending upon the severity of these defects, changes in the drainage structures may result in elevated IOP and thus, aid in the development and progression of POAG. Further research is needed to elucidate the association between *EYA1* and IOP.

The reported associated SNPs and the genes that they are located within or near, may offer biological insight into the development of POAG. Most notably, SNPs rs276550, located between *IL20RA* and *IL22RA2*, rs2303218, located in an intron of *CES2*, and rs4738128, located in an intron of *EYA1*, appear to have relevant biological mechanisms that may influence the risk, development, progression, or treatment of POAG. These genes are candidate genes for further investigation, such that these genes may have an etiological role of a disease or trait. In contrast to GWASs, which scan the entire genome, candidate-gene studies is a more focused approach where genotyping is performed in selected genes with multiple SNPs in a gene. If the results from this study confirm an association with the

trait, researchers may also decide to conduct a fine-mapping study. Compared to both GWASs and candidate-gene studies, where associated SNPs localize a genetic marker of the genome that is either directly or indirectly associated with a given trait, fine mapping attempts to identify the exact location of the genetic variant (Foulkes, 2009). Knowing precisely where a genetic variant is located reduces the possibility of a false positive, due to fewer SNPs, as well as having more concise results.

Associations between SNPs and traits by GWASs have improved the scientific community's knowledge of the genes that contribute to complex diseases. While identifying a significant SNP related to a trait has helped scientists understand the genetic basis of many diseases, some criticize the usefulness of GWAS results and comment that most of the SNPs identified do not have any biological relevance to disease, and have no clinical utility (McClellan & King, 2010). Despite such remarks, it has become more apparent that most complex diseases occur due to the cumulative effect of genes, some of which act together to perform a biological function. As such, a transitional shift occurred that sought to obtain a deeper biological understanding of GWAS results with respect to how associated SNPs mapped to genes interacted with other genes. By looking at sets of genes that are known to be involved in a certain biological function, instead of individual genetic variants, SNPs that do not reach the traditional genome-wide significance cutoff could still influence the etiology or development of a disease. The collapsing of SNPs into genes can result in stronger associations with the phenotype compared to individual genetic variants (Mooney, Nigg, McWeeney, & Wilmot, 2014). In turn, more biological information can be extracted from GWAS results. The process of mapping SNPs to genes and genes to biological pathways is commonly referred to as pathway analysis.

For pathway analysis, SNPs are first mapped to genes. One commonly used program for such a task is Versatile Gene-Based Association Study, VEGAS (J. Z. Liu et al., 2010). By using the individual χ^2 statistics from each SNP, VEGAS calculates gene-based *P* values within a gene and predefined gene boundaries, e.g., ± 50 kilobases (kb) of a gene. Once the SNPs have been mapped to genes, gene sets, lists of either manually or automatically curated collection of genes known to be associated with a biological function, can be tested. The most common gene sets are biological pathways. A biological pathway is a list of genes, and their products, which interact with each other to perform a specific function (Mooney et al., 2014). By comparison, gene ontologies are another form of gene sets in which the database attempts to describe gene function into three hierarchical categories: molecular functions, cellular components, and biological processes. These categories group genes that are associated with similar molecular function, cellular component, or biological process. These groupings, however, do not take into account relationships between genes or their interactions (Mooney et al., 2014). The latter form of pathway analysis is limiting due to the fact that genes associated with a similar function may have different biological endpoints in the human body. In addition to these forms of gene sets, network analysis is another commonly used downstream analysis method to understand the biology of a group of genes. Unlike pathway analysis, where a group of genes interact with each other in a manner that performs a biological function, and gene ontologies, where genes are grouped together that are associated with a similar function, biological networks do not specify a particular biology function or process. Networks describe biological relationships between genes and gene products (Mooney et al., 2014). Although each method differs slightly with regard to the type and quality of information, these methods attempt to further the

knowledge and information that can be extracted from GWAS results. The SNP data from GWASs have been successful in identifying genetic variants associated with diseases, and pathway and network analyses are methods that enable researchers to further uncover the biological mechanisms that drive the development and progression of disease.

