

MOLECULAR GENETICS IN THE CLINICAL PRACTICE OF GLAUCOMA

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Glaucomatous optic neuropathy is the second leading cause of blindness in the world (after cataract). Approximately 67 million people have glaucoma, more than half of whom have open-angle glaucoma, and approximately 7 million individuals have bilateral blindness.⁵¹ Even in populations with good medical access, as many as half or more of the affected individuals are asymptomatic and thus unaware of their disease until its very late stages.⁵¹

Although pressure-lowering treatment is available, medical treatment is expensive and associated with local and systemic side effects. Surgical treatment is also associated with certain risks, some of them vision-threatening. Although both medical and surgical treatments are effective options for lowering eye pressure, they generally do not have a life-long effect.

Glaucoma has a strong hereditary basis. A family history of primary open-angle glaucoma (POAG) is an important risk factor for the disease. A positive family history was reported in 13% to 25% of POAG cases.^{8, 27} The odds ratio for a positive family history in a POAG patient, as opposed to a nonglaucoma one, varied from 2.7 to 4.7.⁶⁷ In this review, we highlight our current understanding of the genetics of glaucoma in an attempt to predict its affect on future clinical practice.

ARE GENETIC TESTS CURRENTLY PART OF AN OPHTHALMOLOGIST'S ARSENAL?

When did you last order genetic testing for a glaucoma patient or suspect? How many of your patients have genetic testing results documented in their charts? The look of bewilderment we received from practitioners to whom we posed these questions led us to conclude that glaucoma genetic testing has not yet penetrated into ophthalmic clinical practice. At this stage, most health care providers do not reimburse expenses for genetic testing of glaucoma.

Genetic tests are ideal screening tests for glaucoma. On the whole, they are precise, being highly sensitive and specific at the molecular level. They are inexpensive, noninvasive, and contrary to most other clinical tests, provide information that does not change throughout the subject's lifetime and does not fluctuate with disease activity; however, to justify their incorporation into clinical practice, a clear relevance to diagnosis, management, or at the minimum, to prognosis must be shown. In addition, it must be demonstrated that they are adequately sensitive and specific to be useful in the clinical arena. Therefore, it is not enough to show that genetic markers are predictive at the molecular level but also predictive to clinical state and outcome.

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This can be demonstrated by an example. Consider an excellent screening test for a hypothetical mutation highly predictive of glaucoma, which is present only in a single family. Furthermore, assume that this hypothetical mutation has a penetrance of 100% so that it is perfectly correlated with a glaucomatous phenotype. Using this test (a molecular probe), the presence of this mutation in every family member with 100% sensitivity and specificity can be established (or excluded). Although this setting is ideal at the molecular level, it is not clinically useful because the mutation does not occur outside this single family, perhaps owing to a founder effect. This example is somewhat extreme, but it points to a discrepancy between the molecular and the clinical levels, a situation with many of the mutations so far implicated in glaucoma.

WHO COULD POTENTIALLY BENEFIT FROM GENETIC TESTING?

At present, glaucomatous optic neuropathy can be diagnosed only when damage in the form of either optic nerve axonal loss or visual function deficits is already present. The use of genetic tools makes possible both early diagnosis, before any irreversible damage has occurred, and early treatment. Also, ruling out the existence of glaucoma can eliminate unnecessary treatment, lifelong follow-up, and perhaps, just as important, lifelong anxiety with its effect on the individual's quality of life. Genetic testing may be particularly relevant to the following groups, who pose difficult diagnostic and therapeutic problems:

1. Ocular hypertensive subjects who have repeated measurements of increased intraocular pressure (IOP) but lack established glaucoma damage. It is currently believed that only a subset of these individuals ever develop glaucoma.
2. Glaucoma suspects, including subjects with suspicious-looking discs and those with stable (or atypical) visual field abnormalities. At present, it is unclear for which of these glaucoma suspects treatment is indicated.
3. Patients with early glaucoma in whom the question arises whether to use aggressive therapy, such as initial trabeculectomy,³⁸ or rather to start the step-wise approach with a single medication. Genetic testing may assist in tailoring treatment to an individual's long-term prog-

nosis. Target IOP is a concept by which a goal IOP is tailored to an individual's susceptibility for future optic nerve damage. Because prognosis assessment is extremely vague, target IOPs are based, at present, only on documented deterioration. In the future, genetic data could be used for determining target IOPs, on the basis of future prognosis rather than past deterioration.

4. Individuals who are at an increased risk for glaucoma due to a positive family history or other risk factors, such as pseudo-exfoliation or pigmentary dispersion syndromes.

FOR WHICH OPHTHALMIC GENETIC DISEASES IS TESTING WORTHWHILE?

