Identifying Genes and Loci for Complex Diseases: Examples from Primary Open Angle Glaucoma and Schizophrenia

by

Jennifer Abigail Woodroffe

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Epidemiological Science)
in The University of Michigan
2009

Doctoral Committee:

Professor Julia E. Richards, Chair
Professor Michael L. Boehnke
Professor Stephen B. Gruber
Professor Patricia A. Peyser
Associate Professor Gonçalo Abecasis

© Jennifer Abigail Woodroffe 2009

Acknowledgements

I would like to thank all the researchers with whom I collaborated. This dissertation would not have been possible without their contributions.

Chapter 2: Gonçalo Abecasis, Joseph Gogos, Maria Karayiorgou, Laura Lundy, and Louw Roos

Chapter 3: Michael Boehnke, Catherine Downs, William Duren, Nobuo Fuse, Charles Krafchak, Paul Lichter, Sayoko Moroi, Julia Richards, and Robert Schertzer

Chapter 4: Brenda Gillespie, Paul Lichter, David Musch, Leslie Niziol, Julia Richards, Kathleen Scott, and the CIGTS Group

Chapter 5: Gonçalo Abecasis, William Bromley, David Burke, Virna Jaramillo-Babb, Jenae Johnson, Theresa Kijek, Paul Lichter, Sayoko Moroi, Christine Ntim-Amponsah, Ebenezer Obeng-Nyarkoh, Julia Richards, Frank Rozsa, Kathleen Scott, Marty Soehnlen, Douglas Vollrath, Alyce Whipp, and Jodi Wilkowski

I would also like to recognize my committee members for their helpful comments and suggestions. I would especially like to thank my advisors: Michael Boehnke, who was a big part of the reason I came to Michigan and who was never too busy for a chat; Gonçalo Abecasis, who was my advisor during my Masters program when I had to learn how little I knew and who continued to let me pester him throughout my PhD; and Julia Richards, my chair, who never failed to listen to my concerns or suggestions, answer my questions, and inspire me to accomplish my goals.

Lastly, I must thank my family and friends who are near and far; they have all supported me throughout this process. Most notably I would like to thank Charles Krafchak, with whom I wish I could have co-authored every paper, you will be missed; Tory Hendry, my roommate of two years who has been amazingly supportive and encouraging; and, of course my husband, Daniel Zitnick, whose patience and understanding has been unwavering, and who always knows when and how to make me laugh.

Table of Contents

Acknowledgements ii
List of Figures iv
List of Tables v
List of Abbreviations vi
Abstract viii
Chapter
1. Introduction 1
   Background and significance 1
   Primary open-angle glaucoma 2
   Schizophrenia 8
   Overview 13
2. High-Density Scan with 1980 Microsatellite Markers Highlights Linkage of Schizophrenia to 13q34 and 1p36 in Afrikaners 14
   Abstract 14
   Introduction 15
   Methods 17
   Results 23
   Discussion 32
3. Ordered Subset Analysis Supports a Glaucoma Locus at GLC1I on Chromosome 15 in Families with Earlier Adult Age at Diagnosis 36
   Abstract 36
   Introduction 37
   Methods 41
   Results 44
   Discussion 47
4. Lack of Association between Glaucoma Severity and Myocilin (MYOC) Promoter SNPs in CIGTS Subjects 52
   Abstract 52
   Introduction 53
   Methods 58
   Results 61
   Discussion 71
5. Conclusions 74
   Summary of findings 74
   Ongoing study 76
   Future directions 92
Bibliography 95

List of Figures

1-1 Anatomy of the eye 3
1-2 Anterior chamber angle of the eye 3

2-1 Plot of mean identity-by-state by standard deviation identity-by-state for a pair of individuals from the same family 19
2-2 Genome-wide information content 24
2-3 MOD score analysis 26
2-4 Principle component analysis of Afrikaners as compared to HapMap YRI, CEU, and JPT+CHB analysis panels 30
2-5 Plot of peak LOD (or MOD) scores and 1-LOD (or 1-MOD) intervals for the four papers reporting LOD (or MOD) scores greater than 2.0 on 13q32-34 .. 33

3-1 (A) Nonparametric LOD scores, for the OSA subset (earlier AAD) (solid line) and LOD scores before subsetting (dashed line). (B) Information plot, for genotypes for families in the maximum OSA LOD score subset 46

4-1 Predicted haplotypes of MYOC promoter SNPs 63

5-1 Fourteen common SNPs and their positions in LMX1B 85

List of Tables

1-1 Risk factors for primary open-angle glaucoma 6
1-2 Genes and loci implicated in primary open angle glaucoma 7
1-3 Risk factors for schizophrenia 10
1-4 Genes and loci implicated in schizophrenia 12

2-1 Summary of informative families 23
2-2 Parametric multipoint MOD scores >1.5 for either schizophrenia status 27
2-3 Nonparametric multipoint LOD scores >1.5 for either schizophrenia status .. 28
2-4 Parametric linkage to chromosome 1p36 29
2-5 Linkage disequilibrium between *G72* SNPs 31
2-6 Association between schizophrenia and *G72* SNPs 31

3-1 Family-specific characteristics 45

4-1 Comparison of subjects genotyped and not genotyped 62
4-2 Genotype frequencies 63
4-3 Exon 3 non-synonomous mutations 65
4-4 Baseline characteristics of exon 3 variants 66
4-5 Linkage disequilibrium between MYOC promoter and common exon 3 SNPs ………... 67
4-6 Phenotype by -1000CG genotype …... 69
4-7 Average mean deviation across the study period 70
4-8 Average IOP across the study period 71

5-1a Power calculation for 99 African American cases and 105 African American controls 78
5-1b Power calculation for 75 European American cases and 116 European American controls 79
5-1c Power calculation for 101 Ghanaian cases and 105 Ghanaian controls 79
5-2 Primers used to PCR amplify and sequence the coding regions of LMX1B ... 80
5-3 Subjects by affection status and ethnicity 83
5-4 Missing data and Hardy-Weinberg equilibrium (HWE) checks 84
5-5 Minor allele frequencies and comparisons across populations 86
5-6 Pair-wise linkage disequilibrium of common SNPs 88
5-7 Association between POAG and common LMX1B SNPs in individual populations 90
5-8 Association between POAG and common LMX1B SNPs in combined populations 91

List of Abbreviations

AA	African American
Af	Affection status
AG	African Ghanaian
CEPH	Centre d'Etude du Polymorphisme Humain
CEU	CEPH in Utah, United States
CHB	Han Chinese in Beijing, China
CI	Confidence interval
cM	Centimorgans
Cntl	Control
CNV	Copy number variant
CV	Cardiovascular
dB	Decibels
DCV	Disease causing variant
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, version IV
Dx	Diagnosis
EA	European American
ETDRS	Early Treatment Diabetic Research Study
Freq	Frequency
GABA	Gamma-aminobutyric acid
GWA	Genome-wide association
HWE	Hardy-Weinberg equilibrium
IOP	Intraocular pressure
JPT	Japanese in Tokyo, Japan
kb	Kilobase
LOD	Logarithm (base 10) of odds
MD	Mean deviation of the visual field
mmHg	Millimeters of mercury
MOD	Maximum LOD
MYOC	Myocilin
µm	Micrometers
N	Number
NMDA	N-methyl D-aspartate
NPS	Nail patella syndrome
OAG	Open angle glaucoma
OPTN	Optineurin
OR	Odds ratio
OSA	Ordered subset analysis
PCR	Polymerase chain reaction

Pen	Penetrance
POAG	Primary open angle glaucoma
Pval	P-Value
RR	Relative risk
SD	Standard deviation
SE	Standard error
SNP	Single nucleotide polymorphism
Unk	Unknown
UTR	Untranslated region
VA	Visual acuity
WDR36	Wd-repeat36
YRI	Yoruban in Irbid, Nigeria

Abstract

Identifying Genes and Loci for Complex Diseases:
Examples from Primary Open Angle Glaucoma and Schizophrenia

by

Jennifer Abigail Woodroffe

Chair: Julia E. Richards

This purpose of this dissertation is to progress towards identification of disease genes and loci for primary open-angle glaucoma (POAG) and schizophrenia. In studying schizophrenia, we conducted a genome-wide linkage scan in 479 subjects from 129 Afrikaner families; Afrikaners are a founder population from South Africa. Conducting a MOD score analysis, we were able to replicate a schizophrenia locus on chromosome 13q34 in subjects with broadly defined schizophrenia, which includes schizoaffective disorder- depressive subtype and schizoaffective disorder- bipolar subtype (MOD score = 3.76). We also detected a locus on chromosome 1p36 in subjects classified with a more narrowly defined form of schizophrenia that include schizoaffective disorder- depressive subtype, but not bipolar subtype (MOD score = 3.21).

We conducted two separate studies looking for POAG genes and loci. In the first study, we replicated the GLC1I locus on chromosomes 15q11-13. We initially tested 167 European American individuals in 25 multiplex open-angle glaucoma families and

detected a LOD score of 1.01 at 14.3 cM. However when we used ordered subset analysis, we found that by including only the 14 families with earlier average ages at diagnosis (average=50.6 years ±5.4 years), there is evidence for linkage to GLC1I (LOD score = 2.09; p-value = 0.021).

In the third and final study, we tested whether glaucoma severity is associated with variants in the promoter region of a known glaucoma gene, myocilin (MYOC). There have been conflicting reports regarding the relationship between glaucoma severity and the -1000CG MYOC promoter SNP, also designated mt1. This study tested for an association in subjects from the Collaborative Initial Glaucoma Treatment Study (CIGTS); a longitudinal study where the subjects were reevaluated every six months. We found that there was not evidence for an association between -1000CG and visual field mean deviation (p=0.98) or intraocular pressure (p=0.52) across the study period in the CIGTS population. There was also no evidence of association between two other MYOC promoter SNPs, -1075GA and -1081AG, and mean deviation or intraocular pressure.

In describing these studies, this dissertation illustrates methods and techniques that can be applied to the study of the genetics of other complex diseases.

Chapter 1

Introduction

BACKGROUND AND SIGNIFICANCE

Genetic studies have identified causal mutations of many simple Mendelian disorders and the focus is shifting to understanding the etiology of complex diseases[1; 2]. Complex diseases are not caused by a single genetic variant, rather they are the result of interactions between multiple genetic and/or environmental factors and it is likely that different combinations produce the same clinical symptoms. It is possible that a common allele increases susceptibility to the disease in many people[3; 4] or there may be many rare variants in the same gene[5] or in different genes[6; 7]. Unfortunately, we typically do not know the true disease model in an individual or in a population[8]; therefore, identifying these variants has been challenging[9; 10]. Complications for studying complex diseases include: locus and allelic heterogeneity, incomplete penetrance, phenocopies, and interactions between genetic (epistatic) and environmental factors[1; 11; 12]. However, complex disease are often severe and may affect a substantial number of people, therefore it is imperative that we understand how to study these diseases.

Since heterogeneity can make elucidating the causal alleles more difficult, methods have been developed to identify a more homogeneous population. One method is to stratify the sample population based on disease-associated characteristics[13]. It may

also be advantageous to stratify the population based on race or to control for race in our models[14]. Race can be determined by self-report, by incorporating information on pedigree structure and affection status[15], or by genotyping unlinked markers to empirically classify individuals into one of multiple populations[16]. Likewise, founder populations may be studied because they are often more genetically and environmentally homogeneous[2; 17; 18]. In the studies described below, we will use these techniques to create more homogeneous populations in which to identify causal variants for open-angle glaucoma and schizophrenia.

PRIMARY OPEN-ANGLE GLAUCOMA

Anatomy of the eye. The outermost layer of the eye is made up of the sclera and cornea. The sclera is sometimes referred to as white of the eye. It encompasses most of the eye posterior to the cornea and helps maintain the shape of the eye. The cornea is the clear, anterior portion through which light passes. Posterior to the cornea, the iris is the colored portion of the eye that adjusts the size of the pupil to regulate the amount of light into the eye. The lens is directly posterior to the pupil and changes shape to focus the light onto the fovea at the back of the eye. The innermost layer of the eye is the retina, the neural layer which converts light energy to nerve impulses. The retinal ganglion cells converge at the optic disc and form the optic nerve. The optic nerve then passes through a connective tissue matrix, the lamina cribrosa, as it leaves the eye before reaching the lateral geniculate nucleus. The anterior chamber of the eye is the region anterior to the iris and the lens, but posterior to the cornea (Figure 1-1). Particularly important in the study of glaucoma is the anterior chamber angle. This is where the iris, sclera, and

cornea meet, and where the aqueous humor drains from the anterior chamber. The structures of the angle include the trabecular meshwork and Schlemm's canal[19].

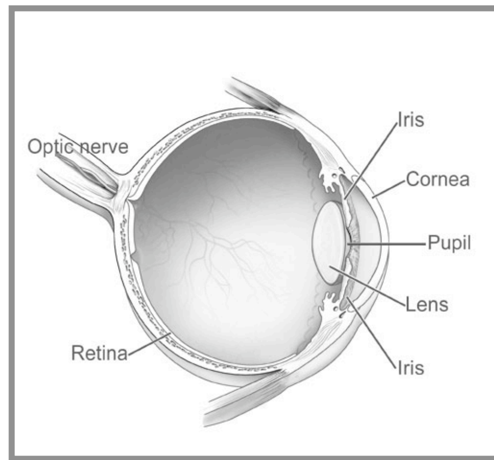

Figure 1-1. Anatomy of the eye.

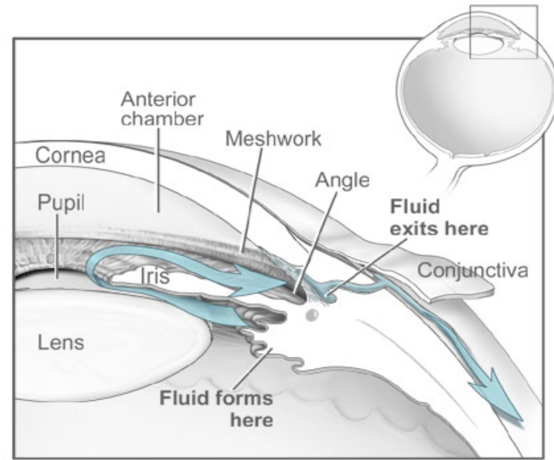

Figure 1-2. Anterior chamber angle of the eye.

Figures courtesy of the National Eye Institute (www.nei.nih.gov)

The aqueous humor nourishes the tissues that are not innervated by the vascular system. It also aids in focusing the light on the fovea. The pressure from the aqueous humor (intraocular pressure) helps maintain the shape of the eye. The humor is produced by the ciliary body and flows between the iris and lens to enter the anterior chamber through the pupil. It exits the chamber through the trabecular meshwork and Schlemm's canal, and is absorbed into the venous system[19; 20] (Figure 1-2).

Definition. Glaucoma affects over 60 million people worldwide and is the second leading cause of blindness worldwide[21; 22]. The prevalence in the United States for individuals over 40 years is almost 2%[23]. Glaucoma is diagnosed based on visual field loss and abnormalities in the optic disc of the retina due to loss of retinal ganglion cells; sometimes intraocular pressure (IOP) is a diagnostic criterion as well. If untreated,

glaucomatous nerve damage may lead to blindness[24]. Primary open angle glaucoma (POAG) is the most common subtype[25] and it occurs when the anterior chamber angle is not physically obstructed and there is no secondary cause of the glaucoma [20].

Pathophysiology. The etiology of glaucoma is not well understood; however the predominant theories suggest damage to nerve fibers caused by mechanical and/or vasculature mechanisms. The mechanical hypothesis suggests that increased IOP causes distortion of the lamina cribrosa. This warping is thought to pinch the nerve cells, causing their death and the characteristic cupping of the optic disc seen in subjects with glaucoma[26; 27].

The vasculature hypothesis suggests that glaucoma is caused by a decrease in blood supply to the retina. This ischemia results in a decrease in ATP, which depolarizes the nerve cell and causes the sodium-glutamate transporter to reverse. The resulting overstimulation of the glutaminergic system is toxic to the cells. The reduced blood flow can be caused by increased IOP or trauma[26-28].

Risk factors. The most consistent risk factors for POAG are older age[29; 30] and increasing IOP[31-33]. The prevalence of POAG for 40-49 year olds in the United States is only about 0.7%, and it increases ten-fold to 7.7% for individuals over 80 years old[23]. Although IOP is an established risk factor, it is neither necessary nor sufficient for development of POAG; 20-40% of patients with POAG have normal ocular pressures (IOP < 22 mmHg)[31; 34; 35] and only 10% of individuals with high IOP will develop POAG within five years[36]. However, lowering IOP slows progression in high-tension[36] and

normal-tension subjects[37]. Increased IOP is also correlated with larger fluctuations in IOP and some studies have suggested that POAG is associated with IOP fluctuation rather than with average IOP[38; 39], but evidence for this is not consistent[40; 41].

POAG prevalence also varies by race, thickness of the central cornea, and family history. People of Asian descent have the lowest prevalence rates of POAG (1-2%), people of European or Hispanic descent have intermediate rates (1.5-3%), and people of African descent have the highest rates (3-6%)[23; 42]. Furthermore, populations of African ancestry have an earlier age at diagnosis and different responses to therapies[43; 44]. Thin central corneas are also associated with POAG in subjects with normal IOP[45] and with high IOP[45; 46]. This association may be partly because the central cornea thins with age[47] and people of African descent have thinner corneas than people of European, Hispanic, or Asian descent[48]. Family history is another significant predictor of POAG[29; 49]. The increased risk for subjects with self-reported family history is estimated as high as nine-fold, compared to subjects without a family history[50]. It has also been suggested that subjects with a positive family history have a more severe disease outcome[51]. Interestingly, siblings appear to be at a higher risk than parents or children of an affected individual[52], possibly due to a more similar environment or gene-gene interactions. It is worth noting that of the five risk factors mentioned above, only IOP is currently modifiable (Table 1-1).

In addition to the generally accepted risk factors, there is suggestive evidence for increased risk of POAG with myopia[53], diabetes[54; 55], systemic hypertension[54; 56], and cigarette smoking[57; 58].

Table 1-1. Risk factors for primary open-angle glaucoma

Not modifiable
Increased age
Thin central corneas
African descent
Family history
Potentially modifiable
Elevated IOP

Genetics. Fourteen named glaucoma loci (GLC1A-GLC1N) and at least two unnamed loci have been mapped[24]; genes have been identified at three of the named loci (Table 1-2). At GLC1A, on chromosome 1q23-q24[59], over 120 different mutations in myocilin (MYOC) have been associated with glaucoma[60-62]. Although primarily resulting in juvenile onset POAG, alterations in MYOC also segregate in families with adult onset cases (age at diagnosis after 35 years). Most MYOC mutations in juvenile-onset glaucoma are missense mutations, while the Gln368stop nonsense mutation is most common among adult-onset families[61; 62]. Expression of MYOC is ubiquitous; it is found in intraocular, extraocular, heart, skeletal, and numerous other tissues. Although the function is still not known, it may be involved in cell-matrix interactions[63; 64]. In transgenic studies, no abnormal ocular phenotype was observed in heterozygous and homozygous null mice[65]. Over-expression of normal Myoc did not result in increased IOP or glaucoma[66], but knock-in mice bearing a known MYOC missense mutation do manifest glaucoma[67]. Therefore, MYOC-associated glaucoma is more likely caused by a gain of function than loss of function[64].

The gene encoding optineurin (OPTN) is located at the GLC1E locus on chromosome 10p15-p14[68]. Mutations in the protein are predominantly linked to normal-tension glaucoma (IOP < 22 mmHg)[69]. OPTN is hypothesized to be a short-lived

secretory protein[70] and is expressed in ocular tissues, including the trabecular meshwork, as well as in non-ocular tissues such as liver, brain, and fibroblast cells[69]. Although the function of OPTN is not clear, it may be involved in neuroprotection[71] since E50K, the most common glaucoma associated mutation in OPTN, has been shown to increase the rate of retinal ganglion cell death[72; 73].

The most recently identified POAG gene is wd-repeat36 (WDR36), located at GLC1G on chromosome 5q22.1[74]. Mutations in this gene segregate in families with the more prevalent adult onset, high-IOP form of POAG and is expressed widely, ocularly and non-ocularly[74]. However, it has been suggested that mutations in WDR36 may not be sufficient to cause glaucoma, rather the mutations may modify the effects of other genes[75]. At present, the function of this protein is not known.

Table 1-2. Genes and loci implicated in primary open angle glaucoma

Chrom	Gene	Locus	Chrom	Gene	Locus
1q23-24	MYOC[60]	GLC1A[59]	7q35-36		GLC1F[76]
2p16-15		GLC1H[77]	8q23		GLC1D[68; 78]
2cen-q13		GLC1B[79]	9q22		GLC1J[80]
3p21-22		GLC1L[81]	10p13	OPTN[69]	GLC1E[68]
3q21-24		GLC1C[82]	15q11-13		GLC1I[83]
5q21-22	WDR36[74]	GLC1G[74]	15q22-24		GLC1N[84]
5q22-32		GLC1M[85]	20p12		GLC1K[80]

It has been estimated that mutations in these three genes account for less than 10% of cases[75; 86; 87]. However, there is debate regarding whether or not all of the reported alleles are causative. A meta-analysis of more than a dozen studies suggests that some of the more prevalent alleles may not be causal[88], so this may be an overestimate.

Linkage and association studies support the hypothesis of further heterogeneity in glaucoma. A genome-wide linkage scan carried out on 182 POAG sibling pairs

identified five additional regions of interest[77]. Also, eight Finnish families with POAG were genotyped at glaucoma loci GLC1A-GLC1F and eight other candidate gene regions. The study did not find evidence for linkage to any of the regions[89]. At least 25 candidate genes have been associated with POAG; however few of these findings have been replicated[71; 90; 91]. A recent genome-wide association scan identified a gene associated with pseudoexfoliative glaucoma, but failed to find a significant association with POAG[92]. Therefore, it is likely that additional genes for POAG remain to be identified.

SCHIZOPHRENIA

Definition. Schizophrenia is a chronic psychological disorder that affects over 50 million people and has an estimated worldwide prevalence of approximately 1%[93-95]. The Diagnostic and Statistical Manual of Mental Disorders, version IV (DSM-IV) defines schizophrenia as a group of associated disorders, the symptoms of which include irregular "thought, perception, affect, behavior, and communication"[96]. Therefore schizophrenia may be a cluster of phenotypically similar disorders each caused by a genetically heterogeneous etiology, rather than a heterogeneous disorder caused by a single mechanism[97; 98]. The effectiveness of treatment for schizophrenia is not consistent across patients and there is no known cure[99].

Pathophysiology. Although there is little definitive knowledge regarding the causal and progressive mechanisms of schizophrenia, there are several hypotheses, all involving abnormal levels of neurotransmitters or hormones. One hypothesis proposes a decrease in the excitatory neurotransmitter glutamate[100; 101]. Administration of ketamine, an

antagonist that decreases neurotransmission of glutamate via the N-methyl D-aspartate (NMDA) receptor, results in increased schizophrenic symptoms in previously affected individuals[102] and schizophrenic-like symptoms in control subjects[103]. Also, a mouse model of schizophrenia has been induced by decreasing expression levels of the NMDA receptor. The schizophrenic behavior of these mice can be decreased by administration of antipsychotic drugs that are used to treat schizophrenia[104]. The decrease in NMDA receptor may result in further dysfunction by decreasing the gamma-aminobutyric acid (GABA) related inter-neurons[105].

Another causal model hypothesizes disruption of the dopamine system[106; 107]. Patients with schizophrenia show an increase in dopamine uptake and have an increased release of dopamine upon inhibition of the dopamine transporter, likely due to increased dopamine synthesis[108]. The effects of drug use also support this hypothesis. Drugs that increase dopamine can elicit schizophrenic-like symptoms in unaffected individuals and can aggravate symptoms in affected individuals. In addition, some anti-psychotic medications used to treat schizophrenia block the dopamine receptor[109].

There is also evidence that damage to the hippocampus due to stress may be associated with schizophrenia. Stress causes an increase in glucocorticoids via the hypothalamus-pituitary-adrenal gland axis that may be harmful. Stress may trigger schizophrenic episodes in previously unaffected individuals and increase the severity of episodes in schizophrenic subjects[109]. Some researchers suggest that schizophrenia is caused by interacting effects from the above systems[110].

Risk factors. The strongest predictor of schizophrenia is having a first-degree relative who is affected. Depending on the nature of the relationship, a first-degree relative with schizophrenia increases the risk 6-17 fold; having a monozygotic twin with schizophrenia increases the risk of schizophrenia nearly 50 times[97; 111]. Further support for a strong genetic role in schizophrenia comes from adoption studies. These studies indicate a four to ten fold increase in the prevalence of schizophrenia in adoptees with schizophrenic birth parents compared to adoptees with unaffected birth parents[112; 113]. There is also evidence for an increased risk for migrants or individuals who live in urban areas, possibly due to increased stress[114]. Other risk factors associated with schizophrenia are related to pregnancy or delivery complications: older paternal age, preeclampsia, maternal infection during pregnancy, and famine during pregnancy[97; 115] (Table 1-3).

Table 1-3. Risk factors for schizophrenia

Not modifiable
Family history
Velo-cardio-facial syndrome (22q11 del)
Older paternal age
Potentially modifiable
Migration
Urbanicity
Preeclampsia
Maternal infection during pregnancy
Famine during pregnancy

Genetics. As indicated above, a positive family history is the strongest predictor of schizophrenia. Numerous genome-wide linkage scans and association studies have identified genes and loci for schizophrenia[116-118], notably disrupted in schizophrenia 1 (DISC1) and trace amine associated receptor 5 (TAAR5/TRAR4) (Table 1-4). DISC1 was originally identified by a balanced translocation between chromosomes 1q42 and

11q14.3 segregating with mental illness in an extended family[119]. Follow-up detected a maximum LOD score of 7.1 on 1q42 in that family[120] and there have been at least six subsequent studies linking schizophrenia to 1q42. Association studies have indicated a possible gender effect; the results are more significant in females[121]. Also, DISC1 knock-out mice have physical and functional characteristics similar to schizophrenics[122].

The region containing TAAR5 was detected in a linkage study of 192 African American and European American families with schizophrenia[123]. A subsequent study identified TAAR5 as the gene of interest and showed that TAAR5 is expressed in regions of the brain where pathological alterations have been identified[124]. However, in replication studies, no association between TAAR5 and schizophrenia was found in either a Japanese[125] or a Han Chinese population[126].

Recently, genome-wide association (GWA) studies have been conducted, using either individual cases and controls[127-129] or pooled samples[130-132]. One study provides considerable evidence for association with ZNF804A at SCZD13[127]; however, there are no overlapping results from these GWA reports. More consistent results have come from looking for genome-wide copy number variants (CNVs). Multiple studies have found an excess of rare CNVs in schizophrenic subjects[133-136].

Table 1-4. Genes and loci implicated in schizophrenia

Chrom	Gene	Locus	Chrom	Gene	Locus
1p36		SCZD12[137]	8p12	NRG1[138]	
1q23	NOS1AP[139]	SCZD9	8p21	PPP3CC[140]	SCZD6
1q42	DISC1[119]		10q22		SCZD11[141]
2q32	ZNF804A[127]	SCZD13	11q14-21		SCZD2[142]
5q11-13		SCZD1[143]	13q32	DAOA[144]	SCZD7
5q33	EPN4[145]		15q15		SCZD10[146]
6p24-22	DTNBP1[147]	SCZD3	18p11		SCZD8[148]
6q23	TAAR6[123]	SCZD5	22q11-13	PRODH[149] COMT[150] ZDHHC8[151]	SCZD4[152]

Founder population. Founder populations are valuable in identifying susceptibility loci for complex diseases as they are often more genetically and environmentally homogeneous[17; 18]. Also, they typically show increased levels of linkage disequilibrium[153], therefore providing more information from the same number of markers. Afrikaners are a founder population descended from mostly Dutch immigrants who settled in South Africa beginning in 1652. They became isolated due to geographic location, as well as language and religious differences[154]. Linkage disequilibrium analyses identified significant disequilibrium extending more than five cM[155]. In addition to their genetic homogeneity, the Afrikaner population is valuable because they have a limited number of initial founders and presently have a large population from which to recruit individuals[154]. Founder mutations have been identified in the Afrikaners for various disorders, such as: Fancomi anemeia[156], pseudoxanthoma elasticum[157], and ovarian/breast cancer[158].

