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Cochlin and glaucoma: A mini-review

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Abstract

Primary open angle glaucoma (POAG) is a leading cause of late onset, progressive, irreversible blindness and, although its etiology is poorly understood, elevated intraocular pressure (IOP) often appears to be a contributory factor. Proteomic and Western analyses of trabecular meshwork (TM) from patients with POAG and age-matched controls originally implicated cochlin as possibly contributing to glaucoma pathogenesis. Cochlin deposits were subsequently detected in glaucomatous but not in control TM and older glaucomatous TM was found to contain higher levels of cochlin and significantly lower amounts of collagen type II. More recently, similar results were reported in DBA/2J mice, which at older ages develop elevated IOP, retinal ganglion cell degeneration, and optic nerve damage. Notably, cochlin was absent in TM from C57BL/6J, CD1, and BALBc/ByJ mice, which do not exhibit elevated IOP or glaucoma. Cochlin was found in the TM of very young DBA/2J mice, prior to elevated IOP, suggesting that over time the protein may contribute to the events leading to increased IOP and optic nerve damage. Here we review these findings and describe how future studies in DBA/2J mice can help resolve whether cochlin plays a causal role in mechanisms of POAG and elevated IOP.

Keywords

Cochlin; Trabecular meshwork; Intraocular pressure; Glaucoma; Optic nerve damage

Introduction

Glaucoma refers to a group of progressive diseases that results in ganglion cell degeneration and permanent vision loss. While current glaucoma therapies are able to slow the disease process, they do not end disease progression and the associated visual impairment (Friedman et al., 2004). Glaucoma affects ~3 million Americans (Wilson & Martone, 1996) and over 70 million people worldwide (Quigley, 1996). Primary open angle glaucoma (POAG) is the most common form of glaucoma and is typically associated with elevated intraocular pressure (IOP). Although changes in IOP could reflect imbalance between aqueous humor production and removal, in glaucoma IOP elevations typically reflect an impediment in outflow (Tomarev, 2001). Aqueous humor is produced by the ciliary epithelium, delivered to the anterior chamber, and then drains through the trabecular meshwork (TM) into Schlemm's canal and the episcleral veins (Morrison & Acott, 2003). The major resistance to aqueous outflow occurs at the TM, which is comprised of a complex extracellular matrix (ECM) of collagen beams lined with endothelial-like cells (Lutjen-Drecoll, 1999, 2000).

Recently, we found that cochlin deposits develop in the TM of patients with POAG (Bhattacharya et al., 2005a) and of glaucomatous DBA/2J mice (Bhattacharya et al., 2005b). In both POAG patients and DBA/2J mice, we observed an age-related increase in these deposits, which were consistently absent in TM samples obtained from normal humans and mouse strains that do not develop glaucoma (Bhattacharya et al., 2005a,b). We hypothesize that cochlin deposits may obstruct aqueous humor circulation through the TM and thereby contribute to the IOP elevations that are associated with glaucoma. Here we review our initial findings regarding cochlin expression in the TM and discuss the results in the context of previous cochlin structure–function studies and the DBA/2J mouse, an animal model of glaucoma that offers promise for determining whether cochlin expression contributes to elevated IOP.

Previous cochlin structure–function studies

Cochlin, the product of the *COCH* (Coagulation factor C Homology) gene, comprises the major noncollagen component of the ECM of the inner ear (Robertson et al., 1994, 2003). Cochlin mutations and cochlear cochlin deposits are associated with DFNA9, an autosomal dominant nonsyndromic auditory and vestibular disorder (Kamarinos et al., 2001; Robertson et al., 2003). In DFNA9, misfolded cochlin is thought to contribute to the formation of cochlear deposits (Robertson et al., 2003). In addition to DFNA9, *COCH* mutations have also been implicated in Ménière's disease and in presbycusis (Kamarinos et al., 2001; Robertson et al., 2003). Ménière's disease is a disorder of the inner ear that causes repeated episodes of vertigo, ringing in the ears (also known as tinnitus), and fluctuating low-frequency sensorineural hearing loss. Patients with presbycusis present with vertigo and a progressive bilateral hearing loss that involves loss of high-frequency sensitivity first (Kimura, 1986).