To warrant genetic testing in clinical practice, a disease must be prevalent, have significant impact on the individual's quality of life, and, most important, be amenable to treatment. Ideally, prophylactic therapy should exist, preventing the disease from reaching a symptomatic stage. It is our conviction that open-angle glaucoma should be near the top of the list of ophthalmic diseases for which genetic screening could become clinically meaningful. POAG is a common disease for which treatment exists, yet early diagnosis is difficult and imprecise. Among inherited eye disorders for which gene identification is known, glaucoma is one of the few for which effective treatments are currently available.¹³

Another disease in which genetic information could be valuable is primary angle-closure glaucoma (PACG), a common disease especially among Asians. Contrary to POAG, prophylactic treatment, in the form of a laser peripheral iridotomy, is cheap, simple, immediate, and far more definite and safe than what POAG patients are currently offered. A genetic marker identifying those at risk of angle closure (both acute and chronic) would be valuable. Unfortunately, such a marker has not been identified yet.

HOW COULD GENETIC INPUT BENEFIT CLINICAL MANAGEMENT OF GLAUCOMA?

Current therapeutic options for ameliorating glaucoma involve lowering of IOP. Al-

though neuroprotective drugs as therapy for glaucoma are promising, the use of this potential treatment option could be enhanced by early diagnosis.

Patients already diagnosed with glaucoma could benefit from genetic screening if the test results could predict the severity and rapidity of disease progression. As treatment options range from mild (a beta-blocker) to aggressive (initial trabeculectomy), predicting future disease behavior could help tailor therapy to the needs of an individual.

FAMILY HISTORY OF GLAUCOMA AS A RISK FACTOR FOR DEVELOPMENT OF THE DISEASE

Clearly, most POAG pedigrees do not show a simple Mendelian pattern of inheritance.¹² One indirect approach to assess the extent to which genetic factors contribute to the glaucomatous phenotype is by quantifying the magnitude of a positive family history as a risk factor for the disease. For good study design, it is required that some key issues be addressed. First, all subjects should, ideally, belong to a single cohort of the population. Second, diagnosis (both glaucoma and nonglaucoma) must be based on a standardized examination routine, including disc appearance, visual fields, and IOP. Third, history data should not be relied upon for diagnosing the index case or family members.

As early as 1966, Francois²³ concluded that simple glaucoma (POAG) has a definite hereditary component. This finding is substantiated by the geographic distribution of the disease; the geographic distribution of the aggressiveness of the disease (as measured by the proportion of affected individuals blinded by it), studies of twins, evidence of outflow compromise and steroid responsiveness in relatives of affected patients, and the observation that in each family glaucoma is usually of the same subtype. A meta-analysis of 10 studies published between 1947 and 1959, in which 3254 cases were analyzed, shows the average percentage of hereditary cases to be 20.3%.²³ Interestingly, although simple glaucoma is assumed to be, by and large, a dominant disease, a recessive mode of inheritance was found in a minority of cases, based on family pedigrees and consanguinity data. A concluding statement even from this early 1966 article, however, points to the fact that "it is not at all

certain that open-angle glaucoma is a monogenic trait."

A population based familial aggregation study⁸⁰ published in 1998, as part of the Rotterdam study, presented a well-designed, yet small, prospective study of first degree relatives of patients with POAG (N = 48) and of control subjects (N = 155). Siblings of POAG patients had a much higher rate of POAG (10.4%) as compared to siblings of controls (0.7%). The overall risk-ratio in first-degree relatives was 9.2, with a wide 95% confidence interval (1.2–73.9) owing to the relatively small sample size. The authors state that the lifetime risk of POAG was 22% in relatives of patients with glaucoma, almost 10 times higher than that in controls.⁸⁰ They conclude that: "at least one sixth of all glaucoma in the general population may be caused by a genetic component." This last statement warrants careful scrutiny. First, based on data presented in this study, it is very likely that nongenetic factors far outweigh genetic factors in their contribution towards a glaucomatous phenotype. Second, the likelihood that a combination of any number of genetic mutations could explain the vast majority of POAG patients seems rather small.

A study based on the Baltimore Eye Survey also looked at family history and risk of POAG.⁶⁷ Of 5308 residents of east Baltimore, 161 cases of POAG were identified by the use of a standardized examination. In this study, data on family history was obtained only by interview. The age adjusted association of POAG with a history of glaucoma was higher in siblings (odds ratio [OR] = 3.7) than in parents (OR = 2.2) or children (OR = 1.1). The OR was between 2–3 times higher for patients with prior knowledge of their glaucoma diagnosis than for those who first learned about their condition at the study examination. This study provides further evidence of a substantial genetic component in POAG.