OVERVIEW

This dissertation will describe three studies aimed at identifying genetic loci or genes for either of the complex diseases POAG and schizophrenia. The first study replicated a locus on chromosome 13q34 for schizophrenia and identified a second locus on chromosome 1p36 in families that did not show linkage to chromosome 13q. The second study replicated the GLC1I locus for POAG on chromosome 15q11-13 in families with an earlier age at diagnosis. The last study failed to find an association between the severity of POAG and the -1000CG SNP in the myocilin promoter.

Chapter 2

High-Density Scan with 1980 Microsatellite Markers Highlights Linkage of Schizophrenia to 13q34 and 1p36 in Afrikaners

ABSTRACT

We report on the results of a high-density genome-wide linkage scan for schizophrenia in a population of Afrikaners, a founder population from South Africa. We tested 1980 microsatellite markers in 479 subjects to identify a genome-wide significant linkage peak on chromosome 13q34. In our analysis, we used an algorithm implemented in LAMP to optimize the frequency of the disease allele and genotype penetrances of our parametric model. The highest LOD score when maximizing over these parameters (MOD score) is 3.76 (empirical p-value = 0.042). We also detected a nonparametric LOD score of 2.66 at that locus. This region of chromosome 13q34 has previously been implicated in linkage scans for schizophrenia and bipolar disorder. Consistent with these studies, the maximum MOD score was identified when we included subjects with schizoaffective disorder, bipolar subtype in our analysis. In addition, when we repeated our analysis in families that did not show linkage to 13q34, we identified a linkage peak on chromosome

---

My role in this study includes checking for genotyping error, validating the marker map, performing linkage analyses, choosing SNPs for follow-up, conducting association analysis, writing the manuscript, and preparing the tables and figures.

1p36 (MOD score = 3.21). We also identified regions on chromosome 21q22 and 9q21 that show suggestive evidence for linkage; MOD scores at these loci are 2.72 and 2.20, respectively.

INTRODUCTION

Schizophrenia is a chronic, psychiatric disorder that has an estimated worldwide prevalence of approximately 1%[93; 159]. The diagnosis of schizophrenia encompasses a group of associated disorders, the symptoms of which include irregular "thought, perception, affect, behavior, and communication"[96]. The effectiveness of schizophrenia treatment is not consistent across patients[99] and it is likely that schizophrenia is not a homogeneous disorder caused by a single mechanism, rather the mechanisms underlying schizophrenia are heterogeneous and include a variety of genetic susceptibility factors and environmental triggers[97; 111]. A molecular dissection of schizophrenia may provide insight into underlying pathological mechanisms that could result in more effective and individualized treatment.

The strongest predictor of schizophrenia is having a first-degree relative who is affected. Depending on the specific relationship involved, risk of disease increases 6-17 fold for first degree relatives of affected individuals; having a monozygotic twin increases the risk of disease even further, nearly 50 fold[97; 111]. Adoption studies indicate a four to ten fold increase in the prevalence of schizophrenia in the biological children of schizophrenic parents compared to the biological children of control parents[112; 113].

Genome-wide linkage scans have been conducted to identify loci harboring genes that increase susceptibility to schizophrenia. Although loci have been identified on almost every chromosome, some regions have been replicated across studies; one region is the long arm of chromosome 13[137; 144; 160-165]. Across studies, the linkage peaks span 13q12 to 13q34. This region has also been linked to bipolar disorder; a meta-analysis of 18 genome-wide scans identified 13q as one of the most promising regions for schizophrenia, as well as for bipolar disorder[166]. However, a more recent meta-analysis of 20 studies, using a different analysis technique, did not find evidence for a schizophrenia locus or a bipolar locus on 13q[167; 168]. Perhaps the most promising candidate genes in this region are *G72* (*DAOA*) and *G30*, which are transcribed on overlapping and opposite strands of DNA. Two recent meta-analyses explored the association between these genes and schizophrenia. The first study found strong evidence for an association, but noted that risk alleles and haplotypes were not consistent across samples[169]. The second meta-analysis concluded a weak association with schizophrenia[170; 171].

Founder populations are valuable for identifying susceptibility loci of complex diseases. These populations are often more genetically and environmentally homogeneous[17; 18] and typically show increased levels of linkage disequilibrium[153; 172]. Afrikaners are a founder population descended from mostly Dutch immigrants who settled in South Africa beginning in 1652. They became isolated due to geographic, language, and religious differences[154]. Analyses of this population identified significant linkage disequilibrium extending more than five cM[155]. In addition to its genetic homogeneity, the Afrikaner population is valuable because they have a limited

number of initial founders and presently have a large population from which to recruit individuals[154]. Founder mutations have been identified in Afrikaners for several disorders with Mendelian inheritance, such as: Fancomi anemeia[156], pseudoxanthoma elasticum[157], and ovarian/breast cancer[158].

Previously, we performed a genome-wide linkage scan using microsatellite markers spaced, on average, nine cM apart in an Afrikaner sample. The maximum nonparametric linkage LOD scores were 2.99 at 1p36 and 2.23 at 13q34. A subject was identified with paternal isodisomy of his chromosome 1, potentially facilitating identification of a chromosome 1 risk haplotype[137]. Here, we present findings from the high-density 2-cM follow-up linkage scan of 1980 microsatellites in 479 genotyped individuals from an expanded sample of 127 families. Our results provide considerable evidence for a schizophrenia locus on chromosome 13q and show that the chromosome 1p linkage signal is strongest in a subset of individuals who do not have evidence of linkage to chromosome 13q. We also identify modest evidence for linkage to 21q22 and 9q21.

METHODS

Study sample. Subjects were recruited from local hospitals, support groups, and through newspaper/magazine advertisements in Pretoria. The clinical evaluation took place at the Weskoppies Hospital in Pretoria. All subjects were confirmed as Afrikaners by tracing ancestry back to the 1800s using state and church records and finally to the initial 2,000 founders through the Genealogies of Old South African Families[173]. Interviews were conducted in person by trained clinicians using the

Diagnostic Interview for Genetic Studies[174] and diagnoses were assigned according to the Diagnostic and Statistical Manual of Mental Disorders, version IV (DSM-IV)[96]. The diagnostic instruments were translated into Afrikaans and back-translated into English to allow all interviews to be conducted in Afrikaans[154]. Affected subjects were classified as either narrowly or broadly affected. The narrow diagnosis includes subjects with schizophrenia or schizoaffective disorder- depressive type; it is the same classification as LCI of the previous paper[137]. The broad diagnosis includes all individuals classified as affected under the narrow definition as well as individuals with schizoaffective disorder- bipolar type. Compared to the classifications in the previous paper, it is more encompassing than LCI, but not as broad as LCII.

Genotyping. DNA from all study participants was extracted from 24 ml of EDTA-treated blood, according to standard procedures[175]. Genotyping for 2005 di-, tri-, and tetra-nucleotide repeat microsatellite markers was performed by deCODE (Reykjavik, Iceland), through their fee-for-service genotyping facility. The deCODE genotyping protocol involves PCR amplification followed by capillary electrophoresis and automated allele calling by deCODE's Allele Caller software. Among all the markers genotyped, 1904 autosomal markers and 76 chromosome X markers passed our internal quality checks, which are described in detail below. Of the 960,395 possible genotypes, 878,046 were successfully called, corresponding to an average (±standard deviation) per marker genotyping rate of 91% (±7%).

Error checking. Prior to linkage analysis, we verified reported relationships using genetic marker data. To do this, we used GRR to examine identity-by-state distributions for all pairs of individuals[176] (Figure 2-1). We identified three instances of non-paternity and one set of individuals who appeared to be switched. Using the X chromosome information, we identified one subject who had the appropriate levels of identity-by-state with their relatives, but was labeled as a male while appearing to be genetically female. The pedigree file was corrected to resolve these discrepancies.

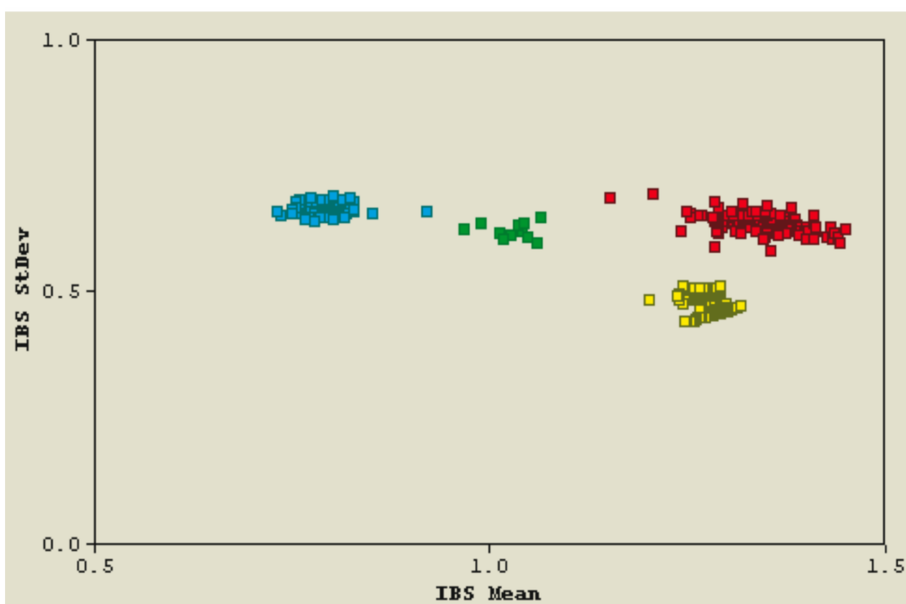

Figure 2-1. Plot of mean identity-by-state by standard deviation identity-by-state for a pair of individuals from the same family. Each square represents a pair of individuals who have been genotyped for at least 900 of the same markers. Blue squares are unrelated individuals, green squares are half-siblings, red squares are full siblings, and yellow squares are parents-offsprings. The plot was created by Graphical Relationship Representation[176].

Additionally, we identified problematic markers that either (1) exhibited more than four Mendelian errors or (2) in a subset of 210 unrelated subjects, deviated from Hardy-Weinberg equilibrium (HWE) at $p<0.01$. These tests help identify markers with

high rates of genotyping error[177]. We excluded 12 markers with more than four Mendelian errors and another 12 markers with evidence for deviations from HWE. Thus, among the 2005 genotyped microsatellites, 1981 (99%) passed these initial quality filters. The error checks were conducted using Pedstats[178].

Genetic map validation. All of our multipoint analyses are based on the deCODE linkage map[179]. For markers that did not have unique positions, we adjusted cM positions slightly according to the UniSTS National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov). This small adjustment avoided problems with likelihood calculations when obligate recombinants are encountered in intervals of length zero. Since the results of multipoint linkage analysis can be sensitive to errors in genetic maps, we checked the agreement of our genotyped data and the published map by comparing the likelihood of the entire genotype set when: (1) all markers were analyzed in their original locations, (2) the order of two consecutive markers was switched, and (3) the position of a specific marker was changed so that it was unlinked to all others (this analysis should identify markers that are mismapped, perhaps due to an error in tracking primers during genotyping). By performing these checks, we identified one marker that appeared to be at the incorrect location on the chromosome (D14S56) (Difference in likelihood is 116.3; average ±standard deviation likelihood difference for all other markers is -14.12 ±16.17). This marker was not included in subsequent analyses, resulting in a total of 1980 analyzed markers.

MOD score parametric linkage analysis. Since the mode of inheritance for schizophrenia is unknown, we performed multipoint and singlepoint parametric maximum LOD (MOD) score linkage analysis using LAMP[180; 181], calculating the MOD scores at 1-cM intervals along the autosomes for the multipoint analysis. Both multipoint and singlepoint analyses were performed because multipoint analysis is more powerful, while singlepoint analysis may be more robust to genotyping errors[182-184]. In contrast to conventional parametric linkage analysis, MOD score analysis does not require the disease allele frequency and penetrance parameters to be specified *a priori*; they are estimated at each location using maximum likelihood. Thus, we expect MOD score analysis to be more powerful than both traditional parametric and nonparametric analysis in situations where the mode of inheritance is uncertain. We initially used the unconstrained (free) model; however since most of our families include a single affected relative pair, we used a multiplicative model that required estimation of only two parameters (disease allele frequency and effect size). We report the multiplicative results unless otherwise specified.

Nonparametric linkage analysis. We also tested for linkage to a schizophrenia locus with multipoint and singlepoint nonparametric linkage using the S_{all} statistic[185]. The S_{all} statistic tests for excess identity-by-descent allele sharing between all pairs of affected individuals within a family. We used the excess-sharing parameterization modified statistic[186] as implemented in Merlin[187]. LOD scores were calculated at 1-cM intervals across all chromosomes for the multipoint analysis. To evaluate evidence for parent-of-origin effects, we defined a nonparametric linkage statistic[179; 185] that measured allele

sharing for maternally inherited alleles only. In each case, these statistics considered all pairs of affected individuals in each pedigree. For each inheritance vector, each pair was scored according to whether they shared their maternally inherited allele (1) or not (0). An overall nonparametric statistic was calculated for each pedigree by summing all pair specific statistics and converted to a LOD score as previously described[179; 185]. An analogous statistic was defined to evaluate sharing of paternally inherited alleles.

Empirical significance levels. To assess the significance of our results, we simulated genotypes for 1000 datasets by gene dropping as implemented in Merlin[187]. The datasets used the same family structures, included the same 1980 markers, and both phenotypes. To determine an empirical distribution of maximum genome-wide MOD (or LOD) scores, we repeated our analysis using the simulated datasets. We then counted the number of times a simulated dataset resulted in a genome-wide maximum MOD (or LOD) score greater than or equal to our experimental genome-wide maximum MOD (or LOD) score. To obtain the empirical p-value, we divided this count by the number of simulations performed.

Follow-up association. The Center for Inherited Disease Research genotyped the following seven SNPs that are located within 10kb of or in *G72* on the Illumina GoldenGate platform: rs1341403, rs1539070, rs3916968, rs7139958, rs701567, rs778294, and rs954580. We tested each SNP for association with schizophrenia in 470 affected and 730 unaffected individuals from 412 multiplex families using LAMP. The association analysis performed by LAMP accounts for pedigree structure and

appropriately corrects for multiple individuals in a family. We used the free model that does not constrain the penetrances for the three genotypes. Although the LAMP algorithm does not control for population stratification, the subjects are members of a founder population and it is unlikely that population heterogeneity is a confounder. We calculated an empirical p-value to correct for the number of SNPs and affection classifications tested.

RESULTS

We genotyped 479 subjects from 127 families. In the 54 families that have at least two affected members, 95 individuals are diagnosed as affected in the narrow category and 120 individuals are classified as broadly affected. There are a total of 60 and 79 affected relative pairs for the narrow and broad affection categories, respectively (Table 2-1); this is 43% more affective relative pairs than were present in the previous study. Of the broadly affected relative pairs, 30 are sibling pairs, 24 are parent-child, 14 are avuncular, six are cousin, three are second cousin, and two are grandparent-grandchild.

Table 2-1. Summary of informative families

	Narrow	**Broad**
Affected individuals	98 (42%)	121 (43%)
Unaffected individuals	135 (58%)	160 (57%)
Affected females	42 (43%)	52 (43%)
Affected males	56 (57%)	69 (57%)
Families with:		
≥1 affected individual	118	127
≥2 affected individuals	44	53
≥3 affected individuals	8	12
Average affected individuals per family with ≥2 affected individuals	2.23	2.28
Number of affected relative pairs	67	87

Among markers that passed quality control, the average inter-marker distance is 1.9 cM (±1.4 cM) and the average heterozygosity for the autosomal markers is .71 (±.12). The high density and heterozygosity of the microsatellites results in an information content across the autosomal chromosomes of .84 (±.046) (Figure 2-2). This increase in information content, as compared to the previous scan (.60 ±.085)[137], improves our ability to detect linkage.

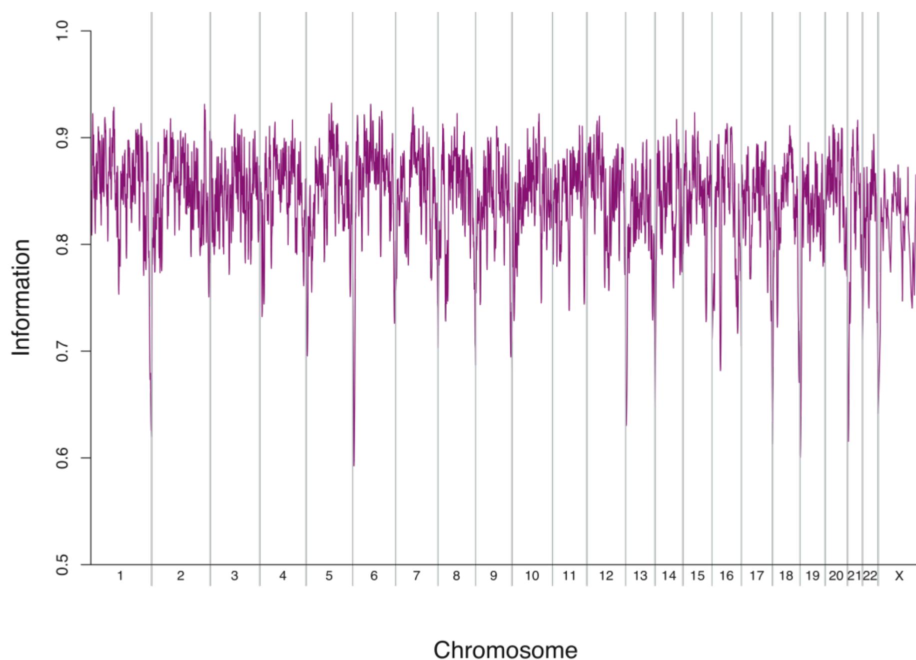

Figure 2-2. Genome-wide information content.

Parametric linkage results. For our multipoint parametric analysis, the maximum MOD scores for both affection classifications are on 13q34. The maximum MOD score for the narrow affection status is 3.13 at 126 cM. When we repeated the linkage analyses on 1000 simulated datasets, we calculated an empirical p-value of .093. For the broad affection status, the highest MOD score is 3.76 at 131 cM, near D13S293,

and the 1-MOD region spans from 115 cM to the q-terminus. The empirical p-value for the broad affection status alone is .025. When we include both the narrow and broad classifications in our simulations, 42 of the datasets resulted in a MOD score greater than or equal to 3.76. This empirical genome-wide p-value of .042 meets the criteria for a significant linkage result[188]. For the broad phenotype, the maximum likelihood estimate for the disease penetrance for an individual with two copies of the disease allele is 1.0, for an individual with one copy is .073, and for an individual with no copies is .005. Although the disease allele frequency is estimated to be fairly rare (f_d = .030), the relative risk is very high (RR = 13.77). Using these two parameters, the attributable risk is estimated to be 0.28, indicating that 28% of the instances of schizophrenia in the population from which the families were ascertained are due to this mutation. In addition to the 13q locus, we identified linkage peaks on chromosomes 21q22 at 46 cM, near D21S1900, with a 1-MOD interval from 41 to 48 cM and on 9q21 at 85 cM, near D9S1877, with a 1-MOD interval from 70 to 96 cM. The linkage signal on 21q22 is much stronger for the narrow classification (MOD=2.72) than for the broad (MOD=1.18). Conversely, the MOD scores on 9q21 are weaker for the narrow (MOD=1.05) than for the broad category (MOD=2.20) (Table 2-2). Genome-wide MOD scores are shown in Figure 2-3 and the correlation between the narrow and broad MOD scores is 0.77. We also carried out single point parametric analysis. These results paralleled the multipoint results; the three highest MOD scores are at 13q34, 9p13, and 21q22.

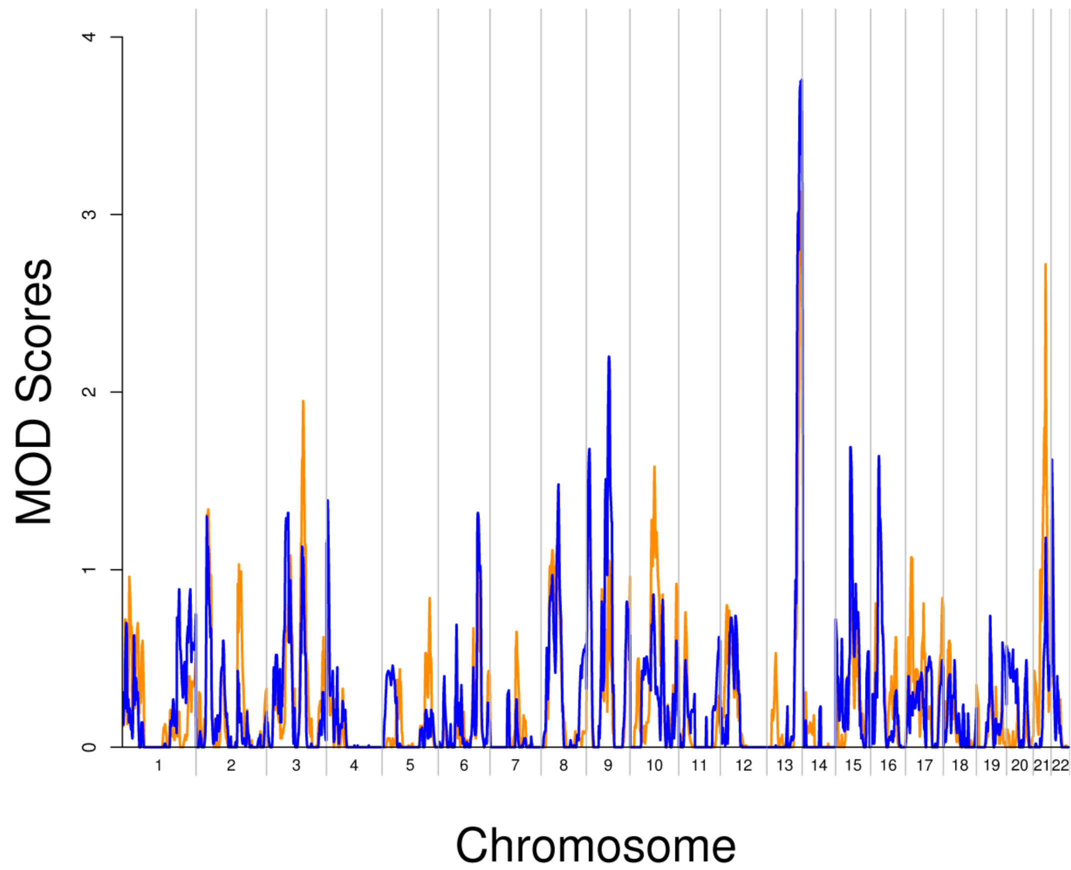

Figure 2-3. MOD score analysis. Green line shows MOD scores for the narrow classification. Blue line shows MOD scores for the broad classification.

Changed the color from green to orange because Steve thought it was hard to tell green from blue.

Table 2-2. Parametric multipoint MOD scores >1.5 for either schizophrenia status (multiplicative model)

Chr	Position (1-MOD)	Nearest Marker	Af	MOD	Freq of A	RR	Pen of AA	Pen of Aa	Pen of aa
3q21	137 cM	D3S1589	N	**1.95**	.39	99.84	.065	.00065	6.5 x10^{-6}
	(128-147)		B	1.13	.53	99.97	.035	.00035	3.5 x10^{-6}
9p24	12 cM	D9S1686	N	1.40	.01	9.86	.77	.078	.0079
	(3-19)		B	**1.68**	.01	11.03	>.99	.091	.0082
9q21	85 cM	D9S1877	N	1.05	<.01	10.44	>.99	.096	.0092
	(70-96)		B	**2.20**	<.01	10.72	>.99	.093	.0087
10q22	92 cM	D10S537	N	**1.58**	.01	11.32	>.99	.088	.0078
	(77-112)		B	0.71	.17	4.04	.072	.018	.0044
13q34	131 cM	D13S293	N	**3.13**	.03	14.76	>.99	.068	.0046
	(115-qter)		B	**3.76**	.03	13.77	>.99	.073	.0053
15q21	56 cM	D15S1022	N	0.87	<.01	10.35	>.99	.097	.0094
	(54-65)		B	**1.69**	<.01	10.39	>.99	.096	.0093
16p13	31 cM	D16S3047	N	1.31	.17	5.60	.10	.018	.0032
	(27-45)		B	**1.64**	.10	5.64	.14	.026	.0046
21q22	46 cM	D21S1900	N	**2.72**	.36	99.95	.073	.00073	7.4 x10^{-6}
	(41-48)		B	1.18	.52	99.86	.037	.00037	3.7 x10^{-6}
22q11	3 cM	D22S420	N	1.47	.44	99.99	.049	.00050	5.0 x10^{-6}
	(2-9)		B	**1.62**	.45	99.99	.048	.00048	4.8 x10^{-6}

'A' is the disease allele, 'a' is the non-disease allele. MOD scores greater than 1.5 are bolded. Abbreviations: 1-MOD, region in which the MOD score is within 1 MOD score of the highest MOD score; Af, affection classification; N, narrowly affected; B, broadly affected; Freq, allele frequency; RR, relative risk based on a 1% prevalence of schizophrenia; Pen, penetrance of schizophrenia for the given genotype. Therefore, Pen of AA is the probability of having schizophrenia, given two copies of the disease allele.

Nonparametric linkage results. The maximum multipoint nonparametric LOD score is located about two cM away from D13S285 at 125 cM on 13q34. At this location, the LOD score for the narrow classification is 2.65; for the broad classification the LOD score is slightly higher at 2.66. The 1-LOD interval around this peak extends from 119 cM to the q-terminus. However, the empirical significance based on 1000 simulations is not significant (p=.25). The nonparametric multipoint analysis also provides evidence for linkage at 21q22. The LOD scores are 2.16 for the narrow classification and 0.83 for the broad classification (Table 2-3). For the nonparametric singlepoint analysis, the highest LOD scores are on 1p36, 13q34, and 21q22. We also conducted

parent of origin analysis genome-wide, but found little evidence for imprinting and we do not present the results. Parametric linkage analysis was only conducted on the autosomal chromosome, however there was no evidence for non-parametric linkage on the X chromosome; the highest LOD score on the X chromosome was 1.03.

Table 2-3. Nonparametric multipoint LOD scores >1.5 for either schizophrenia status

Chr	Position (in cM)	Nearest Marker	1-LOD interval	Narrow LOD	Broad LOD
3q21	137	D3S1589	129-147	**1.61**	0.81
8q11	65	D8S1831	58-78	**1.52**	**1.62**
13q33-34	125	D13S261	119-qter	**2.65**	**2.66**
21q22	46	D21S1900	38-48	**2.16**	0.83

LOD scores greater than 1.5 are bolded.
Abbreviation: 1-LOD, region in which the LOD score is within 1 LOD score of the highest LOD score

Targeted subset analyses. Twenty-five of our families in our sample exhibited a MOD score of < 0.0 at the location of our strongest linkage peak, at 131 cM on chromosome 13q34 near marker D13S293. To examine evidence for additional susceptibility loci, we carried out a series of analysis targeted at these 25 families. When we repeated our genome-wide MOD score analysis, the maximum MOD score in these 25 families occurs on chromosome 1p36 at 35 cM (~1 cM from D1S2644) near the peak that was identified in the previous 9-cM scan. The MOD score for the narrow classification at that position is 3.21; the MOD score for the broad classification is 1.74. The nonparametric LOD scores in the region (both at ~29 cM, near D1S2697) were only 1.32 and 0.54 for the narrow and broad classifications, respectively. The MOD score analysis results suggest a dominant mode of inheritance, with the estimated disease allele frequency of .003 and penetrances/genotype relative risks of 1.0, .93, and .004. In the original analysis,

including all 69 informative families, only modest evidence for linkage was observed in this region, with MOD scores of 1.28 and 0.20 for the narrow and broad classifications, respectively (Table 2-4). Interestingly, the linkage to 13q34 is strongest in the broad affection category while linkage to 1p36 is primarily in the narrow category. The difference in phenotype between the two affection statuses is that the narrow classification does not include schizoaffective disorder, bipolar subtype.

