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ORIGINAL PAPER

Comparative evaluation of the aqueous humor proteome of primary angle closure and primary open angle glaucomas and age-related cataract eyes

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Abstract

Purpose To analyze and compare the total proteome of aqueous humor (AH) from patients having primary angle closure glaucoma (PACG), primary open angle glaucoma (POAG) and age-related cataract.

Materials and methodology Aqueous humor was collected from age-matched PACG, POAG and cataract patients who underwent surgery, and it was immediately stored at − 80 °C until analysis. From each sample, 25 μg of total protein was subjected to trypsin digestion and subsequently LC–MS/MS analysis was performed for the deep proteome analysis. The data acquired after the LC–MS/MS analysis were analyzed using Proteome Discoverer 1.4. The identified peptide matches were validated using percolator, at less than 1% false discovery rates.

Results A total of 625, 594 and 636 proteins were identified in PACG, POAG and cataract groups, respectively ($n = 9$ in each group). The inter-group comparison among all these groups showed that 246 proteins were identified in all the three groups. An average of 236 ± 42, 218 ± 40 and 214 ± 62 proteins from each AH sample of PACG, POAG and cataract, respectively, was identified. There were 53 proteins commonly found in all 9 PACG AH, 59 proteins in POAG AH and 42 proteins in 9 cataracts AH samples. In the individual analysis, there were 28 proteins found in all the samples analyzed representing the "constitutive AH proteome." Spectral counting analysis of 246 proteins identified in all three group types showed significant differences in protein abundance. In proteins unique to PACG AH, 7 proteins viz. ARHGEF12, APC2, WAS, PIK3CG, ITGB1, MSN and PFN1 out of 226 were found in "Regulation of Actin Cytoskeleton" pathway, whereas in POAG 5 out of 206 proteins viz. ADCY2, ITPR1, MAPK3, MAP3K2 and TUBB1 were found in "Gap Junction" pathway.

Conclusions A qualitative as well as a quantitative comparison of proteomes of AH from PACG, POAG and age-related cataract eyes showed significant differences, thus providing clues to the disease pathophysiology.

Keywords Aqueous humor · Proteome · LC–MS/MS · Primary angle closure and open angle glaucoma

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Introduction

Glaucoma is a spectrum of multi-factorial ocular diseases and is one of the leading causes of vision impairment worldwide. It is a chronic progressive optic neuropathy, with raised intraocular pressure (IOP) being a major risk factor. The two commonly occurring glaucoma types are primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG). Pathomechanisms of glaucomatous neuropathy and trabecular abnormalities causing a raised intraocular pressure are still not fully understood, but have been attributed to a number of possible mechanisms such as oxidative stress [1], aging [2], vascular dysregulation, autoimmunity [3]. These processes possibly alter the aqueous humor (AH) flow dynamics within the eye. Aqueous humor composition is dynamic, as AH serves the important functions of supplying nutrients to the cornea, lens, and trabecular meshwork, while maintaining refraction and intraocular pressure (IOP) of the eye. Alterations in the protein composition of aqueous humor provide a window into altered biochemical processes, either leading to dysfunction or reflecting pathological processes in the surrounding tissues, such as the trabecular meshwork.

Several studies have described the protein composition of AH in different ophthalmic conditions including cataract [4, 5], myopia [6], corneal graft rejection [7], age-related macular degeneration [8], primary open angle glaucoma [9] and proteome of AH after glaucoma shunt device implant [10]. There are reports in literature focused on specific families of proteins in PACG aqueous, but the global proteome of PACG aqueous has not been described till date.

The present study compares the total proteome of AH, from patients having PACG, POAG or cataract to further our understanding of the pathophysiology of primary adult glaucomas.

Materials and methods

Iodoacetamide, dithiothreitol, ammonium bicarbonate, formic acid and protease inhibitor cocktail were purchased from SIGMA–Aldrich, USA. Sequencing grade trypsin was obtained from Promega Corp., Madison, WI and BCA protein estimation kit from Pierce (ThermoFisher Scientific, USA).

Subjects and collection of aqueous humor

Consecutive patients having an adult primary glaucoma (PACG or POAG) underwent a thorough clinical examination, including slit-lamp biomicroscopy, fundus examination using + 90D lens, applanation tonometry, gonioscopy and perimetry (Humphrey® Field Analyzer/HFA™ II-*i* Series). Patients were initially advised topical medical therapy. If "target IOP" was not achieved with maximally tolerated topical medications, a trabeculectomy was advised.

The inclusion criteria for this study were patients aged 50–60 years who have advised a trabeculectomy. The diagnostic criteria for the two primary glaucomas were:

Primary open angle glaucoma

An intraocular pressure > 21 mmHg on 3 different occasions with applanation tonometry, in the presence of open angles on gonioscopy, and characteristic optic nerve head and visual field changes suggestive of glaucoma.

Primary angle closure glaucoma

Patients having an "occludable" angle with peripheral anterior synechiae extending over at least 180° on indentation/manipulative gonioscopy, with a chronically raised IOP of over 21 mmHg on at least 3 occasions in the presence of optic nerve head and visual field changes suggestive of glaucoma.

Exclusion criteria for glaucoma patients were a patient having cataract, any other ocular pathology, history of an attack of acute angle closure or secondary glaucomas, prior surgery and patients who were unwilling or had any known systemic disease.

Age-matched individuals undergoing cataract surgery were recruited as control. Exclusion criteria for these patients were a patient having any other ocular pathology, any family history or suspicion of glaucoma, secondary glaucomas, prior surgery, and patients who were unwilling or had any known systemic disease.

The study was approved by our institutional ethics committee and adhered to the Tenets of the Declaration of Helsinki. Written informed consent was obtained from each patient.

Identical topical and systemic medications were used for all glaucoma patients included in this study at the time of the surgery such as timolol drops and tablet acetazolamide. AH was aspirated at the beginning of the surgical procedure through a limbal site, using a 27-gauge needle on a tuberculin syringe, taking special care to avoid blood contamination or the presence of iris pigment. The AH samples were transferred to pre-marked microcentrifuge tubes and kept frozen at − 80 °C.

Protein estimation and in-solution trypsin digestion

The total amount of protein in each sample was estimated by Bicinchoninic Assay (BCA) using a commercial protein estimation kit (ThermoFisher Scientific, USA). An equal amount (25 μg) of total protein from each patient of PACG, POAG and cataract was reduced with 10 mM dithiothreitol for 1 hour at room temperature and subsequently alkylated with 40 mM iodoacetamide for 45 minutes at room temperature. The reduced and alkylated proteins were digested with modified porcine trypsin overnight at 37 °C. The enzyme/total protein ratio was 1:50.

Liquid chromatography and mass spectrometry

Mass spectrometry was performed on a hybrid Orbitrap Velos Pro mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) using the Xcalibur version 2.1 coupled to a Nano LC-1000 HPLC nanoflow system (ThermoFisher Scientific, Waltham, MA, USA) via nanoelectrospray ion source. The tryptic peptide mixture (∼ 2 μg) from each sample (in duplicates) was loaded on to a pre-column (Acclaim Pep Map100-75 μm × 2 cm, C-18, 3 μm, 100A0) which was in line with an analytical column (Acclaim Pep Map RSLC-50 μm × 15 cm, C-18, 2 μm, 100A0). The peptides were eluted from the column with a gradient of 5% Solvent B: 95% acetonitrile in 0.1% formic acid, Solvent A: 5% acetonitrile in 0.1% formic acid to 50% Solvent B over a period of 180 minutes with a flow rate of 300 nL/min. The mass spectra were acquired in a data-dependent acquisition mode allowing the first scan as full scan in FTMS at a resolution of 60,000 and selecting Top-20 precursor ions to fragment, using collision-induced dissociation method in the ion trap. The threshold for switching from MS to MS/MS was 2000 counts.