Cochlin is a secreted protein with three glycosylated isoforms detectable with apparent masses of approximately 40 kDa, 46 kDa, and 60 kDa (Robertson et al., 2003; Ikezono et al., 2004). The protein is highly conserved, with 94% amino acid identity between human and mouse and 79% identity between human and chicken sequences (Robertson et al., 1998). As diagrammed in Fig. 1, the cochlin gene contains a total of 12 exons (Fig. 1A), encoding a protein that contains 550 (human) or 552 (mouse) amino acids and several functional domains (Fig. 1B). In addition to a short predicted signal peptide, cochlin contains an N-terminal factor C homology (FCH) domain and two von Willebrand factor A-like (vWFA) domains. The FCH domain is named after the coagulation factor from horseshoe crabs (*Limulus* factor C) that initiates a host defense coagulation cascade. Mutations in the FCH domain of cochlin have been implicated in DFNA9. vWFA domains exist in a number of ECM and immune system proteins and have been implicated in aggregation of platelets and macrophages. The two vWFA-like domains in cochlin are encoded by exons 8–10 and 11–12, respectively (Robertson et al., 1997, 1998).

The function of cochlin is not known, although cochlin comprises the major noncollagen component of the ECM of the inner ear (Ikezono et al., 2001; Robertson et al., 1998). The overall structure of cochlin resembles two other proteins, vitrin and akhirin, that also contain the FCH domain followed by two vWFA domains and that have been localized to the vertebrate eye (Mayne et al., 1999; Ahsan et al., 2005). Vitrin was originally identified in vitreous of bovine eyes and it is expressed in lens (Mayne et al., 1999; Bishop et al., 2002). Akhirin is expressed in the ciliary marginal zone, lens epithelium as well as in the retinal pigment epithelial layer (Ahsan et al., 2005). Although the functions of vitrin and akhirin are not known, akhirin has been implicated to have a role in cell adhesion (Ahsan et al., 2005). The N-terminal FCH domain of cochlin is also referred as the LCCL domain, named after *Limulus* factor C, cochlear protein (Coch 5b2), and the late gestation lung protein (Lgl1). This domain has also been found in other proteins including, for example, CocoaCrisp (Clark et al., 2003), vitrin

(Mayne et al., 1999), and in the cub1 domain of human bone morphogenetic protein (Hartigan et al., 2003; Leighton & Kadler, 2003). While the function of the FCH domain in cochlin remains to be elucidated, in other proteins this domain is involved in antibody-independent host defense. For example, Lgl1 is expressed in fetal lungs coincident with the production of pulmonary surfactant and it has been suggested that Lgl1-like surfactant proteins SP-A and SP-D serve to protect the lung from pathogens (Kaplan et al., 1999). Limulus factor C is a trypsin-type serine protease, activated upon binding to lipopolysaccharides in its zymogen state. Activation initiates the Limulus coagulation cascade, a host defense mechanism to protect the organism from bacterial infection (Nakamura et al., 1988*a,b*; Liepinsh et al., 2001). Amino acid sequence comparisons, circular dichroism analyses, and nuclear magnetic resonance studies have demonstrated the importance of FCH domains for protein folding (Trexler et al., 2000). As noted above, several of the cochlin FCH mutations causing DFNA9 (Fig. 1B) are thought to interfere with proper protein folding (Liepinsh et al., 2001; Robertson et al., 2003). The FCH domain also includes a potential N-linked glycosylation site located at amino acid residue 100.

Recent cochlin studies

Cochlin is associated with glaucomatous human TM

Human TM samples were obtained from clinically documented control cadaver eyes from the Cleveland Eye Bank and by trabeculectomy at the Cleveland Clinic Foundation (Bhattacharya et al., 2005*a*). Fig. 2 illustrates a TM sample obtained by trabeculectomy. TM protein extracts were subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), gel slices were excised, and proteins were identified using well-established mass spectrometric and bioinformatic methods (Bhattacharya et al., 2005*a*). A total of 368 proteins were identified from 12 TM samples, and of these ~14% were found only in TM from POAG patients. The protein most frequently identified in glaucomatous TM but not in control TM was cochlin (Table 1). Subsequently, by Western analysis, cochlin was identified in 20 out of 20 TM samples from patients with POAG (irrespective of fresh trabeculectomy or cadaver glaucomatous tissue) and in 0 of 20 TM samples from normal control tissue donors (Bhattacharya et al., 2005*a*). Due to antibody specificity, only the higher molecular weight 46-kD and 60-kD isoforms of cochlin have been detected in glaucomatous TM from humans and mice (Bhattacharya et al., 2005*a,b*).