Table 2-4. Parametric linkage to chromosome 1p36 (dominant model)

Family group	Number of families	Position	Nearest Marker	Narrow MOD	Broad MOD
All*	69	35 cM	D1S2826	1.28	0.20
Unlinked to 13q	25	35 cM	D1S2826	**3.21**	1.74
Linked to 13q	43	35 cM	D1S2826	0.12	0.00

*One family had a MOD score of 0.000 and was not used in the subset analysis

***G72* association.** *G72* is a candidate gene for schizophrenia located on 13q33.2 at 104.9 Mb. The gene is more than 25kb, consists of at least five exons, and has several splicing isoforms. To determine if the linkage signal on chromosome 13 is due to a variant in *G72*, we analyzed seven SNPs across the gene and flanking region for association with schizophrenia. To test for stratification in our sample, we compared the 537 unrelated founders from 412 Afrikaner families to the 60 unrelated HapMap YRI, 60 unrelated HapMap CEU, and 90 HapMap JPT+HCB using principle components analysis as implemented in EIGENSTRAT[189]. We found that the

Afrikaners are grouped in a single cluster and are distinguishable from the other three populations, indicating no evidence of stratification (Figure 2-4).

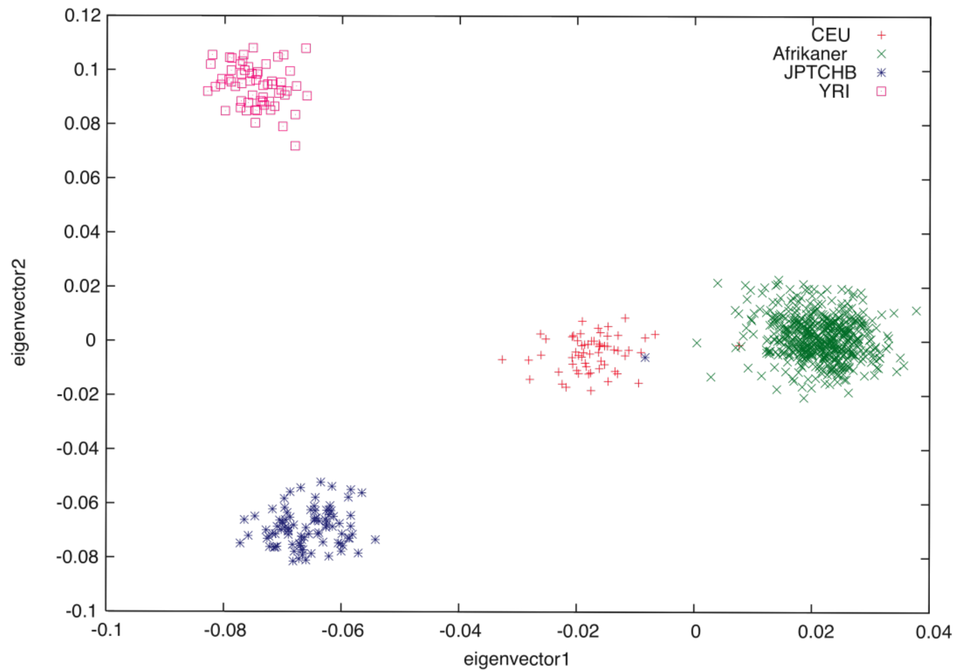

Figure 2-4. Principle component analysis of Afrikaners as compared to HapMap YRI, CEU, and JPT+CHB analysis panels.

When we tested for association in the 412 families, we found an association with two SNPs, rs1539070 and rs954580, that are in moderate linkage disequilibrium (r^2=.51; Table 2-5). For both SNPs the association is stronger in the broad category (p-value of .0066 and .0096 for rs1539070 and rs954580, respectively). However, the SNPs are not significantly associated with schizophrenia when we calculate empirical p-values (.29 and .39, respectively). When we tested 41 of the 43 families that showed linkage to 13q, both SNPs are no longer associated with schizophrenia (Table 2-6).

Table 2-5. Linkage disequilibrium between *G72* SNPs

	rs1341403	rs1539070	rs3916968	rs7139958	rs701567	rs778294	rs954580
rs1341403	-	<.01	.03	.38	.38	.25	.02
rs1539070	.02	-	.44	.12	.15	.09	.51
rs3916968	.32	.97	-	.05	.06	.19	.53
rs7139958	.62	1.00	.45	-	.50	.29	.29
rs701567	.87	.88	.40	1.00	-	.58	<.01
rs778294	.93	1.00	.99	1.00	1.00	-	.16
rs954580	.28	1.00	.77	1.00	<.01	.96	-

R^2 values are above the diagonal, D' values are below the diagonal

Table 2-6. Association between schizophrenia and *G72* SNPs

SNP	kb Position (site in gene)	Allele (Freq)	All Families				Chr 13 Families			
			Narrow		Broad		Narrow		Broad	
			RR	PV/EPV	RR	PV/EPV	RR	PV/EPV	RR	PV/EPV
rs1341403	104,915 (5')	2 (.58)	1.21	-	1.17	-	1.11	-	1.20	-
rs1539070	104,922 (intron2)	1 (.81)	1.69	.018 (.59)	1.99	.007 (.29)	1.10	-	1.22	-
rs3916968	104,926 (intron3)	2 (.67)	1.07	-	1.08	-	1.08	-	1.10	-
rs7139958	104,935 (intron3)	2 (.59)	1.03	-	1.05	-	1.04	-	1.03	-
rs701567	104,940 (intron3)	2 (.41)	1.06	-	1.07	-	1.36	-	1.32	-
rs778294	104,940 (intron3)	1 (.27)	1.10	-	1.13	-	1.56	.005 (.21)	1.47	.018 (.56)
rs954580	104,950 (3')	1 (.68)	1.48	.050 (.86)	1.62	.010 (.39)	1.05	-	1.05	-

Abbreviations: Freq, allele frequency; RR, relative risk based on a 1% prevalence of schizophrenia; PV, p-value; EPV, empirical p-value

DISCUSSION

We conducted a high-density genome-wide linkage scan that uses a microsatellite panel of nearly 2000 markers to test for linkage to schizophrenia in an Afrikaner founder population. In our analysis, we conducted both parametric and nonparametric linkage analyses. Since the mode of inheritance for schizophrenia is not known, for our parametric analyses we used the algorithm implemented in LAMP to estimate the disease allele frequency and genotype penetrances by maximum likelihood. We used the optimized parameters to calculate MOD scores, which are more powerful than LOD scores when the disease parameters are unknown[190].

Given the complexity of schizophrenia, it is unlikely that this disease has a homogeneous etiology, even in the Afrikaner founder population. Our analyses indicate strong evidence for a gene that increases susceptibility to schizophrenia on chromosome 13q34 in our broadly affected individuals. When maximizing the disease parameters in our MOD score analysis, we estimated a MOD score of 3.76, with a genome-wide significant empirical p-value of .042 using the multiplicative model. Although the disease allele is found in only three percent of the individuals in our population, the risk of schizophrenia for those who have a disease allele is more than 13 times higher than the risk for those who do not have an allele. Also, it is noteworthy that two of the three other schizophrenia linkage scans that identified a LOD score greater than 2.0 at 13q32-13q34 included schizoaffective disorder, bipolar type in the affection category (Figure 2-5)[144; 160; 163; 165]. That our broad classification also includes this diagnosis supports the hypothesis that a gene in this region increases susceptibility to schizophrenia and bipolar spectrum disorders.

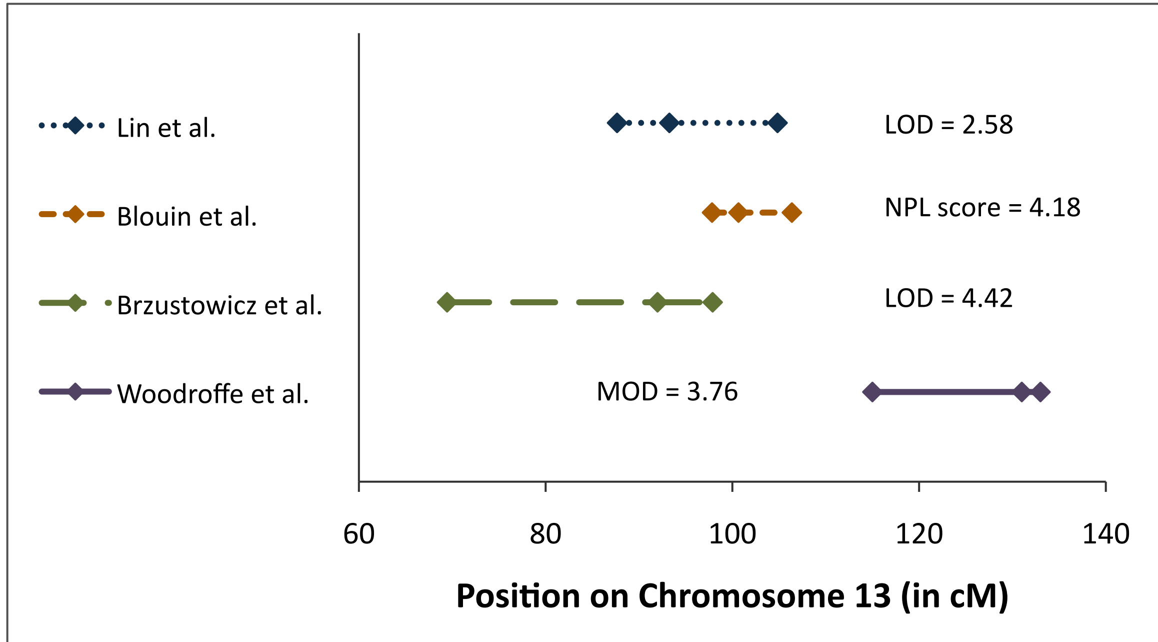

Figure 2-5. Plot of peak LOD (or MOD) scores and 1-LOD (or 1-MOD) intervals for the four papers reporting LOD (or MOD) scores greater than 2.0 on 13q32-34. The diamond in the line is location of maximum LOD (or NPL or MOD) score, the diamonds at the start and end of each line mark the 1-LOD (or 1-NPL or 1-MOD) scores. Blouin et al.[144], Brzustowicz et al.[160], and Woodroffe et al. included schizoaffective disorder, broad type in their phenotype classification; Lin et al.[161] did not.

Based on the results of this study and previous ones, there is considerable evidence for linkage to 13q. However, the causal gene has not been conclusively identified. The gene *G72* is located on 13q and is a candidate gene for both schizophrenia and bipolar disorder. The function of this gene remains controversial. It was originally proposed that the gene activates D-amino acid oxidase but a more recent study suggested an alternative role for G72 in modulating mitochondrial function[191]. In this population of Afrikaners, we found only modest evidence for association between SNPs in *G72* and schizophrenia. The results from this and other studies suggests a possible role for *G72* in schizophrenia etiology, but it seems likely that additional

associated variants would be required to explain the strong linkage signal we observe at this locus.

When we analyzed families that do not show evidence for linkage to chromosome 13q34, we identified another linkage peak on chromosome 1p36. This result was much stronger when we used the narrow definition of schizophrenia that did not include schizoaffective disorder, bipolar subtype. This suggests different causal mechanisms in subjects with and without symptoms of bipolar disorder. The linkage to 1p36 appears to be only in the Afrikaner population, and may be a founder mutation.

The chromosome 1p36 result is particularly interesting since one of the narrowly affected schizophrenic subjects has uniparental disomy (UPD) for all of chromosome 1. However, the evidence for linkage to chromosome 1p36 is strongest when using a dominant model and UPD would be most indicative of a recessive model due to the homozygosity for the susceptibility allele. When we compared the rates of allele sharing with the UPD individual in unrelated affected and unaffected individuals, we found no difference in allele sharing; unaffected individuals carried the UPD allele as often as affected individuals.

There were, however, limitations of this study. By studying a founder population that is more genetically homogeneous, we may have a better chance of identifying a causative gene, but this homogeneity may limit the generalizability of our results to a broader population. Also by using linkage analyses, we are able to overcome allelic heterogeneity, but we are unable to identify susceptibility alleles that have a lower disease penetrance.

To identify these lower penetrant alleles, the next step would be to conduct a genome-wide association scan. Hundreds of thousands of SNPs will be tested and it will likely be difficult to discern which SNPs are truly associated with schizophrenia. The findings from this linkage study can be used to prioritize regions. Using a false discovery rate (FDR) weighted by the linkage results, it is possible to increase power to detect disease loci. This method also does not cause a large decrease in power if there is not a disease gene in the linkage region[192].

In summary, we identified a region on chromosome 13q34 that shows significant linkage to schizophrenia in broadly affected subjects, including those with schizoaffective disorder, bipolar type. This result is particularly interesting because there is previous evidence to suggest that this region may harbor a gene that increases susceptibility to schizophrenia and bipolar disorder. We also found that in the families that did not show linkage to chromosome 13q34, there is evidence for linkage to chromosome 1p36 in narrowly affected individuals. If causative genes are identified in these regions, their functions may give insight into the heterogeneity of schizophrenia and increase our understanding of its phenotypic variation.

Chapter 3

Ordered Subset Analysis Supports a Glaucoma Locus at GLC1I on Chromosome 15 in Families with Earlier Adult Age at Diagnosis[†]

ABSTRACT

Open angle glaucoma (OAG) is a complex disorder with varying etiologies due to multiple genes and environmental effects. This genetic heterogeneity can confound efforts to map loci. Increased homogeneity in a sample can be achieved using either ordered subset analysis (OSA) which groups families, or individual OSA (IOSA), which groups individuals based on disease related covariates. Recently, GLC1I was mapped to 15q11–13 in families with early adult onset of OAG. We tested for linkage to GLC1I in an independent sample of 167 individuals in 25 multiplex OAG families of European descent. We carried out nonparametric linkage analysis on the complete set of 25 families

---

[†]This chapter has been previously published: Woodroffe A.[*], Krafchak C.M.[*], Fuse N., Lichter P.R., Moroi S.E., Schertzer R., Downs C.A., Duren W.L., Boehnke M., Richards J.E. (2006) Experimental Eye Research 82,1068-74. [*]These authors contributed equally to this work.

My role in this study includes selecting markers to genotype, genotyping the markers, checking for genotyping error, performing linkage and ordered subset analyses, writing the introduction and results sections, contributing to the methods and discussion sections, and creating the table.

and obtained a maximum LOD score of 1.00 at 9.0 cM. Using mean age at diagnosis (AAD) across the affected individuals within each family to order the families as a proxy for age at onset, we found a maximum OSA LOD score of 2.09 (p=0.021) at 26.1 cM. The mean (±s.d.) AAD across the 14 earlier AAD families that contributed to the OSA LOD score was 50.6 years (±5.38); the mean AAD for the other 10 later AAD families that did not contribute to the OSA LOD score (the high-AAD) was 61.5 years (±3.72). We also ran IOSA on our families using AAD as our covariate on which to subset affected individuals. The maximum LOD score was 1.01 at 14.3 cM when ordering subjects from early to late AAD. Ordered subset analysis of this sample has provided evidence of linkage close to the previously identified GLC1I glaucoma locus on 15q11–13 in families with middle-aged mean age at diagnosis.

INTRODUCTION

Glaucoma is the second leading cause of blindness in the world affecting over 60 million people[21]. The most common form of glaucoma in individuals of European ancestry is primary open angle glaucoma (POAG) in which intraocular pressure (IOP) is often elevated without a known underlying condition. Family history is a significant predictor of glaucoma[193]. Pedigrees demonstrating different apparent modes of inheritance were available in the literature long before the first glaucoma gene was mapped[194-199].

Elevated IOP and older age are also important risk factors for both occurrence and progression of glaucoma[31; 38; 200]. Although elevated IOP is a risk factor, not all patients with ocular hypertension (OHT) progress to glaucoma[201]. Furthermore, about 30% of

patients with OAG have normal tension glaucoma (NTG) in which IOP has been documented not to be elevated[202]. Although most cases of OAG develop later in life, onset before 35 years of age can be seen in individuals with juvenile open angle glaucoma (JOAG).

The genetic relationship of POAG, NTG, and JOAG is not yet clear since any given OAG family may show one predominant diagnosis while some family members may have one or both of the other diagnoses[61; 74; 203; 204]. Genotype–phenotype studies among glaucoma genes identified so far indicate that in many families, cases of POAG may be identical by descent with the predominant JOAG or NTG cases in those families[61; 74; 203].

To date, it is appreciated that OAG is highly heterogeneous and it is likely that many more glaucoma loci will be mapped. This large number of loci is not unusual for eye disorders[205-207]. An especially dramatic example is retinitis pigmentosa, for which more than 133 loci have been mapped, with at least 75 genes now identified[208; 209]. OAG loci mapped so far include GLC1A (1q23–q24, MIM137750)[59], GLC1B (2cen–q13, MIM606689)[79], GLC1C (3q21–q24, MIM601682)[82], GLC1D (8q23, MIM602429)[78], GLC1E (10p14–p15, MIM602432)[78], GLC1F (7q35–q36, MIM603383)[76], GLC1G (5q22.1)[74], GLC1H (14q11–q13)[77], GLC1I (15q11–13)[83], GLC1J (9q22, also called JOAG2, MIM608695)[80], and GLC1K (20p12, also called JOAG3, MIM608696)[80].

Studies have indicated further heterogeneity in hereditary glaucoma. A genome-wide linkage scan was carried out on 182 affected sibling pairs that identified five additional regions of interest[77] and additional regions of the genome showed moderate evidence for linkage to OAG in a genome scan of participants in the Barbados Eye

Study[210]. Recently, eight Finnish families with POAG were genotyped at glaucoma loci GLC1A–GLC1F and eight other candidate gene regions. Evidence for linkage was not found in any of the tested regions[89].

Three of the mapped OAG genes have so far been identified: myocilin (MYOC, MIM601652) at the GLC1A locus[60; 211], optineurin (OPTN, MIM602432) at the GLC1E locus[69], and WD-repeat36 (WDR36) at GLC1G[74]. MYOC mutations account for about 3% of OAG, with the Gln368stop MYOC nonsense variant accounting for many, but not all, of the adult onset OAG cases caused by mutations at the GLC1A locus[61; 86]. Most of the other MYOC variants are missense mutations usually associated with a JOAG phenotype. A small number of mutations in OPTN are found in families in which most affected individuals have NTG[69]. Mutations in WDR36 are the first to be found segregating through families in which the more prevalent adult onset glaucoma involving elevation of IOP is predominant[74]. Much remains to be understood about the underlying mechanisms by which mutations in these genes predispose to glaucoma and most cases cannot be accounted for by these three genes.

Genetic heterogeneity can confound mapping efforts. That populations of African ancestry show differences in prevalence, average age at diagnosis, and response to therapies suggests that separate evaluation of European and African populations in glaucoma mapping studies might assist in reducing the complex etiology of the disease[43; 44; 212]. Increased homogeneity in a sample can also be achieved by grouping families or individuals based on covariates, such as age at diagnosis or maximum pre-treatment IOP. Where a clinically significant cutoff value for a covariate has not been identified, it may be unclear which families and/or subjects to include in an analysis.

In ordered subset analysis (OSA), pre-specified cutoff values are not necessary. Families are ranked by an ordinal or continuous covariate and nonparametric linkage analysis is performed on the first family. At each subsequent round of analysis a family, or families, is added based on their covariate ranking and the linkage analysis is repeated. The analysis continues until all families have been added. The LOD scores at each step are compared and a maximum score is obtained for a subset of the families[13]. Individual OSA (IOSA) performs a similar analysis except that affected individuals, rather than entire families, are added sequentially based on their covariate values. Both methods attempt to create a more homogeneous sample. In OSA, using a summary statistic across all affected family members may remove some of the within-family variability due to environmental and/or genetic interactions. IOSA may account for phenocopies as the presentation of glaucoma, and thus covariate values, in these sporadic cases may differ from that in familial cases in the same families. In identifying the GLC1I locus, Allingham and colleagues performed OSA by sorting the families based on age at diagnosis (AAD), a proxy for age of onset. The families with an earlier mean AAD (44.1 years ±9.1) were linked to GLC1I, while families with a later mean AAD (61.3 years ±10.4) were not[83].

In this study we tested for a locus on 15q11–13 by subsetting our sample based on AAD. We compared the results obtained via three statistical approaches: OSA, IOSA, and nonparametric linkage analysis of the whole family set. Consistent with the results of Allingham and collegues[83], we report modest evidence for a locus on chromosome 15, with a maximum LOD score of 1.00 at the GLC1I locus, and increased evidence for linkage when we focused on early AAD families identified using OSA, with a maximum

OSA LOD score of 2.09 17 cM from the GLC1I locus.

METHODS

Subjects. Subjects provided informed consent and blood samples according to protocols approved by the Institutional Review Board for human subject research of the University of Michigan medical school. For purposes of this study, the narrow definition of affected POAG status was based on open angles, glaucomatous optic neuropathy, and visual field defects consistent with glaucoma. Glaucomatous optic neuropathy was defined as a narrowed neuroretinal rim, notching of the neuroretinal rim, and/or marked asymmetry in the cup to disc ratio. Glaucomatous visual field defects were based on the Glaucoma Hemifield Test and clinician interpretation. In addition, we considered a broad definition of the term affected that included individuals for whom visual field tests were not available but who presented with open angles and glaucomatous optic neuropathy when diagnosed with POAG according to an ophthalmologist's exam. Those individuals with the above characteristics, but who were reported to have pigment dispersion syndrome or pseudoexfoliation, were considered to have an indeterminate phenotype and not classified as either affected or unaffected in the analysis. We also screened all probands for mutations in MYOC and OPTN and found no causative mutations.

Markers. We carried out initial genotyping using microsatellite markers from the Applied Biosystems MD-10 panel set (Applied Biosystems, Foster City, CA) as well as markers from the Marshfield Clinic map (http://research.marshfieldclinic.org/genetics) which were produced by Invitrogen (Carlsbad, CA). After evaluation of marker quality

and coverage, four chromosome 15 markers from the MD-10 panel set were used in the analysis. To complete coverage and to improve data quality, four markers from the Marshfield map were added to replace poorly performing markers and to map more finely the region reported to contain GLC1I. The final marker coverage in this region on chromosome 15 gave an average intermarker distance of 4.3 cM. The eight markers used in this analysis were: D15S128, D15S822, D15S1002, D15S1048, D15S1007, D15S1040, D15S1042, and D15S994.

Marker amplification. We amplified markers using the TrueAllele PCR Premix from Applied Biosystems according to the manufacturer's instructions. To improve throughput with simultaneous amplification of multiple markers, we also used the Qiagen Multiplex PCR Kit according to manufacturer's instructions (Qiagen, Hilden, Germany). We amplified up to six markers in one reaction using the Perkin–Elmer 9700 or 9600 thermocycler according to manufacturer's instructions.

Genotyping. Allele sizes were read on either an ABI 377 Automated Sequencer or an ABI 3100 Automated Sequencer from Applied Biosystems, with multiplex loading of one panel of markers per sample per run. Allele sizes were called using Genotyper from Applied Biosystems version 3.7 for data generated on the ABI 3100 or version 3.0 for data generated on the ABI 377. Allele sizes were adjusted to reference individual 1347-02 from the Centre d'Etude du Polymorphisme Humain (CEPH) and entered into a database through the Cicada web interface at http://eyegene.ophthy.med.umich.edu. Markers were evaluated for the appropriate bin definition using the histogram editor in

Cicada (http://eyegene.ophthy.med.umich.edu/about_cicada). Data were extracted from the database using Cicada and formatted for use in analysis software.

Error checking. We included an internal duplicate control and a negative control for each marker. We used Pedstats[178] to check the data for Mendelian errors and Merlin[187] to identify possible double recombination events involving closely neighboring markers. We also checked family relationships using GRR[176]. We identified an affected individual who appeared to be a half sibling. As we did not have parental genotypes, we could not determine which parent was incorrectly specified and we removed that subject from the analysis. Based on genotypes from one set of monozygotic twins and our internal control, we estimated an initial genotyping error rate of 1.3% for a larger data set of 382 markers that includes these eight markers from chromosome 15. To reduce the error rate in this chromosome15 data set, a second researcher re-genotyped the eight chromosome 15 markers listed above. The two researchers resolved any discrepancy in allele calls. Each genotype was called independently in the absence of information on individual identities, affected status, or family relationships.

Statistical analysis. We performed nonparametric analyses on the chromosome 15 markers for our complete set of families using Merlin[187]. To identify a potentially more homogeneous subset of families, we subsequently ran OSA and IOSA using AAD as our covariate. The analysis was performed with ascending and descending AAD rankings. To adjust for multiple testing, we calculated an empirical *p*-value by permuting the covariate values across families[13]. We further corrected our significance level by dividing 0.05 by

two for testing both late-to-early and early-to-late ordering, thus giving a *p*-value cutoff of 0.025.

RESULTS

To evaluate whether the GLC1I locus could be detected in a second independent sample, we genotyped markers on chromosome 15 from 167 individuals in 25 multiplex OAG families of European ancestry. Under the narrow affection criteria, 90 people were considered affected; under the broad criteria 107 people were considered affected. For nineteen of the families, we genotyped affected individuals in two or more generations. There were eight cases of JOAG among six families and eighteen cases of NTG among twelve families. When considering the narrow definition of affected status, AAD ranged across subjects from 16 to 86 years, with a median age of 57.5 and a mean age (±s.d.) of 55.6 (±14.5) years. The maximum detected pre-treatment IOP ranged from 14.0 to 51 mmHg with a mean of 26.6 (±6.2) mmHg. When considering the broad definition, the AAD ranged from 16 to 86, years and the median age was 55.3 (±14.2) years. Maximum IOP ranged from 14.0 to 51.0 mmHg with a mean of 26.3 (±6.6) mmHg. The correlation between AAD and maximum IOP across individuals was not significant for either the narrow (r=−0.08 p=0.46) or broad criteria (r=−0.11, p=0.29). Familial mean AAD and mean maximum IOP were not correlated across families for the narrow criteria (r=−0.36, p=0.09), but were negatively correlated for the broad (r=−0.41, p=0.048). Family characteristics are in Table 3-1.

Table 3-1. Family-specific characteristics

Family	AAD	IOP	Cumulative LOD[a]
F0190	35.0	29.5	0.00
F0034	46.3	25.5	0.01
F0046	47.0	31.0	n/a
F0300	48.3	28.0	0.32
F0201	49.0	26.0	0.66
F0096	49.3	28.3	0.53
F0001	51.0	28.8	0.88
F0003	51.0	28.8	–
F0081	51.7	26.4	0.79
F0255	52.6	27.8	1.09
F0060	52.7	30.0	1.33
F0058	53.7	26.3	1.43
F0082	54.0	30.0	1.55
F0029	56.7	29.0	2.09
F0055	56.7	25.3	–
F0048	57.7	25.3	2.07
F0334	58.0	21.3	1.88
F0056	58.5	22.3	1.78
F0333	59.3	26.0	1.45
F0320	59.7	23.7	0.80
F0373	60.8	28.5	0.61
F0070	62.0	26.7	0.72
F0024	65.7	23.0	0.58
F0240	66.3	21.2	0.56
F0094	67.3	30.3	0.5

Abbreviations: AAD, age at diagnosis; IOP, intraocular pressure; Cumulative LOD, cumulative LOD score generated by including families with AAD less than or equal to family value in linkage analysis.
[a]Family F0046 was not included in analysis for cumulative LOD.

