The genetics of open-angle glaucoma: the story of GLC1A and myocilin

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ARTICLE

Abstract

A linkage analysis study was performed on a single large family with juvenile-onset primary open-angle glaucoma (POAG). This led to the recognition that there was a region of chromosome 1q that harboured a gene for juvenile-onset POAG. This chromosomal site was called GLC1A. It was discovered that a gene that produces the protein myocilin resides within this interval and that mutations in myocilin caused most cases of autosomal dominant juvenile-onset POAG. More importantly myocilin mutations also cause up to 4.6% of cases of adult-onset POAG. The prevalence of myocilin mutations is similar regardless of race or geographic location. There are widely variable glaucoma phenotypes depending on the specific mutation in myocilin. Myocilin is expressed in multiple tissues throughout the eye and in many other organs. In the trabecular meshwork the production of myocilin can be induced by the application of topical corticosteroids. The exact function of myocilin in health and disease remains a mystery.

Key words Genetics, Glaucoma, GLC1A, Myocilin, TIGR

Glaucoma is the leading cause of irreversible blindness in the world. It is estimated that approximately 67 million people worldwide are affected with glaucoma and, of these, 6.7 million are bilaterally blind.[1] In Western countries, primary open-glaucoma (POAG) is by far the most common form of glaucoma.[2] The four most important risk factors for the development of POAG include advanced age, black race, elevated intraocular pressure (IOP) and positive family history.[2] The risk of developing glaucoma for individuals with a history of glaucoma among first-degree relatives is up to 8 times higher than the risk for the general population.[2–4] This heritable nature of glaucoma permits the use of molecular genetic techniques to study this important blinding disease.

There are three main approaches to identifying a disease-causing gene (Table 1). The candidate gene approach is useful when there is a known gene whose function makes it a strong suspect. For example rhodopsin was a very reasonable candidate for retinitis pigmentosa and proved to be the disease-causing gene in some cases.[5] Unfortunately there are too many potential candidate genes for POAG, including all the genes involved in the development, structure and function of the trabecular meshwork and optic nerve head.

A second approach is to identify patients who manifest the disease of interest and also have a chromosomal deletion or translocation; the break in the chromosome might be in or near the disease-causing gene. This technique has proved helpful for a variety of developmental glaucomas. Recently Nishimura *et al*.[6] discovered the forkhead transcription factor gene FKHL7 by studying the breakpoints in chromosome 6 of two children with primary congenital glaucoma and chromosomal translocations. Mutations in this gene have been demonstrated to cause a variety of anterior segment abnormalities including Axenfeld anomaly and Rieger anomaly.[6,7]

In the absence of any other clues as to the location and nature of the gene causing a disease, the search typically begins by performing linkage analysis studies on large families affected with the disease. In this technique there is a search for co-segregation between a disease phenotype and polymorphic genetic markers. Unfortunately, POAG is not an ideal disease for linkage studies. Linkage analysis requires large numbers of living affected individuals. Because POAG is a late-onset disease, the parents of affected individuals are almost always deceased, as are many of the siblings. Additionally, the children

Table 1. *Major techniques used in searching for disease-causing genes*

Candidate gene approach
Utilising clues from chromosomal deletions and translocations
Linkage analysis
Combination of the above techniques