Data analysis

The acquired data were processed with Proteome Discoverer 1.4 (ThermoFisher, Waltham, MA, USA) using SEQUEST algorithm with a mass tolerance of 10 ppm for MS and 0.6 Da for MS/MS. The raw files were searched against the human Uniprot database (UP000005640 proteome). A maximum of two missed tryptic cleavage sites were allowed. Carbamidomethyl cysteine was set as fixed modification, while oxidized methionine was kept as variable modification. Percolator available with Proteome Discoverer was used for validation of peptide matches at less than 1% false discovery rates (FDR). Spectral counting of the proteins identified in all three diseases was performed to evaluate the relative abundance of the proteins detected in three diseases.

Results

A total of 75 consecutive glaucoma patients were screened and reviewed. Nine eyes each of PACG and POAG that fulfilled all our inclusion and exclusion criteria underwent a trabeculectomy, and AH was collected. Concurrently, 9 age-matched patients undergoing a cataract surgery had their AH collected. The mean age of patients with PACG was 53.5 ± 3.8 years, POAG was 56.14 ± 2.2 years, and patients undergoing cataract surgery was 54.9 ± 2.4 years with no significant difference among the groups ($p = 0.64$). There were 14 males and 13 females, with no statistical difference in male to female ratio among the groups ($p = 0.81$). The IOP prior to aqueous aspiration was 18.5 ± 2.6, 18.8 ± 3.1, and 15.6 ± 2.3 mmHg, in PACG, POAG and cataract patients, respectively. The protein concentration was 2–5 mg/ml in PACG, 3–8 mg/ml in POAG and 1–3 mg/ml in cataract eye aqueous samples.

Trypsin-digested aqueous humor from individual samples was subjected to LC–MS/MS analysis. The raw data from each of the nine samples from each disease group were analyzed together to identify proteins in each disease type. A total of 625 proteins were identified in PACG AH at less than 1% FDR.

Similarly, 594 and 636 proteins were identified in POAG and cataract AH, respectively. When compared with each other, it was observed that 246 proteins were common in all three disease groups (Fig. 1). On an average, we have identified 236 ± 42, 218 ± 40 and 214 ± 62 proteins from each sample of PACG, POAG and cataract AH, respectively (Table 1). There were few proteins that were common to the 9 samples of each disease group, 53 in PACG (Table 2), 59 in POAG (Table 3) and 42 in cataract (Table 4). Also a total of 28 proteins were present in all 27 analyzed samples which represent the "Constitutive proteome of human aqueous humor" (Table 5).

To investigate the relative, differential protein expression if any, in proteins common to all three groups, spectral counting analysis of all the proteins detected in each disease group was performed and the normalized spectral abundance factor (NSAF) was calculated [11, 12]. The NSAF was then used to compare protein differential rates in the 246 proteins identified in all three groups (NSAF ratios of ≥ 2 and ≤ 0.5 being considered significant). This analysis revealed that 91 proteins in PACG AH and 97 proteins in POAG AH were significantly differentially expressed as compared with age-matched cataract AH. Out of these, 31 proteins were found to be up-regulated in both glaucoma types as compared to cataract AH and 24 proteins were down-regulated, in both subtypes of glaucoma, but were not significantly different as compared to age-matched cataract AH. Functional interaction analysis of these differentially expressed proteins of both glaucoma subtypes was performed using STRING. Gene ontology (GO) biological processes-based enrichment analysis of these proteins showed that proteins involved in tissue and vascular remodeling and immune response pathways were majorly up-regulated in AH of both glaucoma types as compared to cataract AH.

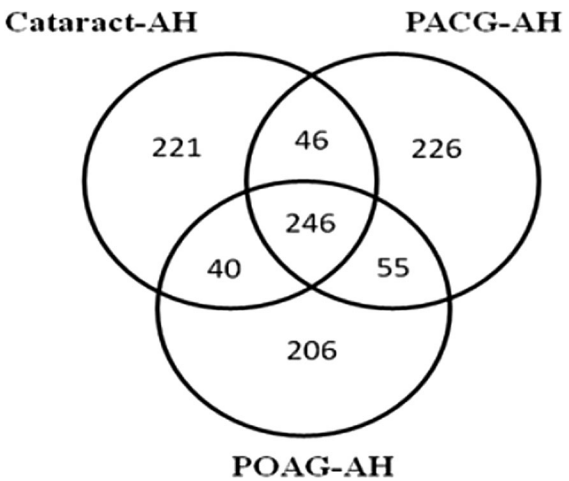

Fig. 1 Common proteins found in three disease groups

Proteins significantly up-regulated in PACG as compared to POAG AH were 72 kDa type IV collagenase, cDNA FLJ77835, highly similar to Homo sapiens complement component 1, s subcomponent (C1S), transcript variant 2, mRNA, Centrosomal protein C10orf90, Complement C1q subcomponent subunit B, DNA excision repair protein ERCC-6-like, Ganglioside GM2 activator, INADL protein, Metalloproteinase inhibitor 1, Monocyte differentiation antigen CD14, Pleiotrophin and Protein-glutamine gamma-glutamyltransferase Z.

Proteins significantly up-regulated in POAG as compared to PACG AH were 40S ribosomal protein S4, X isoform, cDNA FLJ75416, highly similar to Homo sapiens complement factor H (CFH), mRNA, cDNA FLJ78207, highly similar to Human complement protein component C7 mRNA, Complement C1q tumor necrosis factor-related protein 1, Inter-alpha-trypsin inhibitor heavy chain H2, Lipocalin-1, L-lactate dehydrogenase A chain, ProSAAS, Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (Testican) 2, isoform CRA_b, and Ubiquitin carboxyl-terminal hydrolase 40.

Cytoscape tool was used for pathway analysis, with pathway visualization using the KEGG source database. In proteins unique to PACG AH, 7 proteins viz. ARHGEF12, APC2, WAS, PIK3CG, ITGB1, MSN and PFN1 out of 226 were found in "Regulation of Actin Cytoskeleton" pathway. In POAG AH 5 out of 206 proteins viz. ADCY2, ITPR1, MAPK3, MAP3K2 and TUBB1 were found in "Gap Junction" pathway. Four proteins viz. VWF, C1QC, C5 and PROS1 out of a total of 221 proteins were found in the AH of cataract patients that formed part of the pathway known as "Complement and Coagulation Cascade."

Discussion

This study compared the global AH proteome of PACG and POAG with age-related cataract eyes having no other ocular or systemic disease. The proteomes of AH from various glaucomatous eyes,