We carried out nonparametric linkage analysis on the complete set of 25 families. The maximum LOD scores obtained were 1.00 at 9.1 cM for the broad definition of affected status and 0.51 at 26.1 cM for the narrow definition of affected status (Figure 3-1A). One family was not analysed using the broad definition and a second family was not analysed using the narrow definition.

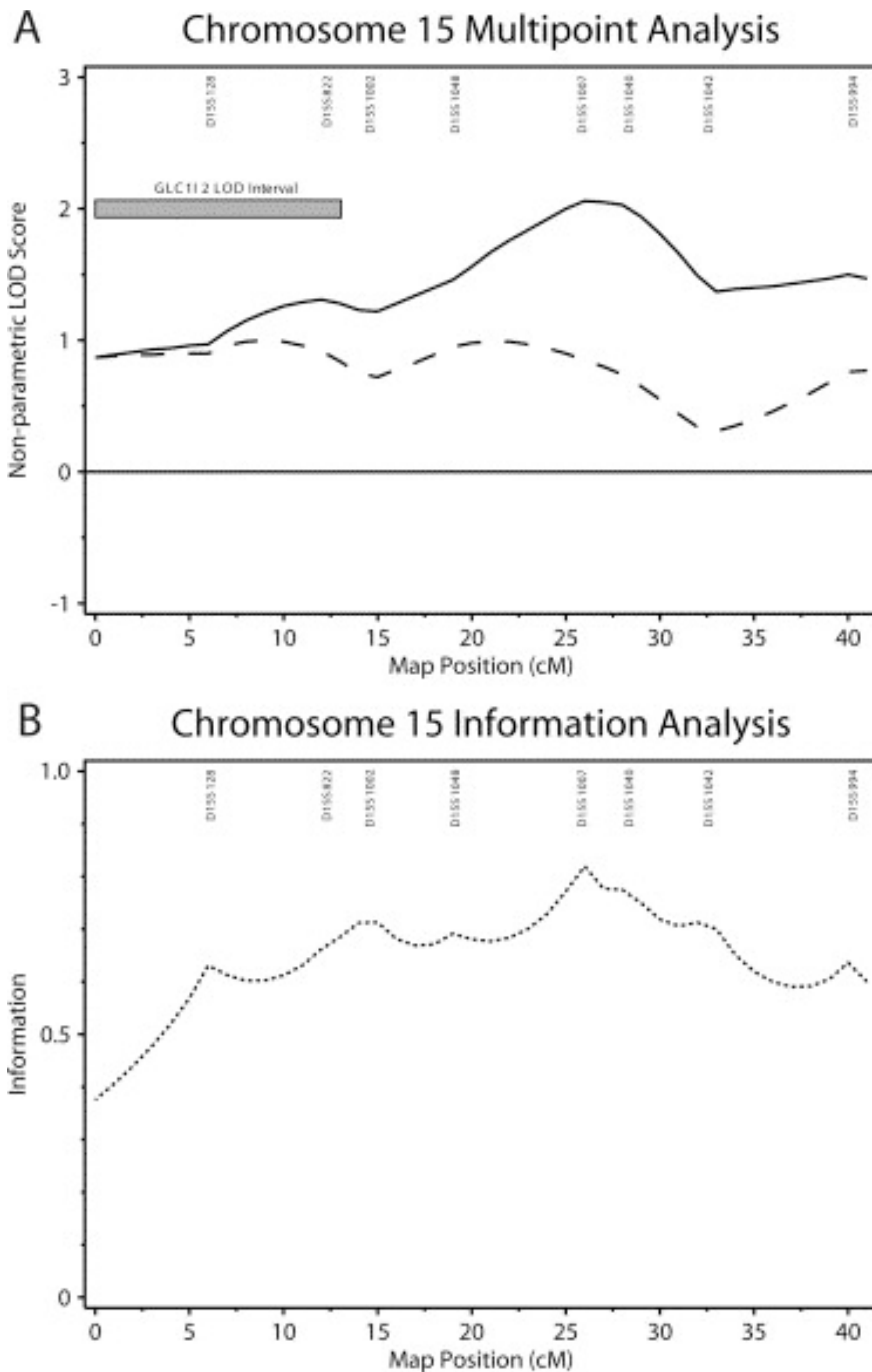

Figure 3-1. (A). Nonparametric LOD scores, for the OSA subset (earlier AAD) (solid line) and LOD scores before subsetting (dashed line). (B) Information plot, for genotypes for families in the maximum OSA LOD score subset.

To identify a more homogeneous subset, we used OSA with mean AAD as the covariate on which the families were ordered. The maximum LOD score obtained from this analysis was 2.09 at 26.1 cM, using mean AAD across affected members of a family as the summary statistic (Figure 3-1A). This LOD score was obtained when the families were ordered from early to late, using the broad affection criteria. The empirical *p*-value for observing this large an increase in LOD score across chromosome 15 was 0.021. The informativeness of our marker data peaked at 25.9 cM and was lowest at 8.1 cM (Figure 3-1B). The mean AAD across the 14 (58%) earlier AAD families that contributed to the maximum OSA LOD score was 50.6 years (±5.38); the mean for the other 10 later AAD families (the high-AAD) was 61.5 years (±3.72).

We also ran IOSA on our subjects using AAD as our covariate on which to subset affected individuals. Using the broad affection criteria, the maximum LOD score was 1.01 at 14.3 cM when ordering subjects from early to late AAD and was 1.05 at 14.3 cM for late to early.

DISCUSSION

The purpose of this study was to explore the role of the recently reported GLC1I OAG locus on chromosome 15[83] in a second independent sample. In addition to evaluating this locus in our set of families, we were interested in comparing the results obtained from non-subsetted multipoint linkage analysis to the results from two different subsetting approaches: OSA which subsets families based on the family's summary covariate and IOSA, which subsets individuals based on the subject's covariate,

independent of family.

Use of ordered subset analysis to detect OAG linkage to 15q. Although standard nonparametric multipoint linkage analysis did not identify this region of chromosome 15 as providing compelling evidence of linkage, use of OSA to subset families based on AAD identified a broad region of interest close to the GLC1I locus (LOD=2.09, *p*=0.021). Thus our data provide confirmatory evidence for linkage to the GLC1I locus in an independent study cohort, but our results are interesting only in context of the previous report[83] and would not be considered adequate to constitute initial identification of a locus.

Our data are consistent with a replication of the GLC1I locus despite a 17 cM separation of the maximum OSA peak LOD score in our study from the peak LOD score in the Allingham study, when positions of markers from both studies are evaluated on the same map. Our strongest evidence for linkage was found at 26 cM on chromosome 15 on the Marshfield map (http://research.marshfieldclinic.org/genetics), with evidence for linkage across a broad region including GLC1I (Figure 3-1A), while Allingham and colleagues reported their maximum LOD score at a marker that sits at approximately 9 cM on the Marshfield map. It has been shown that there can be large variation in the particular location identified for a given locus for complex disorders like OAG. Simulations showed that this effect is accentuated as the number of families considered becomes smaller, and that evidence for a location as far as 20–30 cM from a given locus could indicate confirmation[213]. We have used a relatively small number of families, so the distance of our linkage peak from GLC1I does not exceed reasonable expectations as

a confirmation of GLC1I on 15q11–13, and suggests but certainly does not prove that the same locus may be relevant in both sets of families.

It is worth noting that before subsetting, our greatest evidence for linkage is at 9 cM, in the vicinity of GABRB3, which is where Allingham and colleagues, find their peak OSA score[83]; however, this LOD score is low enough that this location would not normally have been selected in the course of a full genome scan. It is interesting that the LOD increases slightly using OSA, even though it does not end up providing the maximum LOD score. The genotypes in our family subset are less informative near the GLC1I peak at 9 cM than around 26 cM, the location of our peak near D15S1007 (Figure 3-1B). Clearly, additional studies with more informative markers will be needed to optimize localization of GLC1I and to confirm that the two studies are identifying the same locus.

GLC1I and earlier age at diagnosis. Our finding of linkage to the GLC1I region used the same covariate as Allingham and colleges[83], earlier AAD. It should be noted that mean family AAD is strongly negatively correlated with mean family maximum IOP in our study. The mean IOP in the high-AAD families in 24.8 mmHg (±3.07) and is 27.8 mmHg (±1.65) in the low-AAD families. The mean IOPs of the two subsets are significantly different (p=0.005). Thus we have to consider that earlier family mean AAD might be a proxy measurement for high family mean IOP, and that the appropriate covariate to consider is in fact higher IOP. This becomes relevant for evaluation of this locus, and should be considered when choosing individuals for further study.

Using OSA, we were able to subset our data without a priori decisions regarding

which families to classify as earlier or later AAD. A subset of 14 families within our set were identified that, when considered as a group, more than doubled the nonparametric LOD score that was calculated on the complete sample. The simulations performed by OSA demonstrate that if we have randomly selected families to include in the linkage analysis, we would not expect a LOD score as high as the one we observed at this location. Thus, OSA has identified an earlier AAD subset that is significantly younger than the other families

It is prudent to exercise due caution when interpreting results obtained using OSA, as these results could be due to chance. It is important to carefully consider results obtained using OSA in the context of the family data and in the context of data from other researchers. We have tested the specific hypothesis that a previously identified locus on 15q11–13 is influencing risk of glaucoma, however our sample had only 25 families and the evidence for linkage in both studies is modest. Therefore, it remains possible that this is a false positive finding.

Individual ordered subset analysis of the data. OSA attempts to create an etiologically homogeneous sample of families, but there is also within family variation in a complex disease like OAG. Subsetting individuals within families may serve to create more etiologically homogeneous families for analysis, which is what Individual OSA (IOSA) attempts to accomplish. However, IOSA did not provide further information, since subsetting family members on AAD did not substantially improve our scores. It is interesting that although both this study and the Allingham study identified optimal subsets at this location by considering the families with the earliest average AAD within

an adult onset set of OAG families, we did not gain the same effect by considering only the youngest affected individuals in a given family.

In summary, this study has provided evidence to confirm the previously identified GLC1I glaucoma locus through use of OSA to detect linkage of OAG to markers located on 15q11–13. Both studies detected linkage to this region through use of AAD as the covariate for ordering the families; therefore, future studies should be conducted using OAG families with earlier adult age at diagnosis and/or higher IOP. However, comparison of the two studies suggests that the locus as currently defined covers a broad region near the centromere on 15q. Thus, further work is needed to validate and refine the region around GLC1I.

Chapter 4

Lack of Association between Glaucoma Severity and Myocilin (MYOC) Promoter SNPs in CIGTS Subjects

ABSTRACT

Myocilin is the first identified primary open-angle glaucoma (POAG) gene. Mutations in the coding region of myocilin (MYOC) are often associated with juvenile-onset, familial forms of open-angle glaucoma characterized by unusually high intraocular pressure. Previous reports have suggested that the -1000CG SNP, also designated myoc.mt1, in the promoter region of myocilin may be associated with a more severe form glaucoma, but other reports have found no such association. In this study, we tested for an association between three MYOC promoter SNPs (-1000CG, -1075GA, and -1081AG) and more severe visual field loss and higher intraocular pressure (IOP) in subjects from the Collaborative Initial Glaucoma Treatment Study (CIGTS). The CIGTS subjects were recruited into the study at initial glaucoma diagnosis and were followed for nine years, with clinic visits every six months. None of the SNPs were associated with visual field

---

My role in this study includes sequencing exon 3 of myocilin, checking for genotyping errors, performing the bivariate and multiple logistic regression analyses, aiding in the mixed model analysis, writing the manuscript, and creating the figure and tables.

mean deviation (-1000CG, p=0.98; -1075GA, p=0.29; -1081AG, p=0.96) or with IOP (-1000CG, p=0.52; -1075GA, p=0.67; -1081AG, p=0.95) throughout the study period. The results of this study do not support the hypothesis that the -1000CG MYOC SNP, or two other promoter SNPs, are associated with glaucoma severity in the CIGTS population.

INTRODUCTION

Glaucoma is characterized by progressive visual field loss. It affects over 60 million people worldwide and is the second leading cause of blindness[22; 25]. The most common type of glaucoma in the United States is primary open angle glaucoma (POAG), where the anterior chamber angle is physically obstructed and there is no known underlying cause[23]. Although the etiology of POAG is unknown, it is likely that multiple genetic and environmental risk factors play a role in its onset and progression[24; 214]. One of the most consistent risk factors is family history, therefore suggesting underlying genetic causes of glaucoma[29; 215-217]. More than a dozen POAG loci have been mapped; however only three genes related to POAG have been identified: myocilin, optineurin, and wd-repeat36[24; 218]; the first identified and most well-characterized of these is myocilin.

Myocilin was first discovered during a cellular pharmacology experiment designed to mimic biochemical events that occur with steroid-induced ocular hypertension. The investigators treated trabecular meshwork cells with glucocorticoid steroids and identified genes with altered expression levels in response to the steroid exposure. They found that expression of an unknown gene was induced by the steroids[219]. The investigators originally named the gene trabecular meshwork inducible

glucocorticoid response (TIGR) gene, but the official name, myocilin (MYOC), was derived from a report on the presence of this protein in the cilium of the photoreceptor[220]. Independently of these studies, linkage to 1q23-q24 (locus GLC1A) was identified in families with juvenile onset open-angle glaucoma[59]. Subsequently, MYOC was identified as the gene causing the linkage signal to the GLC1A locus, thus making mutation in MYOC the first known genetic cause of POAG[60].

The MYOC gene is located at 1q23-q24 and is composed of three exons and a 5-kb promoter region[211]. Over 190 variants have been identified in the gene and promoter region, and although more than 70 glaucoma-causing mutations have been reported[221], only 2-4% of POAG patients have potentially disease-causing mutations in MYOC[62; 222]. The vast majority of putative disease causing variants are located in the third exon, most often in the olfactomedin domain[223]. In one region of more than 100 residues within the third exon, most of the presumed causative mutations involve a change in charge[61; 224]. Although predominately resulting in high intraocular pressure (IOP) and juvenile-onset POAG, alterations in MYOC have also been associated with adult-onset POAG and normal tension glaucoma[222]. Most MYOC mutations in juvenile-onset glaucoma are missense mutations, while the predominant mutation among adult-onset families is the Gln368stop nonsense mutation[61; 62; 221].

The exact function of the myocilin protein is not known. Recombinant MYOC appears to increase outflow resistance[225] and MYOC carrying a glaucoma-associated mutation forms aggregates in trabecular meshwork cells; over-expression of this mutant MYOC leads to cell death[226]. Some MYOC mutations alter translocational processing of the protein[227], other MYOC mutations bind to peroxisomal targeting signaling 1 receptor

(PTS1R) resulting in increased IOP[228], and two causative mutations are located adjacent to a peroxisome C-terminal targeting signal motif[224]. Thus, there is evidence that different mutations operate via different mechanisms[63].

A common trend is that MYOC mutations appear to act through gain of function rather than loss of function. A missense mutation known to cause POAG in humans, Tyr437His, resulted in a POAG phenotype in a mouse knock-in model[67]. No abnormal ocular phenotype was observed in heterozygous and homozygous null mice[65] and over-expression of Myoc did not result in increased IOP or glaucoma[66]. Humans can be homozygous for a nonsense mutation, Arg46stop, without developing glaucoma by 77 years[229] and a subject who was hemizygous for MYOC did not show increased IOP or optic nerve damage at 29 years[230]. Interestingly, subjects who were homozygous for two different deleterious mutations (Lys423Glu, Gln368stop) in MYOC were phenotypically normal at 43-50 years old[231; 232], while another subject who was homozygous for a third glaucoma-associated mutation (Gln48His) was severely affected[233].

Expression of MYOC is fairly ubiquitous; it is found in intraocular and extraocular tissue, as well as in tissues likely uninvolved with glaucoma such as heart and skeletal muscle[63; 64]. It is interesting to note that the corticosteroid induction of increased expression has been observed only in trabecular meshwork cells[234]. Another study tested for an association between variants in MYOC and change in IOP in response to glucocorticoids. There was no significant difference when responders (whose IOP increased at least 5 mmHg) were compare to non-responders; however, only 70 responders and 23 non-responders were tested[235].

Mutations in the promoter region of MYOC have the potential to alter the level of transcription of the gene. As mentioned above, over-expression of Myoc in mice does not cause glaucoma, but it has been hypothesized that over-expression of MYOC in patients who already have POAG may increase their disease severity or rate of disease progression. A study of 142 French POAG patients and 94 unrelated controls identified a single nucleotide polymorphism (SNP) located in the promoter region, 1000 base pairs upstream from the MYOC transcription start site. The researchers detected no association between the genotypes of -1000CG, also designated MYOC.mt1, and glaucoma status. Subsequently, the researchers conducted a retrospective study to examine glaucoma severity over time. At initial presentation at the clinic (ie. baseline), there was no difference in IOP or age across the genotypes. The subjects also did not differ significantly in length of time between baseline and enrollment in the study. However, glaucoma subjects who carried at least one copy of the rarer G allele (C/G or G/G genotypes) had a higher average IOP at the time of study enrollment than did subjects without the G allele. Upon further analysis, the researchers showed that males of all three genotypes (C/C, C/G, G/G) and females without the G allele (C/C) had significant decreases in IOP at the time of the study compared to baseline, while females with the G allele did not show a significant decrease in IOP. They also reported worse visual field scores in females with the G allele, compared to women without the G allele; a difference that was not seen when comparing men with and without the G allele. However they did not report baseline visual field scores for any study subjects[236].

Since the initial study, four additional studies have explored the relationship between -1000CG and glaucoma severity, as characterized by IOP, cup-to-disc ratio,

and/or visual field loss. Three of the four studies examined their patients cross-sectionally, testing the measure of disease severity (ie. vision loss) either at baseline or at the time of study. None of the three studies found an association between any measure of disease severity and -1000CG genotype when looking in populations of primarily European Americans[86], Chinese[237], and Turks[238]. The fourth study used survival analysis to test for an association with -1000CG. The outcomes were (1) time to a two-step worsening of optic disc and (2) time to a two-step worsening of visual field. The investigators found a main effect for -1000CG association with both outcomes. However, they included interactions of -1000CG by age and -1000CG by medication. Since they standardized age to 35 years old, the interpretation of the main effect reflects the increase in risk with a G allele for subjects who were 35 years old at baseline and not on medications at baseline. The significance of the effect of a G allele on risk in older subjects or on those taking medications is ambiguous[239].

The Collaborative Initial Glaucoma Treatment Study (CIGTS) is a multicenter, clinical trial of newly diagnosed open-angle glaucoma patients. The primary goals of CIGTS are to compare glaucoma outcomes and quality of life between subjects receiving either topical medications or trabeculectomy surgical treatment[240]. Six hundred and seven subjects with POAG, pseudoexfoliative glaucoma, or pigmentary glaucoma were randomized to one of the two treatment groups. The subjects have been re-examined and re-interviewed at regular intervals and standardized information on them has been collected. The results showed that, although IOP was higher in subjects randomized to the surgery arm, there was no significant difference in visual field loss[241]. For subjects

randomized to the surgery arm, there was a decrease in quality of life for a few of the measures, however some of these differences are decreasing over time[242].

CIGTS is an ideal population to test for glaucoma severity because of the thorough baseline assessment, the prospective nature of the study, the large number of subjects, inclusion of both African Americans and European Americans, and the uniformity of the data collected. In this genetics ancillary study, we tested for an association between the -1000CG genotype and (1) visual field loss and (2) IOP across the study period. The results of this study will provide valuable information regarding the relationship between -1000CG and glaucoma severity.

METHODS

Subjects. Recruitment for CIGTS has been described in detail previously[240]. In brief, to be eligible for CIGTS, subjects were required to meet four diagnostic criteria: (1) a diagnosis of POAG, pseudoexfoliative glaucoma, or pigmentary glaucoma in at least one eye; (2) at least one of three combination criteria of visual field changes, optic disc changes, and increased IOP, where less visual field loss requires a higher minimum IOP; (3) a best-corrected visual acuity of 70 or greater on the Early Treatment Diabetic Retinopathy Study scale (approximately 20/40 Snellen equivalent) in both eyes; and (4) between 25 and 75 years at enrollment. Exclusion criteria included eye surgery and use of glaucoma eyedrops for more than two weeks[240].

Over three and a half years, 607 subjects were recruited from 14 clinical centers. Three hundred subjects were randomized to receive surgery and 307 were randomized to receive medication. Blood samples were not required for participation in the study;

however we obtained samples from 467 patients (77%). To obtain a more phenotypically homogeneous population, we excluded subjects who were diagnosed with pseudoexfoliative glaucoma or pigmentary glaucoma. We also excluded subjects whose race was reported as Asian or Other since we had a limited number of subjects in each category and were concerned about possible phenotipic heterogeneity as seen when comparing African Americans with POAG to European Americans with POAG. Therefore, 393 African American or European American subjects with POAG were included in the study. Subjects were monitored for a median of 7.5 years, with a follow-up visit nearly every 6 months.

For this study, the main outcomes are changes in visual field and changes in IOP. Visual field loss is defined by mean deviation (MD) from Humphrey 24-2 visual field test and IOP is measured by a Goldmann applanation tonometery[240]. We also collected baseline data: glaucoma diagnosis, age at diagnosis, sex, self-reported race, diabetes (yes/no), hypertension (yes/no), and immediate family history of glaucoma (yes/no). Visual acuity was also collected at baseline as well as throughout the study.

Genotyping. To determine the genotype at -1000CG, we used PCR to amplify the region of interest (forward primer: ACTGTGTTTCTCCACTCTGG, reverse primer: CTGGGGAACTCTTCTCAGAA). The 25 μL reaction included 1 U AmpliTaq Gold (Applied Biosystems, Foster City, CA), 2 mM $MgCl_2$, 200 μM each dNTP, 20 pmol of each primer, and 100 ng of DNA. The thermal cycling conditions were (1) an initial denaturation step of 95° for ten minutes, (2) 35 cycles of 95° for 45 seconds, 66° for one minute, and 72° for one minute, and (3) an elongation step of 72° for ten minutes. The

PCR product was then purified using the Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced by the University of Michigan DNA Sequencing Core using dideoxynucleotide dye-termination sequencing and read by an ABI 3730 Sequencer. Polymorphisms were detected using Phred and Phrap software[243; 244], and traces were reviewed manually. We identified two other SNPs near -1000CG, designated -1081AG and -1075GA, located 1081 bases and 1075 bases upstream from MYOC, respectively. This appears to be the first identification of -1075GA, most likely because it may be only polymorphic in populations of African descent, whereas -1081AG was previously detected in a Chinese population[237]. We also estimated haplotypes of the three SNPs using the Markov Chain algorithm implemented in MACH[245].

In addition to the promoter region around -1000CG, we also sequenced exon 3 of MYOC to identify potential glaucoma-causing mutations. The PCR protocol was the same as for the promoter region except that we used 2.5 mM $MgCl_2$ and the denaturing temperature was 65°. The forward and reverse primers are CTGGCTCTGCCAAGCTTCCGCATGA and GGCTCTCCCTTCAGCCTGCT, respectively. We defined a disease causing variant as (1) non-synonomous, causing a change in protein and (2) occurring more often in cases than controls, based on a literature review. We also calculated the degree of linkage disequilibrium between -1000CG, -1075GA, -1081AG, and reoccurring disease causing variants (minor allele frequency ≥ 1%).

Statistical analysis. To test for an association between -1000CG and glaucoma-associated variables, we initially performed bivariate analyses. Since our continuous

covariates were not normally distributed, we performed Wilcoxin rank tests and for categorical covariates, we used Fisher's exact tests. Since some of the variables covary, we also established multiple logistic regression models containing the most parsimonious set of covariates associated with each of the promoter SNPs (-1000CG, -1075GA, and -1081AG).

To test for an association between average visual field or IOP over time, we included -1000CG in our established models for MD[246] and IOP[247], respectively. It has been suggested that subjects with MYOC mutations may respond differently to medical or surgical interventions, so we also included an interaction term between -1000CG and initial treatment. Additionally, we tested for progression of visual field using the change in MD after five years. We repeated these analyses replacing -1000CG with -1081AG and -1075GA or the GGC haplotype.

RESULTS

Study population. Of the 607 CIGTS subjects, 512 are African American or European American with POAG; 393 (77%) of them participated in the CIGTS ancillary genetic study. When we compare these 393 subjects to the 119 African American and European American POAG subjects we did not genotype, we find two differences. The genotyped subjects have a worse initial visual field (p=.026) and visual acuity (p=.039) than subjects who were not genotyped. However, using the Bonferroni correction for multiple testing (p-value threshold of 0.0029), these differences are not significant (Table 4-1).

Table 4-1. Comparison of subjects genotyped and not genotyped

	Genotyped (n=393)	Not genotyped (n=119)	P-Value
Socio-demographics			
Age at diagnosis (in years)*	59.3 (10.6)	59.7 (11.1)	.750
Sex (female)^	182 (46%)	50 (42%)	.462
Race (AA)^	167 (42%)	61 (51%)	.094
Marital status (not currently)^	45 (11%)	15 (13%)	.138
(never)	105 (27%)	42 (35%)	
Education*§	4.0 (1.3)	5.0 (1.5)	.150
Glaucoma related			
Treatment (surgery)^	187 (48%)	66 (55%)	.144
Study eye (left)^	196 (50%)	63 (53%)	.601
MD at diagnosis (in dB)*	-4.3 (4.3)	-5.3 (4.1)	**.026**
IOP at diagnosis (in mmHg)*	27.0 (5.4)	27.0 (5.0)	.973
VA (ETDRS) at diagnosis*	86.0 (5.6)	85.0 (6.0)	**.039**
Corneal thickness (in µm)*†	550.0 (46.3)	535.0 (66.7)	.498
Family history (yes)^‡	144 (40%)	38 (35%)	.315
Other medical conditions			
Diabetes (yes)^	70 (18%)	22 (18%)	.892
Hypertension (yes)^	157 (40%)	41 (34%)	.334
Other CV disease (yes)^	62 (16%)	16 (13%)	.662
Drug use			
Ever use tobacco (yes)^	16 (4%)	7 (6%)	.448
Alcohol (servings/week)*	0.0 (7.8)	0.0 (5.0)	.647

Comparison among African American and European American POAG subjects. Abbreviations: AA, African American; MD, mean deviation; IOP, intraocular pressure; VA (ETDRS), Early Treatment Diabetic Research Study visual acuity score; CV, cardiovascular

*Median (standard deviation) and Wilcoxon rank test p-value

^Count (percent) and Fisher's exact test p-value

†Genotyped n=221; not genotyped n=19

‡Genotyped n=357; not genotyped n=109

§Education was measured on a 7 point scale: 1=none, 2=1-6 years, 3=7-11 years, 4=12 years, 5=some college, 6=college degree, 7=graduate education

SNP detection. In sequencing the region around -1000CG, we obtained genotype information on two additional SNPs: -1081AG and -1075GA (Table 4-2). -1000CG, -1081AG, and -1075GA are in linkage disequilibrium with each other; we observe only four of the eight possible haplotypes in our African American population and only three of four possible haplotypes in our European American population (Figure 4-1).

