Myocilin and Glaucoma

A TIGR by the Tail?

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In 1997, Stone and 14 colleagues from 7 laboratories reported the identification of a gene (TIGR) associated with juvenile open-angle glaucoma (JOAG). Screening of adults with primary open-angle glaucoma (POAG) revealed that about 4% also carried a mutation of the coding region of this gene. The mutations were found through genetic linkage analysis of families with JOAG. Juvenile open-angle glaucoma was a logical starting point in the search for genetic causes of open-angle glaucoma: it shows a strong autosomal-dominant inheritance pattern, occurs at an early age, demonstrates obvious phenotypic signs (dramatic elevation of intraocular pressure and subsequent optic nerve damage), and is likely to be found in multiple generations as parents of affected children are still living. These factors, however, also serve to distinguish it from adult-onset POAG, which generally has a lower intraocular pressure and a less severe course. The discovery of the actual gene represented a true advance over previous studies that had mapped the gene to a segment of a chromosome but did not identify the specific gene. How the mutant gene causes glaucoma is unknown and is the subject of intense research. To date, 26 mutations in the TIGR gene sequence (the term TIGR has been replaced by the term myocilin, abbreviated MYOC) have been described, all associated with either JOAG or adult-onset POAG. A correlation between specific mutations in MYOC and the clinical course of glaucoma has been found. Not all cases of JOAG or POAG have mutations in the MYOC gene, however, indicating that more discoveries of other genes are yet to come.

A new door has opened in glaucoma research. The discovery of the myocilin gene and its association with glaucoma provides a key to understanding the physiology of aqueous outflow through the trabecular meshwork, and the pathophysicsiology of glaucoma. Undoubtedly the answers will be far more complex than we can imagine at our current state of knowledge.

WHAT’S IN A NAME?

GLC1A

The region Stone et al1 identified was first given the nonspecific name GLC1A, following the convention for naming genes not yet discovered but surmised because of genetic linkage studies.2 Glaucoma is indicated by GLC, with “1” indicating primary open-angle glaucoma (POAG) and “A” indicating that it was the first gene linkage site found in POAG. As expected, other gene linkages for glaucoma have been identified: 5 additional POAG linkages (GLC1B, GLC1C, GLC1D, GLC1E, and GLC1F), congenital glaucoma (GLC3A and GLC3B), and developmental anomalies or other conditions associated with glaucoma (Rieger syndrome, RIEG 1; pigment dispersion syndrome, PDS).3 Some of the candidate genes are transcription factors that affect different stages of embryonic development.

TIGR

The mutant gene at the GLC1A locus identified by Stone and colleagues for juvenile open-angle glaucoma (JOAG) was found to
be a previously described gene, \textit{TIGR} (trabecular meshwork-induced glucocorticoid response). This gene had been found, sequenced, and named during studies on corticosteroid-induced glaucoma. Polansky et al\textsuperscript{4,5} had found a series of proteins that were induced by long-term dexamethasone treatment in cultured human trabecular cells. One of the proteins, originally called \textit{TIGR}, had the intriguing feature of time-dependent induction: progressively more protein was produced with continued dexamethasone treatment. This time course of induction was similar to that of the clinical situation of corticosteroid-induced glaucoma, occurring over a period of several weeks. Subsequent analysis through a complementary DNA library screening enabled discovery and cloning of the gene.\textsuperscript{4-6}

**Myocilin**

While the study on JOAG and \textit{TIGR} by Stone et al\textsuperscript{1} was in press, Kubota et al\textsuperscript{1} in Japan submitted a study on the discovery of a protein associated with the cytoskeleton in the retina. They termed this protein \textit{myocilin} (MYOC) because it shared homologous regions with myosin.\textsuperscript{7} This was the same gene and protein that Nguyen and colleagues\textsuperscript{8} had described and named \textit{TIGR}, unbeknownst to Kubota. In 1998 the Human Genome Organization Genome Database Nomenclature Committee assigned the gene the name myocilin, and it is now referred to as MYOC rather than \textit{TIGR}. Note the convention of using italics to refer to the gene and standard font to refer to the protein.