The widely fluctuating prevalence of glaucoma among different racial groups is another indication of the importance of genetic factors. The adjusted prevalence rate for POAG has been shown to be at least fourfold higher in blacks than in whites. Although environmental factors may cause this difference, it was suggested that it probably reflects different genetic susceptibilities to POAG.⁶⁸ It is, however, essential to realize that many different factors can influence and modulate expression of genes. In addition, the appearance and timing of symptoms and signs in a each specific individual is probably modified by other genes as

well as unknown nongenetic environmental factors.

TRAITS GENETICALLY ASSOCIATED WITH GLAUCOMA

Several genetically determined traits (phenotypes) were studied in relation to glaucoma. Prior to the 1970s, when the era of molecular genetics began, such studies looked at a "linkage" between these traits and glaucoma. An association of glaucoma and the ability to taste phenylthiocarbamide was suggested.⁹ An association of glaucoma and various red blood cell markers (blood groups) was found,¹⁴ whereas no association with HLA antigens could be demonstrated.⁵⁴ The observation of an association between glaucoma and a rise in IOP in response to topical steroids^{6,7} (so-called steroid-responders) initiated the search that eventually led to the discovery of the TIGR (GLC1A) gene. The Duffy blood group was found to be weakly linked to POAG. This was later explained by the fact that the FY gene coding this blood group is located in the 1q22-q23 region, in the immediate vicinity of the GLC1A region.³⁵ Overall, these studies provided conflicting results, and the association between some of these phenotypes and glaucoma remains questionable.¹² Both diabetes and myopia were found to be associated with glaucoma³¹; however, it is likely that these two conditions exert their effect at the pathophysiologic level, rather than serving merely as genetic markers.

ENVIRONMENTAL (NONGENETIC) FACTORS

The role played by the environment in the development and progression of glaucomatous damage remains obscure. An association of season of birth with later development of glaucoma was proposed,^{73,74} as were the effects of lifestyle.⁶¹ Both heavy alcohol consumption and cigarette smoking showed no correlation with glaucoma.^{28, 52}

IN PURSUIT OF LOCI, GENES, AND MUTATIONS

As the human genome contains some 3.3 billion base pairs, identifying a disease causing mutation is a formidable task. The common

approach to identifying clinically meaningful mutations is by defining a gene locus (see glossary), then pinpointing (and characterizing) the actual gene involved, and finally, identifying various disease-causing mutations. The following section provides some insight into this process.

A standard approach is to start by selecting large family pedigrees containing multiple individuals having the disease of interest and through linkage analysis (see glossary) narrow down the gene locus. For this purpose one must have access to a very large family pedigree or, alternatively, to have several different families with mutations in the same gene. This approach permits gene localization without prior knowledge of the pathophysiology of the disease. Strict diagnostic criteria, however, are crucial for the success of this approach, as any misclassification seriously undermines the ability to prove linkage. Through linkage analysis researchers were able to identify and then narrow down the GLC1A locus from 1q21-q31^{59,77} to its present location at 1q23-25. Once a locus of a gene is narrowed to an interval of about 1 cM (see glossary), physical-mapping techniques can be used to identify the actual gene involved. It should be emphasized that the crucial step of identifying large affected pedigrees is solely in the hands of practicing ophthalmologists.

Chromosomal abnormalities can provide substantial shortcuts, provided that individuals with such abnormalities also show the phenotype of the disease in question. One example is the discovery of a locus for Rieger's syndrome, initiated by the observation that abnormalities of chromosome 4q (the long arm of chromosome 4) were found in patients with Reiger's syndrome.

While genetic linkage analysis begins with a phenotype and ends with a gene locus, a totally different approach is to start by speculating which genes may be involved in the pathophysiology of the disease in question (the candidate gene approach). An attempt is then made to "link" mutations along a candidate gene to the disease phenotype, a process that may substantiate or disprove an association between the two. Whereas such an approach proved highly rewarding in the study of the peripherin RDS gene and its role in retinitis pigmentosa,²⁰ the pathophysiology of glaucoma is, at present, still poorly understood and, as a result, numerous candidate genes exist, making it difficult to take this approach.

A combined approach is to define positional candidate genes along sections of the chromosome found by linkage analysis studies. Thus, candidate genes are not selected from the entire genome, but from a short segment showing linkage to the disease phenotype.

One successful example for the combined approach is the study of the trabecular meshwork-induced glucocorticoid response (TIGR) gene, implicated in the IOP response to corticosteroids. Because the GLC1A interval, defined by linkage studies, was found to contain this gene, it naturally became a positional candidate gene, later proved to be the actual GLC1A gene.

