Recent advances in molecular genetics of glaucomas

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Glaucomas are a heterogeneous group of eye conditions with manifestation from as early as birth to very late age of onset in life. The primary type of these conditions affecting children and juveniles are less frequent, but the prevalence of glaucomas affecting older people of \( \geq 70 \) years progressively rises to \( \sim 5\% \). The molecular genetics of three types of glaucoma have been the subject of investigation in the last few years. As a result, two loci (GLC3A and GLC3B) have been identified for primary congenital glaucoma, one locus (GLC1A) for juvenile-onset primary open angle glaucoma and a further two loci (GLC1B and GLC1C) for late-onset chronic open angle glaucoma. Early this year, the first set of mutations was described in the CYP1B1 (Cytochrome P4501B1) and TIGR (Trabecular meshwork Inducible Glucocorticoid Response Protein) genes for the GLC3A and GLC1A-linked families, respectively. The mapping of different types of glaucoma and mutation identification in these two genes are the focus of this review.

INTRODUCTION

Glaucomas are a group of optic neuropathies that, if untreated, can result in total blindness. The condition is classified according to the etiology (primary versus secondary), anatomy of the anterior chamber (open angle versus closed angle) and time of onset (infantile versus juvenile versus adult) (1). However, a more precise classification of this condition may only be possible when all the etiological factors, including primary defective molecules and other contributing risk factors, are identified.

Different forms of glaucoma share some common clinical manifestations that usually include specific abnormal appearance of the optic nerve head, characteristic loss of the visual field and chronic painless progression. Furthermore, the condition frequently is associated with increased intraocular pressure (IOP), but this elevation is neither necessary nor sufficient for onset or progression of the disease (1).

The manifestation of this group of eye conditions could start at birth or may appear after the age of 80, depending on the type of glaucoma present in an individual. The pediatric form of glaucoma (Buphthalmos) usually occurs at birth and up to the age of three, while juvenile-onset glaucoma may appear somewhere between the ages of 3 and 30 (2–5). The late-onset form of this condition rarely starts before the age of 40 and is the most prevalent type observed in an everyday glaucoma clinic (6–11). The pediatric and juvenile types of glaucoma are generally rare conditions and, while the incidence of the primary congenital type varies between 1 in 1250 (12) and 1 in 10,000 (13), no comparable estimates are available for the juvenile-onset type of glaucoma. Although a large number of affected subjects have no previous family history (14), a significant proportion show a clear familial aggregation with multiply affected subjects in their respective pedigrees (15–21). Whereas the main mode of inheritance for primary congenital glaucoma is autosomal recessive (12,13,22), for juvenile and adult-onset glaucoma the more frequently reported mode of inheritance is autosomal dominant with reduced penetrance (23–26). A significant proportion of other ocular conditions associated with glaucoma are also inherited as autosomal dominant traits (27–31).

In this article, the molecular genetics of different types of glaucoma will be reviewed in order to provide some insight into the molecular etiology of two well studied types of glaucoma, namely, the juvenile-onset primary open angle glaucoma and the pediatric form of primary congenital glaucoma. A brief summary on the current status of other types of ocular conditions associated with glaucoma will also be presented.

PRIMARY OPEN ANGLE GLAUCOMA (POAG)

This is the most common form of this group of eye conditions, usually accompanied with variable severity and phenotypic expressivity (1,9). The clinical manifestation is further complicated with IOP measurements that could vary from 10 to \( >50 \) mm Hg. POAG have been arbitrarily divided into two groups of juvenile and adult with an overlapping clinical presentation and a sliding scale age of onset and IOP values (1). Although there are some differences between the rare form of juvenile-onset open angle glaucoma (JOAG) as compared with the more frequent form of adult-onset chronic open angle glaucoma (COAG), the clinical diagnosis of both groups are based on the presentation of...
visual field loss, glaucomatous changes of the optic nerve and optic nerve damage that is usually accompanied with an increased IOP (9). Apart from the clear differences in the age of diagnosis in these two groups of POAG, the condition in juvenile subjects is more severe, presenting with significantly higher IOPs (i.e., >40–50 mm Hg) that usually does not respond to drug treatment, and, therefore, lowering of the IOPs through multiple surgical interventions is a necessity (3–5). In contrast, the late-onset form has a quite different phenotype, usually with a milder presentation, progressive development, moderate elevation of IOP and medical treatments often yield a satisfactory outcome (6, 7, 32–34). The painless progression often leads to a late diagnosis, when irreversible damage to the optic nerve has already occurred, thus complicating the prognosis of this type of POAG.

Although there is some controversy about the exact mode of inheritance in these two groups of eye condition, pedigree structure of the majority of families used in genetic linkage analysis clearly suggest that inheritance is autosomal dominant with an incomplete penetrance.

**JUVENILE-ONSET PRIMARY OPEN ANGLE GLAUCOMA (JOAG)**

A new page in the molecular genetic study of JOAG was opened in early 1993, when using a single large American family, Sheffield et al. (35) localized the first locus for this type of glaucoma. The locus was mapped to the 1q21–q31 region and named GLC1A (note: use of GLC1 symbol for all types of POAG has been approved by the HUGO Nomenclature Committee; the letters A, B, C, etc. will be assigned to each newly identified locus). After this initial report, the efforts of other investigators from the US and Europe were focused on testing additional glaucoma families from different genetic backgrounds. Confirmation of this initial linkage was soon followed in another American JOAG family (36), families with Irish, British and German backgrounds (37), two French families (38) and one large Danish family (39).