**AN ENDOGENOUS PROTEIN THAT INCREASES DURING STRESS**

Myocilin is normally present in a variety of ocular and nonocular tissues: trabecular meshwork, cornea, retina, optic nerve, ciliary nerves, and the heart, skeletal muscle, stomach, thyroid, bone marrow, prostate, intestine, lung, pancreas, and lymph node.\textsuperscript{8,9} In the eye, MYOC may be produced in greater amounts in times of stress. It is present in increased amounts in cultured anterior segments from human eyes and in monolayer cultured trabecular cells after undergoing dexamethasone treatment, oxidative stress, stretching, and treatment with transforming growth factor \(\beta\).\textsuperscript{4,6,10-12} As such, MYOC is one of a diverse group of stress proteins, the role of which seems to be protective of vital cellular proteins or enzymes.\textsuperscript{13-16}

Another class of stress-induced proteins that have received study are the heat shock proteins, first found in the larva of the fruit fly \textit{Drosophila} when enlargements of certain regions of the chromosomes were noted after heating (hence the term \textit{heat shock proteins}).\textsuperscript{17} Some of the stress proteins are thought to bind to important cellular proteins, preventing them from unfolding or denaturing under stress, earning them the name \textit{molecular chaperones}.\textsuperscript{13-16} Such stress proteins may be endogenously present in a variety of tissues and also may have specific functions of their own: \(\alpha-B\) crystalline is a major component of the crystalline lens and is also present in smaller amounts in other tissue (cardiac, lung, kidney, muscle, brain, and retina) in a presumably protective role as a molecular chaperone.\textsuperscript{15-16,18} Although MYOC is present in the trabecular meshwork of both normal and glaucomatous eyes, it may be present in more regions and have more intense labeling in glaucomatous eyes.\textsuperscript{19} The presence of MYOC in normal eyes is consistent with a physiologic role for MYOC and indicates that the mere presence of MYOC does not by itself cause glaucoma.

**PROPERTIES OF MYOC PROTEIN**

Myocilin is a glycoprotein that exists in glycosylated and nonglycosylated forms, with molecular weights of 66 kd and 55 kd.\textsuperscript{4,6} It is present both intracellularly and extracellularly.\textsuperscript{1,4-6} Myocilin protein consists of 504 amino acids and has 2 major domains: a myosin-like domain near the N terminal and an olfactomedin-like domain near the C terminal (Figure 1).\textsuperscript{4,9} Most of the mutations in MYOC associated with glaucoma are in the olfactomedin domain.\textsuperscript{1,3} Olfactomedins are a family of mucous proteins that are predominately found in nasal mucus.\textsuperscript{20} Myocilin protein has several noteworthy elements, including (1) a “leucine zipper” region: this is a series of 7 leucine molecules (amino acids) in a configuration that might allow it to interact with leucine zippers present in other proteins; using these zippers, MYOC could also form dimers or oligomers with itself; (2) multiple possible sites for glycosylation and phosphorylation; (3) presumed binding sites for hyaluronic acid and heparan sulfate\textsuperscript{4,7,21}; and (4) a “signal sequence” of 32 amino acids at the N terminal of the molecule, which is usually found in molecules secreted extracellularly.

The structure of MYOC protein is conserved through evolution, with 80% or more homogeneity of amino acids between human and mouse MYOC.\textsuperscript{8,9} This cross-species presence is in keeping with a presumed physiologic role for MYOC, though the role is unknown. Comparison of the sequence of MYOC across species can be helpful in understanding which domains are of functional importance. The preservation of the leucine zipper motifs and sites for glycosylation and phosphorylation between mouse and man suggests that these play a vital role in the function of the protein, while regions not conserved across species are presumably not as critical to its function.\textsuperscript{9}

The normal physiologic function of MYOC in the cell is unknown. It may serve a structural function within the cytoplasm, or it may associate with other molecules within the cell, perhaps as a molecular chaperone. Extracellularly, it may be involved in creating resistance to aqueous outflow by binding to other extracellular molecules or to the cell membrane of trabecular cells.

In addition to studies of the MYOC protein and gene, attention has focused on the promoter region of MYOC.
Promoters are gene elements “upstream” from the protein-encoding DNA sequence. They control the amount of protein produced by the gene by regulating transcription of the protein-coding portions of the DNA. The promoter region of MYOC has been identified and contains regions (consensus motifs) that are responsive to glucocorticoids and a number of other hormones.4-6 The presence of this glucocorticoid response element may explain the increase in the amount of normal MYOC protein present in cultured trabecular cells from susceptible patients after corticosteroid treatment.4-6,22

**JOAG, POAG, MYOC, AND STEROIDS**

Juvenile open-angle glaucoma has an earlier age of onset, higher intraocular pressure (IOP), and more severe course than adult-onset POAG. In addition, trabecular meshwork from eyes with JOAG has a markedly different appearance than that of eyes with POAG when examined with the electron microscope. Histologic studies of JOAG report the accumulation of an abnormal basement membrane-like material within the trabecular meshwork, which may fill the meshwork and disrupt Schlemm’s canal.23-25 This differs dramatically from the changes of adult-onset POAG: thickening of tendon and tendon-sheath material within the meshwork.26,27 The changes of JOAG are strikingly similar to those of corticosteroid-induced glaucoma in which the meshwork also contains accumulations of abnormal basement membrane-like material, often in whorls resembling a fingerprint (**Figures 2, 3, and 4**).28,29

Corticosteroid treatment causes the overproduction of MYOC in cultured trabecular cells from some patients.4-6 Could excess MYOC be a link between steroid glaucoma and JOAG? Does the similarity in the ultrastructural appearance of the 2 conditions suggest that...
the abnormal basement membrane–like material consists, at least in part, of MYOC? Myocilin is known to be a sticky molecule with binding sites for several components of the basement membrane.3-6

HOW COULD MYOC CAUSE GLAUCOMA?