Cochlin is associated with glaucomatous mouse TM

In studies of human tissue, we noted that older glaucoma patients had higher levels of cochlin but lower levels of type II collagen (Bhattacharya et al., 2005*a*). This observation suggests that cochlin may disrupt the TM architecture, rendering components like collagen more susceptible to degradation. To evaluate this possibility, we turned to the DBA/2J mouse, a well-defined model of glaucoma (John et al., 1998; Chang et al., 1999; Bayer et al., 2001). Mouse tissues were obtained from breeding colonies established from DBA/2J, BALBc/ByJ, and C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME). By 8 months of age, DBA/2J mice develop anterior segment pigment dispersion associated with optic nerve damage that does not occur in age-matched control C57BL/6J mice (John et al., 1998; Savinova et al., 2001). Optic nerve cross sections obtained from 8-month-old DBA/2J mice (Figs. 3A & 3C) show an overall constriction as well as damage to optic nerve bundles that is lacking in age-matched C57BL/6J mice (Figs. 3B & 3D).

Western blot analyses of cochlin and collagen II expression in mouse TM are presented in Fig. 4. Cochlin was observed in DBA/2J mice as early as 2–3 weeks of age and progressively increased in abundance with age (Figs. 4A–4C). In contrast to this time course, IOP elevations do not occur in DBA/2J mice until approximately 6–8 months of age (Fig. 5; John et al.,

1997,1998;Savinova et al., 2001). Both age and gender have significant effects on IOP. For example, at 6 months of age, IOP levels were considerably higher in female than in male mice (John et al., 1998). We are currently examining whether cochlin expression in DBA/2J mice is also influenced by gender. In comparison, cochlin was not observed in TM from C57BL/6J mice (Figs. 4A–4C), or BALBc/ByJ mice (Fig. 4B) which do not develop glaucoma (Savinova et al., 2001). Type II collagen expression was also measured by Western analysis in DBA/2J and C57BL/6J mice (Figs. 4A & 4C). Similar to the human condition, DBA/2J mice developed a progressive decline in type II collagen expression in the TM (Figs. 4A & 4C). This change is not a feature of normal murine aging, as type II collagen levels remained stable in older C57BL/6J mice (Fig. 4C).

Cochlin forms deposits in glaucomatous TM

Sheath-derived plaques and microfibrillar deposits have been observed in the TM of POAG donors (Lutjen-Drecoll et al., 1986; Ueda et al., 2002). To explore a possible relationship between cochlin and these deposits, we pursued histochemical analyses of human and mouse TM. Analyses of human TM revealed that cochlin, in association with mucopolysaccharide (Bhattacharya et al., 2005a), forms deposits within glaucomatous TM (Fig. 6A) but not control TM (Fig. 6B). Cochlin deposits were observed in TM from ten glaucoma patients all ten glaucoma patients examined, but in none of ten control subjects. In all cases, the regions of large deposits with cochlin appears to be acellular (Figs. 6C & 6D), which is similar to the distribution of cochlin in the cochlea of patients with DFNA9 (Robertson et al., 2003). Comparable analyses of 8-month-old mice also revealed cochlin deposits in DBA/2J TM (Figs. 6E & 6E') but not in C57BL/6J TM (Figs. 6F & 6F').

von Willebrand factor-like domains and cochlin interactions

Most vWFA domains are found in cell adhesion and ECM proteins and integrin receptors that participate in multiprotein complex formation (Tuckwell, 1999). The eponymous vWFA domains of von Willebrand and the homologous inserted or I domains of some integrin subunits are involved in interactions with collagen and other ligands (Whittaker & Hynes, 2002). vWFA domains are present in a variety of ECM components, including cartilage matrix protein and collagen type VI, VII, XII, and XIV (Whittaker & Hynes, 2002). vWFA domains are also involved in shear-induced self-aggregation and adherence to platelets, macrophages, or leukocytes (Ruggeri et al., 1983; Shankaran et al., 2003). vWFA domains have been implicated in defense mechanisms, including hemostasis, and many components of the immune system (complement factor C, factor B, integrins LFA-1, Mac-1, VLA-1 and 2, p150, and p95) harbor vWFA domains (Colombatti et al., 1993). Furthermore, vWFA domains are known to interact with proteins such as collagen, GpIb α , and integrin $\alpha_{IIb}\beta_3$ (Ruggeri et al., 1983; Eble & Tuckwell, 2003; Vanhoorelbeke et al., 2003). It is interesting to note that many of these interactions are involved in different regimes of fluid dynamics (Shankaran et al., 2003) and have been implicated in adherence to platelets and possibly also to macrophages. High fluid shear induces platelet aggregation that involves the shear-induced binding of vWFA to the platelet membrane GpIb/IX/V complex (O'Brien and Salmon, 1987; Kroll et al., 1996; Ikeda et al., 1997). Hydrodynamic forces induce vWFA-based aggregation in suspension and have been suggested as one of the controlling factors in cell-adhesion rates in circulation (Shankaran et al., 2003; Dong et al., 2003).