Morissette et al. (40) reported linkage in a multigeneration French-Canadian family with 142 members of whom 40 were affected. Based on the age of detection, the authors divided the glaucoma patients into two groups of JOAG and COAG. In this study, 36 subjects were diagnosed between the ages of 25 and 35 and, therefore, classified as having JOAG, while four subjects were diagnosed after the age of 40 and considered as COAG. Six other members were diagnosed with ocular hypertension and several other asymptomatic obligate carriers were also identified. The authors concluded that the GLC1A locus is responsible for both juvenile- and adult-onset POAG. A second family also presented with variable age of onset of POAG linked to the GLC1A locus (41). Twenty family members developed glaucoma between 11 and 51 years of age (median 36). A 35 year old healthy female had a severely affected daughter and therefore classified as a case of incomplete penetrance. Nine more normal family members aged from 14 to 66 had also inherited the affected haplotype.

Clinical features in the affected individuals from all of the families reported so far were very similar and conformed to the typical form of juvenile-onset primary open angle glaucoma. Despite the existing variability in the exact age of detection, the majority of the patients were diagnosed with glaucoma in childhood to early adulthood (average 18 years). The IOP was typically very high, often in the range of 40–50 mm Hg. Medical treatment was initially effective, but surgery was required to control the progress of glaucoma.

### Table 1. Chromosomal location for different types of primary glaucoma and other associated ocular conditions

<table>
<thead>
<tr>
<th>Glaucoma type</th>
<th>Locus</th>
<th>Location</th>
<th>Inheritance</th>
<th>Severity</th>
<th>Mutated gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary open angle glaucoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile-onset (JOAG)</td>
<td>GLC1A</td>
<td>1q23–q25</td>
<td>AD</td>
<td>Severe</td>
<td>TIGR</td>
</tr>
<tr>
<td>Adult-onset chronic (COAG)</td>
<td>GLC1B</td>
<td>2cen–q13</td>
<td>AD</td>
<td>mild/mod</td>
<td>unknown</td>
</tr>
<tr>
<td>Adult-onset chronic (COAG)</td>
<td>GLC1C</td>
<td>3q21–q24</td>
<td>AD</td>
<td>mild/mod</td>
<td>unknown</td>
</tr>
<tr>
<td>Primary infantile/congenital glaucoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Buphthalmos)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary congenital glaucoma</td>
<td>GLC3A</td>
<td>2p21</td>
<td>AR</td>
<td>Severe</td>
<td>CYP1B1</td>
</tr>
<tr>
<td>Primary congenital glaucoma</td>
<td>GLC3B</td>
<td>1p36</td>
<td>AR</td>
<td>Severe</td>
<td>unknown</td>
</tr>
<tr>
<td>Developmental anomalies associated with glaucoma</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Rieger syndrome</td>
<td>RIEG 1</td>
<td>4q25</td>
<td>AD</td>
<td>–</td>
<td>RIEG1</td>
</tr>
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<td>13q14</td>
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<tr>
<td>Iridodysgenesis anomaly</td>
<td>IGDA</td>
<td>6p25</td>
<td>AD</td>
<td>–</td>
<td>unknown</td>
</tr>
<tr>
<td>Pigment dispersion syndrome</td>
<td>PDS</td>
<td>7q35–q36</td>
<td>AD</td>
<td>–</td>
<td>unknown</td>
</tr>
</tbody>
</table>

*AD, autosomal dominant; AR, autosomal recessive.  
*mod, moderate.
Figure 1. Mutations in coding regions of the TIGR and CYP1B1 genes. The translated regions in both genes are lightly shaded. Frameshift mutations that are predicted to truncate the open reading frames of these two genes are shown with dark up-arrows below the exons. Missense mutations are indicated with contour down-arrows above the exons. The region of the CYP1B1 gene affected by a large deletion is shown by a bi-directional arrow below the exon line. The missense mutation indicated by an asterisk in exon III of TIGR is one we have recently identified in one JOAG family. The darkly shaded areas in the CYP1B1 gene as presented in 5′→3′ direction are the hinge, K-helix, meander and hem-binding regions, respectively. Note that all the missense mutations identified in our study are in these regions.

Genetic heterogeneity of GLC1A

Evidence for genetic heterogeneity of this condition has also been reported (39,42,43). Graff et al. (39) excluded linkage in a large Swedish family with JOAG and iris hypoplasia. The affected members of this family developed glaucoma before the age of 25 and maintained IOP pressure without treatment in the 28–50 mm Hg range. This may possibly prove to be linked to the 4q25 or 6p25 regions, where other families with both glaucoma and iris hypoplasia have been mapped. In two JOAG families unlinked to the 1q21–q31 region, some differences in the clinical presentation of glaucoma were noted (42). For the first family the age of onset and severe optic nerve damage was before the age of 10, but the IOP was mildly elevated (25–30 mm Hg range). The second family was of African American descent. Further evidence for genetic heterogeneity of this condition has also been reported for French JOAG families (43).