Table 1 List of proteins found only in one disease type

221 proteins detected exclusively in "Cataract"	226 proteins detected exclusively in "PACG"	206 proteins detected exclusively in "POAG"
14-3-3 Protein zeta/delta (Fragment)	28S ribosomal protein S17, mitochondrial (Fragment)	1,2-Dihydroxy-3-keto-5-methylthiopentene dioxygenase
3-Ketoacyl-CoA thiolase, peroxisomal (Fragment)	3′-5′ 'Exonuclease TREX2 long form	39S ribosomal protein L44, mitochondrial
Acetyl-CoA carboxylase 2	3-Keto-steroid reductase	39S ribosomal protein L49, mitochondrial
Acidic leucine-rich nuclear phosphoprotein 32 family member B (Fragment)	60S ribosomal export protein NMD3 (Fragment)	45 kDa calcium-binding protein (Fragment)
Adenylate cyclase type 10	Acetoacetyl-CoA synthetase (Fragment)	A disintegrin and metalloproteinase with thrombospondin motifs 12
Alcohol dehydrogenase class-3 (Fragment)	Acetyl-CoA carboxylase 1	ACAP3 protein (Fragment)
Alpha-crystallin B chain (Fragment)	Activating transcription factor 7-interacting protein 2	Acidic fibroblast growth factor intracellular-binding protein
Alternative protein MFHAS1	Adenosine deaminase, RNA-specific, isoform CRA_b	All-trans-retinol 13,14-reductase (Fragment)
Alternative protein PLEKHA5	Alternative protein WIPI2	Alpha-parvin (Fragment)
Alternative protein TMPRSS7	Anion exchanger-1 variant	Alternative protein APAF1
ANKS1A protein (Fragment)	AP-2 complex subunit beta (Fragment)	Alternative protein C8orf38
Ankyrin repeat and SOCS box protein 16	AP-3 complex subunit beta-1	Alternative protein GFRA1
Annexin (Fragment)	Aryl hydrocarbon receptor repressor	Alternative protein PNKD
Anti-HER3 scFv (Fragment)	ATP-binding cassette sub-family F member 1 (Fragment)	ANKDD1A protein (Fragment)
Anti-streptococcal/anti-myosin immunoglobulin lambda light chain variable region (Fragment)	ATR-interacting protein (Fragment)	ANKRD12 protein (Fragment)
Archaemetzincin-2	Autophagy-related protein 16-2	Ankyrin repeat and LEM domain-containing protein 1
Arfaptin-2	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3	Annexin A2 (Fragment)
Arginine/serine-rich coiled-coil protein 2	Beta-crystallin A4	Apolipoprotein F
ATP-binding cassette sub-family A member 10	Beta-enolase	ATP-binding cassette sub-family A member 12
Beta-globin (Fragment)	Brain acid soluble protein 1	ATP-dependent RNA helicase DHX29
Beta-hexosaminidase subunit beta (Fragment)	Bromodomain adjacent to zinc finger domain protein 2A (Fragment)	Beta-globin
Bile acyl-CoA synthetase	C2 domain-containing protein 3	Calcium-activated potassium channel subunit alpha-1 (Fragment)
C1orf2 protein	Cartilage intermediate layer protein 1	Calcium-dependent secretion activator 1 (Fragment)
Calmodulin-like protein 5	Caspase recruitment domain family, member 14, isoform CRA_d	Caveolin (Fragment)
Cation channel sperm-associated protein 3	CCDC144B protein	cDNA FLJ23951 fis, clone HEP08954
cDNA FLJ11167 fis, clone PLACE1007257, highly similar to Protein diaphanous homolog 2	CCR4-NOT transcription complex subunit 10 (Fragment)	CDNA FLJ27201 fis, clone SYN03133

Table 1 continued

221 proteins detected exclusively in "Cataract"	226 proteins detected exclusively in "PACG"	206 proteins detected exclusively in "POAG"
cDNA FLJ32530 fis, clone SMINT2000185, highly similar to TRANSKETOLASE (EC 2.2.1.1)	CDK5 regulatory subunit-associated protein 2 (Fragment)	cDNA FLJ27339 fis, clone TMS09615, highly similar to TFIIH basal transcription factor complex helicase subunit (EC 3.6.1.-)
cDNA FLJ34132 fis, clone FCBBF3010586, weakly similar to Microtubule-associated protein orbit (Fragment)	cDNA FLJ10286 fis, clone HEMBB1001384, highly similar to COP9 signalosome complex subunit 4	cDNA FLJ30874 fis, clone FEBRA2004329, moderately similar to MYOSIN HEAVY CHAIN, CARDIAC MUSCLE ISOFORM
cDNA FLJ34277 fis, clone FEBRA2003524	cDNA FLJ30924 fis, clone FEBRA2006521, highly similar to Junctophilin-3	cDNA FLJ35087 fis, clone PLACE6005546, highly similar to Polymerase I and transcript release factor
cDNA FLJ36001 fis, clone TESTI2015213, highly similar to Homo sapiens target of EGR1, member 1 (nuclear) (TOE1), mRNA	cDNA FLJ39849 fis, clone SPLEN2014711, highly similar to Homo sapiens hect domain and RLD 6 (HERC6), transcript variant 1, mRNA	cDNA FLJ35722 fis, clone TESTI2001697, highly similar to Homo sapiens spermatogenesis associated 16 (SPATA16), mRNA
cDNA FLJ39243 fis, clone OCBBF2008283, highly similar to Protein NDRG1	cDNA FLJ40508 fis, clone TESTI2045850, highly similar to AP-3 complex subunit beta-2	cDNA FLJ35943 fis, clone TESTI2011750
cDNA FLJ51255, moderately similar to NKG2D ligand 3	cDNA FLJ45314 fis, clone BRHIP3005142, highly similar to Proteasome-associated protein ECM29 homolog	cDNA FLJ37350 fis, clone BRAMY2021825, highly similar to Homo sapiens voltage-gated channel like 1 (VGCNL1), mRNA
cDNA FLJ51288, highly similar to Kinesin heavy chain	cDNA FLJ45547 fis, clone BRTHA2035743, highly similar to Activated CDC42 kinase 1 (EC 2.7.10.2)	cDNA FLJ39132 fis, clone NTONG2008365, highly similar to Homo sapiens tetratricopeptide repeat domain 21B (TTC21B), mRNA
cDNA FLJ53570, highly similar to Keratin, type I cytoskeletal 16	cDNA FLJ45596 fis, clone BRTHA3017791, highly similar to F-box only protein 41	cDNA FLJ39219 fis, clone OCBBF2006849, weakly similar to Transmembrane protein 131
cDNA FLJ54162, moderately similar to CDP-diacylglycerol–inositol3-phosphatidyltransferase (EC 2.7.8.11)	cDNA FLJ51627, highly similar to Rho guanine nucleotide exchange factor 12	cDNA FLJ39664 fis, clone SMINT2007219, highly similar to SH2 domain-containing protein 3C
cDNA FLJ54342, highly similar to Heat shock 70 kDa protein 1	cDNA FLJ52922, highly similar to Serine/threonine-protein kinase WNK4 (EC 2.7.11.1)	cDNA FLJ39696 fis, clone SMINT2011033, highly similar to Sorting and assembly machinery component 50 homolog
cDNA FLJ54378, highly similar to Golgin subfamily B member 1 (Fragment)	cDNA FLJ52935, highly similar to CMRF35-like-molecule 1	cDNA FLJ41945 fis, clone PLACE6019676, highly similar to Coatomer subunit gamma
cDNA FLJ55295, highly similar to Nuclear factor of activated T-cells, cytoplasmic 4	cDNA FLJ53478, highly similar to Galectin-3-binding protein	cDNA FLJ42001 fis, clone SPLEN2029912
cDNA FLJ56286, highly similar to Homo sapiens meiosis defective 1 (MEI1), mRNA	cDNA FLJ54656, highly similar to Aryl hydrocarbon receptor nuclear translocator	cDNA FLJ43122 fis, clone CTONG3003737, highly similar to Leucine-rich repeat-containing protein 15
cDNA FLJ56823, highly similar to Protein-glutamine gamma-glutamyltransferase E (EC 2.3.2.13)	cDNA FLJ55023, highly similar to Adapter-related protein complex 1 sigma- 1B subunit	cDNA FLJ43785 fis, clone TESTI2052211