Protein and cell aggregation is a common theme in the pathogenesis of diverse diseases, including atherosclerosis, Parkinson's disease, and Alzheimer's (Masliah & Hashimoto, 2002). Previous studies suggest that glaucoma is part of this disease group (McKinnon, 2003; Parisi, 2003). In POAG, the increased IOP is related to altered fluid shear and hydrodynamic forces. As a consequence, cochlin vWFA domains may respond abnormally to the different fluid-flow regimes in the TM as IOP fluctuates, or to local alterations in ion

concentration or oxidation within the TM. In this scenario, cochlin may undergo multimeric aggregation, and begin the process of deposit formation and collagen degradation. Consistent with this hypothesis, purified cochlin was found to aggregate after sheer stress and, in primary cultures of TM, to induce cell aggregation (Bhattacharya et al., 2005a). Future research will be needed to determine whether multimeric aggregates of wild-type full-length cochlin are biologically active and capable of eliciting cell aggregation and to determine whether cochlin vWFA-like domains are involved in the aggregation process.

It is possible that cochlin may have other effects on the TM. For example, increased levels of cochlin may change the manner in which fibrillar collagens interact with other TM components and thereby trigger collagen degradation (Pareti et al., 1987; Marchant et al., 1996). Furthermore, the prolonged presence of cochlin in glaucomatous TM may lead to perturbations in collagen fibrillar assembly and loss of tissue-specific morphology (Marchant et al., 1996). It is also possible that cochlin may disrupt the integrity of the TM extracellular matrix, rendering it more susceptible to proteolytic degradation, collapse, and debris deposition, resulting in elevated IOP from the obstruction of aqueous outflow. Presently, it is not known whether cochlin expression and deposition in the TM is a cause of increased IOP or a secondary effect.

The DBA/2J mouse model of glaucoma

The DBA/2J mouse offers significant advantages for determining whether cochlin plays a role in glaucoma pathophysiology because this mouse develops many of the hallmarks of human glaucoma, including age-dependent elevated IOP, ganglion cell degeneration, and optic nerve damage (John et al., 1998; Chang et al., 1999; Anderson et al., 2001; Danias et al., 2003). In DBA/2J mice, elevated IOP appears to develop secondary to iris pigment dispersion and iris stromal atrophy (John et al., 1998; Anderson et al., 2001), which have been attributed to mutations in the *Gpnmb* and *Tyrp1b* genes, respectively (Anderson et al., 2002). However, the hypopigmented DBA/2J-pe mouse strain does not exhibit iris pigment dispersion, iris stromal atrophy, or glaucoma despite homozygosity for the *Gpnmb* and *Tyrp1b* mutations (Anderson et al., 2002). Thus, all of the factors contributing to elevated IOP in DBA/2J mice remain to be identified. Also the average IOP varies between 10–20 mm of Hg across different mouse strains (John et al., 1997; Savinova et al., 2001) and the reasons for this variation among different strains is not understood. In the DBA/2J mouse, cochlin was of particular interest since it was detectable in the TM shortly after birth (Bhattacharya et al., 2005a,b), well before the earliest signs of elevated IOP at 6–8 months of age (Fig. 5). Interestingly, DBA/2J mice also show late onset and progressive hearing loss (Willott & Erway, 1998; Jeskey & Willott, 2000).