Physical mapping and refinement of the GLC1A locus

To further refine the mapping of the GLC1A region, Sunden et al. (44) analyzed new members from the family used in the original linkage study together with an additional large pedigree also segregating with the JOAG phenotype. Radiation hybrid mapping and construction of a YAC contig indicated that the candidate region is <5 Mb and flanked by DNA markers of D1S3665 and D1S3664. These authors identified four genes (SELE, SELL, TXGP1 and APT1LG1) within and another three genes (LAMC1, NPR1 and CNR2) outside of the critical region. A similar contig map of the region has also been reported by another group (45). A more comprehensive physical map of GLC1A has recently been published (46). These researchers narrowed down the GLC1A critical region to ~2.8 Mb cytogenetically located at band 1q24 with an unusually low gene content. These investigators also completed a 4.7 Mb YAC contig of the surrounding GLC1A region and further excluded a total of 15 important candidate genes from the GLC1A susceptibility region. Construction of a PAC contig from the GLC1A region spanning ~5 Mb between markers D1S196 and D1S242 has also been reported recently (47).

Mutation studies in the GLC1A-linked families

Recently, three different mutations in the TIGR (Trabecular meshwork Inducible Glucocorticoid Response Protein) gene have been described (Fig. 1A) in a group of 10 familial and three isolated cases with POAG, as well as in one normal subject (48). Of the 10 familial cases with mutations, at least one has been indicated to be of the adult-onset type. A predominantly reported mutation is the Gln368STOP observed in six familial, three sporadic and one normal healthy subject. The mutation truncates the TIGR protein by 136 amino acids prematurely (note that due to an earlier error in the cDNA sequence of this gene, the amino acid numeration has now changed). Identification of these mutations has opened up a new possibility that the TIGR gene may not only be involved in the juvenile-onset type of POAG but also in a certain portion of other adult-onset POAG cases as well.

The TIGR gene was originally identified and isolated by induction of primary cultured cells of trabecular meshwork (TM) tissue with glucocorticoids (DDBJ/EMBL/GenBank accession no. U85257). The responding protein was named TIGR and subsequently shown to be present not only in the TM but also in the ciliary body, the two major tissues that are involved in the production and regulation of aqueous humor which normally nourishes ocular tissues and controls the intraocular pressures (49). This protein has also been independently isolated (DDBJ/EMBL/GenBank accession no. AF001620), cloned (named CBS-670) and characterized from a human ciliary body library (50). These authors showed that, in the ocular tissues, the TIGR
transcript is abundantly present in iris and ciliary body, but in low or undetectable levels in cornea, lens and retinal pigment epithelium. In the non-ocular tissues, the transcript preferentially hybridizes to heart and skeletal muscle RNA, but is undetectable in brain, placenta, lung, liver, kidney and pancreas. Therefore, it was suggested that TIGR is restricted preferentially to muscle in ocular and non-ocular tissues (50).

The TIGR gene encodes for a polypeptide that is 504 amino acids long. The gene consists of three exons (604, 126 and 782 bp, respectively) that are separated by two large introns (Table 2 and Fig. 1 A). The TIGR protein consists of a N-terminus hydrophobic region (i.e., cleavage site) at position 32 (VGA\textsubscript{RT}), a myosin-like domain (residues 63–221), a leucine zipper-like motif at every seven positions (residues 117–166), five arginine residues at every 11 positions (residues 125–169) and an olfactomedin (OLFM)-like domain at the C-terminus (50). The leucine-rich region contain an \( \alpha \)-helical conformation and on its opposite sites, five arginine residues form a positively charged domain (Fig. 2) suggesting that these leucine and arginine repeats may be involved in protein–protein interactions (50). At the C-terminus part of this protein, there is a domain (residues 246–501) with 40\% identity with olfactomedin (Fig. 2), a major component of the extracellular matrix of the olfactory neuroepithelium. Interestingly, this region contains all the known TIGR mutations (48) and has been suggested as possibly being involved in the cell binding activity of this protein (49). Therefore, mutations in this region may interfere with uptake or metabolism of the protein leading to its accumulation, obstruction of aqueous outflow and increased IOPs. This abnormal IOP regulation and control would eventually result in damage of the vulnerable optic nerve tissues, thus leading to glaucoma and possible blindness.

Recently, molecular cloning, tissue expression and chromosomal mapping of a new novel myosin-like protein named Myocilin (MYOC; DDBJ/EMBL/GenBank accession no. D88214) that maps to the 1q23–q24 region has been described (51). The nucleotide sequence of the human myocilin cDNA is identical to that of the TIGR gene with only one exception. The difference is in the presence of an additional 'GA' at position 107–108 of the MYOC gene that has shifted the initiation codon of 'ATG' to position 109. The TIGR initiation codon is located at position 65 of the MYOC gene. It has been recently confirmed that these two genes are identical (N. Shimizu, personal communication).
The Myocilin gene was cloned from the human retina cDNA library and shown to encode an acidic protein (isolectric point 5.2) that is localized preferentially in the ciliary rootlet and basal body of the connecting cilium of photoreceptor cells (51). These authors suggest that this is a new cytoskeletal protein involved in the morphogenesis of ciliated neuroepithelium such as photoreceptor cells. Northern blot analysis with a cDNA clone corresponding to nt 277–1217 (i.e., a.a. 57–370) of the MYOC gene (corresponding to TIGR a.a. 71–384) showed only a single mRNA of 2.3 kb, predominantly in the human retina and mildly in skeletal muscle. However, no signals were detected in other tissues including heart, brain, placenta, lung, liver, kidney and pancreas. These data indicate that this gene is predominantly expressed in only limited types of highly differentiated tissues including retina and perhaps muscle. These authors also showed that Myocilin is actively produced in the ellipsoid of the rod inner segment where most protein synthesis takes place, and it is apparently transported to the basal apparatus of the connecting cilium. They further speculated that the hydrophobic regions at the N-terminus may act as glue connecting the microtubules or, alternatively function as a connector between the nucleus and the basal body. Based on the characteristic nature of this gene, the authors also suggested that myocilin may be a candidate gene for an inherited retinal disease such as Usher syndrome (51). However, the precise localization of the MYOC in the rootlet and basal body of connecting cilium and specific tissue expression of this protein should provide further understanding of the role and function of this gene (or TIGR) in patients affected with POAG.