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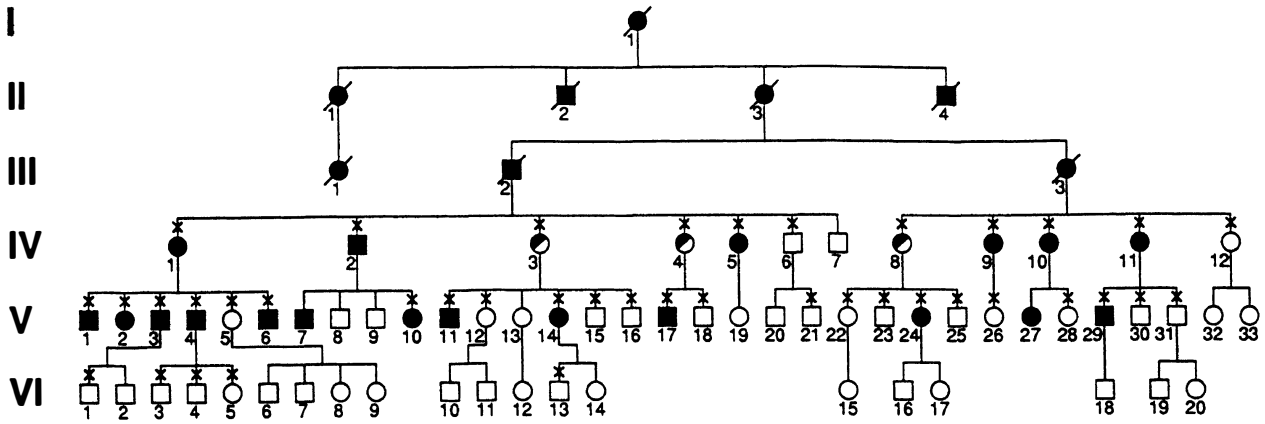

Fig. 1. *Pedigree of the proband's family. Individuals with glaucoma are indicated with filled symbols. Those individuals indicated with half-filled symbols have ocular hypertension and are known to have the disease-causing mutation because they have affected children. Reproduced with permission from Johnson* et al.[13]

are often too young to manifest signs of the disease. Therefore, obtaining large numbers of affected individuals in a single extended family is very difficult.

Often a combination of the techniques mentioned above is used to find a disease-causing gene. Murray *et al.*[8] found that a gene on the long arm of chromosome 4 could cause Rieger syndrome by performing linkage analysis on relatively small families. They were able to use small families because they had narrowed the region of search by targeting a small portion of the genome using clues from the coexistence of Rieger syndrome and translocations and deletions in chromosome 4q.[8]

This article will discuss the story behind the discovery of the first linkage for POAG (GLC1A) and ultimately the gene at this locus (myocilin). Mutations in myocilin have been demonstrated to be capable of causing juvenile-onset POAG,[9] adult-onset POAG[9] and normal tension glaucoma.[10] The discovery was made through the use of linkage analysis[11] followed by evaluation of candidate genes in the linked interval.[9] This approach has been called the positional candidate approach.[12] Because most of the papers about this discovery have appeared in the basic science, genetic and general medical literature it is useful to review them for an ophthalmology audience.

The proband and his family

As with all genetics studies, this story begins with a patient. The proband presented to the University of Iowa Department of Ophthalmology in 1986 requesting glaucoma filtration surgery. He was 28 years old and had been aware that he had glaucoma since age 16. At the time we first examined him he was using timolol maleate, dipivefrine HCl and pilocarpine gel. His IOPs were 24 mmHg in the right eye and 25 mmHg in the left. He supported the request for filtration surgery by presenting a simple pedigree that he had constructed. Half his relatives were affected by a form of glaucoma that had a very early age of onset, was resistant to medical therapy, was resistant to laser trabeculoplasty, and generally did well after filtration surgery. He ultimately had bilateral trabeculectomies with resultant excellent control of his IOP and stability of his optic nerves and visual fields.

In 1993, Johnson described this family in detail.[13] The family was of German ancestry. Of 59 individuals in five generations who were at risk for the disease, 30 were affected (Fig. 1). They had a POAG with a very early age of onset (mean 18 years) and very high IOPs (mean 45 mmHg). A sampling of the age of onset and IOPs of some of the more dramatic cases is shown in Table 2. Eighteen (82%) of 22 living affected family members had

Table 2. *Examples of the early age of onset and dramatically high IOP found in members of this pedigree*

Pedigree no.	Age at diagnosis (years)	Peak IOP (mmHg)	
		Right eye	Left eye
IV-9	17	54	54
IV-11	16	66	58
V-2	18	62	63
V-3	27	59	53
V-11	22	57	56
V-24	16	52	56
V-27	16	52	56
V-29	8	50	43