A. **Strengths**

There were several strengths in this study. First, this study used the largest population of genotyped Latinos with ocular data available. Secondly, population stratification was properly accounted for in the current study. Latinos are typically an admixture of African, European, and Native American ancestries, and given the differences in POAG prevalence and IOP among various ethnic populations, taking into account genetic ancestry is crucial to avoid false positives. The third strength was that this study was able to replicate previously reported SNPs that have been associated with IOP. While replication studies are usually conducted in the same population, the replication of these SNPs suggests that some of the genetic variants associated with IOP similarly affect this phenotype across different ethnic populations. Fourth, the data for this analysis, both the genotype and phenotype data, went through vigorous quality control in order to ensure the data were clean of any genotyping errors and phenotype recording errors, thus providing valid and robust results. The fifth and last strength is that several of the associated SNPs identified in this study have strong biological relevance that are suggestive of a probable role in the elevated IOP, and potentially the pathogenesis of POAG.

B. **Weaknesses**

This study, however, is not without limitations. The first limitation of this study is that this study did not include rare variants. The chip used in the studies analyzed common variants, and thus rare variants associated with IOP were not included in the study. A second drawback to this study is it did not include a replication set. In order to reduce the number of both false positives and false negatives, and at the same time increase the number of true positives, a replication set is often used. Thirdly, no expression analysis was performed for the top genetic locus. Recently, GWASs have performed expression analysis, in which investigators examine the expression of transcripts surrounding an identified genetic locus in a related tissue (Gao et al., 2013). Through such analysis, robust biological evidence further supports the statistical findings in a GWAS. And lastly, the SNPs analyzed in this study represent only a small proportion of the entire human genome. As such, further studies are needed to examine other parts of the genome not previously studied to identify additional genetic variants associated with IOP.

C. **Conclusion**

This study represents the first genome-wide association study conducted of Latinos for IOP. While further studies are needed in order to replicate these findings, this study confirms similar genetic variants that are associated with IOP that span different ethnic populations, and has identified potentially new genetic variants that are unique to the Latino population. As the only modifiable risk factor for POAG, understanding and identifying genetic variants that are associated with IOP may provide insight into the genetic architecture of POAG.

The role of human genetics has many implications in the field of public health. Genetics can help in the role of primary prevention by identifying individuals who have a genetic variant that may increase their risk of developing a disease. Furthermore, understanding interactions between genes and the environment will help to stratify individuals based on environmental exposures which may increase their risk of a disease. In such situations, avoiding known environmental exposures, and altering modifiable lifestyles, will enable individuals to reduce their risk of disease. Additionally, genetic variants may serve as diagnostic tests for early detection of disease for secondary prevention. For example, a biomarker maybe developed for IOP or POAG that can be tested in a blood draw to determine the presence of either phenotype and proper medical treatment can be given, such as the use of pressure-lowering eye drops. And lastly, gene therapy and tailored medication, specific to an individual's genome, maybe explored as potential tertiary prevention strategies.

While POAG is an irreversible blinding disorder that affects millions of people each year, it is preventable through early detection and through the lowering of elevated IOP. By understanding the human genome, researchers will be better able to dissect the role genetics has on human disease. However, to fully translate genetic discoveries into medical and public health use, the collaboration of epidemiologists, statistical geneticists, environmental and occupational researchers, health policy and administration workers, community health science workers, and physicians are required to gather and analyze the data, to implement changes in current work conditions and policies, to establish programs to educate the public, and to provide medical care and treatment. The findings from this

study may help to shed light on the pathogenesis of POAG and to be an initial step in preventing irreversible blindness.

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VITA

NAME: Drew Robert Nannini

EDUCATION: B.A., Chemistry, University of Illinois at Champaign-Urbana, Urbana, 2011

M.A., Bioethics, Kansas City University of Medicine and Biosciences, Kansas City, 2012

HONORS: 19th Summer Institute in Statistical Genetics Scholarship, 2014

PUBLICATIONS: Aghababaei N, Wasserman J, Nannini D. (2014). The religious person revisited: cross-cultural evidence from the HEXACO model of personality structure. *Mental Health Religion & Culture*. 17(1): 24-29.

Wasserman J, Aghababaei N, Nannini D. (2015). Culture, Personality, and Attitudes Toward Euthanasia: A Comparative Study of University Students in Iran and the United States. *OMEGA – Journal of Death and Dying*. 0(0): 1-24.