In summary, the gene that is mutated in patients with POAG has been independently cloned and isolated from trabecular meshwork (49), ciliary body (50) and retina (51).

**Recent developments**

A number of new mutations in the TIGR gene were presented recently (Annual Meeting of ARVO, The Association for Research in Vision and Ophthalmology, 1997). A total of 14 mutations in exon III and a number of new polymorphisms in exon I and/or 3' end of this gene were reported (52). Additional mutations were also identified by other groups in JOAG families from Italy (53), Germany (54), France (55) and Canada (French-Canadian; 56–57). We have also identified a mutation in one family from Edinburgh, Scotland (58). The only deletion of 3 bp in exon III was reported in four families from a small southern Italian village where all individuals carry a common haplotype. This mutation most likely resulted from a founder effect with a common ancestral mutation (53). However, most of the reported sequence changes in the TIGR gene are missense mutations that are clustered in exon III.

One other interesting patient has been described who is hemizygous for the GLC1A region (59). This patient is 24 years of age and has no clinical evidence of glaucoma. The deletion of DNA markers in this subject included a 10 cM interval that contained the GLC1A locus. Therefore, it was indicated that the loss of function of one of the copies of the GLC1A gene (i.e., haploinsufficiency) in this patient would not be the cause of this type of glaucoma. However, it is still likely that this patient will develop glaucoma at a later stage of life and that the haploinsufficiency detected in this subject may still be adequate to cause the phenotype, a phenomena that has recently been reported for the chromosome 4q-linked Rieger syndrome (60).

Another interesting piece of evidence is presented in a large French-Canadian pedigree that has previously been shown to be segregating for the GLC1A locus in which four subjects born to two affected parents inherited two copies of the affected GLC1A haplotype from their parents, but all were clinically normal for glaucoma (56). These patients were aged between 41 and 49 and they have already produced two clinically affected offspring who have inherited only a single affected haplotype. This observation indicated that heteroallelic complementation of the affected haplotypes is a likely mechanism to account for this phenotypically normal homozygous affected carrying haplotype subjects. If these patients do not share the same TIGR mutation with other branches of the pedigree, a separate mutation in another part of the genome or mutation in one of the closely located genes within the GLC1A critical region could be responsible for this observation. This is likely as in the same pedigree, another two affected subjects (one with low-tension and another with angle-closure glaucoma) were described (57) neither of whom carry the common affected haplotype. Therefore, this may indicate that for such a large pedigree, one should expect to see phenocopies for the glaucoma phenotype, perhaps more than once.

**ADULT-ONSET CHRONIC OPEN ANGLE GLAUCOMA (COAG)**

The adult-onset chronic open angle glaucoma (COAG) has the highest prevalence/incidence in Western societies (10). In these patients angle of the anterior chamber is normal and the optic nerve undergoes a characteristic atrophy that results in visual field loss and eventual blindness (9). Some patients have elevated IOP, but other confirmed open angle glaucoma patients have a 'screening' IOP within the statistically normal range (7, 61–64). For this form of glaucoma, the onset is usually after the age of 40, but the majority of sufferers exhibit the disease even at later stages of life, usually after the ages of 50 or 60. This has had a serious implication for diagnosis and proper treatment. As a consequence, it has been difficult to determine the exact mode of inheritance for this type of glaucoma, as the majority of these patients are either isolated cases, or by the time of first presentation, their parents are deceased and, therefore, no accurate clinical data or systematic ophthalmic information is available. Therefore, the disease is often diagnosed after a definite visual field defect has developed.
available from previous generations. Therefore, autosomal domin-
ant, autosomal recessive, X-linked and multifactorial modes of inheritance have been suggested for this condition (16,19,20,65,66). However, in the majority of families that have been systematically studied, the autosomal dominant mode of inheritance with reduced penetrance has been suggested (15,65,67–70).

The first COAG locus (GLC1B) maps to the 2cen–q13 region

Last year, our group presented clinical, two-point linkage and haplotype transmission data that assigned the first locus (GLC1B) for adult-onset primary open angle glaucoma to the 2cen–q13 region (67). Six families previously excluded from the GLC1A locus (and 215 other DNA markers) were found to be linked to a group of STRP markers located on 2p11–2q13. The highest lod score was obtained with marker D2S113 (Z = 6.48). Four critical cross overs in affected individuals placed the GLC1B locus to a 11.2 cM region that is flanked by D2S2161 and D2S176. Recently, we have extended this initial linkage data and identified another 12 families that segregated with this region of chromosome 2 by utilizing a combination of two-point LOD score, analysis of affected meioses only, haplotype construction and transmission data, APM analysis (affected pedigree members method of linkage analysis that requires no prior mode of inheritance), and multipoint linkage segregation. Overwhelming evidence of linkage with LOD scores of 12.97 (D2S274) and 12.13 (D2S113) were obtained in these families. When only the affected meioses of these families were used, the corresponding LOD scores of 6.42 and 7.00 were obtained respectively for these two markers. However, the newly generated linkage data did not help to reduce the critical region of the GLC1B locus any further.