Table 1 continued

221 proteins detected exclusively in "Cataract"	226 proteins detected exclusively in "PACG"	206 proteins detected exclusively in "POAG"
cDNA FLJ57480, moderately similar to Platelet endothelial cell adhesion molecule	cDNA FLJ55124, highly similar to Plasma serine protease inhibitor	cDNA FLJ45304 fis, clone BRHIP3003984, highly similar to IkappaB kinase complex-associated protein
cDNA FLJ59064, highly similar to UGA suppressor tRNA-associated protein	cDNA FLJ57044, highly similar to Homo sapiens fetal Alzheimer antigen (FALZ), transcript variant 1, mRNA	cDNA FLJ45819 fis, clone NT2RP8001363, highly similar to Mus musculus signal peptide, CUB domain, EGF-like 1 (Scube1)
cDNA FLJ60999, highly similar to Centromere protein I	cDNA FLJ57396, highly similar to Lipoprotein lipase (EC 3.1.1.34)	cDNA FLJ50835, highly similar to Retinoic acid-induced protein 2
cDNA FLJ61084, highly similar to Angiopoietin-1	cDNA FLJ57840, highly similar to Chromodomain-helicase-DNA-binding protein 7 (EC 3.6.1.-)	cDNA FLJ52634, highly similar to G-protein-coupled receptor 120
cDNA FLJ61480	cDNA FLJ58131, highly similar to Secretogranin-1	cDNA FLJ53503, highly similar to Plasma serine protease inhibitor
cDNA FLJ61743, highly similar to Class B basic helix-loop-helix protein 3	cDNA FLJ58169, highly similar to Anaphase-promoting complex subunit 2 (APC2)(Cyclosome subunit 2)	cDNA FLJ54510, highly similar to Adenylate cyclase type 2 (EC 4.6.1.1)
cDNA FLJ75522, highly similar to Homo sapiens von Willebrand factor (VWF), mRNA (Fragment)	cDNA FLJ58989, moderately similar to ADP-ribosylation factor-like protein 2	cDNA FLJ54546, highly similar to Homo sapiens sarcolemma associated protein (SLMAP), mRNA
cDNA FLJ75846, highly similar to Homo sapiens cytochrome P450, family 19, subfamily A, polypeptide 1 (CYP19A1), transcript variant 1, mRNA	cDNA FLJ60265, highly similar to Adenylate cyclase type 2 (EC 4.6.1.1)	cDNA FLJ54912, highly similar to 60 kDa heat shock protein, mitochondrial
cDNA FLJ77092, highly similar to Homo sapiens phosphatidylinositol transfer protein, cytoplasmic 1 (PITPNC1), transcript variant 2, mRNA	cDNA FLJ60975	cDNA FLJ55364, highly similar to CRSP complex subunit 6
cDNA FLJ77399, highly similar to Homo sapiens cofactor required for Sp1 transcriptional activation, subunit 2, 150 kDa (CRSP2), mRNA	cDNA FLJ61016	cDNA FLJ56520, highly similar to CG1 protein
cDNA FLJ90719 fis, clone PLACE1008744, highly similar to Mus musculus sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1, mRNA	cDNA FLJ61075, highly similar to Mineralocorticoid receptor	cDNA FLJ58224, highly similar to Calpain-2 catalytic subunit (EC 3.4.22.53)
cDNA PSEC0206 fis, clone HEMBA1002913, weakly similar to CARBOXYPEPTIDASE H (EC 3.4.17.10)	cDNA FLJ61371, highly similar to Phosphatidylinositol 3-kinase regulatory subunit alpha	cDNA FLJ58776, highly similar to Nidogen-2
cDNA, FLJ79515, highly similar to Kinesin-like protein KIF2	cDNA FLJ61630, highly similar to Bardet–Biedl syndrome 10 protein	cDNA FLJ59550, highly similar to Homo sapiens amyloid beta (A4) protein, transcript variant 3, mRNA
cDNA, FLJ93389, highly similar to Homo sapiens multiple inositol polyphosphate histidine phosphatase, 1 (MINPP1), mRNA	cDNA FLJ61714, highly similar to Tripeptidyl-peptidase 2 (EC 3.4.14.10) (Fragment)	cDNA FLJ59628, highly similar to Etoposide-induced protein 2.4

Table 1 continued

221 proteins detected exclusively in "Cataract"	226 proteins detected exclusively in "PACG"	206 proteins detected exclusively in "POAG"
cDNA, FLJ93695, highly similar to Homo sapiens serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4 (SERPINA4), mRNA	cDNA FLJ76059, highly similar to Homo sapiens dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4 (DYRK4), mRNA	cDNA FLJ61351, highly similar to Fanconi anemia group A protein
cDNA, FLJ95242, highly similar to Homo sapiens L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain (HADHSC), mRNA	cDNA FLJ77260, highly similar to Homo sapiens phosphoinositide-3-kinase, catalytic, gamma polypeptide (PIK3CG), mRNA	cDNA FLJ75171, highly similar to Homo sapiens amyloid beta (A4) protein-binding, family B, member 2 (Fe65-like) (APBB2), mRNA
cDNA, FLJ95441, highly similar to Homo sapiens zinc finger protein 347 (ZNF347), mRNA	cDNA FLJ78137, highly similar to Homo sapiens uronyl-2-sulfotransferase (UST), mRNA	cDNA FLJ76468, highly similar to Homo sapiens cullin 7 (CUL7), mRNA
cDNA, FLJ95817, highly similar to Homo sapiens ZYG homolog (ZYG), mRNA	cDNA FLJ78318, highly similar to Homo sapiens sestrin 1 (SESN1), mRNA	cDNA FLJ76647, highly similar to Homo sapiens zinc finger protein 198 (ZNF198), mRNA (Fragment)
Cell adhesion molecule 3	cDNA, FLJ79037, highly similar to Myosin-10	cDNA, FLJ79457, highly similar to Insulin-like growth factor-binding protein complex acid labile chain
CENPC1 protein	cDNA, FLJ79139, highly similar to Alkyl dihydroxyacetone phosphate synthase, peroxisomal (EC 2.5.1.26)	cDNA, FLJ92530, highly similar to Homo sapiens chromogranin B (secretogranin 1) (CHGB), mRNA
Centrosome and spindle pole-associated protein 1	cDNA, FLJ79240, moderately similar to Neutrophil cytosol factor 1	cDNA, FLJ92723, highly similar to Homo sapiens Down syndrome critical region gene 1-like 1 (DSCR1L1), mRNA
Chromodomain Y-like protein	cDNA, FLJ79538, highly similar to Prominin-1	cDNA, FLJ95135
Chromosome 10 open reading frame 47, isoform CRA_b	cDNA, FLJ94688, highly similar to Homo sapiens solute carrier family 10 (sodium/bile acid cotransporter family), member 1 (SLC10A1), mRNA	cDNA, FLJ96567, highly similar to Homo sapiens propionyl Coenzyme A carboxylase, alpha polypeptide(PCCA), mRNA
C-Jun-amino-terminal kinase-interacting protein 3	cDNA, FLJ95281, highly similar to Homo sapiens a disintegrin-like and metalloprotease (reprolysintype) with thrombospondin type 1 motif, 1 (ADAMTS1), mRNA	cDNA: FLJ21924 fis, clone HEP04086
Cofilin-1 (Fragment)	Cell adhesion molecule 4	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 4 (Fragment)
Coiled-coil domain-containing protein 154	Centromere protein F	Coiled-coil domain-containing protein 3
Coiled-coil domain-containing protein 24	cGMP-dependent 3′,5′-cyclic phosphodiesterase (Fragment)	Coiled-coil domain-containing protein 41
Collagen alpha-2(XI) chain	Coiled-coil domain-containing protein 38	Coiled-coil domain-containing protein 67
Complement C1q subcomponent subunit C	Collagen alpha-1(XXI) chain (Fragment)	Cyclin-dependent kinase 4 inhibitor C
Complement component 5 variant (Fragment)	Complement C5	Cystatin-F
Complement factor H-related protein 1	Complement component C8 gamma chain	Dickkopf-related protein 3
Cystatin-11	CROCC protein (Fragment)	Disintegrin and metalloproteinase domain-containing protein 11 (Fragment)
Cystatin-A	Cytochrome P450 2W1	DNA polymerase epsilon catalytic subunit A