Adapted with permission from Johnson *et al.*[13]
These are some of the most striking examples from this pedigree.

undergone filtering surgery. Affected members of the family were usually myopic (mean refractive error −4.06 D) but otherwise had normal ocular anatomy.[13]

It was felt that juvenile-onset POAG could serve as a model for the adult-onset disease because it shared the clinical phenotype, except for the following differences: a very early age of onset, a striking autosomal dominant inheritance pattern with high penetrance, and very high IOPs.[13] Otherwise, the diseases were identical; the iridocorneal angle was open and the trabecular meshwork was normal in appearance.

Linkage analysis

DNA samples were obtained from 22 affected individuals and tested for linkage with 90 short tandem repeat polymorphism (STRP) markers scattered across the entire human genome. These markers, also called microsatellites, are areas of normal variability in the DNA sequence. There are thousands of these markers located on all chromosomes. These portions of the DNA can be amplified by polymerase chain reaction (PCR) and assayed to see whether one of the possible variations in DNA sequence (alleles) segregates with the affected phenotype.

In this family there was linkage between the affected status and markers on the long arm of chromosome 1 (1q21–q31) (Fig. 2).[11] After this linkage was reported it was confirmed in populations in the United States[14,15] and throughout the world.[16–18] The majority of large autosomal dominant juvenile-onset POAG families link to chromosome 1q.[19] A large family with both juvenile-onset and adult-onset POAG was also found to link to chromosome 1q.[18]

This locus on chromosome 1 was assigned the name GLC1A. 'GLC' stands for glaucoma, '1' stands for POAG, 'A' stands for the first linkage for this disease.

Fine mapping

Identifying the chromosomal locus was a major step in finding a gene for POAG. Unfortunately, within the interval described were hundreds of genes (mostly unidentified) and millions of base pairs. Characterising and screening all the genes in this interval for mutations would have been unrealistic. Therefore, further families were identified and more markers tested in order to reduce the linked interval to the smallest possible region. Sunden *et al.*[20] reported narrowing the interval to 3 centimorgans (cM) in 1996. A centimorgan is a unit of DNA length. One centimorgan is that length of DNA in which one would expect a 1% chance of recombination in the offspring. Through a variety of techniques and assumptions the interval was finally narrowed to approximately 1 cM.[9] Within this region were two known genes: APPT1LG1 and TXGP1.[9]

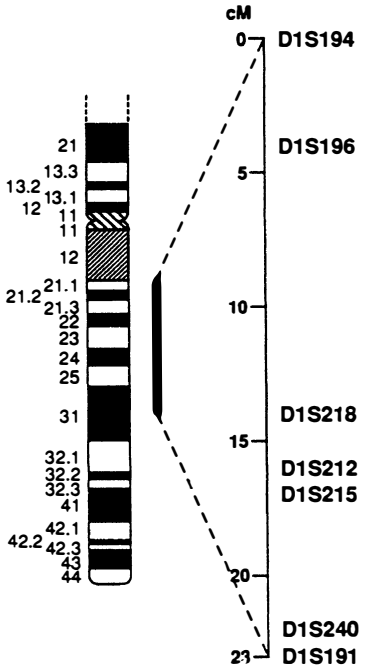

Fig. 2. *Location of the disease-causing gene on chromosome 1. The heavy vertical bar to the right of the chromosome drawing denotes the interval q21–q31, which contains the gene. Reproduced with permission from Sheffield* et al.[11]

Trabecular meshwork inducible glucocorticoid receptor protein

During the years that these family studies were in progress Drs Nguyen and Polansky were performing an independent and seemingly unrelated series of experiments. Their work was built upon the recognition that POAG can be induced in humans by the topical application of corticosteroids. In the laboratory they treated cultured trabecular meshwork cells with fairly high doses of dexamethasone. They then determined the changes in gene expression of the trabecular meshwork cells by comparing corticosteroid-treated cells with control cells. They discovered a protein that was markedly increased when the trabecular meshwork cells were exposed to corticosteroids. They named the protein trabecular meshwork inducible glucocorticoid receptor protein (TIGR).[21]

Because of the expression of this protein in the trabecular meshwork and its reponse to corticosteroids, the gene producing the TIGR protein was viewed as an attractive candidate gene for glaucoma. Stone *et al.*[9] discovered that the TIGR gene was in the interval containing GLC1A, further increasing the interest in this gene.