PRIMARY OPEN-ANGLE GLAUCOMA

POAG is the most prevalent subtype of glaucoma. Because POAG is diagnosed by exclusion, it is a difficult glaucoma subtype to diagnose definitively in clinical practice. In contrast to the Mendelian inheritance with high penetrance found in the majority of juvenile open-angle glaucoma (JOAG) families, the heredity of adult POAG seems more complex. Study of POAG offers several challenges. First, being a late onset disease implies that parents of index cases are in most cases already deceased, whereas children may not be affected yet. Second, in a subset of the pedigree individuals, diagnosing (or excluding) POAG with certainty at a given point in time often is difficult. Third, a complex mode of inheritance of an apparent genetically heterogeneous disorder is by far more difficult to unravel than a disorder with a simple Mendelian inheritance.

SPECIFIC LOCI ASSOCIATED WITH PRIMARY OPEN-ANGLE GLAUCOMA

The TIGR-MYOC (GLC1A) Gene

A five generation family pedigree with 22 living affected individuals was studied to first identify the GLC1A locus.^{26, 59} Most GLC1A-linked families have been characterized by a severe form of open-angle glaucoma with an early onset, usually before age 40, peak IOPs greater than 30 mm Hg, and severe damage to the optic nerve. Many individuals required filtering procedures.¹³ GLC1A was initially described in association with autosomal dominant JOAG and only later implied in a small subset of adult-onset POAG cases.

The TIGR (trabecular meshwork-induced glucocorticoid response) gene, situated at the 1q23-25 GLC1A locus,⁶⁵ was first identified and isolated by induction of primary cultured cells of trabecular meshwork tissue with glucocorticoids.⁴⁸ This gene was later shown to be identical to the MYOC gene originally described as a gene expressed in the connecting cilium of photoreceptors.^{29, 30} Mutations in the TIGR gene were found to be associated with juvenile glaucoma families^{26, 53, 59} and later also with adult-onset chronic glaucoma.⁴⁰ Recently, mutations in the coding sequence (exons) of this gene were associated with a small subset (3%–4%) of POAG patients.^{2, 22, 65} Although the overall incidence of these coding region TIGR mutations was quite similar in five different populations around the world, the actual mutations varied significantly.²²

Because high specificity was present (a low mutation rate found in control subjects), this group of structural mutations in the TIGR gene qualifies as the genetically most recognizable form of blindness, despite a modest yield (sensitivity) of only 3% to 4% for structural mutations in this gene.³³

The TIGR/Myocilin gene consists of three exons and encodes a protein that is 501 amino acids in length. Rozsa et al,⁵⁵ in 1998, demonstrated the clustering of coding region defects in specific portions of the gene's coding regions, the olfactomedin homology domain. The TIGR gene product has been proposed to cause increased IOP by obstruction of the aqueous outflow.^{49, 50} It is possible that once excreted, the TIGR protein forms oligomeric complexes.^{39, 44} Another area of the TIGR gene currently investigated is the promoter region, a noncoding portion of the gene. Sequence variants along this region may have potential disease consequences through regulation of the gene product.

GLC1B-GLC1F

Five other loci, besides GLC1A, were implicated in certain families as associated with glaucoma. The actual gene has not been identified for any of the POAG loci except GLC1A. Consequently, data regarding these additional loci are limited to descriptive clinical features (phenotype). Because additional families with glaucoma do not localize to any of these regions, it is quite certain that additional loci exist.

GLC1B

This is the second locus found to be linked to the POAG phenotype.⁶⁴ In contrast with GLC1A families and individuals (many of which are juvenile POAG), those with the GLC1B locus appear to be associated with a milder phenotype, lower peak-IOP, and older age of onset. Fifty percent of GLC1B-affected individuals never have had a measured IOP higher than 22 mm Hg, whereas most of the remaining showed maximal elevations in the range of 22 to 30 mm Hg. Onset was usually in the late forties, with a good response to medical therapy. This phenotype raises the question of whether normal tension glaucoma (NTG) is associated with this locus. If this is the case, the GLC1B gene, once identified, may shed light on pathophysiologic mechanisms underlying the susceptibility of some optic nerves to damage at normal IOPs.

GLC1C

Affected family members in the single pedigree shown to harbor the GLC1C gene have glaucoma with high pressures, late onset, and a moderate response to medication.⁷⁸ Although apparently a rare mutation, the phenotype presented by GLC1C patients is more typical of POAG as opposed to the younger onset of GLC1A and the low IOPs of GLC1B. Thus, it is possible that the GLC1C gene will provide insight into the pathophysiologic mechanism of the more typical high IOP, late-onset, POAG.