MYOC Alone

If excess MYOC accumulated in the trabecular meshwork, whether from overproduction or a decrease in degradation, glaucoma could develop. As discussed earlier, corticosteroid treatment causes the overproduction of normal MYOC protein in susceptible trabecular meshwork cells. This MYOC protein is found both intracellularly and extracellularly.4-6 Excess extracellular MYOC could bind to the aqueous outflow pathways and increase outflow resistance, similar to the mechanism of retained hyaluronic acid (Healon; Pharmacia & Upjohn Inc, Kalamazoo, Mich) or other viscoelastic material increasing IOP after cataract surgery. Preliminary results with recombinant MYOC protein in my laboratory, in conjunction with the studies of Nguyen et al8 and Polansky et al,4,5 confirm that recombinant MYOC protein can indeed elevate IOP in eyes in perfusion organ culture of the anterior segment. If synthesis of a mutant MYOC protein escapes control of normal feedback regulation, then excess amounts of MYOC or its mutant form could be produced and thus increase outflow resistance.

Alternatively, the mutant form of MYOC may be unable to fulfill the presumed physiologic role of MYOC protein. In this scenario, some mutations would cause more problems than others. This is supported by the finding of a clinical correlation among the various mutations and the onset and severity of glaucoma. The most common mutation, Gln368STOP, is associated with an older age of onset and less elevation of IOP than the reported missense mutations described in the younger-onset groups.7 This correlation between genotype and phenotype and the delayed onset of glaucoma suggest that a truncated form of the MYOC protein may still serve some physiologic function, while those mutant forms with variations in the amino acid sequence are more problematic and cause glaucoma at an earlier age. One could imagine that proper dimerization or oligomerization is necessary for the function of MYOC and that certain mutations prevent proper interactions of this protein. The resultant increase in monomeric forms of the protein could be associated with increased resistance in the trabecular meshwork. Not all changes in the MYOC gene sequence have been associated with glaucoma; those apparently nondisease-causing sequence variations are termed polymorphisms.

Another interesting possibility raised by the finding of Morissette et al30 is that patients with the same MYOC mutation in both chromosomes (maternal and paternal) do not develop glaucoma while patients with a mutation in only 1 of 2 chromosomes will develop glaucoma. This implies that the interaction between the MYOC protein molecules produced by each chromosome may be important to the function of MYOC. In the usual case of glaucoma associated with a MYOC mutation, only 1 chromosome carries the mutant gene, and presumably 1 mutant MYOC molecule and 1 normal MYOC molecule are produced. The mutant MYOC protein may have a different shape (secondary or tertiary protein structure) that prevents its interaction with the normal MYOC molecule. Morissette and colleagues have hypothesized that if both copies of MYOC are mutant (homozygous), the MYOC proteins may interact and no glaucoma would result.30

MYOC Plus

None of these speculations account for the relatively delayed onset of glaucoma even in the juvenile-onset cases: Why is glaucoma not present at birth or in the first few years of life, given that the mutation is present at birth? In addition, not all patients with mutant MYOC have glaucoma. At least 10 cases of adult patients with the Gln368STOP mutation who do not have glaucoma have been reported.3,31 Both of these lines of evidence suggest that a second factor may be involved in causing glaucoma or that there may be a cascade of events leading to glaucoma. This second factor could be genetic or environmental: it could be an abnormality in the promoter region, an abnormality in other molecules with which MYOC presumably interacts, changes in the outflow pathway by light-induced oxidation, or the breakdown of a redundant pathway that had been compensating for the physiologic function of mutant MYOC protein.

Despite the unanswered questions, the discovery of the MYOC gene and its link to open-angle glaucoma represent a true step forward in the search for the pathogenesis of glaucoma. Study of MYOC and its function in health and disease will lead to a better understanding of the mechanism of aqueous outflow and ultimately to therapy that could correct the genetic problems associated with glaucoma.

Accepted for publication March 27, 2000.

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A look at the past...

Cyclodialysis as an operation for the relief of increased intraocular pressure has gained in favor with the staff of the ophthalmologic clinic at the University of Iowa until at present it is our operation of choice for chronic noncongestive wide angle or narrow angle glaucoma, hydropthalmos and certain types of secondary glaucoma, notably that after cataract extraction.