Cloning the gene

The TIGR gene was screened for mutations by a combination of single-strand conformational polymorphism analysis (SSCP) and direct sequencing. These techniques are designed to determine whether there are any alterations in the genetic coding sequence of the base pairs that make up the DNA. If a sequence

Table 3. *Prevalence of myocilin mutations in five populations*

Population studied	POAG patients studied	POAG patients (%) with mutations	Normals studied	Normals with mutations
USA whites (Iowa)	727	31 (4.3%)	91	0
USA blacks (New York)	312	8 (2.6%)	40	0
Australia	390	11 (2.8%)	58	0
Canada	157	5 (3.0%)	0	
Japan	107	3 (2.8%)	49	0

Adapted with permission from Fingert *et al.*[27]

variation is discovered that changes an amino acid in the resultant protein it is suspicious for being a disease-causing mutation, especially if the sequence variation is seen much more commonly in patients than in unaffected individuals.

The proband and his family were found to have a single base pair change that caused the amino acid histidine to be produced instead of tyrosine at codon 437 (TYR437HIS).[9] Of eight families with juvenile-onset POAG, all were found to have mutations in this gene at GLC1A.[22] Mutations have now been found in a large number of patients in populations throughout the world.[22–32]

Nomenclature

The terminology of genetics terms can be confusing. GLC1A is the assigned name for the chromosomal linkage, as described above. Nguyen and Polansky called the gene TIGR for the reasons mentioned earlier. At approximately the same time that Nguyen and Polansky were describing TIGR, Kubota and co-workers described a protein in the photoreceptors of the retina that had some sequence homology to non-muscle myosin and olfactomedin; they called this protein myocilin.[33] Myocilin and TIGR are identical. The international nomenclature committee, HUGO, has chosen the name myocilin for this protein. In this paper, GLC1A will refer to the chromosomal locus and myocilin to the gene and resultant protein.

Myocilin mutations in adult-onset POAG

In the first paper reporting that mutations in myocilin could cause juvenile-onset POAG, Stone *et al.*[9] screened the DNA of adult-onset POAG patients as well. Myocilin mutations were found in 10 (4.4%) of 227 individuals whose DNA had been stored because of a family history of glaucoma and 3 (2.9%) of 103 unselected POAG patients from a glaucoma clinic. None of 91 normal volunteers had mutations. Only one (0.3%) of 380 individuals from the general population, whose affection status was not known, had a mutation.[9]

In larger screen of 716 Midwestern American probands with adult-onset POAG, 33 patients (4.6%) were found to have one of 16 myocilin mutations.[22] To determine whether the prevalence of mutations in myocilin was unique to the Midwestern American population, 1703 probands from five populations were evaluated. The study included primarily Caucasian patients from the Midwestern United States (Iowa), Canada and Australia; African-American patients from the United States (New York City); and Asian patients from Japan.[27] A similar prevalence of myocilin mutations was found in these diverse populations, ranging from 2.6% to 4.3% (Table 3).[27] The fact that African-American patients have a similar percentage of myocilin mutations to Caucasian-Americans demonstrates that the increased prevalence of POAG among African-Americans is not caused by a higher prevalence of myocilin mutations.[27]

Mutations phenotypes

Of 16 mutations found in the screen of 716 Midwestern American probands, six were most prevalent (Table 4).[22] The majority of mutations in myocilin cause a juvenile-onset glaucoma with a mean age at diagnosis ranging from 20 to 37 years and mean peak IOPs as high as 44 mmHg. One mutation (GLN368STOP) stands out because, unlike the other mutations, it causes a typical adult-onset form of POAG with a mean age of onset of 59 years and a mean peak IOP of 30 mmHg – a phenotype indistinguishable from ordinary adult-onset POAG.