GLC1D

This locus was identified through a four-generation family. Little is known about the GLC1D locus. To date, only a single family is known to harbor this 8q23 mutation.⁷⁰ The glaucoma phenotype within this family is variable.

GLC1E

This was identified in a large British family presenting a classical form of NTG. The age at diagnosis ranged from 23 to 65 years.⁵⁷

GLC1F

This locus, which was identified in a single family with 10 affected individuals spanning four generations enabled the localization of

yet another POAG locus, at 7q35-36.⁷⁹ In these individuals IOP ranged from 22 to 38 mm Hg. Interestingly, a gene for pigment dispersion syndrome was recently shown to occupy the same 7q35-36 locus.⁴ One may only speculate on whether the same gene or different close-by genes are involved in these seemingly very different phenotypes.

JUVENILE OPEN-ANGLE GLAUCOMA (JOAG)

JOAG is characterized by onset before the age of 30 (often younger), high IOPs (often 30 to 50 mm Hg), large diurnal variations, and a poor response to medical⁷² and laser therapy. Affected individuals are often myopic and often have a strong family history of the disease. Juvenile POAG was once thought to show complete penetrance, but more recent studies suggest that penetrance is in the range of 80% to 95%.¹²

Mutations in the GLC1A gene were identified in a large proportion of the JOAG families studied. Only later was the GLC1A locus also implied in adult POAG. As previously discussed the current classification of open-angle glaucoma into two distinct entities, JOAG and adult-onset POAG, based on age at onset, seems questionable.

CONGENITAL GLAUCOMA

Patients with primary congenital glaucoma often present during the first year of life. The classic clinical triad consists of photophobia, epiphora (tearing), and blepharospasm. Its incidence varies from 1:12,000 to 1:22,000.³⁷ It is thought to be a result of abnormal development of anterior chamber angle structures, which leads to increased resistance to aqueous outflow.²⁵ Approximately 10% of cases are familial and usually show an autosomal recessive mode of inheritance.¹⁹ Penetrance may range from 40% to 100%, depending on the pedigree.⁷¹

Two loci for congenital glaucoma were identified, designated GLC3A⁵⁶ and GLC3B.¹

GLC3A

The locus was mapped using linkage analysis on 17 Turkish families, 11 of which were mapped to the GLC3A locus. CYP1B1, a gene

coding for cytochrome P4501B1,^{62,63} was shown to be almost uniformly responsible for the disease in a large series of Saudi Arabian families in whom three distinct mutations segregated with the phenotype in 24 of 25 tested families.¹¹ Disease causing mutations identified in the CYP1B1 gene either truncate the protein product prematurely or alter highly conserved regions of the protein.

GLC3B

Six Turkish families that did not map to GLC3A (and two additional families) were used to identify a second locus for congenital glaucoma, the GLC3B. Because only one half of these families mapped to this locus at 1p36, it seems almost certain that additional loci will be identified in the future.

PIGMENT DISPERSION AND PIGMENTARY GLAUCOMA

Pigment dispersion is characterized by changes within the iris pigment epithelium, which results in peripheral iris spokelike transillumination defects and pigment deposition throughout the anterior segment, including on the corneal endothelium (Krukenberg's spindle) and in the trabecular meshwork.²¹ Iris pigment is thought to be released secondary to mechanical friction between a posteriorly bowed iris and the anterior most zonular bundles.¹⁵ When associated with optic nerve damage and visual field loss, it is referred to as pigmentary glaucoma. It remains unclear precisely how pigment dispersion syndrome and pigmentary glaucoma are related.²¹

An autosomal dominant inheritance pattern was suggested for both pigmentary dispersion syndrome and pigmentary glaucoma.^{34,66} Both POAG and pigmentary glaucoma have been described in the same family, suggesting that they may be genetically related³¹ or else that some cases of POAG may actually represent remitted pigmentary glaucoma.¹⁰ It is interesting that 4% to 26% of patients with pigment dispersion syndrome and pigmentary glaucoma have a family history of POAG.