The phenotype of glaucoma patients linked to 2cen–q13 is generally less severe compared with GLC1A-linked families. The mean age of onset/diagnosis in these families was 53 years. Of the affected subjects in these families, 33% had intraocular pressures of <22 mm Hg, and the remaining 67% had moderate to high pressures that varied between 22 and 40 mm Hg. With the exception of one family that showed very high IOP values, the remaining families had IOPs of 22–34 mm Hg suggesting that the GLC1B locus on chromosome 2q is responsible for families with moderate tension COAG. Furthermore, other large families with IOP values constantly <22 mm Hg (i.e., classical low-tension glaucoma) did not segregate with this region of chromosome 2, suggesting that IOP values are not a significant pre-determinant factor for linkage of other COAG families to the GLC1B locus. Of the 52 affected subjects in the GLC1B-linked families, a total of 22 (42%) had ocular surgery to control their pressures.

We have also excluded another 22 COAG families that do not show any linkage to the 2cen–q13 region and, therefore, provide evidence for genetic heterogeneity of this group of conditions. The families linked to the GLC1B locus may represent a distinct entity with further genetic heterogeneity.

The second COAG locus (GLC1C) maps to the 3q21–q24 region

The second locus (GLC1C) for adult-onset COAG has recently been described in a single American family that maps to the 3q21–24 region (68). The GLC1C locus is flanked by two DNA markers of D3S3637 and D3S1744, within a region of ∼11.1 cM. Nine living affecteds, seven possible affecteds and another four healthy members of this pedigree share a common affected-bearing haplotype. Another nine offspring of affected subjects who showed no sign of the COAG disease also inherited a normal haplotype from their respective parents. A maximum LOD score of 3.02 was reported with D3S1535 for the full family, with a corresponding value of 2.12 when only the affected subjects were included in the linkage analysis. The GLC1C region has recently been narrowed to 9.6 cM that is flanked by D3S3637 and D3S1550. The patients in this study presented with a phenotype characteristic of the common form of glaucoma including onset after the age of 35, high intraocular pressures, compromised disc-to-cup ratio and vision loss. Three of the ten affected individuals (30%) have had to resort to surgery to control their pressures (M. Wirtz, personal communication).

The clinical presentation of affected subjects segregating for GLC1B or GLC1C loci are very similar. The average age of onset in the affected subjects are 53 and 55 for GLC1B and GLC1C, respectively. Ocular surgery was required in 42% of GLC1B-linked patients compared with 30% of the GLC1C-linked affected subjects. However, the IOP values presented for the GLC1C-linked affected subjects are not directly comparable with those reported for the GLC1B-linked families, as these values (i.e., 18–32 mm Hg) are presented for individuals after the medical treatment initiated and/or diagnosis was made. There are no other differences between the clinical presentation of the GLC1B and GLC1C-linked affected subjects.

Both the GLC1B and GLC1C loci still remain to be confirmed by other investigators. However, at least two other families with adult-onset POAG have already been excluded (69,70) from the GLC1A region, the site for the juvenile-onset POAG.

Therefore, genetic linkage studies of different types of COAG have clearly indicated that this group of glaucomas are heterogeneous and a very large number of loci are expected to be involved in the etiology. What has already been observed for Retinitis Pigmentosa (RP) in which more than 30 loci identified in the syndromic and non-syndromic RP types (71), may also be the case for glaucoma phenotypes.

**PRIMARY CONGENITAL GLAUCOMA (PCG) OR BUPHTHALMOS**

Primary congenital or infantile glaucoma (gene symbol, GLC3) is a specific inherited eye disorder that manifests itself in early childhood, usually within the first year of life, but may emerge up to the age of 3 (2,12,13). The incidence is 1:1250 in the Gypsy population of Slovakia (12), 1:2500 in the Middle Eastern (72) and between 1:5000 and 1:10 000 in the Western countries (13). Although the familial forms of this condition have an autosomal recessive mode of inheritance, some apparent vertical transmission in some families may be explained by pseudo-dominance (73). Association of congenital glaucoma with chromosomal abnormalities of at least 17 different autosomes has also been reported in the literature (74). Glaucoma in these instances is accompanied by dysmorphic features, multi-system abnormalities, developmental delay and abnormal results of cytogenetic studies. Therefore, it is likely that the nature of glaucoma in these subjects is secondary or the observed association of congenital glaucoma with these chromosomal abnormalities is coincidental. Furthermore, genetic linkage study of a group of highly
informative DNA markers selected from the reported regions with chromosomal abnormalities did not show any evidence for existence of a site in a group of families segregating for primary congenital glaucoma (22,75).

The first locus for PCG (GLC3A) maps to the 2p21 region

In 1995, we used a group of 17 PCG families with multiply affected subjects and mapped the first locus (named GLC3A) for this condition to the 2p21 region (22). Eleven families showed no recombination with three tightly linked markers of D2S177, D2S1346 and D2S1348 with a combined haplotype LOD score of 11.50. Inspection of haplotype and heterogeneity analysis confirmed that six other families were not linked to the 2p21 region, thus providing the first proof of genetic heterogeneity for this phenotype. Initial mapping concluded that the disease gene is between D2S1325 and D2S1356 (a region of ∼8 cm), but subsequent recombinational events together with conservation of the smallest segment of homozygosity in the consanguineous families suggested that the GLC3A locus is flanked by D2S2186 and D2S1346, within a genetic distance of ∼2.5 cM (73).