Table 4. *The phenotypes of patients with the six most common mutations in myocilin*

	Mutation identified in proband					
Variable	GLY364VAL	GLN368STOP	THR377MET	386INS397	TYR437HIS	ILE477ASN
No. of families	2	15	2	1	2	1
No. affected	16	22	15	6	27	13
Age at diagnosis (years)						
Range	22–48	36–77	20–60	19–31	8–41	12–41
Mean	34	59	37	25	20	21
Highest IOP (mmHg)						
Range	15–65	21–56	20–50	12–60	14–77	20–52
Mean	36	30	31	40	44	40

Adapted with permission from Alward *et al.*[22]

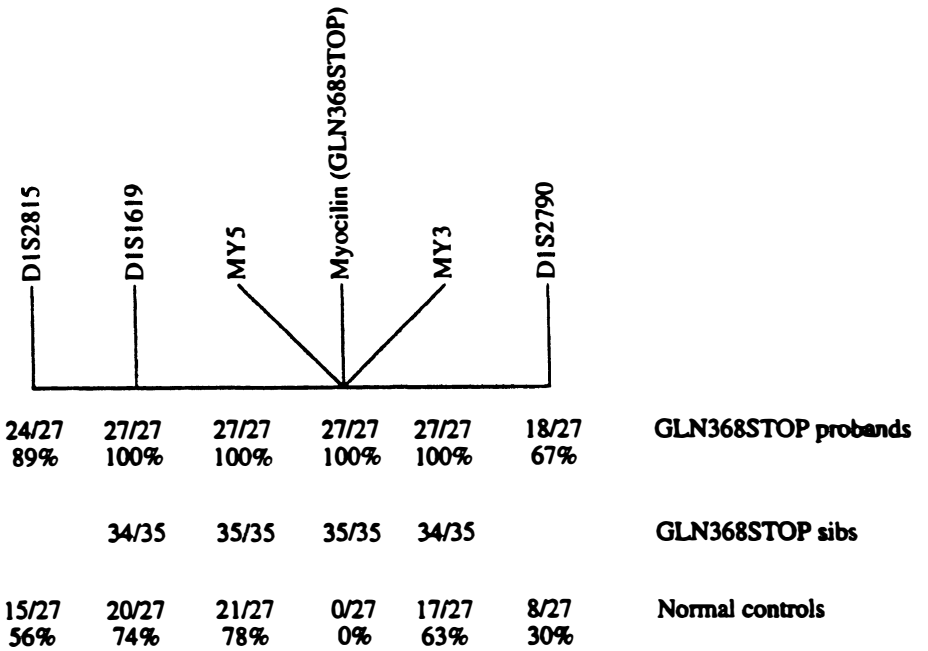

Fig. 3. *Genotypic evidence of a founder effect causing the high prevalence of the GLN368STOP mutation. Twenty-seven probands and 35 sibs with the GLN368STOP mutation as well as 27 ethnically matched controls were genotyped at five markers closely flanking the myocilin gene. The fraction of subjects that shared a common allele of each marker is indicated. Reproduced with permission from Fingert* et al.[27]

There has even been a report of one patient with normal tension glaucoma who has been found to have the GLN368STOP mutation.[10]

In screens of adult-onset POAG, the GLN368STOP mutation is by far the most prevalent.[22,27] GLN368STOP mutations were found in four of the five populations reported by Fingert *et al.*[27] – every population but the Japanese. The high frequency of GLN368STOP mutations suggested a possible founder effect. Fingert *et al.* genotyped markers in and around the myocilin gene and found a very high degree of allele sharing between 27 GLN368STOP probands from four populations when compared with ethnically matched controls (Fig. 3).[27] This confirmed the theory that most patients with GLN368STOP myocilin mutations are descended from a common founder.[27] In the Midwestern United States, 2.1% of all adult-onset POAG patients had this one mutation.[22] If a similar prevalence of GLN368STOP