The first identified locus linked to the pigment dispersion syndrome phenotype, PDS1, is located at 7q35-36.⁴ A second locus, PDS2, was mapped to 18q11-q22.⁵

PSEUDOEXFOLIATION AND PSEUDOEXFOLIATIVE GLAUCOMA

Pseudoexfoliation syndrome, a systemic disease, manifests in a proportion of affected individuals with elevated IOP and glaucomatous damage. Pseudoexfoliation is primarily a disease of the elderly and appears to have wide variation in its prevalence rate in different parts of the world. Despite that, it is rarely visible in a patient before age 55, some two-generation families with pseudoexfoliation have been described.¹⁸ A locus on chromosome 2p16,⁷⁶ and maternal inheritance, perhaps suggestive of a mitochondrial locus,⁶⁰ have been reported. Others have questioned this.¹⁷

AXENFELD-RIEGER ANOMALY, SYNDROME, AND IRIS HYPOPLASIA

Rieger's syndrome is an autosomal dominant condition characterized by ocular involvement (malformations of the anterior segment) as well as systemic involvement (facial and dental abnormalities). When the systemic features are lacking, it is termed Rieger (or Axenfeld-Rieger) anomaly. Although Axenfeld-Rieger anomaly and Rieger syndrome share similar ocular features, they are distinct entities on the genetic level. Axenfeld-Rieger anomaly, mapped to 6p25, is associated with mutations in the FKHL7 gene that codes a transcription factor containing a fork-head domain.^{36,45}

Initial localization of the RIEG1 gene, responsible for Rieger syndrome, was aided by cases of 4q-deletion that also demonstrated the Rieger's phenotype.^{32,41} Pedigree analysis further narrowed the locus to the 4q25 region. RIEG1 was found to be a homeobox gene, namely a gene containing a sequence of about 180 DNA base pairs that are highly conserved throughout evolution.⁵⁸ These genes are thought to play an important role in development. A second locus involved in the Rieger phenotype was mapped to 13q14.⁴⁷ It is interesting to note that a gene for iridogoniodysgenesis, distinct from FKHL7, but in close proximity to it was identified at the 6p25 locus.³⁶

Iris hypoplasia, a developmental disorder isolated to the eyes, possesses some clinical overlap with Rieger's anomaly. It is characterized by attenuation of the anterior iris stroma and is highly associated with glaucoma.⁷⁵ Additional findings include excess trabecular tissue and abnormal vascularity, as evident on

gonioscopy. Iris hypoplasia was mapped to the 4q25 (RIEG1) region. It is possible that these two phenotypes are allelic variants, namely expressions of different mutations in the same gene.

PAX6

This gene, situated at 11p13, has been implicated in a heterogeneous group of anterior segment anomalies, including Peter's anomaly, autosomal dominant keratitis, congenital cataract with late onset corneal dystrophy, isolated foveal hypoplasia and aniridia.²⁴ Recent work suggests that PAX6 is a master control gene for eye formation throughout the animal kingdom, regulating the expression of other genes in time and space during embryogenesis.⁶⁹

The remarkable heterogeneous phenotype resulting from abnormalities in the PAX6 gene led us to classify this entity under the gene, rather than under any specific disease entity. This trend of classification by genotype, rather than by phenotype, will probably increase as we learn more about mutations underlying various diseases. A shift from phenotype to genotype has been the case in the fields of corneal dystrophies⁴³ and with various retinal degenerations.

POSTERIOR POLYMORPHOUS DYSTROPHY AND CONGENITAL HEREDITARY ENDOTHELIAL DYSTROPHY

Both primarily corneal conditions posterior polymorphous dystrophy, (PPMD) and congenital hereditary endothelial dystrophy (CHED) can manifest with glaucoma. PPMD is an autosomal dominant disorder in which vesicles and plaques form on the corneal endothelium. This abnormal endothelium often proliferates over the trabecular meshwork and iris, resulting in glaucoma that is found in 15% to 40% of PPMD patients.¹⁶ In a large PPMD pedigree, the 20q11 locus showed linkage to the PPMD phenotype. Understanding the pathophysiology of this disease may elucidate the mechanism by which normally dormant corneal endothelium cells start proliferating.³ Corneal endothelium is in many respects similar to trabecular endothelium, as both are derived from neural crest cells.

CHED is another endothelial dystrophy that results in corneal opacification. Glaucoma is

infrequently encountered.^{42, 46} Significant overlap was demonstrated between CHED and PPMD. Furthermore, in a large British pedigree, CHED was linked to the 20q11 locus, the previously known PPMD locus.

SUMMARY

This review summarizes molecular genetic advances in the field of glaucoma. We chose to focus our discussion on the clinical relevance of these major genetic breakthroughs and explain why these significant discoveries have not yet had a meaningful impact on our clinical approach and management of glaucoma.

Why Are Genetic Tests Currently Not Part of the Clinician's Arsenal?

To translate genetic data to better patient care, it is not enough to establish loci for specific conditions. Several other important issues need to be addressed. First, it is crucial to isolate the actual gene and then to identify the common disease-causing mutations.