We have found an age-dependent increase in cochlin expression in the TM of DBA/2J mice. In comparison to C57BL/6J, CD1, or BALBc/B7J mice, which do not develop glaucoma and do not develop detectable levels of cochlin in the TM, cochlin is present in DBA/2J mice as early as 2–3 weeks of age, and increases progressively to about age 8 months. Our preliminary analyses suggest little difference in cochlin levels between 8- and 10-month-old DBA/2J mice, an age when their IOP becomes significantly elevated (data not shown). This suggests that the gradual increase in cochlin content in the TM may influence IOP. Along with the increased cochlin content, cochlin deposits were observed in the TM of 8-month-old animals, coincident with the reported onset of increased IOP and optic nerve damage (John et al., 1997, 1998). Furthermore, in both DBA/2J mice and human patients with POAG, age-dependent increases of cochlin in the TM were accompanied by decreases in type II collagen (Bhattacharya et al., 2005a,b).

In comparing results obtained in C57BL/6J and DBA/2J mice, it is important to bear in mind that there are many other differences between these two strains, derived from the original B6 and D2 strains, respectively (Festing, 1979; Crawley et al., 1997). For example, in comparison to C57BL/6J mice, oxidative protein modifications are elevated in the TM of DBA/2J mice at 8 months of age (Annangudi et al., 2005), suggesting an increased susceptibility to oxidative damage in the DBA/2J strain. DBA/2J mice also display other behavioral differences including, for example, a lower preference for ethanol than C57BL/6J mice (Camarini & Hodge, 2004; Treadwell et al., 2004), which genetic studies have attributed to protein expression differences in the brain (Treadwell et al., 2004). Such protein expression differences also likely render DBA/2J mice more susceptible to development of elevated IOP. Overall, the differences between B6- and D2-derived strains support a genetic contribution to glaucoma pathology in DBA/2J mice.

Future directions

Further studies with mouse mutants offer substantial promise for understanding the role that cochlin may play in glaucoma. In particular, it will be important to determine whether cochlin is elevated in other mouse models with elevated IOP (Anderson et al., 2001; Libby et al., 2003; Mabuchi et al., 2004). Manipulation of cochlin expression in mice is another approach that should help to determine if cochlin plays a role in modulating IOP. We are in the process of crossing a cochlin knockout allele (Rodriguez et al., 2004) onto the DBA/2J background. If cochlin accumulation and deposition is an important step in developing elevated IOP and glaucomatous damage, then these pathophysiological changes should be ameliorated in the absence of cochlin.

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Fig. 1.

Schematic representation of the cochlin gene and protein. A: Schematic drawing of human cochlin genomic structure showing intron-exon organization of the gene. Exons are indicated by boxes, Limulus factor C homology (FCH) spans exons 4–6. The von Willebrand factor type A-like domain (vWFA)1 is contained in exons 8–10; vWFA2 is in exons 11 and 12. B: Schematic representation of cochlin protein. SP: signal peptide; FCH: factor C homology domain; vWFA1 and vWFA2: von Willebrand factor A homology domains 1 and 2; the reported mutations in FCH domain associated with DFNA9 are indicated. The G represents N-linked glycosylation sites at residue 100 and 221. [Reproduced from Robertson et al. (1998) with permission of Nature publishing group].

Fig. 2.
Representative example of surgically excised TM tissue obtained from trabeculectomy surgery in a patient with glaucoma. Scale divisions indicate 1 mm.

Fig. 3. Histochemical assessment of mouse optic nerve. Hematoxylin-stained retinal cross sections passing through the optic nerve head of an eight month-old DBA/2J mouse (A) or an age-matched C57BL/6J mouse (B). Optic nerve cross sections made (lamina cribrosa region) in an eight-month-old DBA/2J mouse (C) and an age-matched C57BL/6J mouse (D). Alternate sections of mouse eyes were subjected to hematoxylin staining for assessment of optic nerve damage. Scale bars indicate 100 μ m.

Fig. 4.

Cochlin and collagen type II levels in mouse TM. A: Representative quantitative Western analysis of DBA/2J and C57BL/6J mice with chicken anti-cochlin antibody and IR 800 (anti-chicken) or IR 700 (anti-mouse) coupled secondary antibodies. B: Cochlin expression in TM from DBA/2J mice ranging in age from 3 to 20 weeks, and a 20-week-old mouse from the C57BL/6J or BALBc/ByJ strain. C: Quantitative summary of multiple Western analyses as in panel A with normalization to GAPDH staining intensity. Levels of cochlin (squares, solid line) and type II collagen (circles, dashed lines) expression in DBA/2J (filled symbols) and C57BL/6J (open circles) mice. Data points indicate the average ($\pm$ S.D.) results from three different mice at each age. [Reproduced from Bhattacharya et al. (2005b) with permission of Elsevier].