The localization of the GLC3A locus to the 2p21 region has been confirmed in a group of 25 Saudi Arabian families (76) and in the Gypsy population of Slovakia (77). We also have evidence for linkage of families from other geographical populations (unpublished). Therefore, confirmation of linkage in different ethnic populations has now verified our original speculation that the GLC3A site on the 2p21 region is a major locus for primary congenital glaucoma, perhaps accounting for 85–90% of all familial cases.

Causative mutations in cytochrome P4501B1 in the GLC3A-linked families

Heritable mutations in cytochrome P4501B1 (CYP1B1) in five GLC3A-linked families have been identified (73). One consanguineous and one non-consanguineous family showed a 13 bp homozgyous deletion that removed nucleotides 1410–1422 (in exon III) of the CYP1B1 gene. This mutation resulted in a frameshift that truncated the open reading frame (ORF) by creating a premature stop codon (TGA), 203 bp downstream from this deletion, thus resulting in a product that lacks 189 amino acids from the C-terminus. One of these two families had an apparent pseudo-dominant inheritance. Similarly, a single base homozygous cytosine insertion in a stretch of six cytosines at nucleotide positions 1209–1214 (in exon II) in another two consanguineous families has created a premature stop codon, 106 bp downstream from the site of this insertion, thus resulting in a truncated product that lacks a total of 254 amino acids. The third homozygous mutation observed in a consanguineous family was a large deletion affecting intron II and the 5′ end of exon III. As the 3′ splice acceptor site of intron II has been deleted, this mutation is expected to interfere with the normal splicing of the CYP1B1 gene, resulting in synthesis of either truncated protein, or null allele (Fig. 1B).

The CYP1B1 genomic region spans >12 kb and has three exons (78). Its ORF consists of 1629 bp, organized in exons II and III that encodes for a protein of 543 amino acids (Fig. 1B). At the C-terminus of this protein, the conserved structural core domains are shared by all the cytochrome P450 molecules. Among them is the haem-binding region which is easily identified by the presence of invariant cysteine residue (i.e., C-470) which provides the axial haem ligand (79). The truncating mutations described above are expected to eliminate this essential region and, therefore, most probably result in functional null alleles. Subsequent analysis of the translated regions of the CYP1B1 gene in 14 more PCG families (unpublished) resulted in the identification of 11 new mutations (Fig. 1B). Five of them are predicted to truncate the ORF by introducing frameshifts and creating premature stop codons that eliminate the haem-binding region. The remaining six are missence mutations, exclusively identified in the conserved regions of this gene (Fig. 1B).

In summary, we have identified 14 different mutations in 19 familial and sporadic cases from three different populations: Turkish, French-Canadian and Pakistani (73; unpublished). So far, no predominant mutation has been identified. Only one of these mutations has been observed in four unrelated Turkish families. Therefore, it seems that a significant CYP1B1 allelic heterogeneity must exist in the affected patients with this condition. However, since the majority of these mutations are clustered in the 5′ end of exon III, the task of mutation screening in the general population for detection of gene carriers will be a permissible exercise.

CYP1B1 is expressed in at least 15 non-ocular normal human tissues (79). This gene is also expressed in the trabecular meshwork, ciliary body, iris and retina (unpublished). CYP1B1 belongs to a group of P450 superfamily multigene expression that consists of over 300 members. These are monomeric mixed-function monooxygenase genes that are responsible for the phase 1 metabolism of a wide range of structurally diverse substrates. The P450s usually insert one atom of atmospheric oxygen into the target substrate molecule, thereby creating a new functional group (e.g., -OH, -NH₂, COOH). Therefore, it is likely that these drug-metabolizing enzymes are responsible for controlling the steady-state levels of small bio-organic oxygenated molecules that act as ligands in the receptor-mediated signal transduction pathways (80–81). In this case it is reasonable to expect that CYP1B1 participates in the metabolism of a molecule important for the normal development and function of the anterior eye segment. Steroids and derivatives of the arachidonic acid are one of the possible targets. Recently, it has been shown that CYP1B1 hydroxylases the 17β-estradiol at position 4 (82). Estradiol receptors have also been found in the eye (83). On the other hand, Schwartzman et al. (84–85) implicated a cytochrome P450-dependent arachidionate metabolite that inhibits Na⁺, K⁺-ATPase in the cornea and regulates corneal transparency and aqueous humor secretion. This finding is consistent with the clouding of the cornea and increased intraocular pressure, the two major diagnostic criteria for primary congenital glaucoma.

The second locus for PCG (GLC3B) maps to the 1p36 region

Positional mapping of eight families unrelated to the GLC3A locus has revealed linkage to a number of DNA markers that are located at the 1p36 region (86). Only four of these families were linked to this region of chromosome 1, indicating that at least one more locus for this condition must exist in the genome. The two markers of DIS2834 and DIS402 showed no recombination in the linked families and provided the maximum LOD score values of 4.510 and 4.157, respectively. This locus (GLC3B) is confined within a 3 cM interval that is flanked by two groups of tightly
linked markers of (D1S597/D1S489/D1S228) and (D1S1176/D1S507/ D1S407).