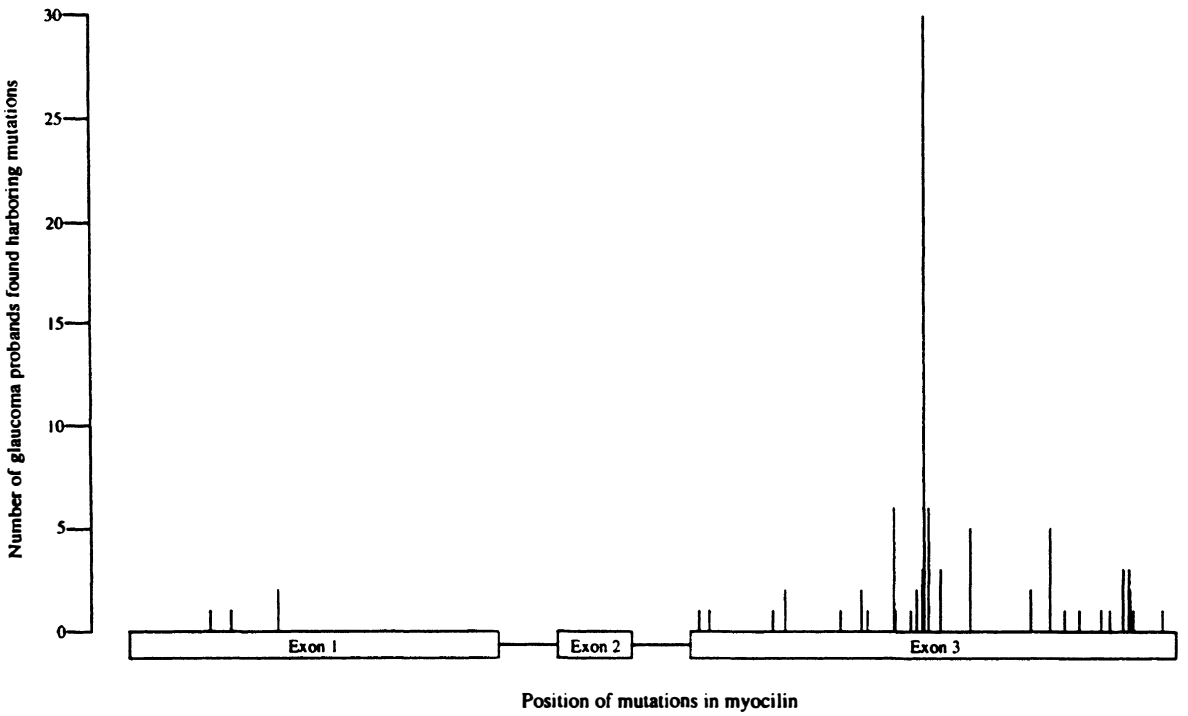

Fig. 4. *Distribution of mutations across the myocilin gene. The boxes represent the three exons. The location of known mutations is indicated by a vertical line. The height of the line represents the total number of glaucoma probands with that particular mutation. The highest line is at GLN368STOP. These mutations are from a variety of reports.*[9,22,23,27,30–32,46–51] *Reproduced with permission from Fingert* et al.[27]

Table 5. *The known chromosomal loci for primary open-angle glaucoma genes*

Locus	Chromosome	Reference
GLC1A	1q21–31	11
GLC1B	2cen–q13	41
GLC1C	3q21–24	42
GLC1D	8p23	43
GLC1E	10p15–14	44
GLC1F	7q35–36	45

mutations held across the population, this family could account for tens of thousands of cases of POAG in the United States alone.