Table 4-2. Genotype frequencies

SNP	Major/minor alleles	Homozygote – major allele	Heterozygote	Homozygote – minor allele
-1000CG	C/G	333 (85%)	59 (15%)	1 (0%)
-1075GA†	G/A	370 (94%)	23 (6%)	0 (0%)
-1081AG	A/G	223 (57%)	145 (37%)	25 (6%)

Comparison among African American and European American POAG subjects; reported count (percentage).
† -1075GA is not polymorphic in European Americans; all have the G/G genotype

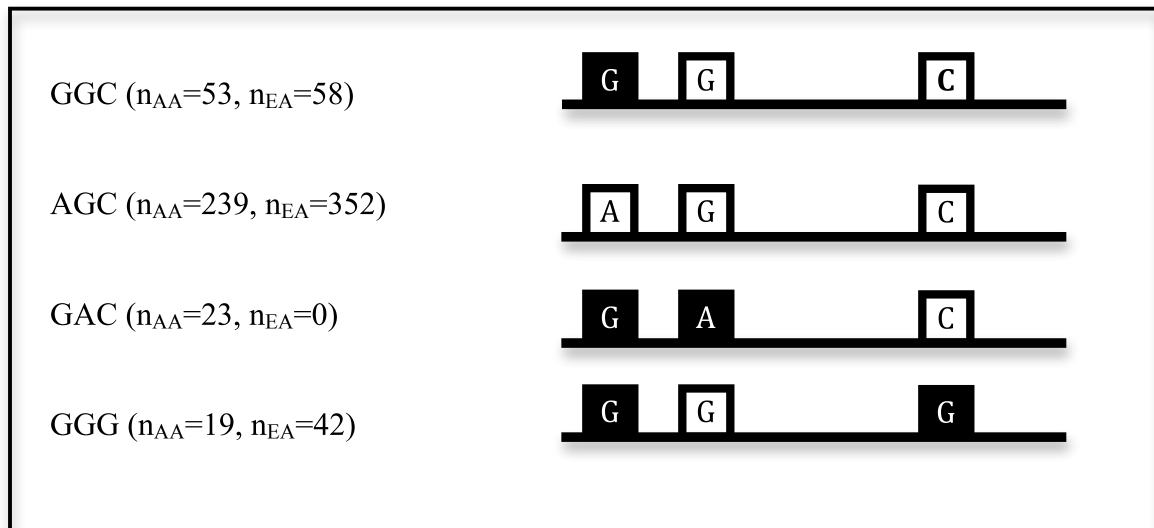

Figure 4-1. Predicted haplotypes of MYOC promoter SNPs. Squares represent -1081AG, -1075GA, and -1000CG alleles, respectively. White squares are the common alleles; black squares are the rare alleles. Abbreviations: n_{AA}, number of African Americans with the haplotype; n_{EA}, number of European Americans with the haplotype.

Almost all disease causing mutations in MYOC occur in the third exon. When we sequenced exon 3 in our samples, we identified 25 subjects with non-synonymous mutations (Table 4-3). One of these subjects has two non-synonymous mutations; one of the mutations (Lys398Arg) does not appear to be associated with POAG, however the other mutation (Arg272Gly) is likely causative. In another subject, we identified a novel SNP, Phe430Ser, that was not previously reported in dbSNP nor at myocilin.com[221]. When we compare subjects with reoccurring mutations (Glu352Lys, Gln368stop, and Lys398Arg) and subjects with a rare mutation to subjects without any mutations in exon 3, we were not able to detect any genotype-phenotype correlations. However the mutation frequencies vary by race. Seven of the eight individuals with Glu352Lys are African Americans (87.5%). This racial composition is significantly different than subjects without an exon 3 mutation; only 36% of them are African Americans (p=.0034). Gln368stop and Lys398Arg occur in only one African American each, and this is not different from subjects without an exon 3 mutation (p=.74, .51, respectively). All four subjects with a rare mutation are European American, this is also not different from subjects without an exon 3 mutation (p=.14; Table 4-4).

Table 4-3. Exon 3 non-synonomous mutations

Mutation(s)	DCV	Dx	Age at dx (in years)	Race	Median MD (in dB)	MD at dx (in dB)	IOP at dx (in mmHg)	VA at dx	Family history
Arg272Gly/ Lys398Arg	Yes	POAG	40.6	EA	-0.75	-2.5	37	77	Yes
Thr293Lys	Unk	POAG	56.6	EA	-4.21	-3.5	22	91	Unkn
Glu352Lys	Unk	POAG	64.8	AA	-5.29	-4.5	25	76	No
Glu352Lys	Unk	POAG	59.0	AA	-8.46	-5.8	20	86	Yes
Glu352Lys	Unk	POAG	56.5	AA	-10.09	-5.3	23	85	Yes
Glu352Lys	Unk	POAG	69.7	AA	-4.90	-4.6	28	85	No
Glu352Lys	Unk	POAG	52.0	AA	-1.84	-2.7	30	89	Unkn
Glu352Lys	Unk	POAG	55.4	Other	-1.87	-5.5	30	94	Unkn
Glu352Lys	Unk	POAG	55.7	AA	-3.37	-2.0	29	89	Yes
Glu352Lys	Unk	POAG	58.5	AA	-0.66	-0.7	22	95	No
Gly367Arg	Yes	POAG	39.7	EA	-18.25	-15.2	47	82	Yes
Gln368stop	Yes	POAG	42.1	EA	-0.69	-2.4	27	97	No
Gln368stop	Yes	POAG	66.4	EA	-5.43	-4.9	44	81	Unkn
Gln368stop	Yes	POAG	71.2	EA	-9.35	-9.7	25	86	Yes
Gln368stop	Yes	POAG	43.9	EA	-11.79	-9.3	31	81	Yes
Gln368stop	Yes	POAG	49.9	AA	-6.11	-5.3	23	82	Yes
Lys398Arg	No	POAG	58.1	EA	-1.95	-4.5	25	87	Yes
Lys398Arg	No	POAG	63.9	EA	-2.44	-5.8	30	80	No
Lys398Arg	No	POAG	42.8	EA	0.46	-1.0	32	83	Yes
Lys398Arg	No	POAG	71.8	AA	-5.40	-6.6	21	83	Yes
Lys398Arg	No	POAG	67.4	EA	-2.18	-4.2	26	85	No
Lys398Arg	No	POAG	56.0	EA	-3.97	-4.4	22	85	Yes
Lys398Arg	No	Pigm	45.3	EA	-18.51	-17.2	25	76	No
Phe430Ser*	Unk	POAG	67.5	Other	-3.67	0.0	29	80	Yes
Ala445Val	Unk	POAG	65.3	EA	-8.85	-2.4	28	81	No

Abbreviations: DCV, disease causing variant; dx, glaucoma diagnosis; MD, mean deviation; IOP, intraocular pressure; VA, Early Treatment Diabetic Research Study visual acuity score; Unk, unknown; Pigm, pigmentary glaucoma; EA, European American; AA, African American

Table 4-4. Baseline characteristics of exon 3 variants

Mutation	DCV	Age at dx (in years)	EA race	Median MD (in dB)	MD at dx (in dB)	IOP at dx (in mmHg)	VA at dx	Family history
Glu352Lys (n=7)	Unk	58.5 (6.0)	0* (0%)	-4.9 (3.4)	-4.5 (1.9)	25 (3.8)	86 (5.8)	3 (43%)
Gln368stop (n=5)	Yes	49.9 (13.3)	4 (80%)	-6.1 (4.2)	-5.3 (3.1)	27 (8.4)	82 (6.8)	3 (60%)
Lys398Arg (n=6)	No	61.0 (10.2)	5 (83%)	-2.3 (2.0)	-4.5 (1.9)	26 (4.3)	84 (2.4)	4 (67%)
Rare variant (n=4)	Unk	48.6 (12.5)	4 (100%)	-6.5 (7.6)	-3.0 (6.2)	33 (10.9)	82 (5.9)	2 (50%)
No exon 3 variants (n=371)	n/a	59.6 (10.6)	213 (57%)	-3.8 (5.1)	-4.3 (4.4)	27 (5.3)	86 (5.7)	132 (36%)

Comparison among POAG subjects with and without exon 3 variants. For dichotomous variables counts (percentages) of subjects with European American and positive family history are reported. For continuous subjects, medians (standard deviations) are reported. Abbreviations: DCV, disease causing variant; dx, glaucoma diagnosis; MD, mean deviation; IOP, intraocular pressure; VA, Early Treatment Diabetic Retinopathy Study visual acuity score; Unk, unknown; EA, European American; n/a, not applicable
*Significantly different from subjects with no exon 3 mutation (p=.0028)

We also found that the three common mutations (Glu352Lys, Gln368stop, Lys398Arg) are all in linkage equilibrium with -1000CG, -1075GA, and -1081AG, as well as each other (Table 4-5). Based on a review of the literature, the frequency of the Lys398Arg mutation does not appear to be different in glaucoma cases compared to controls, so we do not consider it to be a disease-causing variant. Subjects with this mutation were included in our analyses, however we excluded the 18 subjects with other non-synonymous mutations from further analyses because the mutations are either associated with glaucoma or there is insufficient data to determine the relationship.

Table 4-5. Linkage disequilibrium between MYOC promoter and common exon 3 SNPs

	-1081AG	-1075GA	-1000CG	E352K	Q368stop	K398R
-1081AG	-	.083	.250	.000	.000	.000
-1075GA	1.000	-	.002	.000	.000	.000
-1000CG	1.000	1.000	-	.000	.000	.000
E352K	.000	.000	.000	-	.000	.000
Q368stop	.000	.000	.000	.000	-	.000
K398R	.000	.000	.000	.000	.000	-

R^2 values are above the diagonal, D' values are below the diagonal. SNPs are ordered along chromosome. All genotyped subjects are included in the calculations.

Association analyses. We tested for an association between baseline characteristics related to glaucoma and the three promoter SNPs using separate multiple logistic regressions. We found that -1000CG is associated with baseline visual acuity; individuals carrying at least one copy of the G allele have higher visual acuity than those who have the C/C genotype (OR=1.11, p=.0008). We also found that subjects carrying the G allele have a lower odds of cardiovascular disease than those with the C/C genotype (OR=0.31; p=.024; Table 4-6). Adjusting for multiple tests using the

Bonferroni correction, our significance threshold is .0045. Therefore only the association with visual acuity meets this level of significance, however the Bonferroni correction assumes that all tests are independent and therefore may be overly conservative.

When analyzing -1075GA, we restricted our sample to only African Americans because the SNP is not polymorphic European Americans. We did not find any association between -1075GA and the glaucoma related variables (Table 4-6).

For -1081AG, we found associations with sex (p=.019), self-reported family history (.0015), and hypertension (p=.0062). Subjects with at least one G have lower odds of being female (OR=0.82), higher odds of reporting family history (OR=2.13), and higher odds of hypertension (OR=1.87) than subjects with an A/A genotype. When we compare to a Bonferroni corrected significance threshold, the association with reported family history remains significant (Table 4-6).

We were also interested in a possible association with the 3-SNP haplotype. Of the four haplotypes we observed, three of them are perfectly tagged by one of the individual SNPs. The only untested haplotype is the GGC haplotype. When we test this haplotype, we identify an association with reported family history (p=.0002); subjects with at least one copy of the GGC haplotype have a higher odds of reported family history (OR=2.67). After Bonferroni correction, reported family history of glaucoma is still significantly associated with the GCC haplotype (Table 4-6).

We also conducted bivariate analyses for each MYOC promoter SNP and the GGC haplotype, to test associations without controlling for other variables. The bivariate analyses provided very similar results to the ones described above (results not shown).

Table 4-6. Phenotype by -1000CG genotype

	-1000CG	PV	-1075GA	PV	-1081AG	PV	CCG hap	PV
Age at diagnosis (in years)	1.03 (1.00, 1.06)	.076	1.04 (0.99, 1.10)	.113	1.03 (1.00, 1.05)	.054	1.01 (0.98, 1.03)	.521
Sex (female)	0.52 (0.27, 1.00)	.051	0.77 (0.29, 2.06)	.606	0.82 (0.53, 1.28)	**.019**	1.13 (0.69, 1.84)	.631
Race (AA)	0.52 (0.26, 1.02)	.058	-	-	1.13 (0.71, 1.79)	.383	1.04 (0.62, 1.75)	.874
MD at diagnosis (in dB)	0.94 (0.88, 1.01)	.087	0.95 (0.86, 1.06)	.343	0.98 (0.93, 1.04)	.613	1.02 (0.96, 1.08)	.590
IOP at diagnosis (in mmHg)	1.00 (0.94, 1.06)	.906	0.99 (0.90, 1.08)	.750	1.00 (0.96, 1.04)	.540	0.99 (0.94, 1.03)	.585
VA (ETDRS) at diagnosis	1.11 (1.05, 1.18)	**<.001**	1.10 (0.99, 1.22)	.082	1.03 (0.99, 1.07)	.908	0.96 (0.92, 1.00)	.055
Corneal thickness (in µm)†	0.99 (0.99, 1.00)	.108	0.99 (0.97, 1.01)	.377	1.00 (0.99, 1.00)	.180	1.00 (0.99, 1.01)	.584
Family history (yes) ‡	0.80 (0.41, 1.57)	.524	2.35 (0.84, 6.58)	.104	2.13 (1.34, 3.40)	**.002**	2.67 (1.60, 4.43)	**<.001**
Diabetes (yes)	2.66 (1.23, 5.75)	.013	1.34 (0.45, 4.00)	.597	1.59 (0.90, 2.86)	.118	0.95 (0.50, 1.79)	.866
Hypertension (yes)	1.09 (0.58, 2.06)	.785	2.30 (0.84, 6.29)	.103	1.87 (1.20, 2.93)	**.006**	1.59 (0.96, 2.61)	.069
Other CV disease (yes)	0.31 (0.11, 0.86)	**.024**	0.78 (0.22, 2.81)	.704	0.60 (0.32, 1.09)	.095	0.95 (0.49, 1.84)	.885

Logistic regression results of African American and European American POAG subjects without exon 3 potentially disease causing variants. The number of individuals for -1000CG, C/C=320 and C/G+G/G=57; for -1075GA, G/G=137 and G/A=22; for -1081AG, AA=212 and A/G+G/G=165; for CCG haplotype, 0 copies=279 and at least 1 copy=98

Abbreviations: AA, African American; MD, mean deviation; IOP, intraocular pressure; VA (ETDRS), Early Treatment Diabetic Retinopathy Study visual acuity score; CV, cardiovascular

†For -1000CG, C/C=175 and C/G+G/G=39; for -1075GA, G/G=74 and G/A=14; for -1081AG, AA=111 and A/G+G/G=103; for CCG haplotype, 0 copies=156 and at least 1 copy=58

‡For -1000CG, C/C=294 and C/G+G/G=50; for -1075GA, G/G=124 and G/A=19; for -1081AG, AA=198 and A/G+G/G=146; for CCG haplotype, 0 copies=255 and at least 1 copy=89

Additionally, we tested for an association between the MYOC promoter variants and average visual field over time, as measured by MD. When we included -1000CG in a previously published regression model[246], -1000CG was not a significant predictor of MD (p=.979), nor were -1081AG (p=.988), -1075GA (p=.294), or the GGC haplotype (p=.880; Table 4-7). These results were consistent within each racial group (results not shown). Since it has been suggested based on anecdotal evidence that individuals with MYOC glaucoma-causing mutations may respond better to surgery than medicine, we stratified our analysis by treatment. We identified no differences in average visual field loss (results not shown).

Table 4-7. Average mean deviation across the study period

SNP / haplotype	Genotype	N	Average IOP (SE)	P-Value
-1000CG	C/C	320	-6.55 (.21)	.979
	C/G or G/G	57	-6.56 (.33)	
-1075GA†	G/G	137	-8.28 (.38)	.294
	G/A	22	-7.56 (.68)	
-1081AG	A/A	212	-6.56 (.23)	.958
	A/G or G/G	165	-6.55 (.24)	
GGC	0 copies of GGC	279	-6.55 (.21)	.880
	≥1 copy of GGC	98	-6.59 (.28)	

Comparison of average mean deviation for years 2-9 of CIGTS, controlling for mean deviation at glaucoma diagnosis (baseline), for complete model see Musch et al[246]. Comparison among African American POAG subjects without exon 3 potentially disease causing variants for -1000CG, -1075GA, and GGC.
†Comparison among African Americans only for -1075GA since all European Americans have G/G genotype.

We also did not find an association between average IOP throughout the study[247] and -1000CG (p=.517), -1075GA (p=.666), -1081AG (p=.953), or the GGC haplotype (p=.965; Table 4-8). We also did not find any differences when we stratified by treatment (results not shown).

Table 4-8. Average IOP across the study period

SNP / haplotype	Genotype	N	Average IOP (SE)	P-Value
-1000CG	C/C	320	16.1 (.17)	.517
	C/G or G/G	57	15.8 (.32)	
-1075GA†	G/G	137	16.9 (.62)	.666
	G/A	22	16.7 (.39)	
-1081AG	A/A	212	16.0 (.20)	.953
	A/G or G/G	165	16.0 (.22)	
GGC	0 copies of GGC	279	16.0 (.18)	.965
	≥1 copy of GGC	98	16.0 (27)	

Comparison of average IOP for years 2-9 of CIGTS, controlling for IOP at glaucoma diagnosis (baseline), for complete model see Musch et al[247]. Comparison among African American POAG subjects without exon 3 potentially disease causing variants for -1000CG, -1075GA, and GGC. †Comparison among African Americans only for -1075GA since all European Americans have G/G genotype.

DISCUSSION

Previous reports have suggested that subjects who carry a G allele at the MYOC promoter SNP, -1000CG, may have more severe glaucoma[236; 239]. Other studies have looked at glaucoma severity at a given point in time and found no association with -1000CG[86; 237; 238]. Here, we examined the relationship between glaucoma severity and three MYOC promoter SNPs (-1000CG, -1075GA, and -1081AG) separately and jointly in newly diagnosed African American and European American POAG subjects from CIGTS. We used IOP and visual field MD as our measures of glaucoma severity. We found no association between any of the MYOC promoter variants with characteristics associated with POAG at diagnosis nor did we find an association with visual field or IOP across the study period. Calculations indicate adequate power for detecting association with visual field (power=0.90) and IOP (power=0.71).

We also screened all of the CIGTS subjects who donated blood samples, regardless of glaucoma diagnosis or race, for variants in exon 3 of MYOC. We did not find any phenotype-genotype correlations. We note that, not surprisingly, the allele frequencies of the SNPs varied substantially by race. We also identified a subject with a novel variant, Phe430Ser.

An interesting result is the association between -1081AG and hypertension. There is evidence for a positive correlation between IOP and systolic blood pressure, in both individuals with POAG and in unaffected individuals. Several of the studies report a positive, but weaker, correlation between IOP and diastolic blood pressure as well. However, the association between POAG and blood pressure is more conflicting[248]. It may be that blood pressure increases IOP, but without other risk factors, the individual does not develop POAG. In this population of subjects already diagnosed with POAG, IOP did not differ by hypertension status (p=.57) nor did it vary with the -1081AG genotype. A second noteworthy association identified in this study is between the GGC haplotype and a self-reported family history of glaucoma.

There were some limitations to our study. Since our subjects were being treated, we do not know the natural history of the disease. It is not possible to assess what the progression of glaucoma would have been if these subjects had not been treated. Also, although these subjects were all newly diagnosed, we were unable to determine their true age at onset. Therefore, some subjects entered the study at a more advanced stage than others. In addition, we were not able to include all CIGTS subjects in the genetics ancillary study. However, the genotyped subjects were similar to those we did not

genotype. Lastly, as with most prospective studies, we did not have complete data on all subjects, since some subjects were lost to follow-up.

Our study has several strengths. Because the CIGTS subjects were followed prospectively, we have data from baseline diagnosis and over time from regular clinic visits. Also the subjects' clinical examinations occurred approximately every six months; therefore the data are fairly complete across subjects and give an informative picture about glaucoma progression. Although there were multiple centers, the treatment and data collection were standardized so that we are confident in evaluating the subjects as one group. Another strength of the study is that we included both European Americans and African Americans in our study. To our knowledge this was the first study to look at -1000CG in African Americans; this is noteworthy since African Americans often have more severe glaucoma outcomes and are sometimes under-represented in studies of glaucoma genetics.

Consistent with other studies that looked at the association with -1000CG and glaucoma severity at one point in time, we did not find an association between the genetic variants and glaucoma-related variables at diagnosis. Our results differ from the two longitudinal studies in that we did not find an association with glaucoma severity over time either. As mentioned above, there are limitations that may have prevented us from identifying an association; however based on our results there does not appear to be an association between any of the variants we tested and glaucoma severity in our population.

Chapter 5

Conclusions

SUMMARY OF FINDINGS

Elucidating the etiologies of complex disorders is the latest challenge for researchers in the field of genetics. Often when studying complex disease genetics, investigators will employ either linkage or association analyses to determine which genes or loci are involved in the disease. In traditional linkage analysis, co-segregation of the disease and a variant is tested within a family with multiple affected individuals. Typically, association analysis tests for variants to be more common in subjects with the disease of interest, compared to subjects without the disease. In this dissertation, we used both linkage and association, but applied modifications specific to each study.

In chapter 2, we performed a genome-wide linkage analysis. However, instead of performing nonparametric linkage analysis or parametric analysis using pre-specified disease parameters, we used a maximum LOD (MOD) score technique. We used this method because, in studying schizophrenia, we do not have reliable estimates for disease penetrances at each genotype nor for disease allele frequency; the MOD score approach estimates these parameters from the data. Using this technique, we were able to identify a region on chromosome 13q that was linked to schizophrenia in our sample of Afrikaners. This linkage result provided further support for this region, given that other

linkage studies had also shown evidence for linkage to that region.

The findings in chapter 3 were also obtained using linkage analysis, however, here we used ordered subset analysis (OSA). With OSA, we aim to create a more homogeneous subset of families on whom we perform linkage analysis. The rationale is that POAG is a heterogeneous disorder; therefore different genes and environmental factors will be playing different roles in each family. Individuals in whom genes are more strongly involved often have an earlier age of onset and a more severe phenotype. In our sample, we found just that; families linked to the GLC1I locus were identified by their earlier age at diagnosis. Interestingly, the families' average ages at diagnosis were inversely correlated with the families' average IOP. One interpretation is that families with earlier age at diagnosis have more severe glaucoma, which would be consistent with an underlying genetic cause of POAG.

In chapter 4, we used association analysis to test the relationship between promoter variants in the known glaucoma gene myocilin and glaucoma severity. Here, the research question was not if the SNPs were associated with the presence/absence of the disease, but rather if the SNPs were associated with any of the categorical or continuous glaucoma-related traits in POAG subjects. We were not able to identify an association between any of the SNPs in the promoter region and glaucoma characteristics at baseline or over time in the CIGTS population.

ONGOING STUDY

In addition to the three completed reports that make up the body of this thesis, it is worth detailing an additional ongoing project. The study is designed to test for an association between POAG and variants in the LMX1B gene; its progress is described here.

Background. An appealing candidate gene for POAG is LMX1B, a LIM-homeodomain transcription factor. Mutations in this gene are known to cause nail patella syndrome (NPS), a disorder that is characterized by underdevelopment of fingernails, kneecaps, and joints[249]. NPS is a highly penetrant, autosomal dominant disorder with phenotypic variation that includes the presence of glaucoma. In four European American families with NPS and open angle glaucoma (OAG), all 13 subjects with confirmed OAG had NPS; and the 15 subjects who had NPS, but not OAG, were generally younger. In two of the four families, NPS was caused by a different premature stop codon, in the third there was a deletion producing a truncated protein. All three mutations result in LMX1B haploinsufficiency. In the fourth family, a missense transversion in a conserved residue that forms a Zn(II)-binding site was identified as the causal mutation for NPS. Since it is unlikely that all four NPS mutations are co-segregating with a glaucoma mutation in a neighboring gene, it was hypothesized that the mutations in LMX1B predisposed family members to OAG[249; 250]. Mouse studies further support this claim; they have shown that LMX1B is expressed in ocular tissues, including the ciliary body and the trabecular meshwork, and that homozygous mutant mice have abnormally developed anterior eye segments, as well as iris and ciliary body hypoplasia[251].

In this study, we are testing for an association between POAG and polymorphisms in LMX1B. Our study population included African Americans, European Americans, and Ghanaians. Since populations of African ancestry show differences in prevalence, average age at diagnosis, and response to therapies, separate evaluation of European and African populations might assist in reducing the heterogeneity of the disease[43; 44]. Therefore, we are testing each of these populations separately and combining them, but controlling for differences in allele frequencies. We are also using a two-stage strategy that allows us to use data on many SNPs in the first stage to select a smaller sample of SNPs for use in second stage testing.

Subjects. Through the Kellogg Eye Center at the University of Michigan and the University of Ghana Medical School, using a protocol approved by the Institutional Review Boards of the University of Michigan and the University of Ghana, we recruited subjects diagnosed with POAG and unrelated controls. Blood samples were collected on all subjects and an eye examination was preformed when possible. The examination tested for glaucomatous optic neuropathy using a slit-lamp and glaucomatous visual fields using the Glaucoma Hemifield Test. Open angles were determined using a gonioscopic lens. A diagnosis of POAG was defined as: evidence of optic nerve damage, glaucomatous visual field, and open angles. Optic nerve damage includes either two primary indicators of damage (cup to disc ratio ≥0.7 and increase in cup to disc ratio of ≥0.2) or one primary indicator and at least one secondary indicator (notching of the neuroretinal rim, hemorrhaging, or asymmetry ≥0.2 between the cup to optic disc ratios of the two eyes). For subjects not examined by the ophthalmologists at the University of

Michigan or the University of Ghana, diagnoses were confirmed by reviewing their medical records. Where visual fields were not available, we accepted optic nerve damage with an IOP >= 19 and evidence that the subject has been treated for glaucoma. We excluded individuals with pigmentary dispersion syndrome, pseudoexfoliation, or high myopia.

The study was carried out in two stages. For Stage 1, the complete LMX1B coding sequence and flanking splice sites were sequenced on 208 subjects. For Stage 2 of the study, we recruited 137 additional cases and 173 additional controls. However, none of these additional controls were African American. Therefore, we also included 83 African American subjects from the Coriell Human Variation Panel into our Stage 2 population. We do not know the affection status of the Coriell individuals. Assuming that the glaucoma prevalence in the Coriell subjects is typical of African Americans (6%)[23; 43] power calculations do not indicate that this potential misclassification will decrease our power substantially (Table 5-1a).

Table 5-1a. Power calculation for 99 African American cases and 105 African American controls (assuming disease prevalence of 6%)

Risk Allele/Haplotype Freq	.1	.2	.5	.1	.2	.5	.1	.2	.5
Heterozygote GRR	1.3	1.3	1.3	1.5	1.5	1.5	1.7	1.7	1.7
Homozygote GRR	1.6	1.6	1.6	2.0	2.0	2.0	2.4	2.4	2.4
Power with confirmed controls	.14	.20	.24	.27	.40	.43	.43	.60	.60
Power with population controls	.13	.18	.21	.25	.36	.39	.39	.54	.55

Table 5-1b. Power calculation for 75 European American cases and 116 European American controls (assuming disease prevalence of 2%)

Risk Allele/Haplotype Freq	.1	.2	.5	.1	.2	.5	.1	.2	.5
Heterozygote GRR	1.3	1.3	1.3	1.5	1.5	1.5	1.7	1.7	1.7
Homozygote GRR	1.6	1.6	1.6	2.0	2.0	2.0	2.4	2.4	2.4
Power with confirmed controls	.12	.17	.20	.24	.34	.37	.38	.52	.52

Table 5-1c. Power calculation for 101 Ghanaian cases and 105 Ghanaian controls (assuming disease prevalence of 6%)

Risk Allele/Haplotype Freq	.1	.2	.5	.1	.2	.5	.1	.2	.5
Heterozygote GRR	1.3	1.3	1.3	1.5	1.5	1.5	1.7	1.7	1.7
Homozygote GRR	1.6	1.6	1.6	2.0	2.0	2.0	2.4	2.4	2.4
Power with confirmed controls	.14	.20	.24	.28	.40	.44	.44	.60	.61

Stage1 genotyping and SNP detection. We used five primer pairs synthesized by the University of Michigan (Ann Arbor, MI) or Research Genetics (Huntsville, AL) to amplify the exons and flanking regions in the Stage 1 cases and controls (Table 5-2). We performed PCR on a Perkin-Elmer 9600 or 9700 thermal cycler using the AmpliTaq Gold (Applied Biosystems, Foster City, CA) or PFU (Stratagen, La Jolla, CA) enzyme and purified the DNA using Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany). Sequencing was conducted at the University of Michigan DNA Sequencing Core by using standard dideoxynucleotide dye-termination protocol and evaluating fragment sizes on an ABI 3730 sequencer. Sequence alignments and allele assignments were done using the SeqMan software (DNASTAR, Madison, WI) and alleles were manually confirmed.