A second prerequisite is that clinical diagnosis upon initial presentation (or suspicion) is not straightforward. For example, although genes for Rieger's anomaly and congenital glaucoma have been identified, these conditions are often readily diagnosed in clinical practice. The same applies to aniridia. Furthermore, a mutation underlying pigment dispersion syndrome would hardly be as clinically meaningful as a genetic marker, which would predict which of the pigmentary dispersion subjects are at increased risk for future development of glaucomatous optic neuropathy.

Third, to be of help in clinical practice, the genetic data should be translatable into improved decision making in the management of individuals. Finally, this genetic data should ideally address the more common forms of glaucoma, namely POAG, PACG, pseudoexfoliation, and pigmentary glaucoma. With the present trend of cost-containment in clinical practice, data that can be inexpensively gathered and yet useful in allocating resources to individuals at high risk for developing glaucoma would be valuable in routine clinical practice.

Browsing the current list of known loci and genes associated with glaucoma (Table 1), the one that is likely to have the most significant

Table 1. LOCI (AND ACTUAL GENES, WHEN KNOWN) ASSOCIATED WITH GLAUCOMA

<p>1. Open-angle glaucoma GLC1A: 1q23-25: MYOC/TIGR gene. Myocilin/TIGR gene is composed of three exons, the olfactomedin domain harbors the structural mutations associated with glaucoma. The TIGR protein, an extracellular matrix protein, is found in the trabecular meshwork, ciliary body, retina, and sclera. It is thought that the gene product influences resistance in the uveoscleral outflow pathway. TIGR mutations encompass both juvenile and adult-onset glaucoma cases. GLC1B: 2cen-q13 is associated with normal tension glaucoma. GLC1C: 3q21-24 is associated with late onset, high-pressure glaucoma. GLC1D: 8q23 LGC1E: 10p15-14 is associated with normal tension glaucoma. GLC1F: 7q35-36</p> <p>2. Pigment dispersion syndrome and pigmentary glaucoma PDS1: 7q36 PDS2: 18q22</p> <p>3. Pseudoexfoliation syndrome and glaucoma PEX1: 2p A mitochondrial locus?</p> <p>4. Primary congenital glaucoma GLC3A: 2p21. CYP11B1 gene. This gene codes for cytochrome P45011B1. Null mutations were identified, implying that loss of function of this enzyme probably underlies this form of congenital glaucoma.</p>	<p>GLC3B: 1p36</p> <p>5. Anterior dysgenesis syndromes <i>Axenfeld-Rieger's anomaly:</i> 6p25. FKHL7 gene. This gene codes for a transcription factor containing a fork-head domain. <i>Iridogoniodysgenesis</i> IRID1: 6p25 (distinct from FKHL7, but in close proximity to it.³⁶). IRID2 (see below PITX2) <i>Rieger Syndrome</i> FKHL7/IRID2: 4q25. PITX2 (Solurshin) gene. This gene codes for a bicoid homeobox transcription factor. RIEG2: 13q14 PAX6: 11p13. Aniridia and other anterior segment developmental anomalies. This gene codes for a transcription factor regulating the expression of other genes during embryogenesis. Nearly all known mutations lead to loss of function of the protein. <i>Anterior segment mesenchymal dysgenesis:</i> 10q25. PITX3 gene.</p> <p>6. Nanophthalmos and primary closed angle glaucoma (NNO1): 11p.</p> <p>7. Nail-patella syndrome and glaucoma NPS: 9q34. LMX1B gene (LIM-homeodomain gene).</p> <p>8. Posterior polymorphous dystrophy/congenital hereditary endothelial dystrophy 20q11</p>
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impact on clinical practice is the TIGR-MYOC gene. Recently, 3% to 4% of unselected POAG patients were found to have one of several mutations in coding regions of the gene.² A more extensive epidemiologic study, looking at racial variations, failed to show populations in which the overall rate was significantly higher, even though each specific mutation did show considerable prevalence variation in different populations around the world.²² Whether a prevalence rate of 3% to 4% justifies large-scale screening is debatable and should be best determined through a cost-benefit analysis; however, because TIGR-MYOC seems to us only marginally beneficial for diagnosis and prognosis purposes, we must conclude that genetic tests for glaucoma are justifiably not yet part of a clinician's arsenal.

Thirty-five years ago in his 1965 Jackson Memorial Lecture, Shaffer suggested a classification shift from phenotypic to genotypic emphasis. The recent advances in molecular genetics leave us optimistic that this will indeed occur in the next decade or two.

Existing data on the genetics of glaucoma suggest that the actual number of glaucoma-associated genes is much greater than the

number of genes currently known. This is analogous to the situation with retinitis pigmentosa, in which more than 22 genes have already been implicated in the phenotype. As more glaucoma-related genes are discovered, a better understanding of the pathophysiology of the disease on the molecular level can be expected. This, in turn, may lead to better modes of therapy and even, perhaps, glaucoma prevention.