The 1p36 region has been subjected to an intensive investigation and cloning bias as many tumour suppressor genes are known to be located in this region (87). This part of genome has also come to attention because it contains a high concentration of G+C rich DNA suggesting an accumulation of genes in this portion of chromosome. A large number of genes have been reported in the 1p36.2–36.1 band, but none of these can be directly attributed as a possible cause of congenital glaucoma. Moreover, the 1p36 region has frequently been involved in chromosomal aberrations that result in various malignancies, but none of these abnormalities has been reported to segregate with the congenital glaucoma phenotype. The linkage study of the GLC3B-linked families established a candidate region of ~3 cM that extends from D1S228 to D1S507. However, as the neighboring region of D1S228 has been indicated to be a hot spot for recombination (88), the critical interval of GLC3B is expected to be considerably less than 3 cM (89).

**GLAUCOMA AND OTHER DEVELOPMENTAL ANOMALIES**

A number of ocular conditions that are generally postulated to be the result of an abnormal differentiation of neural crest cells are also reported to be associated with glaucomas. Usually these conditions exhibit variable expressivity with high degree of penetrance among the members of the families studied.

A number of these conditions have already been mapped. One form of Rieger syndrome (RS1) together with an autosomal dominant form of iris hypoplasia that is associated with early onset glaucoma have been mapped to the 4q25 region (27–28). RS1 has been shown to be the result of mutations in a homeobox transcription factor, the RIEG gene (90). A second locus for Rieger syndrome (RS2) has recently been mapped to the 13q14 region (30). Although both RS1 and RS2 share common malformation of eyes and teeth together with other clinical presentations, the RS2-linked patients do not exhibit redundancy of the periumbilical skin as has been described for the RS1-linked families.

One other autosomal dominant condition that has recently been mapped to the 6p25 region (29) is iridogoniodygenesis (IGDA). This is a condition with clinical presentation of iris hypoplasia, goniodysgenesis (iridocorneal angle defects), elevated intraocular pressures and juvenile glaucoma. The IGDA locus has been mapped to a region of ~3 cM that extends from D6S477. Interestingly, this region has also been implemented as being the site for Axenfeld–Rieger anomaly (91) as well as one form of juvenile-onset primary open angle glaucoma with or without abnormalities of the iris and iridocorneal angle (92). Furthermore, linkage of another large family comprising 20 adult and two juvenile-onset POAG, of whom five had iris hypoplasia, has also been reported with the same region (93). Moreover, these authors suggested that IGDA and the locus for this family could be allelic. These observations together with previously reported chromosomal rearrangements in Rieger syndrome and congenital glaucoma clearly suggest that either a single gene or a cluster of genes in this region of 6p are involved in eye development.

Recently, Anderson *et al.* (31) described a new locus for pigment dispersion syndrome (PDS) on the 7q35–q36 region. The authors identified four families, one of which had 16 affected subjects in four generations. Most of the linkage data presented in this paper come from this one large kindred and the remaining families contributed little to the overall conclusion. These authors used phenocopy as a possible explanation for two affected subjects who were recombinants for the entire region. They also excluded six normal subjects from their analysis who were under the age of 45, although the age of onset in their affected subjects were reported to be between 18 and 37. Since no haplotype data were presented for these normal subjects, it is not clear how many of them (if any) inherited the affected haplotype. Moreover, for smaller pedigrees, they reported recombination in two affected subjects within the same spot (i.e., between D7S2546 and D7S550, a genetic distance of 5 cM); one recombinant cen tromerically and another telomERICALLY. Therefore, it would be interesting to see if these two affected subjects actually recombined in a different interval when additional markers (i.e., D7S1823) are genotyped from this region.

Use of phenocopy in the affected subjects for establishing linkage of a particular condition to a given chromosomal region should be treated with caution. While for the common forms of glaucoma this may be true, it should only be used when linkage has already been established in other families, otherwise this exercise may provide a false positive result.

**SUMMARY AND CONCLUSIONS**

Recently, positional mapping and cloning of different types of glaucoma has taken a significant leap forward and provided the foundation for understanding the basic molecular biology and underlying pathogenic mechanisms of this group of ocular conditions. Of the five known glaucoma loci, the defective molecules have been identified only for the first two that were originally mapped in 1993 (GLC1A) and 1995 (GLC3A) (48,73). We would anticipate that mutations in the other three loci will follow soon.

Identification of a number of mutations in the TIGR gene in families with POAG provided a link between researchers studying the cellular and molecular pharmacology of ocular tissues involved in glaucoma and those using a reversal approach by employing a direct positional mapping and cloning of the putative gene. This gene was cloned independently from morphologically differentiated TM cell cultures (TIGR) and subtracted cDNA libraries constructed from ciliary body (CBS-670) and retina (MYOC). The encoded protein was implicated in controlling the aqueous humor outflow through the trabecular meshwork. TIGR overexpression was observed in patients with glaucoma. The increased TIGR expression in these groups of patients (49) could both result from environmental stimuli or genetic susceptibility (J.Polansky, personal communication). As a result of screening for the TIGR coding sequences in JOAG families, >25 mutations have been identified so far, all clustered in exon III. This therefore suggests that this region of the molecule may be a hot spot for mutation. As the current hypothesis for the role of TIGR protein is in its involvement in outflow obstruction in glaucoma (J.Polansky, personal communication), given the hot spot nature of the region, one would expect to observe more affected JOAG subjects in a given population. However, both increased IOP without optic nerve damage, and optic nerve damage without elevated IOP are documented in POAG patients. Therefore, these are likely to be independent components of the disease phenotype resulting perhaps from mutations at different loci. Therefore, it is likely that a second mutation in either the regulatory elements of the TIGR promoter
or alternatively in a closely mapped neighboring gene (for JOAG) and/or other gene in a different chromosomal location (for COAG) could contribute to the phenotype. It has also been suggested that oxidative stress factors, hormonal signals, as well as susceptibility and protective factors may have relevant effects on the TIGR promoter (49; J Polansky, personal communication) and expression of this gene. Therefore, complementary changes in other extracellular matrix molecule(s) either produced by mutation in other gene(s) or influenced by direct alteration of the function, gene expression and/or life cycle of the TIGR molecule may be involved in subjects affected with POAG. Further study on the role of TIGR and its interaction with other ocular molecules will be required before its significance in the development of glaucoma is fully understood.