Table 1 continued

221 proteins detected exclusively in "Cataract"	226 proteins detected exclusively in "PACG"	206 proteins detected exclusively in "POAG"
Cytidine and dCMP deaminase domain-containing protein 1	Cytoplasmic dynein 1 heavy chain 1	DNA topoisomerase
Desmocollin 1, isoform CRA_b	Developmental pluripotency-associated protein 4	Dombrock blood group protein (Fragment)
Desmoglein-1	Diacylglycerol kinase, gamma 90 kDa variant (Fragment)	Dyad symmetry binding protein (Fragment)
Desmoplakin	Disks large-associated protein 3	Dynein heavy chain 6, axonemal
Dimethylaniline monooxygenase [N-oxide-forming] 1	DNA polymerase (Fragment)	Dystroglycan
Dipeptidyl-peptidase II variant (Fragment)	Dynein heavy chain 3, axonemal	E3 ubiquitin-protein ligase RNF133
DNA replication complex GINS protein SLD5	E3 ubiquitin-protein ligase TRIP12	E3 ubiquitin-protein ligase RNF25
DNA-directed RNA polymerase III subunit RPC2 (Fragment)	Egl nine homolog 2	E3 ubiquitin-protein ligase UBR3 (Fragment)
Doublecortin domain-containing protein 5 (Fragment)	Enolase	Ectonucleoside triphosphate diphosphohydrolase 7
Dual specificity protein phosphatase 15	Ephrin type-A receptor 10	EF-hand calcium-binding domain-containing protein 5
Dynein heavy chain 5, axonemal	Ephrin-A5	FAM166B protein
Dynein heavy chain 7, axonemal	ERI1 exoribonuclease 2	Fanconi anemia group M protein (Fragment)
Dynein intermediate chain 2, axonemal	F-box/LRR-repeat protein 5 (Fragment)	F-box only protein 2
Dynein light chain roadblock-type 1	FEX1	FBXO18 protein (Fragment)
E3 ubiquitin-protein ligase CBL	Full-length cDNA clone CS0DI068YF21 of Placenta of Homo sapiens (human)	FERM domain-containing protein 7
E3 ubiquitin-protein ligase UBR5	Gamma-aminobutyric acid type B receptor subunit 1	Fetuin-B (Fragment)
EGF-containing fibulin-like extracellular matrix protein 2 (Fragment)	Gamma-interferon-inducible lysosomal thiol reductase	Fibrillin-2
ELM2 and SANT domain-containing protein 1 (Fragment)	Gastric inhibitory polypeptide	Fibrinogen beta chain, isoform CRA_e
Exophilin-5 (Fragment)	Glutamate receptor ionotropic, kainate 3	Fibulin-1 (Fragment)
FAST kinase domain-containing protein 1 (Fragment)	Glycine decarboxylase P-protein (Fragment)	Forkhead box protein J3 (Fragment)
Fatty acid-binding protein, epidermal	GTF2IRD2B protein (Fragment)	Frizzled-9
F-box only protein 15 (Fragment)	Hemicentin-2	Galectin-4 (Fragment)
F-box only protein 27 (Fragment)	Hemoglobin beta subunit variant (Fragment)	Glutaryl-CoA dehydrogenase, mitochondrial
F-box only protein 40	HHIP-like protein 2	Glutathione S-transferase P
FCH domain only protein 2	Histone H4 (Fragment)	Glyceraldehyde-3-phosphate dehydrogenase (Fragment)
Fibrinogen silencer-binding protein	Insulin-like growth factor 1 receptor (Fragment)	Golgi membrane protein 1
Filaggrin-2	Insulin-like growth factor-binding protein 4	G-protein-coupled receptor 3
Follistatin-related protein 5	Integrin beta-1	G-protein-coupled receptor-associated sorting protein 1

Table 1 continued

221 proteins detected exclusively in "Cataract"	226 proteins detected exclusively in "PACG"	206 proteins detected exclusively in "POAG"
G protein-coupled receptor family C group 5 member B variant 2	Integrin beta-like protein 1	HCG17955, isoform CRA_a
Gametocyte-specific factor 1	Interleukin-17 receptor A	HCG1816139
Gastric intrinsic factor	IQ motif and SEC7 domain-containing protein 3	Hemoglobin alpha-1 globin chain variant (Fragment)
Glycosyltransferase-like protein LARGE1 (Fragment)	Kaptin	Homeo box A3
Golgin-45 (Fragment)	Kinesin-like protein KIF1C	Huntingtin
G-protein-coupled receptor 98	Kinesin-like protein KIF26B	Inhibitor of growth protein 5
H/ACA ribonucleoprotein complex subunit 4 (Fragment)	Latent-transforming growth factor-beta-binding protein 2	TNF (Fragment)
HCG2015269, isoform CRA_c	LATS homolog 1 variant (Fragment)	Inositol 1,4,5-trisphosphate receptor type 1 variant (Fragment)
Heat shock protein beta-1	Leucine-rich repeat-containing protein 45	Inositol polyphosphate 1-phosphatase (Fragment)
Hemoglobin beta chain variant Hb.Sinai-Bel Air (Fragment)	Lipase member N	Insulin-like growth factor 2
Hereditary hemochromatosis protein (Fragment)	Liver carboxylesterase 1	Integrator complex subunit 3
HERV-K_5q33.3 provirus ancestral Gag polyprotein	Macrophage erythroblast attacher (Fragment)	Josephin-1 (Fragment)
Hexaribonucleotide binding protein 3	Macrophage-capping protein (Fragment)	KIR2DL1
Histone deacetylase 9 (Fragment)	MAML2 protein	LIM domain only protein 7 (Fragment)
Histone-lysine N-methyltransferase NSD3	Moesin	L-lactate dehydrogenase
Histone-lysine N-methyltransferase SETD2 (Fragment)	MOZ/CBP protein (Fragment)	LO AKAP12 92160
Hyaluronan-binding protein 2	Myelin-associated glycoprotein	LRP2 binding protein, isoform CRA_a
Immunoglobulin superfamily containing leucine-rich repeat protein	NADH-ubiquinone oxidoreductase chain 2	MAP kinase-activated protein kinase 3 (Fragment)
Insulin-like growth factor-binding protein 2	Natural killer cell immunoglobulin-like receptor (Fragment)	Mediator of RNA polymerase II transcription subunit 8
Insulinoma-associated protein 1	Nebulin (Fragment)	Microtubule-associated tumor suppressor candidate 2
Junction plakoglobin	Nephrocystin-3	Mitochondrial coenzyme A transporter SLC25A42
LanC-like protein 1 (Fragment)	Neuroblastoma-amplified sequence (Fragment)	Mitogen-activated protein kinase kinase 2, isoform CRA_d
Layilin (Fragment)	Neuronal acetylcholine receptor alpha 4 subunit (Fragment)	Mitogen-activated protein kinase kinase kinase 15
Leucine-rich repeat, immunoglobulin-like domain and transmembrane domain-containing protein 1	NRCAM protein	Monocyte differentiation antigen CD14, urinary form (Fragment)
Lipolysis-stimulated lipoprotein receptor	Nuclear receptor coactivator 1	Myb/SANT-like DNA-binding domain-containing protein 2
LMTK2 protein (Fragment)	Out at first protein homolog	Myosin-IIIa
Lysophospholipid acyltransferase 5	Oxidoreductase-like domain-containing protein 1	N-acetylglucosaminyl-phosphatidylinositol de-N-acetylase
Macrophage migration inhibitory factor	Oxysterols receptor LXR-beta	NACHT, LRR and PYD domains-containing protein 9