Table 5-2. Primers used to PCR amplify and sequence the coding regions of LMX1B

Exon Amplified	Direction	Primer
Exon 1	Forward	ATATAGCAACAGGTCCCGAG
Exon 1	Reverse	CCCATTTCCTTTATCCGTTG
Exon 2	Forward	CTGACGGCCGGGCTTTC
Exon 2	Reverse	AAGACGCGCAGCTCTCGGAA
Exon 3	Forward	TGGGAGGGACTTCTGAGCAC
Exon 3	Reverse	GATATGCATGTACCATCTG
Exons 4,5,6	Forward	CTTATCCTGGGCCACTGGGGAGCC
Exons 4,5,6	Reverse	ATCCCGGGCCCCTTTGTCCCTAGC
Exons 7,8	Forward	CCTGGGGAAGGGGCTGGGGAGTC
Exons 7,8	Reverse	GGTCAGGCCCAGCTGGCCGAGGG

Stage 2 genotyping. For Stage 2, we chose to follow-up the five common exonic SNPs because they may be functionally important and the five common intron 3 SNPs because they were statistically interesting based on the Stage 1 data (results not shown). We used a medium throughput technique to genotype these ten SNPs in the Stage 2 individuals, with accuracy of the method monitored by including 34 individuals from Stage 1 in the Stage 2 genotyping. The medium throughput genotypes all SNPs on one array. First, a multiplex PCR is performed to amplify locus-specific regions from the genomic DNA. Next, a normalization PCR amplifies each locus to uniform levels. To degrade the residual Taq polymerase which can interfere with subsequent steps, the enzyme is digested by a protease. The digestion is followed by a heating step to inactivate the protease. In the final multiplex ligation step, two adjacent oligonucleotides probes are hybridized with the PCR product, flanking the site of the SNP polymorphism. The two possible alleles of the SNP each have a matched sequence oligonucleotide, differing at the variant base and in the total oligonucleotide length. Following ligation, each of the two SNP alleles for each locus generates a ligated product of unique, known size. The

multiple ligated products are electrophoresed and the allele genotypes are determined by the size of the product.

SNP checking. To verify the quality of our data, we tested both markers and subjects for missing genotypes. We also tested our markers for departures from Hardy-Weinberg equilibrium. Since we used different genotyping methods for Stage 1 and Stage 2, we compared the genotyping calls for subjects run on both platforms. To determine the degree of linkage disequilibrium between the identified SNPs, we used the LDMax component of the GOLD statistical package[252]. The program uses an expectation-maximization algorithm to estimate haplotype frequencies and reports the degree of linkage disequilibrium (as pair-wise R^2 and D' values) between two SNPs.

Single SNP analysis. To identify population heterogeneity, we conducted Fisher's exact tests to compare the allele frequencies of common SNPs across the African American, European American, and Ghanaian populations; here, common refers to a SNP with a minor allele frequency greater than 2% in at least one population. We also used Fisher's exact tests to compare the allele frequencies in cases and controls, assessing the association between individual SNPs and POAG. We performed these analyses separately for each of the three populations. We also analyzed the populations together, including an indicator for each population to control for differences in allele frequencies.

Haplotype analysis. We will use a Markov Chain algorithm implemented in MACH[245] to estimate haplotypes for each subject. To test for association, we will

compare the haplotype frequencies in cases and controls using a chi-square test. For the haplotypes with a frequency less than 5%, we will pool them into a haplotype category denoted “rare haplotypes”. An α-level of .05 is our significance threshold.

Automated haplotype search. Since there is likely redundancy in the 14 common SNP haplotypes, we will search for more parsimonious haplotypes using a systematic backwards selection method. In the first round of the algorithm, each SNP in a 14-SNP haplotype is removed to create thirteen 14-SNP haplotypes and we calculate a p-value for each of the 13-SNP haplotypes. The haplotype that is the most significant becomes the test haplotype for the next round and the SNP that was removed is excluded from further testing. The algorithm then proceeds to test for significantly associated 12-SNP haplotypes. The elimination is repeated until the empirical p-values for all haplotypes in a given round are above the .05 threshold for alpha.

Description of study population. In Stage 1 of the study, a total of 208 individuals were sequenced, 138 (66%) cases and 70 (34%) unrelated controls. When we include the subjects from Stage 2, there are 275 (46%) cases and 326 (54%) controls. Of these 601 subjects, 204 (34%) are self-reported African Americans (AA), 206 (34%) are African Ghanaians (AG), and 191 (32%) are European Americans (EA); the proportion of cases and controls in each ethnic population is not significantly different (p=0.093). The mean age (±SD) in our total populations is 60.7 (±17.1); this is different in our cases and controls (p=0.001). Proportionally more of the females are controls (58%) while more of the males are cases (51%; p=0.026; Table 5-3).

Table 5-3. Subjects by affection status and ethnicity

	Stage 1			Stage 1 + Stage 2		
	POAGs	Controls	P-Value	POAGs	Controls*	P-Value
Ethnicity			0.030			0.093
AA	61 (73%)	22 (27%)		99 (49%)	105 (51%)	
AG	18 (49%)	19 (51%)		101 (49%)	105 (51%)	
EA	59 (67%)	29 (33%)		75 (39%)	116 (61%)	
Gender			0.768			0.026
Female	77 (65%)	41 (35%)		142 (42%)	198 (58%)	
Male	61 (68%)	29 (32%)		133 (51%)	128 (49%)	
Age	64.8 (12.4)	63.9 (10.6)	0.646	63.3 (13.1)	58.5 (19.6)	0.001
Total	138 (66%)	70 (34%)		275 (46%)	326 (54%)	

Abbreviations: POAG, primary open-angle glaucoma; AA, African American; AG, African Ghanaian; EA, European American
*Includes 83 Coriell African American population controls

SNP detection. The LMX1B gene maps to 9q34 and is approximately 82 kb. The coding sequence is made up of eight exons and is 1119 bases long; most of the gene is contained within the 80-kb intron 2. In sequencing the eight exons and flanking intronic regions, we identified 22 SNPs. All of the SNPs were genotyped on at least 95% of the subjects attempted. We tested the controls in each population for HWE, to identify possible population stratification. We identified two SNPs in the African American population and two SNPs in the Ghanaian population that were not in equilibrium at the significance level of 0.05. However, none of the SNPs attained the Bonferroni corrected level of significance of 0.0023 (Table 5-4). We also found that only three of 267 genotypes (1.1%) differed when successfully genotyped by sequencing and by the medium throughput technique.

Table 5-4. Missing data and Hardy-Weinberg equilibrium (HWE) checks

SNP	Allele	Genotyping rates	HWE in controls AA (n=105)	AG (n=105)	EA (n=116)
intron2					
int2+7^	G/C	311 (95%)	.538	1.00	.121
exon3					
E124E	A/G	596 (99%)	.234	1.00	.673
S155S	C/A	598 (>99%)	1.00	.071	1.00
intron3					
int3+20	G/A	599 (>99%)	1.00	**.033**	1.00
int3-87	A/G	571 (95%)	1.00	1.00	1.00
int3-61*	C/T	208 (100%)	-	-	-
int3-49	C/T	570 (95%)	1.00	.069	1.00
int3-28	A/C	571 (95%)	1.00	.257	1.00
int3-19	C/T	590 (98%)	1.00	1.00	1.00
exon4					
S219S	C/G	571 (95%)	**.028**	1.00	.423
intron6					
int6+18*	C/T	208 (100%)	-	-	-
int6+38*	G/A	208 (100%)	1.00	-	-
int6-26*	G/T	208 (100%)	.270	.663	1.00
exon7					
T287T	G/A	571 (95%)	1.00	**.018**	1.00
intron7					
int7+27*	G/A	208 (100%)	-	-	-
int7+28*	C/T	208 (100%)	**.023**	-	-
int7-43*	C/A	208 (100%)	-	-	-
exon8					
S346S	C/T	582 (97%)	.554	1.00	1.00
F377F*	C/T	208 (100%)	-	-	-
3'UTR					
3U+27*	G/A	208 (100%)	1.00	.318	-
3U+51*	A/G	208 (100%)	1.00	.345	-
3U+79*	G/A	208 (100%)	1.00	.345	-
Rare SNP*	no/yes	208 (100%)	-	-	-

Major (more common) allele is listed first. Data is missing where the controls in that ethnic population were not polymorphic. Abbreviations: AA, African American; AG, African Ghanaian; EA, European American

*Genotyped on stage 1 samples only: AA=83 subjects/22 controls, AG=37 subjects/19 controls, EA=88 subjects/29 controls

^Genotyped on stage 2 samples for EA and stage 1 samples for AA and AG: AA=83 subjects/22 controls, AG=37 subjects/19 controls, EA=191 subjects/116 controls

When we calculate the allele frequencies in the three populations separately (African Americans, European Americans, and Ghanaians) using all available genotype data, 14 SNPs have a minor allele frequency greater than 2% in at least one group. Thirteen of the 14 common SNPs are located near the 3' end of the gene (Figure 5-1).

Figure 5-1. Fourteen common SNPs and their positions in LMX1B; common SNPs are defined as those with minor allele frequencies ≥ 2% in at least one population. Solid bars are exons and lines are introns. I indicates an intronic marker, E indicates an exonic marker, U indicates marker in 3' UTR.

Of these common SNPs, all are polymorphic in both of the African populations, but three do not vary in the European Americans. Significant allele frequency differences between the populations were detected in 11 of the 22 SNPs (Table 5-5).

Table 5-5. Minor allele frequencies and comparisons across populations

SNP	Alleles	AA (n=204)	AG (n=206)	EA (n=191)	AA v AG p-value	AA v EA p-value	AG v EA p-value
intron2							
int2+7^	G/C	.169	.054	.295	.014	**<.0001**	**<.0001**
exon3							
E124E	A/G	.455	.408	.359	.180	**<.0001**	**<.0001**
S155S	C/A	.022	.027	-	.822	**.0039**	**.0010**
intron3							
int3+20	G/A	.071	.126	-	**.010**	**<.0001**	**<.0001**
int3-87	A/G	.013	.020	.006	.416	.458	.113
int3-61*	C/T	-	.014	-	.311	-	.296
int3-49	C/T	.058	.072	.080	.470	.246	.679
int3-28	A/C	.032	.046	.006	.363	**.0090**	**.0005**
int3-19	C/T	.010	.019	.005	.172	.688	.112
exon4							
S219S	C/G	.408	.421	.363	.719	.229	.114
intron6							
int6+18*	C/T	.006	-	-	1.00	.485	-
int6+38*	G/A	.012	-	-	1.00	.235	-
int6-26*	G/T	.295	486	.057	**.0054**	**<.0001**	**<.0001**
exon7							
T287T	G/A	.032	.070	-	**.016**	**.0004**	**<.0001**
intron7							
int7+27*	G/A	.006	-	.023	1.00	.372	.322
int7+28*	C/T	.012	-	-	1.00	.235	-
int7-43*	C/A	-	-	.006	-	1.00	1.00
exon8							
S346S	C/T	.191	.238%	.003	.123	**<.0001**	**<.0001**
F377F*	C/T	-	-	.006	-	1.00	1.00
3'UTR							
3U+27*	G/A	.205	.365	.006	**.010**	**<.0001**	**<.0001**
3U+51*	A/G	.235	.351	.006	.083	**<.0001**	**<.0001**
3U+79*	G/A	.235	.351	.006	.083	**<.0001**	**<.0001**
Rare SNP	no/yes	4.2%	.054	.017	.742	.208	.200

Major (more common) allele is listed first. Abbreviations: AA, African American; AG, African Ghanaian; EA, European American

*Genotyped on Stage 1 samples only: AA=83 subjects, AG=37 subjects, EA=88 subjects

^Genotyped on stage 2 samples for EA and stage 1 samples for AA and AG

The average R^2 (±SD) for all SNP pairs is 0.12 (± 0.20) and the average D' (±SD) is 0.70 (±0.36). The three 3'UTR SNPs are all in high linkage disequilibrium with each other; 3'UTR +51 and 3'UTR +79 are completely redundant (R^2 = 1.00), and 3'UTR +27 is tightly linked with both of them (R^2 = 0.82; D'=0.94; Table 5-6). Due to the high level of genotype completeness, lack of Hardy-Weinberg disequilibrium, and high degree of agreement between sequencing and medium throughput genotyping technique we are confident in the quality of the data.

Table 5-6. Pair-wise linkage disequilibrium of common SNPs

	int2+7	E124E	S155S	int3+20	int3-87	int3-49	int3-28	S219S	int6-26	T287T	int7+27	S346S	3U+27	3U+51	3U+79
int2+7^	-	0.05	0.00	0.01	0.00	0.01	0.01	0.04	0.08	0.01	0.00	0.03	0.04	0.05	0.05
E124E	0.48	-	0.02	0.03	0.00	0.06	0.01	0.54	0.28	0.03	0.01	0.15	0.17	0.20	0.20
S155S	0.46	1.00	-	0.26	0.02	0.00	0.49	0.02	0.01	0.05	0.00	0.12	0.01	0.01	0.01
int3+20	0.74	0.69	1.00	-	0.09	0.00	0.36	0.09	0.07	0.32	0.00	0.02	0.07	0.07	0.07
int3-87	1.00	0.10	0.14	0.53	-	0.00	0.42	0.03	0.06	0.00	0.00	0.01	0.00	0.00	0.00
int3-49	1.00	1.00	1.00	1.00	0.04	-	0.00	0.09	0.19	0.00	0.00	0.00	0.00	0.00	0.00
int3-28	1.00	0.40	1.00	0.82	0.85	0.45	-	0.05	0.04	0.02	0.00	0.06	0.01	0.00	0.00
S219S	0.49	0.87	1.00	1.00	1.00	1.00	1.00	-	0.19	0.04	0.01	0.04	0.10	0.10	0.10
int6-26*	1.00	0.90	0.39	0.60	1.00	1.00	0.61	0.63	-	0.09	0.00	0.31	0.54	0.61	0.61
T287T	1.00	1.00	0.30	0.81	1.00	0.04	0.14	1.00	1.00	-	0.00	0.00	0.16	0.14	0.14
int7+27*	0.02	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	0.00	0.00	0.00	0.00
S346S	1.00	1.00	1.00	0.20	0.26	0.00	0.49	0.43	0.85	0.06	1.00	-	0.58	0.51	0.51
3U+27*	1.00	0.92	0.29	0.48	0.19	0.11	0.17	0.59	0.96	1.00	1.00	0.90	-	0.82	0.82
3U+51*	1.00	0.96	0.27	0.47	0.24	0.00	0.02	0.56	0.98	1.00	1.00	0.87	0.94	-	1.00
3U+79*	1.00	0.96	0.27	0.47	0.24	0.00	0.02	0.56	0.98	1.00	1.00	0.87	0.94	1.00	-

R^2 values are above the diagonal, D' values are below the diagonal

*Genotyped on Stage 1 samples only: 83 African American subjects, 37 Ghanaian subjects, 88 European American subjects

Association analysis. We tested for associations between POAG and the frequencies of the 14 common SNP alleles and an indicator variable representing presence/absence of a rare polymorphism. No SNPs were significant at the α-level of 0.05 in either the African American, African Ghanaian, or European American populations (Table 5-7). We also tested for an association with POAG in the overall population and in the African population (African Americans and Ghanaians), controlling for race. We did not find any significant associations (Table 5-8).

Table 5-7. Association between POAG and common LMX1B SNPs in individual populations

SNP	AA POAG (n=198)	AA Cntl (n=210)	AA p-value	AG POAG (n=202)	AG Cntl (n=210)	AG p-value	EA POAG (n=150)	EA Cntl (n=232)	EA p-value
intron2									
int2+7^	20 (16%)	8 (18%)	.816	3 (8%)	1 (3%)	.351	39 (27%)	74 (37%)	.063
exon3									
E124E	93 (47%)	91 (44%)	.485	90 (43%)	78 (39%)	.423	47 (31%)	88 (39%)	.154
S155S	4 (2%)	5 (2%)	1.00	6 (3%)	5 (2%)	1.00	-	-	-
intron3									
int3+20	19 (10%)	10 (5%)	.081	30 (14%)	22 (11%)	.373	-	-	-
int3-87	1 (1%)	4 (2%)	.375	6 (3%)	2 (1%)	.284	1 (1%)	1 (0%)	1.00
int3-49	8 (4%)	15 (7%)	.282	14 (7%)	14 (7%)	1.00	9 (6%)	19 (9%)	.424
int3-28	5 (3%)	8 (4%)	.580	11 (6%)	7 (4%)	.470	1 (1%)	1 (0%)	1.00
exon4									
S219S	73 (38%)	90 (43%)	.415	81 (41%)	84 (43%)	.838	46 (32%)	81 (39%)	.176
intron6									
int6-26*	37 (30%)	12 (27%)	.847	17 (47%)	19 (50%)	.821	7 (6%)	3 (5%)	1.00
exon7									
T287T	8 (4%)	5 (2%)	.402	17 (8%)	11 (6%)	.330	-	-	-
intron7									
int7+27*	1 (1%)	0 (0%)	1.00	-	-	-	4 (3%)	0 (0%)	.304
exon8									
S346S	35 (18%)	42 (20%)	.704	43 (21%)	53 (27%)	.198	0 (0%)	1 (0%)	1.00
3'UTR									
3U+27*	27 (22%)	7 (16%)	.514	13 (36%)	13 (34%)	1.00	1 (1%)	0 (0%)	1.00
3U+51*	31 (25%)	8 (18%)	.409	13 (36%)	13 (34%)	1.00	1 (1%)	0 (0%)	1.00
3U+79*	31 (25%)	8 (18%)	.409	13 (36%)	13 (34%)	1.00	1 (1%)	0 (0%)	1.00
Rare SNP*	3 (2%)	4 (9%)	.081	1 (3%)	3 (8%)	.615	3 (3%)	0 (0%)	.552

Gives minor allele counts (and percentages) for cases and controls. Significantly associated SNPs are in bold. Abbreviations: AA, African American; AG, African Ghanaian; EA, European American; POAG, primary open angle glaucoma; Cntl, control

*Genotyped on stage 1 subjects only: AA=83 subjects, AG=37 subjects, EA=88 subjects

Table 5-8. Association between POAG and common LMX1B SNPs in combined populations

SNP	AA+AG OR	AA+AG CI	AA+AG p-val	All OR	All CI	All p-val
intron2						
int2+7*	0.92	(0.39, 2.14)	.839	1.28	(0.76, 2.15)	.358
exon3						
E124E	0.99	(0.86, 1.14)	.928	0.96	(0.85, 1.08)	.459
S155S	0.86	(0.35, 2.10)	.738	-	-	-
intron3						
int3+20	1.04	(0.83, 1.31)	.724	-	-	-
int3-87	1.81	(0.94, 3.46)	.074	0.62	(0.35, 1.11)	0.107
int3-49	1.14	(0.85, 1.51)	.386	0.86	(0.68, 1.08)	0.197
int3-28	1.55	(0.74, 3.24)	.243	0.68	(0.33, 1.38)	0.283
exon4						
S219S	0.94	(0.71, 1.25)	.673	1.15	(0.90, 1.45)	.264
intron6						
int6-26*	1.04	(0.58, 1.87)	.888	1.06	(0.62, 1.81)	.834
exon7						
T287T	0.95	(0.69, 1.30)	.757	-	-	-
intron7						
int7+27†	-	-	-	-	-	-
exon8						
S346S	0.94	(0.80, 1.12)	.495	1.05	(0.89, 1.25)	.541
3'UTR						
3U+27*	0.90	(0.66, 1.25)	.544	0.89	(0.65, 1.23)	.481
3U+51*	1.15	(0.84, 1.57)	.396	1.16	(0.85, 1.59)	.347
3U+79*	0.87	(0.64, 1.20)	.396	0.86	(0.63, 1.18)	.347
Rare SNP*	0.27	(0.07, 1.00)	.050	0.51	(0.17, 1.52)	.224

Gives minor allele counts (and percentages) for cases and controls. Abbreviations: OR, odds ratio; CI, confidence intervals; pval, p-value
*Genotyped on stage 1 subjects only: AA=83 subjects, AG=37 subjects, EA=88 subjects
†Logistic regression results are invalid for int7+27 because of perfect separation of phenotype by genotype

Next steps. We are currently recruiting more subjects to include in our study. As shown in the power calculations, our population specific analyses are underpowered to detect an association (Table 5-1a-c). Therefore, our goal is to increase the number of cases and expand the number of controls so that each of the three racial/ethnic groups will have a 1:1 ratio of cases to controls. We will also genotype all the common SNPs on the Stage 2

samples. Additionally, we will be expanding the region in and around LMX1B to include SNPs in the intronic regions, 5' region, and 3' region. Lastly, we will evaluate association of POAG with haplotypes, as well as individual SNPs.

FUTURE DIRECTIONS

There are follow-up studies that could be conducted for each of the projects of this dissertation. One of the most exciting types of genetics studies being conducted is the genome-wide association study. To follow-up the schizophrenia linkage scan, we are conducting a genome-wide association study to test for association between SNPs and copy number variants (CNVs) across the genome. CNVs are particularly relevant to schizophrenia because microdeletions on chromosome 22 have been found to increase susceptibility to schizophrenia[253] and rare CNVs are more common in subjects with schizophrenia[133-136].

For the GLC1I region, the next step would be to identify the gene producing the linkage signal. To do this, candidate genes would be selected from microarray experiments comparing expression profiles of trabecular meshwork cells from young, healthy (low-risk) eyes to trabecular meshwork cells from eyes with risk factors for glaucoma (high-risk); trabecular meshwork cells are part of the anterior chamber angle and are components of the aqueous humor outflow mechanism[20]. The high-risk eyes would either be treated with dexamethasone, a corticoisteroid that can induce glaucoma[254], or from elderly subjects. Cells from the trabecular meshwork would be extracted from the eyes and hybridized to an Affymetrix U133A microarray GeneChip containing 22,215 probes across the genome. Four possible criteria for identification of potentially interesting probes are:

1. Test each probe for a difference in expression levels between low-risk and high-risk cells.

2. Prioritize probes that have a higher signal level. If an alteration were to occur in a highly expressed protein, the effect may be more deleterious than if the protein is present at low levels.
3. Evaluate tissue specificity of expression for the genes represented by the probes.
4. Among probes that meet condition (1), (2), or (3), evaluate their gene product function to determine if the protein participates in biochemical pathways related to glaucoma pathology.

Because few probes would be expected to meet all of the conditions, evaluation should consider combinations of criteria and probes to determine which genes are of the greatest interest. In a previous study of the GLC1C region, none of the genes in the interval showed expression specific to the trabecular meshwork. However, one gene showed a statistically significant change in expression in response to both dexamethasone and aging, as well as a high overall level of expression and potentially relevant gene function[255].

The results from the study looking at myocilin promoter SNPs are somewhat more ambiguous. There is evidence that there is no association between variations in the promoter and glaucoma progression. However, one future project would be to further explore the promoter region in the CIGTS subjects. The phased HapMap chromosomes, indicate that -1000CG falls between two blocks of linkage disequilibrium. Therefore, we would choose a region including both blocks that extends from about 169,865 kb to 169,920 kb (http://genome.ucsc.edu). There are 397 SNPs in this region; these SNPs and haplotypes composed of these SNPs would be tested for association with visual field and IOP throughout CIGTS. Another approach would be to perform functional analyses comparing the -1000C allele to the -1000G allele.

This dissertation has illustrated how gene mapping techniques can be used to identify regions harboring susceptibility genes or to provide evidence against previously suggested regions. These are, however, only a few examples of the types of studies that are being conducted. There remain many questions regarding the genetics of complex disease to be answered and a multitude of ways to answer them.

BIBLIOGRAPHY

1. Botstein, D., and Risch, N. (2003). Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. Nature genetics 33 Suppl, 228-237.
2. Hirschhorn, J.N., and Daly, M.J. (2005). Genome-wide association studies for common diseases and complex traits. Nature reviews 6, 95-108.
3. Chakravarti, A. (1999). Population genetics--making sense out of sequence. Nature genetics 21, 56-60.
4. Lander, E.S. (1996). The new genomics: global views of biology. Science 274, 536-539.
5. Pritchard, J.K., and Cox, N.J. (2002). The allelic architecture of human disease genes: common disease-common variant...or not? Hum Mol Genet 11, 2417-2423.
6. Bodmer, W., and Bonilla, C. (2008). Common and rare variants in multifactorial susceptibility to common diseases. Nat Genet 40, 695-701.
7. Ropers, H.H. (2007). New perspectives for the elucidation of genetic disorders. Am J Hum Genet 81, 199-207.
8. Ober, C., Abney, M., and McPeek, M.S. (2001). The genetic dissection of complex traits in a founder population. American journal of human genetics 69, 1068-1079.
9. Risch, N.J. (2000). Searching for genetic determinants in the new millennium. Nature 405, 847-856.
10. Baron, M. (2001). The search for complex disease genes: fault by linkage or fault by association? Molecular psychiatry 6, 143-149.
11. Kendler, K.S., and Diehl, S.R. (1993). The genetics of schizophrenia: a current, genetic-epidemiologic perspective. Schizophrenia bulletin 19, 261-285.
12. Cardon, L.R., and Bell, J.I. (2001). Association study designs for complex diseases. Nature reviews 2, 91-99.
13. Hauser, E.R., Watanabe, R.M., Duren, W.L., Bass, M.P., Langefeld, C.D., and Boehnke, M. (2004). Ordered subset analysis in genetic linkage mapping of complex traits. Genetic epidemiology 27, 53-63.
14. Curtis, D., and Sham, P.C. (1996). Population stratifications can cause false positive linkage results if founders are untyped. Annals of human genetics 60, 261-263.
15. Skol, A.D., Xiao, R., and Boehnke, M. (2005). An algorithm to construct genetically similar subsets of families with the use of self-reported ethnicity information. American journal of human genetics 77, 346-354.
16. Pritchard, J.K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics 155, 945-959.
17. Sheffield, V.C., Stone, E.M., and Carmi, R. (1998). Use of isolated inbred human populations for identification of disease genes. Trends Genet 14, 391-396.
18. Neuhausen, S.L. (2000). Founder populations and their uses for breast cancer genetics. Breast Cancer Res 2, 77-81.
19. Remington, L.A. (2005). Clinical Anatomy of the Visual System. Elsevier, St. Louis, MO.