Fig. 5.

Intraocular pressure (IOP) as a function of age and gender in DBA/2J mice. Each data point indicates an individual mouse tested at 3, 6, 9, or 19 months of age. Note that IOP levels are increased in female mice by 6 months of age, and in male mice by 9 months of age. [Reproduced from John et al. (1998) with permission of the Association for Research in Vision and Ophthalmology, Inc.].

Fig. 6. Histochemical localization of cochlin in TM samples obtained from humans (A–D) or mice (E,E',F,F') using a chicken polyclonal cochlin antibody (Robertson et al., 2001) and a rhodamine-conjugated anti-chicken secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Fixed human TM tissues were obtained from The Eye Donor Program of the Foundation Fighting Blindness (Owings Mills, MD). Histochemical analyses were performed (Bhattacharya et al., 2005a) with paraffin embedded 12- μ m tissue sections, and a Nikon EFD-3 fluorescence microscope attached to a CCD camera. Asterisks indicate Schlemm's canal. (A) Representative TM section obtained from a 77-year-old female Caucasian donor with POAG. Arrow indicates representative localized deposit of cochlin

immunoreactivity. (B) Representative TM section obtained from a 75-year-old female Caucasian donor without glaucoma. Note absence of cochlin immunoreactivity. (C) TM section obtained from another 77-year-old female Caucasian donor with POAG. Arrow indicates cochlin immunoreactivity. (D) Same section as in (C) stained with DAPI, to show that cochlin immunoreactivity [indicated by arrow from (C)] does not colocalize with nuclear distribution indicative of presence of cell. (E,E') Representative section obtained from an 8-month-old DBA/2J mouse and imaged with the red channel where cochlin-specific immunofluorescence is expected (E) or the green channel to evaluate nonspecific background immunofluorescence (E'). Note the presence of cochlin deposits, which are specific to the red channel and more easily seen in the inset to each panel, which indicates a higher (2×) power image of anterior chamber encompassing TM. (F,F') Representative section obtained from an 8-month-old C57BL/6J mouse, which is also imaged with both the red (F) and green (F') channels. In the absence of cochlin in the TM of C57BL/6J mice, there is little difference between the images shown in (F) and (F'). Scale bars indicate = 100 μm. [Parts A and B reproduced from Bhattacharya et al. (2005a) with permission of American Society for Biochemistry and Molecular Biology and Parts E, E'–F, F' reproduced from Bhattacharya et al. (2005b) with permission of Elsevier].

Table 1
Select proteins uniquely identified in glaucomatous TM

Protein	Accession number ^a	Peptide matches	Donors ^a
Cochlin	O43405	8	5
Keratin, type II	P48668	3	4
Fibrinogen beta	P02675	2	4
Myocilin	Q99972	1	3
BiP protein	P55107	2	2
HNOEL-iso protein	Q9NRN5	2	2
KIAA1420 protein	Q9P2C7	1	2
Sushi repeat-containing protein SRPX	P78539	2	2
Transcription factor E2F6	O75461	1	2
Helicase SKI2W	Q15477	1	2
Histone H4	P02304	2	2
Cullin homolog 4A	Q13619	1	2
Fibulin 5	Q9UBX5	3	2
Proline-rich protein 4	Q16378	2	2
Protein kinase PRP4 homolog	Q13523	1	2
Small proline-rich protein 3	Q9UBC9	2	2
Thimet oligopeptidase	P52888	1	2
Serine protease	Q92743	2	2
Neuromedin-B receptor	P28336	1	2
Voltage-dependent anion channel 3	Q9Y277	1	2
Collagen alpha 2 (VI)	P12110	1	2
Collagen, pro alpha 1(I)	O76045	3	2
Angiopoietin-like factor	O43827	3	2
Myosin heavy chain	P11055	1	2
Opticin	Q9UBM4	1	2

Selected Proteins identified by LC MS/MS from trabecular meshwork from six normal and six glaucomatous donors. (Bhattacharya et al., 2005a). Peptide matches indicate the maximum number of peptides identified.

^a Donors indicate the number of donors exhibiting the indicated protein.

^b Swiss-Prot database accession numbers are shown.