The structure and function of myocilin

Now that it is known that mutations in myocilin can cause adult-onset POAG, a great deal of attention has been turned to the function of myocilin. The myocilin protein is 57 kDa in size and contains three coding regions (exons) (Fig. 4).[27] Most of the mutations are found in exon 3, a part of myocilin with olfactomedin homology.[27] Olfactomedin is a major component of the extracellular matrix of the olfactory neuroepithelium.[34]

Myocilin is expressed in the trabecular meshwork,[21] and is in higher concentrations in glaucomatous eyes than in normal eyes.[35] As mentioned, the amount of myocilin in the trabecular meshwork can be increased with the application of corticosteroids. Besides the trabecular meshwork, myocilin is expressed in the ciliary body, retina, and 17 of 23 other organ system studied.[36] Swiderski *et al.*[37] have demonstrated expression in several regions of the mouse brain and Clark *et al.*[38] have demonstrated myocilin in the optic nerve head.

The function of myocilin in health and disease is not known and has become the subject of intense research. Unlocking the role of myocilin should improve our understanding of the normal regulation of trabecular function. It should help us to understand how IOP becomes elevated in glaucoma and thereby develop therapeutic interventions.

Other POAG loci

Since the description of GLC1A several other linkages for POAG have been described (Table 5). These have been discovered through linkage analysis studies in large families. All cause adult-onset POAG. The GLC1E locus has been associated with a normal tension glaucoma phenotype. No genes have been identified at these loci to date.

Discussion

A linkage analysis study in a single large family with juvenile-onset POAG led to the discovery of a locus on chromosome 1q (GLC1A) containing a gene that caused glaucoma in this family.[11] As other families from around the world were found to link to this site, it became apparent that this locus harboured a gene that caused glaucoma in the majority of families with autosomal dominant juvenile-onset disease.[15–18] After narrowing the disease-causing interval, candidate genes were evaluated and it was discovered that mutations in myocilin were responsible for the majority of families with autosomal dominant juvenile-onset POAG.[9] More importantly, different mutations in this same gene were shown to cause some cases of typical adult-onset POAG.[9] Myocilin mutations are found in 2.6–4.6% of adult-onset POAG patients in varying racial and ethnic groups.[22,27]

One particular mutation (GLN368STOP) is especially interesting because it is the most prevalent mutation, because it has a phenotype indistinguishable from ordinary adult-onset POAG, and because most patients with this mutation around the world are related through a common founder.[22,27]

The structure of myocilin has been established and much is known about the expression of myocilin in various tissues.[33,36] It is expressed in the trabecular meshwork and is inducible by the application of corticosteroids.[21] It is also found in many other tissues throughout the eye and has been detected in the majority of other organs tested.[33,36] How myocilin functions remains a mystery.

An understanding of the molecular genetics of glaucoma holds much promise for the future of glaucoma diagnosis and management. Once the function of myocilin is determined it will provide pivotal information regarding the function of the trabecular meshwork. Understanding the physiology could lead to the discovery of other genes involved in trabecular function. Molecular genetic techniques will one day allow the earliest possible diagnosis of a population at risk for glaucoma, a group upon which we should target the limited screening resources. We can already determine which members of a family with myocilin mutations are at nearly 100% risk of developing the disease and which members are at no higher risk than the general population. At the present time population-wide screening for myocilin mutations is not practical because this gene accounts for only about 3% of POAG. However, as more mutations are discovered one can envision a day when a simple blood test or cheek swab will determine with high certainty the individuals at the highest risk for glaucoma.

Lastly we will, it is hoped, be able to develop better therapies for glaucoma from the knowledge of the molecular genetics. There is the ultimate prospect of gene replacement therapy, and indeed the technology exists to introduce genes into trabecular meshwork cells.[39,40] Perhaps a more likely therapeutic application will be the development of drugs based on an understanding of the disease at a molecular level. One might develop a drug that inhibits the production of myocilin and therefore prevents the disease from developing. This drug might be highly effective and safe for glaucoma patients with myocilin mutations but may not be useful at all for patients with other forms of glaucoma.

Several other linkages for open-angle glaucoma have been discovered. To date, no genes have been discovered at these loci. As the genes at these loci are found and studied we should have a clearer picture of glaucoma and better tools with which to fight this blinding disease.

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