Glossary

Nomenclature

11p13: the convention for documenting a certain location in the genome. In this case, chromosome "11"; the short arm ("p" for petit, as opposed to "q" for the longer arm); the "13th" segment, as determined by a banding pattern along the stained chromosome.

GLC1E: the convention for naming new loci associated with a disease. In this case "GLC" stands for glaucoma, "1" for open angle ("2" is for closed angle and "3" stands for congenital glaucoma); "E" stands for the fifth loci discovered, regardless of its location or importance. Hence, by convention, the first angle-closure glaucoma locus to be discovered will be named **GLC2A**.

Glossary

Allele: any one of a series of two or more different genes that occupy the same locus on a specific chromosome. The way autosomal chromosomes are paired, each autosomal gene is represented twice in normal somatic cells. If the same allele appears in each of the two paired chromosomes, the individual, or cell, is homozygous for this allele. If the alleles are different, the individual, or cell, is heterozygous for both alleles. With the advent of DNA technology, the allele has become the focus of intense scrutiny, as molecular biologists attempt to track down genes responsible for physical and behavioral traits. Alleles may serve as probes that allow for the identification of such genes.

Allelic Heterogeneity: when several sequence variants of the gene exist.

Centimorgan (cM): a unit used in linkage studies, signifying an interval of about 1 million base pairs along the DNA molecule, with a mean content of 20 to 50 genes. For each 1 cM interval along the chromosome, there is about a 1% chance of recombination occurring during meiosis.

Gene: a functional unit of heredity that occupies a specific place (locus) on a chromosome and directs the formation of a protein. Genes normally occur in pairs in all cells except gametes, as a consequence of the fact that all chromosomes are paired (except the sex chromosomes, X and Y, of the male).

Genetic Heterogeneity: when several different genotypes result in an identical (or similar) phenotype, clinically indistinguishable.

Genotype: the genetic constitution of an individual.

Human Genome: coded on 23 pairs of chromosomes, contains approximately 100,000 genes, and a total of about 3.3 billion base pairs.

Linkage Analysis: a method used to determine the location, within the human genome, of a gene responsible for a particular disease trait. Linkage implies that two alleles in near proximity to each other are inherited together, so that one is not present without the other in a particular pedigree. Linkage can ascertain the approximate location of an unknown gene by assessing its proximity to various markers whose position along the chromosome is known. The classical concern is with estimating recombination fractions (see LOD score). Currently, about five affected living family members are usually required to undertake a genetic analysis to identify a disease-associated haplotype¹³; however, larger families, with 11 or more affected, or at risk, individuals are much more useful for linkage studies.³¹

Locus: the actual physical position that a gene occupies on a chromosome.

LOD Score: a measure that provides information on the likelihood of linkage, which is an estimate of how close two DNA sequences are, along the chromosome. It actually represents the probability of linkage versus no linkage. A LOD score

above three (odds being greater than 1000:1) is usually the statistical proof of linkage between an unknown phenotype and a known genetic marker, whereas a LOD score below -2 rules out linkage with the marker. Mathematically it is the \log_{10} of the likelihood ratio of a marker being linked versus being nonlinked to the phenotype. A high LOD score (>3) implies that the chosen genetic marker is in the direct vicinity of the unknown DNA location of the studied phenotype (disease).

Markers: DNA sequences that are known to be present along the DNA molecule and can serve as landmarks along an otherwise unfamiliar DNA sequence. Frequently, short tandem repeat polymorphisms are used as reliable markers.

Mendelian Inheritance: the case when a single mutation underlies the disease phenotype. Thus, stable characters controlled entirely or overwhelmingly by a single genetic locus are transmitted over many generations. Mendelian inheritance can be autosomal versus X-linked, and dominant versus recessive.

Mutation: a change in the sequence of base pairs in the chromosomal DNA molecule.

PCR (Polymerase Chain Reaction): an enzymatic laboratory method for repeated copying and amplification of a particular DNA sequence. The laboratory technique known as PCR exploits the capacity of DNA polymerase to assemble new DNA. The polymerase is added to a mixture of free nucleotides and primers. Two primers flank the short sequence of DNA to be amplified. Once the reaction begins, the polymerase churns out multiple copies of the target sequence, which can then be recovered for analysis. Kary B. Mullis was awarded a Nobel Prize for the discovery of PCR, first reported in 1985. The impact of PCR on scientific progress in molecular biology is often compared to the impact of Watson and Crick's "double helix".

Phenotype: the actual clinical manifestation of a trait. Phenotype is the result of one or more genes, possibly influenced by environmental factors.

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