Table 1 continued

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Mast/stem cell growth factor receptor variant (Fragment)	P2X purinoceptor 7	NADH-ubiquinone oxidoreductase chain 5
MCM2 protein (Fragment)	Palmitoyl-protein thioesterase 1	Natriuretic peptides B
MIA2 protein (Fragment)	PDE8A protein (Fragment)	Neuroblastoma breakpoint family member 10
Misshapen-like kinase 1 (Fragment)	Phenylalanine–tRNA ligase, mitochondrial	Neuronal pentraxin-2
Mitochondrial ribonuclease P protein 1 (Fragment)	Phospholipid transfer protein	N-methyl-D-aspartate receptor subunit 2D variant (Fragment)
Mitogen-activated protein kinase kinase kinase 19 (Fragment)	PNAS-101	Notchless protein homolog 1 (Fragment)
MSTP128	Poly(A) polymerase beta	Nuclear pore complex protein Nup133
Neurabin-1 (Fragment)	Polycomb complex protein BMI-1 (Fragment)	Nuclear receptor coactivator-6 gamma
Neuralized-like protein 4 (Fragment)	Potassium channel subfamily U member 1	Nuclear receptor subfamily 0 group B member 1
Nuclear pore membrane glycoprotein 210-like	Probable guanine nucleotide exchange factor MCF2L2	Nucleoredoxin-like protein 1
Olfactory receptor 52B2	Profilin-1	OK/SW-CL.19
Olfactory receptor 56B1	Protein FAM26D	Olfactory receptor 14J1
Outer dense fiber protein 2-like (Fragment)	Protein FAM57B	Oncogene
Oxysterol-binding protein-related protein 11	Protein MROH8	Osteopontin-C (Fragment)
Paired immunoglobulin-like type 2 receptor alpha (Fragment)	Protein scribble homolog (Fragment)	Ovostatin homolog 2
Peptidyl-prolyl cis–trans isomerase FKBP5	Protein TANC2	Peflin
PHD finger protein 14	Protein unc-119 homolog B	Peroxisome proliferator-activated receptor gamma coactivator 1 alpha transcript variant B5-NT
Phosphatidate phosphatase LPIN2	Protocadherin beta-1	Plasma membrane calcium-transporting ATPase 1
Phosphoglycerate kinase	Protocadherin Fat 3	POC1 centriolar protein homolog B
PLA2G4F protein	Protocadherin Fat 4	Poly [ADP-ribose] polymerase 12 (Fragment)
Plakophilin-1	PRSS12 protein	Potassium voltage-gated channel subfamily D member 1
Plasma alpha-L-fucosidase	Pseudopodium-enriched atypical kinase 1	Potassium voltage-gated channel subfamily V member 2
PML-RARA regulated adaptor molecule 1, isoform CRA_b	Putative collagen homolog protein-b	PP4664
Polyamine-modulated factor 1-binding protein 1 (Fragment)	Putative DYT3 protein	PPP1R9A protein
Polymerase (Fragment)	Putative olfactory receptor 51H1	Pregnancy-specific beta-1-glycoprotein 5
Polypyrimidine tract-binding protein 2	Putative tripartite motif-containing protein 64C	PRO1770

Table 1 continued

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PREDICTED: filaggrin variant (Fragment)	Putative uncharacterized protein	Probable 2-oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial (Fragment)
Probable ATP-dependent RNA helicase DHX37	Putative uncharacterized protein (Fragment)	Proliferation-inducing protein 33
Probable G-protein-coupled receptor 75	Putative uncharacterized protein DKFZp434G1930 (Fragment)	Proline-rich basic protein 1
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	Putative uncharacterized protein EVC (Fragment)	Proteasome activator complex subunit 4
Prostaglandin E2 receptor EP1 subtype	Putative uncharacterized protein FLJ43826	Protein C9orf3
Protein S100-A8	Putative uncharacterized protein MGAT5 (Fragment)	Protein disulfide-isomerase (Fragment)
Protein SCAF11 (Fragment)	RAD21L	Protein LOC100128326
Protocadherin-15-CD3 isoform 1	Ral GTPase-activating protein subunit alpha-2	Protein MROH8 (Fragment)
Purkinje cell protein 4	Rearranged VKA17 V gene segment (Fragment)	Protein NDRG3
Putative homeodomain transcription factor 1	Retinoic acid receptor responder protein 1	Protein odr-4 homolog
Putative interleukin-17 receptor E-like	Rheumatoid factor RF-IP14 (Fragment)	Protein S100-A10
Putative tyrosine-protein phosphatase auxilin	Rho guanine nucleotide exchange factor 33	Putative heat shock protein HSP 90-alpha A4
Putative uncharacterized protein	Rho-related BTB domain-containing protein 3 (Fragment)	Putative hydroxypyruvate isomerase (Fragment)
Putative uncharacterized protein DKFZp686E18167	Ribonuclease P/MRP protein subunit POP5	Putative uncharacterized protein
Putative uncharacterized protein DKFZp686K18196 (Fragment)	Secretogranin-1	Putative uncharacterized protein CENTG2 (Fragment)
Putative uncharacterized protein DKFZp686N10185 (Fragment)	Serine protease 44	Putative uncharacterized protein DKFZp686H16106 (Fragment)
Putative uncharacterized protein DKFZp761D0223 (Fragment)	Serine/threonine-protein kinase Nek11	Putative uncharacterized protein DKFZp686O061
Putative uncharacterized protein DKFZp761L1623 (Fragment)	Serine/threonine-protein kinase pim-1	Putative uncharacterized protein FLJ46235
Putative uncharacterized protein LAPTM4A (Fragment)	Serum amyloid A-1 protein	Putative zinc-alpha-2-glycoprotein-like 1
Putative uncharacterized protein MCM6 (Fragment)	Signal peptide peptidase-like 2C	Ras-related protein Rap-2a
Putative zinc finger protein	Single-chain Fv (Fragment)	Ras-responsive element-binding protein 1
Pyruvate carboxylase, mitochondrial	Sodium channel protein type 10 subunit alpha	REV25-2 (Fragment)
Rab proteins geranylgeranyltransferase component A 1	Spectrin, alpha, erythrocytic 1 (Elliptocytosis 2), isoform CRA_c	Rhox homeobox family member 1
RGS9 protein	Stanniocalcin-1	RING finger protein 208
Rheumatoid factor RF-IP9 (Fragment)	Tankyrase-2	SCO-spondin
Riboflavin kinase	Target of Nesh-SH3	Serine/threonine-protein kinase Nek11
RNA-binding protein 28	Testis-expressed sequence 11 protein	Serine/threonine-protein kinase Nek8
RNF19B protein	Thrombin light chain	Solute carrier family 35 member G2