One other important development has recently been reported: cytochrome P4501B1 (CYP1B1) was discovered to be the mutated gene in patients with primary congenital glaucoma (PCG). Further to the first three mutations that were originally described in five PCG families, 11 new mutations have now been identified in the CYP1B1 gene (unpublished). These mutations have been detected in both isolated and familial cases from different geographical areas. The GLC3A locus on chromosome 2p21 has now emerged as a major location for the congenital type of glaucoma. Genetic linkage data from published papers and abstracts indicated that the majority of the pedigrees ascertained from ethnic groups with high incidence of the disease, namely Turkish, Saudi Arabian and Slovakian gypsies, map to the same region on chromosome 2p21. Preliminary estimates suggested that the CYP1B1 mutations would be responsible for 85–90% of cases with this condition. This obviously has significant implications in the management, diagnosis and prognosis of this condition. Study of CYP1B1 mutations in one particular family with an apparent pseudo-dominant inheritance indicated that this pedigree is in fact another example of autosomal recessive disease. Furthermore, we have now identified mutations in both normal parents of sporadic cases, strongly indicating that the mode of inheritance for the PCG phenotype, even in isolated cases, is autosomal recessive. The observed mutations have exclusively been identified in regions highly conserved among the members of the cytochrome P450 superfamily. Furthermore, six polymorphic sites detected in this gene so far are exclusively in the non-conserved regions. Numerous members of the cytochrome P450 family of proteins act as intermediate catalysts in metabolic pathways such as the steroidogenesis and metabolism of archidonic acid. It is therefore reasonable to assume that CYP1B1 participates in the metabolism of molecule(s) that are important for the proper development and function of the anterior chamber angle of the eye. Although it has been shown that CYP1B1 can catalyze the 4-hydroxylation of 17β-estradiol (E2), its substrate specificity is not completely characterized. It is quite interesting that both genes implicated in the pathogenesis of two different types of glaucomas are related to steroid metabolism, with CYP1B1 acting upon steroid intermediates, and expression of TIGR being induced by steroids. Is it possible that CYP1B1 metabolizes a steroid molecule which could influence the expression of TIGR protein? This is a very exciting possibility which deserves to be investigated further.

For the adult-onset primary open angle glaucoma, two new loci have recently been identified. GLC1B is mapped to the 2cen–q13 (67) and GLC1C to the 3q21–q24 (68) region. For both of these loci, genetic heterogeneity has been observed. While the initial linkage in GLC1B-linked families has now been extended to a number of new families, no other confirmation of linkage has been reported for the GLC1C locus. Mutation screening of the candidate genes located in these two regions would hopefully identify other genes/molecules that could shed some light on the disease mechanism in this group of POAG families. Given the high prevalence of this group of ocular conditions and the result of genetic linkage studies so far, one would anticipate that a large number of loci are involved in the etiology of adult-onset primary open angle glaucoma.

Two other abstracts presented at the ARVO meeting in May 1997 (92, 93) suggested that a new locus for POAG is mapped to 6p25, where previously iridogoniodysgenesis (IGDA) has been mapped. As these families also presented with iris hypoplasia, it is likely that the IGDA and POAG-related phenotype in these families is the result of mutations in the same gene. Alternatively, these may be separate conditions and/or allelic.

Early this year, a new locus for the pigment dispersion syndrome (PDS) was described (31). This locus was mapped to the 7q35–q36 region. Although PDS is considered to be a secondary type of glaucoma, identification of mutations causing this condition will have direct effect on our understanding of the pathogenic mechanism of POAG. However, localization of this locus to chromosome 7 was based on the use of phenocopies in a large family that provided most of the linkage data. Although phenocopies are quite likely to occur in a given large pedigree, the use of this in establishing linkage to a new locus must be treated cautiously.

Although in the beginning of this year, two separate genes were reported to be involved in the etiology of juvenile-onset primary open angle glaucoma (TIGR) and primary congenital glaucoma (CYP1B1), the real challenge would be in the positional mapping and cloning of the more prevalent form of adult-onset primary open angle glaucoma. A number of investigators in the US and Europe are now actively ascertaining families with multiply affected subjects. Positional mapping of late-onset POAG would require a large number of affected subjects, as usually extended families with this phenotype are very limited. In the next several years, it is anticipated that a number of these loci will be identified and cloned. However, the possibility of identifying a major locus/gene that would be responsible for the majority of adult-onset POAG families is less likely.

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