20. Stamper, R.L., Lieberman, M.F., and Drake, M.V. (1999). Diagnosis and Therapy of the Glaucomas. Mosby, Inc, St. Louis, MO.
21. Quigley, H.A. (1996). Number of people with glaucoma worldwide. Br J Ophthalmol 80, 389-393.
22. Quigley, H.A., and Broman, A.T. (2006). The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90, 262-267.
23. Friedman, D.S., Wolfs, R.C., O'Colmain, B.J., Klein, B.E., Taylor, H.R., West, S., Leske, M.C., Mitchell, P., Congdon, N., and Kempen, J. (2004). Prevalence of open-angle glaucoma among adults in the United States. Archives of ophthalmology 122, 532-538.
24. Wiggs, J.L. (2007). Genetic etiologies of glaucoma. Arch Ophthalmol 125, 30-37.
25. Resnikoff, S., Pascolini, D., Etya'ale, D., Kocur, I., Pararajasegaram, R., Pokharel, G.P., and Mariotti, S.P. (2004). Global data on visual impairment in the year 2002. Bull World Health Organ 82, 844-851.
26. Fechtner, R.D., and Weinreb, R.N. (1994). Mechanisms of optic nerve damage in primary open angle glaucoma. Survey of ophthalmology 39, 23-42.
27. Weinreb, R.N., and Khaw, P.T. (2004). Primary open-angle glaucoma. Lancet 363, 1711-1720.
28. Osborne, N.N., Wood, J.P., and Chidlow, G. (2005). Invited review: Neuroprotective properties of certain beta-adrenoceptor antagonists used for the treatment of glaucoma. J Ocul Pharmacol Ther 21, 175-181.
29. Doshi, V., Ying-Lai, M., Azen, S.P., and Varma, R. (2008). Sociodemographic, family history, and lifestyle risk factors for open-angle glaucoma and ocular hypertension. The Los Angeles Latino Eye Study. Ophthalmology 115, 639-647 e632.
30. Coleman, A.L., and Miglior, S. (2008). Risk factors for glaucoma onset and progression. Surv Ophthalmol 53, S3-S10.
31. Klein, B.E., Klein, R., Sponsel, W.E., Franke, T., Cantor, L.B., Martone, J., and Menage, M.J. (1992). Prevalence of glaucoma. The Beaver Dam Eye Study. Ophthalmology 99, 1499-1504.
32. Le, A., Mukesh, B.N., McCarty, C.A., and Taylor, H.R. (2003). Risk factors associated with the incidence of open-angle glaucoma: the visual impairment project. Invest Ophthalmol Vis Sci 44, 3783-3789.
33. Leske, M.C., Wu, S.Y., Hennis, A., Honkanen, R., and Nemesure, B. (2008). Risk factors for incident open-angle glaucoma: the Barbados Eye Studies. Ophthalmology 115, 85-93.
34. Dielemans, I., Vingerling, J.R., Wolfs, R.C., Hofman, A., Grobbee, D.E., and de Jong, P.T. (1994). The prevalence of primary open-angle glaucoma in a population-based study in The Netherlands. The Rotterdam Study. Ophthalmology 101, 1851-1855.
35. Sommer, A., Tielsch, J.M., Katz, J., Quigley, H.A., Gottsch, J.D., Javitt, J., and Singh, K. (1991). Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. The Baltimore Eye Survey. Arch Ophthalmol 109, 1090-1095.
36. Kass, M.A., Heuer, D.K., Higginbotham, E.J., Johnson, C.A., Keltner, J.L., Miller, J.P., Parrish, R.K., 2nd, Wilson, M.R., and Gordon, M.O. (2002). The Ocular

Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. Arch Ophthalmol 120, 701-713; discussion 829-730.

37. Anderson, D.R. (2003). Collaborative normal tension glaucoma study. Curr Opin Ophthalmol 14, 86-90.
38. Nouri-Mahdavi, K., Hoffman, D., Coleman, A.L., Liu, G., Li, G., Gaasterland, D., and Caprioli, J. (2004). Predictive factors for glaucomatous visual field progression in the Advanced Glaucoma Intervention Study. Ophthalmology 111, 1627-1635.
39. Hong, S., Seong, G.J., and Hong, Y.J. (2007). Long-term intraocular pressure fluctuation and progressive visual field deterioration in patients with glaucoma and low intraocular pressures after a triple procedure. Arch Ophthalmol 125, 1010-1013.
40. Medeiros, F.A., Weinreb, R.N., Zangwill, L.M., Alencar, L.M., Sample, P.A., Vasile, C., and Bowd, C. (2008). Long-term intraocular pressure fluctuations and risk of conversion from ocular hypertension to glaucoma. Ophthalmology 115, 934-940.
41. Bengtsson, B., Leske, M.C., Hyman, L., and Heijl, A. (2007). Fluctuation of intraocular pressure and glaucoma progression in the early manifest glaucoma trial. Ophthalmology 114, 205-209.
42. Rudnicka, A.R., Mt-Isa, S., Owen, C.G., Cook, D.G., and Ashby, D. (2006). Variations in primary open-angle glaucoma prevalence by age, gender, and race: a Bayesian meta-analysis. Invest Ophthalmol Vis Sci 47, 4254-4261.
43. Tielsch, J.M., Sommer, A., Katz, J., Royall, R.M., Quigley, H.A., and Javitt, J. (1991). Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey. JAMA 266, 369-374.
44. Ederer, F., Gaasterland, D.A., Dally, L.G., Kim, J., VanVeldhuisen, P.C., Blackwell, B., Prum, B., Shafranov, G., Allen, R.C., and Beck, A. (2004). The Advanced Glaucoma Intervention Study (AGIS): 13. Comparison of treatment outcomes within race: 10-year results. Ophthalmology 111, 651-664.
45. Aghaian, E., Choe, J.E., Lin, S., and Stamper, R.L. (2004). Central corneal thickness of Caucasians, Chinese, Hispanics, Filipinos, African Americans, and Japanese in a glaucoma clinic. Ophthalmology 111, 2211-2219.
46. Gordon, M.O., Beiser, J.A., Brandt, J.D., Heuer, D.K., Higginbotham, E.J., Johnson, C.A., Keltner, J.L., Miller, J.P., Parrish, R.K., 2nd, Wilson, M.R., et al. (2002). The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. Archives of ophthalmology 120, 714-720; discussion 829-730.
47. Brandt, J.D., Gordon, M.O., Beiser, J.A., Lin, S.C., Alexander, M.Y., and Kass, M.A. (2008). Changes in central corneal thickness over time: the ocular hypertension treatment study. Ophthalmology 115, 1550-1556, 1556 e1551.
48. Shimmyo, M. (2004). Intraocular pressure, Goldmann applanation tension, corneal thickness, and corneal curvature in Caucasians, Asians, Hispanics, and African Americans. Am J Ophthalmol 137, 1170.
49. Green, C.M., Kearns, L.S., Wu, J., Barbour, J.M., Wilkinson, R.M., Ring, M.A., Craig, J.E., Wong, T.L., Hewitt, A.W., and Mackey, D.A. (2007). How significant is a

family history of glaucoma? Experience from the Glaucoma Inheritance Study in Tasmania. Clin Experiment Ophthalmol 35, 793-799.
50. Wolfs, R.C., Klaver, C.C., Ramrattan, R.S., van Duijn, C.M., Hofman, A., and de Jong, P.T. (1998). Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. Archives of ophthalmology 116, 1640-1645.
51. Wu, J., Hewitt, A.W., Green, C.M., Ring, M.A., McCartney, P.J., Craig, J.E., and Mackey, D.A. (2006). Disease severity of familial glaucoma compared with sporadic glaucoma. Arch Ophthalmol 124, 950-954.
52. Tielsch, J.M., Katz, J., Sommer, A., Quigley, H.A., and Javitt, J.C. (1994). Family history and risk of primary open angle glaucoma. The Baltimore Eye Survey. Archives of ophthalmology 112, 69-73.
53. Mitchell, P., Hourihan, F., Sandbach, J., and Wang, J.J. (1999). The relationship between glaucoma and myopia: the Blue Mountains Eye Study. Ophthalmology 106, 2010-2015.
54. Sommer, A. (1996). Glaucoma risk factors observed in the Baltimore Eye Survey. Current opinion in ophthalmology 7, 93-98.
55. Pasquale, L.R., Kang, J.H., Manson, J.E., Willett, W.C., Rosner, B.A., and Hankinson, S.E. (2006). Prospective study of type 2 diabetes mellitus and risk of primary open-angle glaucoma in women. Ophthalmology 113, 1081-1086.
56. Mitchell, P., Lee, A.J., Rochtchina, E., and Wang, J.J. (2004). Open-angle glaucoma and systemic hypertension: the blue mountains eye study. Journal of glaucoma 13, 319-326.
57. Klein, B.E., Klein, R., and Ritter, L.L. (1993). Relationship of drinking alcohol and smoking to prevalence of open-angle glaucoma. The Beaver Dam Eye Study. Ophthalmology 100, 1609-1613.
58. Kang, J.H., Pasquale, L.R., Rosner, B.A., Willett, W.C., Egan, K.M., Faberowski, N., and Hankinson, S.E. (2003). Prospective study of cigarette smoking and the risk of primary open-angle glaucoma. Archives of ophthalmology 121, 1762-1768.
59. Sheffield, V.C., Stone, E.M., Alward, W.L., Drack, A.V., Johnson, A.T., Streb, L.M., and Nichols, B.E. (1993). Genetic linkage of familial open angle glaucoma to chromosome 1q21-q31. Nature genetics 4, 47-50.
60. Stone, E.M., Fingert, J.H., Alward, W.L., Nguyen, T.D., Polansky, J.R., Sunden, S.L., Nishimura, D., Clark, A.F., Nystuen, A., Nichols, B.E., et al. (1997). Identification of a gene that causes primary open angle glaucoma. Science 275, 668-670.
61. Shimizu, S., Lichter, P.R., Johnson, A.T., Zhou, Z., Higashi, M., Gottfredsdottir, M., Othman, M., Moroi, S.E., Rozsa, F.W., Schertzer, R.M., et al. (2000). Age-dependent prevalence of mutations at the GLC1A locus in primary open-angle glaucoma. Am J Ophthalmol 130, 165-177.
62. Fingert, J.H., Heon, E., Liebmann, J.M., Yamamoto, T., Craig, J.E., Rait, J., Kawase, K., Hoh, S.T., Buys, Y.M., Dickinson, J., et al. (1999). Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. Hum Mol Genet 8, 899-905.
63. Polansky, J.R. (2003). Current perspectives on the TIGR/MYOC gene (Myocilin) and glaucoma. Ophthalmol Clin North Am 16, 515-527, v-vi.

64. Resch, Z.T., and Fautsch, M.P. (2008). Glaucoma-associated myocilin: A better understanding but much more to learn. Exp Eye Res.
65. Kim, B.S., Savinova, O.V., Reedy, M.V., Martin, J., Lun, Y., Gan, L., Smith, R.S., Tomarev, S.I., John, S.W., and Johnson, R.L. (2001). Targeted Disruption of the Myocilin Gene (Myoc) Suggests that Human Glaucoma-Causing Mutations Are Gain of Function. Molecular and cellular biology 21, 7707-7713.
66. Gould, D.B., Miceli-Libby, L., Savinova, O.V., Torrado, M., Tomarev, S.I., Smith, R.S., and John, S.W. (2004). Genetically increasing Myoc expression supports a necessary pathologic role of abnormal proteins in glaucoma. Molecular and cellular biology 24, 9019-9025.
67. Zhou, Y., Grinchuk, O., and Tomarev, S.I. (2008). Transgenic mice expressing the Tyr437His mutant of human myocilin protein develop glaucoma. Invest Ophthalmol Vis Sci 49, 1932-1939.
68. Sarfarazi, M., Child, A., Stoilova, D., Brice, G., Desai, T., Trifan, O.C., Poinoosawmy, D., and Crick, R.P. (1998). Localization of the fourth locus (GLC1E) for adult-onset primary open-angle glaucoma to the 10p15-p14 region. American journal of human genetics 62, 641-652.
69. Rezaie, T., Child, A., Hitchings, R., Brice, G., Miller, L., Coca-Prados, M., Heon, E., Krupin, T., Ritch, R., Kreutzer, D., et al. (2002). Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science 295, 1077-1079.
70. Sarfarazi, M., and Rezaie, T. (2003). Optineurin in primary open angle glaucoma. Ophthalmology clinics of North America 16, 529-541.
71. Allingham, R.R., Liu, Y., and Rhee, D.J. (2008). The genetics of primary open-angle glaucoma: A review. Exp Eye Res.
72. Park, B.C., Shen, X., Samaraweera, M., and Yue, B.Y. (2006). Studies of optineurin, a glaucoma gene: Golgi fragmentation and cell death from overexpression of wild-type and mutant optineurin in two ocular cell types. Am J Pathol 169, 1976-1989.
73. Chalasani, M.L., Radha, V., Gupta, V., Agarwal, N., Balasubramanian, D., and Swarup, G. (2007). A glaucoma-associated mutant of optineurin selectively induces death of retinal ganglion cells which is inhibited by antioxidants. Invest Ophthalmol Vis Sci 48, 1607-1614.
74. Monemi, S., Spaeth, G., DaSilva, A., Popinchalk, S., Ilitchev, E., Liebmann, J., Ritch, R., Heon, E., Crick, R.P., Child, A., et al. (2005). Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. Hum Mol Genet 14, 725-733.
75. Hauser, M.A., Allingham, R.R., Linkroum, K., Wang, J., LaRocque-Abramson, K., Figueiredo, D., Santiago-Turla, C., del Bono, E.A., Haines, J.L., Pericak-Vance, M.A., et al. (2006). Distribution of WDR36 DNA sequence variants in patients with primary open-angle glaucoma. Invest Ophthalmol Vis Sci 47, 2542-2546.
76. Wirtz, M.K., Samples, J.R., Rust, K., Lie, J., Nordling, L., Schilling, K., Acott, T.S., and Kramer, P.L. (1999). GLC1F, a new primary open-angle glaucoma locus, maps to 7q35-q36. Arch Ophthalmol 117, 237-241.
77. Wiggs, J.L., Allingham, R.R., Hossain, A., Kern, J., Auguste, J., DelBono, E.A., Broomer, B., Graham, F.L., Hauser, M., Pericak-Vance, M., et al. (2000).

Genome-wide scan for adult onset primary open angle glaucoma. Human molecular genetics 9, 1109-1117.
78. Trifan, O.C., Traboulsi, E.I., Stoilova, D., Alozie, I., Nguyen, R., Raja, S., and Sarfarazi, M. (1998). A third locus (GLC1D) for adult-onset primary open-angle glaucoma maps to the 8q23 region. Am J Ophthalmol 126, 17-28.
79. Stoilova, D., Child, A., Trifan, O.C., Crick, R.P., Coakes, R.L., and Sarfarazi, M. (1996). Localization of a locus (GLC1B) for adult-onset primary open angle glaucoma to the 2cen-q13 region. Genomics 36, 142-150.
80. Wiggs, J.L., Lynch, S., Ynagi, G., Maselli, M., Auguste, J., Del Bono, E.A., Olson, L.M., and Haines, J.L. (2004). A genomewide scan identifies novel early-onset primary open-angle glaucoma loci on 9q22 and 20p12. American journal of human genetics 74, 1314-1320.
81. Baird, P.N., Foote, S.J., Mackey, D.A., Craig, J., Speed, T.P., and Bureau, A. (2005). Evidence for a novel glaucoma locus at chromosome 3p21-22. Hum Genet 117, 249-257.
82. Wirtz, M.K., Samples, J.R., Kramer, P.L., Rust, K., Topinka, J.R., Yount, J., Koler, R.D., and Acott, T.S. (1997). Mapping a gene for adult-onset primary open-angle glaucoma to chromosome 3q. American journal of human genetics 60, 296-304.
83. Allingham, R.R., Wiggs, J.L., Hauser, E.R., Larocque-Abramson, K.R., Santiago-Turla, C., Broomer, B., Del Bono, E.A., Graham, F.L., Haines, J.L., Pericak-Vance, M.A., et al. (2005). Early adult-onset POAG linked to 15q11-13 using ordered subset analysis. Invest Ophthalmol Vis Sci 46, 2002-2005.
84. Wang, D.Y., Fan, B.J., Chua, J.K., Tam, P.O., Leung, C.K., Lam, D.S., and Pang, C.P. (2006). A genome-wide scan maps a novel juvenile-onset primary open-angle glaucoma locus to 15q. Invest Ophthalmol Vis Sci 47, 5315-5321.
85. Pang, C.P., Fan, B.J., Canlas, O., Wang, D.Y., Dubois, S., Tam, P.O., Lam, D.S., Raymond, V., and Ritch, R. (2006). A genome-wide scan maps a novel juvenile-onset primary open angle glaucoma locus to chromosome 5q. Mol Vis 12, 85-92.
86. Alward, W.L., Kwon, Y.H., Khanna, C.L., Johnson, A.T., Hayreh, S.S., Zimmerman, M.B., Narkiewicz, J., Andorf, J.L., Moore, P.A., Fingert, J.H., et al. (2002). Variations in the myocilin gene in patients with open-angle glaucoma. Arch Ophthalmol 120, 1189-1197.
87. Alward, W.L., Kwon, Y.H., Kawase, K., Craig, J.E., Hayreh, S.S., Johnson, A.T., Khanna, C.L., Yamamoto, T., Mackey, D.A., Roos, B.R., et al. (2003). Evaluation of optineurin sequence variations in 1,048 patients with open-angle glaucoma. American journal of ophthalmology 136, 904-910.
88. Ayala-Lugo, R.M., Pawar, H., Reed, D.M., Lichter, P.R., Moroi, S.E., Page, M., Eadie, J., Azocar, V., Maul, E., Ntim-Amponash, C., et al. (in preparation). Variation in optineurin (OPTN) allele frequencies between and within populations.
89. Lemmela, S., Ylisaukko-oja, T., Forsman, E., and Jarvela, I. (2004). Exclusion of 14 candidate loci for primary open angle glaucoma in Finnish families. Molecular vision [electronic resource] 10, 260-264.
90. Fan, B.J., Wang, D.Y., Lam, D.S., and Pang, C.P. (2006). Gene mapping for primary open angle glaucoma. Clinical biochemistry 39, 249-258.

91. Hewitt, A.W., Craig, J.E., and Mackey, D.A. (2006). Complex genetics of complex traits: the case of primary open-angle glaucoma. Clinical & experimental ophthalmology 34, 472-484.
92. Thorleifsson, G., Magnusson, K.P., Sulem, P., Walters, G.B., Gudbjartsson, D.F., Stefansson, H., Jonsson, T., Jonasdottir, A., Stefansdottir, G., Masson, G., et al. (2007). Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. Science 317, 1397-1400.
93. Regier, D.A., Farmer, M.E., Rae, D.S., Myers, J.K., Kramer, M., Robins, L.N., George, L.K., Karno, M., and Locke, B.Z. (1993). One-month prevalence of mental disorders in the United States and sociodemographic characteristics: the Epidemiologic Catchment Area study. Acta psychiatrica Scandinavica 88, 35-47.
94. Jablensky, A. (1997). The 100-year epidemiology of schizophrenia. Schizophrenia research 28, 111-125.
95. McGrath, J., Saha, S., Chant, D., and Welham, J. (2008). Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 30, 67-76.
96. APA. (1994). DSM-IV: diagnostic and statistical manual of mental disorders. American Psychiatric Association, Washington, DC.
97. Tsuang, M. (2000). Schizophrenia: genes and environment. Biological psychiatry 47, 210-220.
98. Sawa, A., and Snyder, S.H. (2002). Schizophrenia: diverse approaches to a complex disease. Science 296, 692-695.
99. Gaebel, W., Janssen, B., and Riesbeck, M. (2003). Modern treatment concepts in schizophrenia. Pharmacopsychiatry 36 Suppl 3, S168-175.
100. Konradi, C., and Heckers, S. (2003). Molecular aspects of glutamate dysregulation: implications for schizophrenia and its treatment. Pharmacology & therapeutics 97, 153-179.
101. Robins, L.N., and Regier, D.A. (1991). An introduction to psychiatric disorders in America. The Free Press, New York, NY.
102. Lahti, A.C., Koffel, B., LaPorte, D., and Tamminga, C.A. (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13, 9-19.
103. Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers, M.B., Jr., and Charney, D.S. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Archives of general psychiatry 51, 199-214.
104. Mohn, A.R., Gainetdinov, R.R., Caron, M.G., and Koller, B.H. (1999). Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell 98, 427-436.
105. Coyle, J.T. (2006). Glutamate and schizophrenia: beyond the dopamine hypothesis. Cell Mol Neurobiol 26, 365-384.
106. Stone, J.M., Morrison, P.D., and Pilowsky, L.S. (2007). Glutamate and dopamine dysregulation in schizophrenia--a synthesis and selective review. J Psychopharmacol 21, 440-452.

107. Di Forti, M., Lappin, J.M., and Murray, R.M. (2007). Risk factors for schizophrenia--all roads lead to dopamine. Eur Neuropsychopharmacol 17 Suppl 2, S101-107.
108. Laruelle, M., Abi-Dargham, A., van Dyck, C.H., Gil, R., D'Souza, C.D., Erdos, J., McCance, E., Rosenblatt, W., Fingado, C., Zoghbi, S.S., et al. (1996). Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci U S A 93, 9235-9240.
109. Keshavan, M.S., Kennedy, J.L., and Murray, R.M. (2004). Neurodevelopment and schizophrenia. Cambridge University Press, Cambridge.
110. Thompson, J.L., Pogue-Geile, M.F., and Grace, A.A. (2004). Developmental pathology, dopamine, and stress: a model for the age of onset of schizophrenia symptoms. Schizophr Bull 30, 875-900.
111. Cardno, A.G., and Gottesman, II. (2000). Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. American journal of medical genetics 97, 12-17.
112. Kety, S.S., Wender, P.H., Jacobsen, B., Ingraham, L.J., Jansson, L., Faber, B., and Kinney, D.K. (1994). Mental illness in the biological and adoptive relatives of schizophrenic adoptees. Replication of the Copenhagen Study in the rest of Denmark. Archives of general psychiatry 51, 442-455.
113. Tienari, P. (1991). Interaction between genetic vulnerability and family environment: the Finnish adoptive family study of schizophrenia. Acta psychiatrica Scandinavica 84, 460-465.
114. McGrath, J., Saha, S., Welham, J., El Saadi, O., MacCauley, C., and Chant, D. (2004). A systematic review of the incidence of schizophrenia: the distribution of rates and the influence of sex, urbanicity, migrant status and methodology. BMC Med 2, 13.
115. Compton, M.T. (2004). Considering schizophrenia from a prevention perspective. American journal of preventive medicine 26, 178-185.
116. Sullivan, P.F. (2005). The genetics of schizophrenia. PLoS medicine 2, e212.
117. Williams, H.J., Owen, M.J., and O'Donovan, M.C. (2009). New findings from genetic association studies of schizophrenia. J Hum Genet.
118. Gogos, J.A., and Gerber, D.J. (2006). Schizophrenia susceptibility genes: emergence of positional candidates and future directions. Trends Pharmacol Sci 27, 226-233.
119. Millar, J.K., Wilson-Annan, J.C., Anderson, S., Christie, S., Taylor, M.S., Semple, C.A., Devon, R.S., Clair, D.M., Muir, W.J., Blackwood, D.H., et al. (2000). Disruption of two novel genes by a translocation co-segregating with schizophrenia. Human molecular genetics 9, 1415-1423.
120. Blackwood, D.H., Fordyce, A., Walker, M.T., St Clair, D.M., Porteous, D.J., and Muir, W.J. (2001). Schizophrenia and affective disorders--cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. American journal of human genetics 69, 428-433.

121. Porteous, D.J., Thomson, P., Brandon, N.J., and Millar, J.K. (2006). The genetics and biology of DISC1--an emerging role in psychosis and cognition. Biological psychiatry 60, 123-131.
122. O'Tuathaigh, C.M., Babovic, D., O'Meara, G., Clifford, J.J., Croke, D.T., and Waddington, J.L. (2007). Susceptibility genes for schizophrenia: characterisation of mutant mouse models at the level of phenotypic behaviour. Neurosci Biobehav Rev 31, 60-78.
123. Cao, Q., Martinez, M., Zhang, J., Sanders, A.R., Badner, J.A., Cravchik, A., Markey, C.J., Beshah, E., Guroff, J.J., Maxwell, M.E., et al. (1997). Suggestive evidence for a schizophrenia susceptibility locus on chromosome 6q and a confirmation in an independent series of pedigrees. Genomics 43, 1-8.
124. Duan, J., Martinez, M., Sanders, A.R., Hou, C., Saitou, N., Kitano, T., Mowry, B.J., Crowe, R.R., Silverman, J.M., Levinson, D.F., et al. (2004). Polymorphisms in the trace amine receptor 4 (TRAR4) gene on chromosome 6q23.2 are associated with susceptibility to schizophrenia. American journal of human genetics 75, 624-638.
125. Ikeda, M., Iwata, N., Suzuki, T., Kitajima, T., Yamanouchi, Y., Kinoshita, Y., Inada, T., and Ozaki, N. (2005). No association of haplotype-tagging SNPs in TRAR4 with schizophrenia in Japanese patients. Schizophrenia research 78, 127-130.
126. Duan, S., Du, J., Xu, Y., Xing, Q., Wang, H., Wu, S., Chen, Q., Li, X., Li, X., Shen, J., et al. (2006). Failure to find association between TRAR4 and schizophrenia in the Chinese Han population. J Neural Transm 113, 381-385.
127. O'Donovan, M.C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., Nikolov, I., Hamshere, M., Carroll, L., Georgieva, L., et al. (2008). Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet.
128. Sullivan, P.F., Lin, D., Tzeng, J.Y., van den Oord, E., Perkins, D., Stroup, T.S., Wagner, M., Lee, S., Wright, F.A., Zou, F., et al. (2008). Genomewide association for schizophrenia in the CATIE study: results of stage 1. Mol Psychiatry 13, 570-584.
129. Lencz, T., Morgan, T.V., Athanasiou, M., Dain, B., Reed, C.R., Kane, J.M., Kucherlapati, R., and Malhotra, A.K. (2007). Converging evidence for a pseudoautosomal cytokine receptor gene locus in schizophrenia. Mol Psychiatry 12, 572-580.
130. Kirov, G., Zaharieva, I., Georgieva, L., Moskvina, V., Nikolov, I., Cichon, S., Hillmer, A., Toncheva, D., Owen, M.J., and O'Donovan, M.C. (2008). A genome-wide association study in 574 schizophrenia trios using DNA pooling. Mol Psychiatry.
131. Shifman, S., Johannesson, M., Bronstein, M., Chen, S.X., Collier, D.A., Craddock, N.J., Kendler, K.S., Li, T., O'Donovan, M., O'Neill, F.A., et al. (2008). Genome-wide association identifies a common variant in the reelin gene that increases the risk of schizophrenia only in women. PLoS Genet 4, e28.
132. Mah, S., Nelson, M.R., Delisi, L.E., Reneland, R.H., Markward, N., James, M.R., Nyholt, D.R., Hayward, N., Handoko, H., Mowry, B., et al. (2006). Identification

of the semaphorin receptor PLXNA2 as a candidate for susceptibility to schizophrenia. Mol Psychiatry 11, 471-478.
133. Stefansson, H., Rujescu, D., Cichon, S., Pietilainen, O.P., Ingason, A., Steinberg, S., Fossdal, R., Sigurdsson, E., Sigmundsson, T., Buizer-Voskamp, J.E., et al. (2008). Large recurrent microdeletions associated with schizophrenia. Nature 455, 232-236.
134. Walsh, T., McClellan, J.M., McCarthy, S.E., Addington, A.M., Pierce, S.B., Cooper, G.M., Nord, A.S., Kusenda, M., Malhotra, D., Bhandari, A., et al. (2008). Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science 320, 539-543.
135. Xu, B., Roos, J.L., Levy, S., van Rensburg, E.J., Gogos, J.A., and Karayiorgou, M. (2008). Strong association of de novo copy number mutations with sporadic schizophrenia. Nat Genet 40, 880-885.
136. Consortium, I.S. (2008). Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 455, 237-241.
137. Abecasis, G.R., Burt, R.A., Hall, D., Bochum, S., Doheny, K.F., Lundy, S.L., Torrington, M., Roos, J.L., Gogos, J.A., and Karayiorgou, M. (2004). Genomewide scan in families with schizophrenia from the founder population of Afrikaners reveals evidence for linkage and uniparental disomy on chromosome 1. American journal of human genetics 74, 403-417.
138. Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., Brynjolfsson, J., Gunnarsdottir, S., Ivarsson, O., Chou, T.T., et al. (2002). Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 71, 877-892.
139. Brzustowicz, L.M., Simone, J., Mohseni, P., Hayter, J.E., Hodgkinson, K.A., Chow, E.W., and Bassett, A.S. (2004). Linkage disequilibrium mapping of schizophrenia susceptibility to the CAPON region of chromosome 1q22. Am J Hum Genet 74, 1057-1063.
140. Kendler, K.S., MacLean, C.J., O'Neill, F.A., Burke, J., Murphy, B., Duke, F., Shinkwin, R., Easter, S.M., Webb, B.T., Zhang, J., et al. (1996). Evidence for a schizophrenia vulnerability locus on chromosome 8p in the Irish Study of High-Density Schizophrenia Families. The American journal of psychiatry 153, 1534-1540.
141. Fallin, M.D., Lasseter, V.K., Wolyniec, P.S., McGrath, J.A., Nestadt, G., Valle, D., Liang, K.Y., and Pulver, A.E. (2003). Genomewide linkage scan for schizophrenia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 10q22. American journal of human genetics 73, 601-611.
142. Gill, M., McGuffin, P., Parfitt, E., Mant, R., Asherson, P., Collier, D., Vallada, H., Powell, J., Shaikh, S., Taylor, C., et al. (1993). A linkage study of schizophrenia with DNA markers from the long arm of chromosome 11. Psychological medicine 23, 27-44.
143. Sherrington, R., Brynjolfsson, J., Petursson, H., Potter, M., Dudleston, K., Barraclough, B., Wasmuth, J., Dobbs, M., and Gurling, H. (1988). Localization of a susceptibility locus for schizophrenia on chromosome 5. Nature 336, 164-167.