Table 1 continued

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RPS6KA4 protein (Fragment)	TOX high mobility group box family member 3	SRSF protein kinase 1
S protein	Transcription initiation factor TFIID subunit 6 (Fragment)	Sushi, nidogen and EGF-like domain-containing protein 1
SBF1 protein (Fragment)	Transient receptor potential cation channel subfamily A member 1 (Fragment)	TBC1 domain family member 15 (Fragment)
Selenophosphate synthetase 2	Transient receptor potential cation channel subfamily V member 2 (Fragment)	Tetratricopeptide repeat protein 40
Selenoprotein P (Fragment)	Transmembrane protein 243	Titin
Sentrin-specific protease 1	Trefoil factor 1	TNPO2 variant protein (Fragment)
Serine/threonine-protein kinase BRSK1	Trichohyalin-like protein 1	Transmembrane protein 251
Serpin B4 (Fragment)	Tripartite motif-containing protein 51	tRNA-dihydrouridine(16/17) synthase [NAD(P)(+)]-like (Fragment)
SH3 and cysteine-rich domain-containing protein 2	tRNA pseudouridine(38/39) synthase	Tubulin beta-1 chain
Signal transduction protein CBL-C	Truncated beta-globin	Type I inositol 1,4,5-trisphosphate 5-phosphatase (Fragment)
Ski-like protein	TTC29 protein	Uncharacterized protein C2orf71
SLCO1A2 protein	Tuftelin-interacting protein 11	Uncharacterized protein C9orf174
Small proline-rich protein 2E	Ubiquitin carboxyl-terminal hydrolase 40	Uncharacterized protein KIAA1109 (Fragment)
Sphingosine-1-phosphate lyase 1 (Fragment)	Ubiquitously transcribed tetratricopeptide repeat protein Y-linked transcript variant 69	Urea transporter 2
Synapse differentiation-inducing gene protein 1	UBX domain-containing protein 7 (Fragment)	Vacuolar protein sorting-associated protein 51 homolog
Synaptojanin-1 (Fragment)	UDP-glucuronosyltransferase 1-6 (Fragment)	Vascular endothelial growth factor C
Tcell alpha chain (Fragment)	UGCGL2 protein (Fragment)	Vesicular integral-membrane protein VIP36 (Fragment)
Transcription factor E2F5 (Fragment)	Uncharacterized protein	WD repeat-containing protein 48
Transcription factor SOX-13 (Fragment)	Uncharacterized protein (Fragment)	Xin actin-binding repeat-containing protein 2
Trans-golgi network protein 2, isoform CRA_a	Uncharacterized protein (Fragment)	Xyloside xylosyltransferase 1
Transmembrane protein 101	Uncharacterized protein (Fragment)	Zinc finger homeobox protein 3
TTLL5 protein (Fragment)	Uncharacterized protein C1orf94	Zinc finger protein 180
Tubulin polyglutamylase TTLL7	Uncharacterized protein C9orf9	Zinc fingers and homeoboxes protein 3 (Fragment)
Ubiquitin-60S ribosomal protein L40 (Fragment)	Uncharacterized protein ENSP00000382033	
UDP-glucuronosyltransferase 2 family, polypeptide B4, isoform CRA_a	UPF0606 protein KIAA1549	
Ugl-Y3	UPF0679 protein C14orf101 (Fragment)	
UNC13B protein	Vesicle-associated membrane protein 1	

Table 1 continued

221 proteins detected exclusively in "Cataract"	226 proteins detected exclusively in "PACG"	206 proteins detected exclusively in "POAG"
Uncharacterized protein	Vesicular integral-membrane protein VIP36	
UPF0317 protein C14orf159, mitochondrial	Vimentin	
Uracil phosphoribosyltransferase homolog	Vitamin K-dependent protein S (Fragment)	
V_segment translation product (Fragment)	Wiskott-Aldrich syndrome protein	
Vacuolar-sorting protein SNF8 (Fragment)	Yorkie homolog	
VH3 protein (Fragment)	Zinc finger CCHC domain-containing protein 14 (Fragment)	
VK3 protein (Fragment)	Zinc finger FYVE domain-containing protein 26	
Zinc finger CW-type PWWP domain protein 1 (Fragment)	Zinc finger protein 202 (Fragment)	
Zinc finger MYND domain-containing protein 19	Zinc finger protein 408	
Zinc finger protein 395 (Fragment)	Zinc finger protein 425 (Fragment)	
Zinc finger protein 836	Zinc finger protein 480 (Fragment)	
	Zinc finger protein 606 (Fragment)	
	Zinc finger protein 782 (Fragment)	
	Zinc finger protein 90 homolog	
	Zinc transporter 6	
	Zinc transporter ZIP8	

POAG, operated eyes, etc., have been described earlier [4, 10]; however, to the best of our knowledge, this is the first report of the global AH proteome from PACG eyes.

In earlier studies, different methods were used to identify individual protein in aqueous humor of glaucoma. [13–15]. However, due to low sample volume and low protein concentration in aqueous humor, wide ranges of the protein studies were limited using traditional methods. But our study compares the total proteome of AH, from patients having PACG, POAG or cataract. In our study, the relative quantitative proteomic analysis of 246 proteins identified in all three disease groups, showed a significant differential regulation of more than 100 proteins in glaucoma as compared to cataract (NSAF ratios of ≥ 2 and ≤ 0.5). Fifty-five proteins were identified in both glaucoma groups, but not from cataract AH in this study. The identification of proteins seen in all aqueous samples of both groups of glaucoma patients in our study points to some common pathways which might be involved in the glaucomatous process. The GO Biological processes-based enrichment analysis using STRING showed that proteins involved in tissue and vascular remodeling and proteins from immune response pathways were majorly up-regulated in AH of both glaucoma types as compared to cataract AH. The up-regulated proteins involved in "Immune system processes/immune response pathways" include CD14, CD59, CFD, RIRREL3, APOA4, CHGA and MYB. The proteins involved in "blood coagulation/regulation of body fluids" pathways were also up-regulated in glaucoma AH, and these include TIMP1, CFD, CD59 and MYB. In addition, proteins involved in "response to light stimulus" pathway were also up-regulated and include MFAP4, AGRN, APOA4 and APOC3. Out of these proteins APOA4, AGRN and C3 are also part of "photo-transduction and retinoic metabolic process" pathways. The finding that proteins involved in the above-mentioned pathways were up-regulated in both PACG and POAG eyes could allow a better understanding of the

Table 2 List of common proteins present in all nine samples of PACG AH

Number	Accession	Description	Molecular function	Cellular component	Biological process
1	A8K050	cDNA FLJ75376, highly similar to Homo sapiens peptidoglycan recognition protein L (PGLYRP) mRNA OS = Homo sapiens PE = 2 SV = 1—[A8K050_HUMAN]	Metal ion binding; catalytic activity; receptor activity; signal transducer activity	Extracellular; membrane	Metabolic process; cell communication; defense response; regulation of biological process; response to stimulus; cell differentiation; development
2	A8KAJ3	cDNA FLJ77823, highly similar to Homo sapiens EGF-containing fibulin-like extracellular matrix protein 1, transcript variant 3, mRNA OS = Homo sapiens PE = 2 SV = 1—[A8KAJ3_HUMAN]	Catalytic activity; receptor activity; signal transducer activity; protein binding; metal ion binding	Extracellular; cell surface; membrane	Metabolic process; regulation of biological process; cell communication; response to stimulus; cell organization and biogenesis; cell differentiation; development
3	B3KXD3	cDNA FLJ45230 fis, clone BRCAN2021325, highly similar to Carboxypeptidase E (EC 3.4.17.10) OS = Homo sapiens PE = 2 SV = 1—[B3KXD3_HUMAN]	Catalytic activity; metal ion binding; protein binding	Extracellular; nucleus; cytoplasm; Golgi; membrane	Development; metabolic process; cell communication; regulation of biological process; response to stimulus; cell organization and biogenesis
4	B4E1B2	cDNA FLJ53691, highly similar to Serotransferrin OS = Homo sapiens PE = 2 SV = 1—[B4E1B2_HUMAN]	Protein binding; metal ion binding	Extracellular; cytoplasm; mitochondrion; endosome; membrane; cell surface; organelle lumen	Transport; cellular homeostasis; coagulation; response to stimulus
5	B4E1B3	cDNA FLJ53950, highly similar to Angiotensinogen OS = Homo sapiens PE = 2 SV = 1—[B4E1B3_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; cytoplasm	Development; reproduction; cell growth; cell organization and biogenesis; regulation of biological process; metabolic process; response to stimulus; transport; cellular homeostasis; cell communication; cell differentiation; cell proliferation; cellular component movement; cell death; defense response
6	D9IWP9	Beta-2-glycoprotein I (Fragment) OS = Homo sapiens PE = 2 SV = 1—[D9IWP9_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cell surface	Cell proliferation; regulation of biological process; metabolic process; coagulation; response to stimulus; cellular component movement; development; cell death; transport
7	F6KPG5	Albumin (Fragment) OS = Homo sapiens PE = 2 SV = 1—[F6KPG5_HUMAN]	DNA binding; metal ion binding; protein binding; antioxidant activity; catalytic activity	Extracellular; nucleus; membrane; cytoplasm; organelle lumen	Transport; metabolic process; response to stimulus; coagulation; cell communication; cell death; regulation of biological process