144. Blouin, J.L., Dombroski, B.A., Nath, S.K., Lasseter, V.K., Wolyniec, P.S., Nestadt, G., Thornquist, M., Ullrich, G., McGrath, J., Kasch, L., et al. (1998). Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. Nature genetics 20, 70-73.
145. Pimm, J., McQuillin, A., Thirumalai, S., Lawrence, J., Quested, D., Bass, N., Lamb, G., Moorey, H., Datta, S.R., Kalsi, G., et al. (2005). The Epsin 4 gene on chromosome 5q, which encodes the clathrin-associated protein enthoprotin, is involved in the genetic susceptibility to schizophrenia. Am J Hum Genet 76, 902-907.
146. Stober, G., Saar, K., Ruschendorf, F., Meyer, J., Nurnberg, G., Jatzke, S., Franzek, E., Reis, A., Lesch, K.P., Wienker, T.F., et al. (2000). Splitting schizophrenia: periodic catatonia-susceptibility locus on chromosome 15q15. American journal of human genetics 67, 1201-1207.
147. Wang, S., Sun, C.E., Walczak, C.A., Ziegle, J.S., Kipps, B.R., Goldin, L.R., and Diehl, S.R. (1995). Evidence for a susceptibility locus for schizophrenia on chromosome 6pter-p22. Nature genetics 10, 41-46.
148. Schwab, S.G., Hallmayer, J., Albus, M., Lerer, B., Hanses, C., Kanyas, K., Segman, R., Borrman, M., Dreikorn, B., Lichtermann, D., et al. (1998). Further evidence for a susceptibility locus on chromosome 10p14-p11 in 72 families with schizophrenia by nonparametric linkage analysis. American journal of medical genetics 81, 302-307.
149. Gogos, J.A., Santha, M., Takacs, Z., Beck, K.D., Luine, V., Lucas, L.R., Nadler, J.V., and Karayiorgou, M. (1999). The gene encoding proline dehydrogenase modulates sensorimotor gating in mice. Nat Genet 21, 434-439.
150. Walker, H.A., Danielson, E., and Levitt, M. (1976). Catechol-O-methyltransferase activity in psychotic children. J Autism Child Schizophr 6, 263-268.
151. Mukai, J., Liu, H., Burt, R.A., Swor, D.E., Lai, W.S., Karayiorgou, M., and Gogos, J.A. (2004). Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. Nat Genet 36, 725-731.
152. Pulver, A.E., Karayiorgou, M., Wolyniec, P.S., Lasseter, V.K., Kasch, L., Nestadt, G., Antonarakis, S., Housman, D., Kazazian, H.H., Meyers, D., et al. (1994). Sequential strategy to identify a susceptibility gene for schizophrenia: report of potential linkage on chromosome 22q12-q13.1: Part 1. American journal of medical genetics 54, 36-43.
153. Kruglyak, L. (1999). Genetic isolates: separate but equal? Proceedings of the National Academy of Sciences of the United States of America 96, 1170-1172.
154. Karayiorgou, M., Torrington, M., Abecasis, G.R., Pretorius, H., Robertson, B., Kaliski, S., Lay, S., Sobin, C., Moller, N., Lundy, S.L., et al. (2004). Phenotypic characterization and genealogical tracing in an Afrikaner schizophrenia database. Am J Med Genet B Neuropsychiatr Genet 124, 20-28.
155. Gordon, D., Simonic, I., and Ott, J. (2000). Significant evidence for linkage disequilibrium over a 5-cM region among Afrikaners. Genomics 66, 87-92.
156. Tipping, A.J., Pearson, T., Morgan, N.V., Gibson, R.A., Kuyt, L.P., Havenga, C., Gluckman, E., Joenje, H., de Ravel, T., Jansen, S., et al. (2001). Molecular and genealogical evidence for a founder effect in Fanconi anemia families of the

Afrikaner population of South Africa. Proceedings of the National Academy of Sciences of the United States of America 98, 5734-5739.
157. Le Saux, O., Beck, K., Sachsinger, C., Treiber, C., Goring, H.H., Curry, K., Johnson, E.W., Bercovitch, L., Marais, A.S., Terry, S.F., et al. (2002). Evidence for a founder effect for pseudoxanthoma elasticum in the Afrikaner population of South Africa. Human genetics 111, 331-338.
158. Reeves, M.D., Yawitch, T.M., van der Merwe, N.C., van den Berg, H.J., Dreyer, G., and van Rensburg, E.J. (2004). BRCA1 mutations in South African breast and/or ovarian cancer families: evidence of a novel founder mutation in Afrikaner families. International journal of cancer 110, 677-682.
159. Jablensky, A. (2000). Epidemiology of schizophrenia: the global burden of disease and disability. Eur Arch Psychiatry Clin Neurosci 250, 274-285.
160. Brzustowicz, L.M., Honer, W.G., Chow, E.W., Little, D., Hogan, J., Hodgkinson, K., and Bassett, A.S. (1999). Linkage of familial schizophrenia to chromosome 13q32. Am J Hum Genet 65, 1096-1103.
161. Lin, M.W., Curtis, D., Williams, N., Arranz, M., Nanko, S., Collier, D., McGuffin, P., Murray, R., Owen, M., Gill, M., et al. (1995). Suggestive evidence for linkage of schizophrenia to markers on chromosome 13q14.1-q32. Psychiatr Genet 5, 117-126.
162. Shaw, S.H., Kelly, M., Smith, A.B., Shields, G., Hopkins, P.J., Loftus, J., Laval, S.H., Vita, A., De Hert, M., Cardon, L.R., et al. (1998). A genome-wide search for schizophrenia susceptibility genes. Am J Med Genet 81, 364-376.
163. Camp, N.J., Neuhausen, S.L., Tiobech, J., Polloi, A., Coon, H., and Myles-Worsley, M. (2001). Genomewide multipoint linkage analysis of seven extended Palauan pedigrees with schizophrenia, by a Markov-chain Monte Carlo method. Am J Hum Genet 69, 1278-1289.
164. Faraone, S.V., Skol, A.D., Tsuang, D.W., Bingham, S., Young, K.A., Prabhudesai, S., Haverstock, S.L., Mena, F., Menon, A.S., Bisset, D., et al. (2002). Linkage of chromosome 13q32 to schizophrenia in a large veterans affairs cooperative study sample. Am J Med Genet 114, 598-604.
165. Lin, M.W., Sham, P., Hwu, H.G., Collier, D., Murray, R., and Powell, J.F. (1997). Suggestive evidence for linkage of schizophrenia to markers on chromosome 13 in Caucasian but not Oriental populations. Hum Genet 99, 417-420.
166. Badner, J.A., and Gershon, E.S. (2002). Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol Psychiatry 7, 405-411.
167. Lewis, C.M., Levinson, D.F., Wise, L.H., DeLisi, L.E., Straub, R.E., Hovatta, I., Williams, N.M., Schwab, S.G., Pulver, A.E., Faraone, S.V., et al. (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. Am J Hum Genet 73, 34-48.
168. Segurado, R., Detera-Wadleigh, S.D., Levinson, D.F., Lewis, C.M., Gill, M., Nurnberger, J.I., Jr., Craddock, N., DePaulo, J.R., Baron, M., Gershon, E.S., et al. (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: Bipolar disorder. Am J Hum Genet 73, 49-62.
169. Detera-Wadleigh, S.D., and McMahon, F.J. (2006). G72/G30 in schizophrenia and bipolar disorder: review and meta-analysis. Biol Psychiatry 60, 106-114.

170. Li, D., and He, L. (2007). G72/G30 Genes and Schizophrenia: A Systematic Meta-analysis of Association Studies. Genetics 175, 917-922.
171. Shi, J., Badner, J.A., Gershon, E.S., and Liu, C. (2008). Allelic association of G72/G30 with schizophrenia and bipolar disorder: a comprehensive meta-analysis. Schizophr Res 98, 89-97.
172. Service, S., DeYoung, J., Karayiorgou, M., Roos, J.L., Pretorious, H., Bedoya, G., Ospina, J., Ruiz-Linares, A., Macedo, A., Palha, J.A., et al. (2006). Magnitude and distribution of linkage disequilibrium in population isolates and implications for genome-wide association studies. Nature genetics 38, 556-560.
173. Pama, C. (1981). C.C. de Villiers' Genealogies of Old South African Families. Balkema, Cape Town.
174. Nurnberger, J.I., Jr., Blehar, M.C., Kaufmann, C.A., York-Cooler, C., Simpson, S.G., Harkavy-Friedman, J., Severe, J.B., Malaspina, D., and Reich, T. (1994). Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. Arch Gen Psychiatry 51, 849-859; discussion 863-844.
175. Ciulla, T.A., Sklar, R.M., and Hauser, S.L. (1988). A simple method for DNA purification from peripheral blood. Anal Biochem 174, 485-488.
176. Abecasis, G.R., Cherny, S.S., Cookson, W.O., and Cardon, L.R. (2001). GRR: graphical representation of relationship errors. Bioinformatics 17, 742-743.
177. Douglas, J.A., Boehnke, M., and Lange, K. (2000). A multipoint method for detecting genotyping errors and mutations in sibling-pair linkage data. American journal of human genetics 66, 1287-1297.
178. Wigginton, J.E., and Abecasis, G.R. (2005). PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. Bioinformatics 21, 3445-3447.
179. Kong, A., Gudbjartsson, D.F., Sainz, J., Jonsdottir, G.M., Gudjonsson, S.A., Richardsson, B., Sigurdardottir, S., Barnard, J., Hallbeck, B., Masson, G., et al. (2002). A high-resolution recombination map of the human genome. Nature genetics 31, 241-247.
180. Li, M., Boehnke, M., and Abecasis, G.R. (2005). Joint modeling of linkage and association: identifying SNPs responsible for a linkage signal. Am J Hum Genet 76, 934-949.
181. Li, M., Boehnke, M., and Abecasis, G.R. (2006). Efficient study designs for test of genetic association using sibship data and unrelated cases and controls. Am J Hum Genet 78, 778-792.
182. Kruglyak, L., Daly, M.J., Reeve-Daly, M.P., and Lander, E.S. (1996). Parametric and nonparametric linkage analysis: a unified multipoint approach. American journal of human genetics 58, 1347-1363.
183. Abecasis, G.R., Cherny, S.S., and Cardon, L.R. (2001). The impact of genotyping error on family-based analysis of quantitative traits. Eur J Hum Genet 9, 130-134.
184. Sullivan, P.F., Neale, B.M., Neale, M.C., van den Oord, E., and Kendler, K.S. (2003). Multipoint and single point non-parametric linkage analysis with imperfect data. Am J Med Genet B Neuropsychiatr Genet 121, 89-94.

185. Whittemore, A.S., and Halpern, J. (1994). A class of tests for linkage using affected pedigree members. Biometrics 50, 118-127.
186. Kong, A., and Cox, N.J. (1997). Allele-sharing models: LOD scores and accurate linkage tests. American journal of human genetics 61, 1179-1188.
187. Abecasis, G.R., Cherny, S.S., Cookson, W.O., and Cardon, L.R. (2002). Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30, 97-101.
188. Lander, E., and Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature genetics 11, 241-247.
189. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A., and Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. Nature genetics 38, 904-909.
190. Greenberg, D.A., Abreu, P., and Hodge, S.E. (1998). The power to detect linkage in complex disease by means of simple LOD-score analyses. Am J Hum Genet 63, 870-879.
191. Kvajo, M., Dhilla, A., Swor, D.E., Karayiorgou, M., and Gogos, J.A. (2007). Evidence implicating the candidate schizophrenia/bipolar disorder susceptibility gene G72 in mitochondrial function. Mol Psychiatry.
192. Roeder, K., Bacanu, S.A., Wasserman, L., and Devlin, B. (2006). Using linkage genome scans to improve power of association in genome scans. Am J Hum Genet 78, 243-252.
193. Wilson, M.R., Hertzmark, E., Walker, A.M., Childs-Shaw, K., and Epstein, D.L. (1987). A case-control study of risk factors in open angle glaucoma. Arch Ophthalmol 105, 1066-1071.
194. Beiguelman, B., and Prado, D. (1963). Recessive Juvenile Glaucoma. J Genet Hum 12, 53-54.
195. Teikari, J.M. (1990). Genetic influences in open-angle glaucoma. Int Ophthalmol Clin 30, 161-168.
196. Bell, J. (1932). On some hereditary structural anomalies of the eye and on the inheritance of glaucoma. Vol. 2. Cambridge University Press, London.
197. Berg. (1932). Erbliches jugendliches glaukom. Acta Ophthalmol Scand 10, 568-587.
198. Combie, A.L., and Cullen, J.F. (1964). Hereditary glaucoma: occurrence in five generations of an Edinburgh familiy. Br J Ophthalmol 48, 143-147.
199. Stokes, W.H. (1940). Hereditary primary glaucoma. Arch Ophthalmol 24, 885-909.
200. Wilson, M.R. (1990). Epidemiological features of glaucoma. Int Ophthalmol Clin 30, 153-160.
201. Quigley, H.A., Enger, C., Katz, J., Sommer, A., Scott, R., and Gilbert, D. (1994). Risk factors for the development of glaucomatous visual field loss in ocular hypertension. Arch Ophthalmol 112, 644-649.
202. Bonomi, L., Marchini, G., Marraffa, M., Bernardi, P., De Franco, I., Perfetti, S., Varotto, A., and Tenna, V. (1998). Prevalence of glaucoma and intraocular pressure distribution in a defined population. The Egna-Neumarkt Study. Ophthalmology 105, 209-215.

203. Morissette, J., Cote, G., Anctil, J.L., Plante, M., Amyot, M., Heon, E., Trope, G.E., Weissenbach, J., and Raymond, V. (1995). A common gene for juvenile and adult-onset primary open-angle glaucomas confined on chromosome 1q. Am J Hum Genet 56, 1431-1442.
204. Richards, J.E., Lichter, P.R., Herman, S., Hauser, E.R., Hou, Y.C., Johnson, A.T., and Boehnke, M. (1996). Probable exclusion of GLC1A as a candidate glaucoma gene in a family with middle-age-onset primary open-angle glaucoma. Ophthalmology 103, 1035-1040.
205. Vincent, A.L., Rootman, D., Munier, F.L., and Heon, E. (2003). A molecular perspective on corneal dystrophies. Dev Ophthalmol 37, 50-66.
206. Tuo, J., Bojanowski, C.M., and Chan, C.C. (2004). Genetic factors of age-related macular degeneration. Prog Retin Eye Res 23, 229-249.
207. Hejtmancik, J.F., and Kantorow, M. (2004). Molecular genetics of age-related cataract. Exp Eye Res 79, 3-9.
208. Rivolta, C., Sharon, D., DeAngelis, M.M., and Dryja, T.P. (2002). Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns. Hum Mol Genet 11, 1219-1227.
209. Daiger, S.P. (2004). Identifying retinal disease genes: how far have we come, how far do we have to go? Novartis Found Symp 255, 17-27; discussion 27-36, 177-178.
210. Nemesure, B., Leske, M.C., He, Q., and Mendell, N. (1996). Analyses of reported family history of glaucoma: a preliminary investigation. The Barbados Eye Study Group. Ophthalmic Epidemiol 3, 135-141.
211. Nguyen, T.D., Chen, P., Huang, W.D., Chen, H., Johnson, D., and Polansky, J.R. (1998). Gene structure and properties of TIGR, an olfactomedin-related glycoprotein cloned from glucocorticoid-induced trabecular meshwork cells. J Biol Chem 273, 6341-6350.
212. Lotufo, D., Ritch, R., Szmyd, L., Jr., and Burris, J.E. (1989). Juvenile glaucoma, race, and refraction. JAMA 261, 249-252.
213. Roberts, S.B., MacLean, C.J., Neale, M.C., Eaves, L.J., and Kendler, K.S. (1999). Replication of linkage studies of complex traits: an examination of variation in location estimates. Am J Hum Genet 65, 876-884.
214. Rivera, J.L., Bell, N.P., and Feldman, R.M. (2008). Risk factors for primary open angle glaucoma progression: what we know and what we need to know. Curr Opin Ophthalmol 19, 102-106.
215. Leske, M.C., Connell, A.M., Wu, S.Y., Hyman, L.G., and Schachat, A.P. (1995). Risk factors for open-angle glaucoma. The Barbados Eye Study. Arch Ophthalmol 113, 918-924.
216. Weih, L.M., Nanjan, M., McCarty, C.A., and Taylor, H.R. (2001). Prevalence and predictors of open-angle glaucoma: results from the visual impairment project. Ophthalmology 108, 1966-1972.
217. Mitchell, P., Rochtchina, E., Lee, A.J., and Wang, J.J. (2002). Bias in self-reported family history and relationship to glaucoma: the Blue Mountains Eye Study. Ophthalmic Epidemiol 9, 333-345.

218. Moroi, S.E., Raoof, D.A., Reed, D.M., Zollner, S., Qin, Z., and Richards, J.E. (2009). Progress toward personalized medicine for glaucoma. Expert Review of Ophthalmology In press.
219. Polansky, J.R., Fauss, D.J., Chen, P., Chen, H., Lutjen-Drecoll, E., Johnson, D., Kurtz, R.M., Ma, Z.D., Bloom, E., and Nguyen, T.D. (1997). Cellular pharmacology and molecular biology of the trabecular meshwork inducible glucocorticoid response gene product. Ophthalmologica 211, 126-139.
220. Kubota, R., Noda, S., Wang, Y., Minoshima, S., Asakawa, S., Kudoh, J., Mashima, Y., Oguchi, Y., and Shimizu, N. (1997). A novel myosin-like protein (myocilin) expressed in the connecting cilium of the photoreceptor: molecular cloning, tissue expression, and chromosomal mapping. Genomics 41, 360-369.
221. Hewitt, A.W., Mackey, D.A., and Craig, J.E. (2008). Myocilin allele-specific glaucoma phenotype database. Hum Mutat 29, 207-211.
222. Gong, G., Kosoko-Lasaki, O., Haynatzki, G.R., and Wilson, M.R. (2004). Genetic dissection of myocilin glaucoma. Hum Mol Genet 13 Spec No 1, R91-102.
223. Fingert, J.H., Stone, E.M., Sheffield, V.C., and Alward, W.L. (2002). Myocilin glaucoma. Surv Ophthalmol 47, 547-561.
224. Rozsa, F.W., Shimizu, S., Lichter, P.R., Johnson, A.T., Othman, M.I., Scott, K., Downs, C.A., Nguyen, T.D., Polansky, J., and Richards, J.E. (1998). GLC1A mutations point to regions of potential functional importance on the TIGR/MYOC protein. Mol Vis 4, 20.
225. Fautsch, M.P., Bahler, C.K., Jewison, D.J., and Johnson, D.H. (2000). Recombinant TIGR/MYOC increases outflow resistance in the human anterior segment. Invest Ophthalmol Vis Sci 41, 4163-4168.
226. Liu, Y., and Vollrath, D. (2004). Reversal of mutant myocilin non-secretion and cell killing: implications for glaucoma. Hum Mol Genet 13, 1193-1204.
227. Zimmerman, C.C., Lingappa, V.R., Richards, J.E., Rozsa, F.W., Lichter, P.R., and Polansky, J.R. (1999). A trabecular meshwork glucocorticoid response (TIGR) gene mutation affects translocational processing. Mol Vis 5, 19.
228. Shepard, A.R., Jacobson, N., Millar, J.C., Pang, I.H., Steely, H.T., Searby, C.C., Sheffield, V.C., Stone, E.M., and Clark, A.F. (2007). Glaucoma-causing myocilin mutants require the Peroxisomal targeting signal-1 receptor (PTS1R) to elevate intraocular pressure. Hum Mol Genet 16, 609-617.
229. Lam, D.S., Leung, Y.F., Chua, J.K., Baum, L., Fan, D.S., Choy, K.W., and Pang, C.P. (2000). Truncations in the TIGR gene in individuals with and without primary open-angle glaucoma. Invest Ophthalmol Vis Sci 41, 1386-1391.
230. Wiggs, J.L., and Vollrath, D. (2001). Molecular and clinical evaluation of a patient hemizygous for TIGR/MYOC. Arch Ophthalmol 119, 1674-1678.
231. Morissette, J., Clepet, C., Moisan, S., Dubois, S., Winstall, E., Vermeeren, D., Nguyen, T.D., Polansky, J.R., Cote, G., Anctil, J.L., et al. (1998). Homozygotes carrying an autosomal dominant TIGR mutation do not manifest glaucoma. Nat Genet 19, 319-321.
232. Hewitt, A.W., Bennett, S.L., Dimasi, D.P., Craig, J.E., and Mackey, D.A. (2006). A myocilin Gln368STOP homozygote does not exhibit a more severe glaucoma phenotype than heterozygous cases. Am J Ophthalmol 141, 402-403.

233. Chakrabarti, S., Kaur, K., Komatireddy, S., Acharya, M., Devi, K.R., Mukhopadhyay, A., Mandal, A.K., Hasnain, S.E., Chandrasekhar, G., Thomas, R., et al. (2005). Gln48His is the prevalent myocilin mutation in primary open angle and primary congenital glaucoma phenotypes in India. Mol Vis 11, 111-113.
234. Lo, W.R., Rowlette, L.L., Caballero, M., Yang, P., Hernandez, M.R., and Borras, T. (2003). Tissue differential microarray analysis of dexamethasone induction reveals potential mechanisms of steroid glaucoma. Invest Ophthalmol Vis Sci 44, 473-485.
235. Fingert, J.H., Clark, A.F., Craig, J.E., Alward, W.L., Snibson, G.R., McLaughlin, M., Tuttle, L., Mackey, D.A., Sheffield, V.C., and Stone, E.M. (2001). Evaluation of the myocilin (MYOC) glaucoma gene in monkey and human steroid-induced ocular hypertension. Invest Ophthalmol Vis Sci 42, 145-152.
236. Colomb, E., Nguyen, T.D., Bechetoille, A., Dascotte, J.C., Valtot, F., Brezin, A.P., Berkani, M., Copin, B., Gomez, L., Polansky, J.R., et al. (2001). Association of a single nucleotide polymorphism in the TIGR/MYOCILIN gene promoter with the severity of primary open-angle glaucoma. Clin Genet 60, 220-225.
237. Fan, B.J., Leung, Y.F., Pang, C.P., Fan, D.S., Wang, D.Y., Tong, W.C., Tam, P.O., Chua, J.K., Lau, T.C., and Lam, D.S. (2004). Polymorphisms in the myocilin promoter unrelated to the risk and severity of primary open-angle glaucoma. J Glaucoma 13, 377-384.
238. Ozgul, R.K., Bozkurt, B., Orcan, S., Bulur, B., Bagiyeva, S., Irkec, M., and Ogus, A. (2005). Myocilin mt1 promoter polymorphism in Turkish patients with primary open angle glaucoma. Mol Vis 11, 916-921.
239. Polansky, J.R., Juster, R.P., and Spaeth, G.L. (2003). Association of the myocilin mt.1 promoter variant with the worsening of glaucomatous disease over time. Clin Genet 64, 18-27.
240. Musch, D.C., Lichter, P.R., Guire, K.E., and Standardi, C.L. (1999). The Collaborative Initial Glaucoma Treatment Study: study design, methods, and baseline characteristics of enrolled patients. Ophthalmology 106, 653-662.
241. Lichter, P.R., Musch, D.C., Gillespie, B.W., Guire, K.E., Janz, N.K., Wren, P.A., and Mills, R.P. (2001). Interim clinical outcomes in the Collaborative Initial Glaucoma Treatment Study comparing initial treatment randomized to medications or surgery. Ophthalmology 108, 1943-1953.
242. Janz, N.K., Wren, P.A., Lichter, P.R., Musch, D.C., Gillespie, B.W., Guire, K.E., and Mills, R.P. (2001). The Collaborative Initial Glaucoma Treatment Study: interim quality of life findings after initial medical or surgical treatment of glaucoma. Ophthalmology 108, 1954-1965.
243. Ewing, B., Hillier, L., Wendl, M.C., and Green, P. (1998). Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res 8, 175-185.
244. Ewing, B., and Green, P. (1998). Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 8, 186-194.
245. Li, Y., Willer, C.J., Ding, J., Scheet, P., and Abecasis, G.R. Rapid Markov Chain Haplotyping and Genotype Inference. Submitted.

246. Musch, D.C., Gillespie, B.W., Lichter, P.R., Niziol, L.M., and Janz, N.K. (2008). Visual Field Progression in the Collaborative Initial Glaucoma Treatment Study The Impact of Treatment and Other Baseline Factors. Ophthalmology.
247. Musch, D.C., Gillespie, B.W., Niziol, L.M., Cashwell, L.F., and Lichter, P.R. (2008). Factors associated with intraocular pressure before and during 9 years of treatment in the Collaborative Initial Glaucoma Treatment Study. Ophthalmology 115, 927-933.
248. Deokule, S., and Weinreb, R.N. (2008). Relationships among systemic blood pressure, intraocular pressure, and open-angle glaucoma. Can J Ophthalmol 43, 302-307.
249. Vollrath, D., Jaramillo-Babb, V.L., Clough, M.V., McIntosh, I., Scott, K.M., Lichter, P.R., and Richards, J.E. (1998). Loss-of-function mutations in the LIM-homeodomain gene, LMX1B, in nail-patella syndrome. Hum Mol Genet 7, 1091-1098.
250. Lichter, P.R., Richards, J.E., Downs, C.A., Stringham, H.M., Boehnke, M., and Farley, F.A. (1997). Cosegregation of open-angle glaucoma and the nail-patella syndrome. American journal of ophthalmology 124, 506-515.
251. Pressman, C.L., Chen, H., and Johnson, R.L. (2000). LMX1B, a LIM homeodomain class transcription factor, is necessary for normal development of multiple tissues in the anterior segment of the murine eye. Genesis 26, 15-25.
252. Abecasis, G.R., and Cookson, W.O. (2000). GOLD--graphical overview of linkage disequilibrium. Bioinformatics 16, 182-183.
253. Karayiorgou, M., and Gogos, J.A. (2004). The molecular genetics of the 22q11-associated schizophrenia. Brain Res Mol Brain Res 132, 95-104.
254. Rozsa, F.W., Reed, D.M., Scott, K.M., Pawar, H., Moroi, S.E., Kijek, T.G., Krafchak, C.M., Othman, M.I., Vollrath, D., Elner, V.M., et al. (2006). Gene expression profile of human trabecular meshwork cells in response to long-term dexamethasone exposure. Molecular vision [electronic resource] 12, 125-141.
255. Rozsa, F.W., Scott, K.M., Pawar, H., Samples, J.R., Wirtz, M.K., and Richards, J.E. (2007). Differential expression profile prioritization of positional candidate glaucoma genes: the GLC1C locus. Arch Ophthalmol 125, 117-127.