Table 2 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
8	G3V2V8	Epididymal secretory protein E1(Fragment) OS = Homo sapiens GN = NPC2 PE = 2 SV = 1—[G3V2V8_HUMAN]			
9	G3V357	Ribonuclease pancreatic OS = Homo sapiens GN = RNASE1 PE = 2 SV = 1—[G3V357_HUMAN]	Catalytic activity		Metabolic process
10	M0R3H1	B cell receptor CD22 (Fragment) OS = Homo sapiens GN = CD22 PE = 4 SV = 1—[M0R3H1_HUMAN]	Protein binding	Membrane; cell surface	
11	O60888	Protein CutA OS = Homo sapiens GN = CUTA PE = 1 SV = 2—[CUTA_HUMAN]	Protein binding	Membrane; extracellular	Response to stimulus; cell organization and biogenesis
12	P00747	Plasminogen OS = Homo sapiens GN = PLG PE = 1 SV = 2—[PLMN_HUMAN]	Catalytic activity; metal ion binding; protein binding	Extracellular; membrane; cell surface; cytoplasm; organelle lumen	Transport; metabolic process; cell death; coagulation; response to stimulus; cell proliferation; regulation of biological process; cell organization and biogenesis; cell differentiation; cellular homeostasis; development; cellular component movement
13	P00748	Coagulation factor XII OS = Homo sapiens GN = F12 PE = 1 SV = 3—[FA12_HUMAN]	Catalytic activity; protein binding	Extracellular; membrane	Defense response; metabolic process; response to stimulus; regulation of biological process; coagulation
14	P01008	Antithrombin-III OS = Homo sapiens GN = SERPINC1 PE = 1 SV = 1—[ANT3_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; membrane	Response to stimulus; coagulation; metabolic process; regulation of biological process; defense response
15	P01009	Alpha-1-antitrypsin OS = Homo sapiens GN = SERPINA1 PE = 1 SV = 3—[A1AT_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; endoplasmic reticulum; Golgi; organelle lumen	Response to stimulus; transport; defense response; coagulation; metabolic process; regulation of biological process
16	P01011	Alpha-1-antichymotrypsin OS = Homo sapiens GN = SERPINA3 PE = 1 SV = 2—[AACT_HUMAN]	DNA binding; enzyme regulator activity; protein binding	Extracellular; nucleus	Defense response; response to stimulus; metabolic process; regulation of biological process
17	P01023	Alpha-2-macroglobulin OS = Homo sapiens GN = A2 M PE = 1 SV = 3—[A2MG_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; cytosol; organelle lumen	Defense response; metabolic process; regulation of biological process; response to stimulus; transport; cell communication; coagulation; cell organization and biogenesis; cell differentiation

Table 2 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
18	P01024	Complement C3 OS = Homo sapiens GN = C3 PE = 1 SV = 2—[CO3_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; membrane	Defense response; regulation of biological process; response to stimulus; metabolic process; cell communication; transport; development; cell organization and biogenesis
19	P01034	Cystatin-C OS = Homo sapiens GN = CST3 PE = 1 SV = 1—[CYTC_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; vacuole; endosome; endoplasmic reticulum; membrane; nucleus	Development; response to stimulus; cell death; defense response; reproduction; cell proliferation; regulation of biological process; metabolic process; cell organization and biogenesis; cell differentiation
20	P01861	Ig gamma-4 chain C region OS = Homo sapiens GN = IGHG4 PE = 1 SV = 1—[IGHG4_HUMAN]	Protein binding	Extracellular; membrane	Response to stimulus; metabolic process; regulation of biological process; cell communication; transport; defense response
21	P02647	Apolipoprotein A-I OS = Homo sapiens GN = APOA1 PE = 1 SV = 1—[APOA1_HUMAN]	Transporter activity; protein binding; enzyme regulator activity	Extracellular; nucleus; cytoplasm; endosome; endoplasmic reticulum; organelle lumen; cytosol; membrane	Metabolic process; regulation of biological process; cell proliferation; transport; response to stimulus; cell communication; coagulation; cell organization and biogenesis; cell differentiation; development; cellular component movement; defense response
22	P02649	Apolipoprotein E OS = Homo sapiens GN = APOE PE = 1 SV = 1—[APOE_HUMAN]	Transporter activity; protein binding; antioxidant activity; catalytic activity; metal ion binding; enzyme regulator activity	Extracellular; nucleus; cytoplasm; endosome; Golgi; cytoskeleton; membrane; cell surface; organelle lumen	Response to stimulus; metabolic process; cell proliferation; regulation of biological process; transport; cellular homeostasis; cell organization and biogenesis; cell communication; cell death; coagulation; cell differentiation; development; cell growth; cellular component movement; defense response
23	P02652	Apolipoprotein A-II OS = Homo sapiens GN = APOA2 PE = 1 SV = 1—[APOA2_HUMAN]	Transporter activity; protein binding; enzyme regulator activity	Extracellular; cytoplasm; endosome; endoplasmic reticulum; organelle lumen; cytosol	Metabolic process; defense response; response to stimulus; regulation of biological process; transport; cell communication; cell organization and biogenesis; development
24	P02760	Protein AMBP OS = Homo sapiens GN = AMBP PE = 1 SV = 1—[AMBP_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; membrane; cell surface	Reproduction; metabolic process; regulation of biological process; cell communication; response to stimulus

Table 2 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
25	P02763	Alpha-1-acid glycoprotein 1 OS = Homo sapiens GN = ORM1 PE = 1 SV = 1—[A1AG1_HUMAN]	Protein binding	Extracellular	Regulation of biological process; transport; defense response; response to stimulus
26	P02765	Alpha-2-HS-glycoprotein OS = Homo sapiens GN = AHSG PE = 1 SV = 1—[FETUA_HUMAN]	Enzyme regulator activity	Extracellular	Development; transport; defense response; response to stimulus; reproduction; metabolic process; regulation of biological process; cell growth; cell organization and biogenesis; cell communication
27	P02766	Transthyretin OS = Homo sapiens GN = TTR PE = 1 SV = 1—[TTHY_HUMAN]	Protein binding	Extracellular; cytoplasm	Metabolic process; transport; cell communication; regulation of biological process; response to stimulus; cell organization and biogenesis
28	P02768	Serum albumin OS = Homo sapiens GN = ALB PE = 1 SV = 2—[ALBU_HUMAN]	DNA binding; metal ion binding; protein binding; antioxidant activity; catalytic activity	Extracellular; nucleus; cytoplasm; organelle lumen	Transport; metabolic process; response to stimulus; coagulation; cell communication; cell death; regulation of biological process
29	P02787	Serotransferrin OS = Homo sapiens GN = TF PE = 1 SV = 3—[TRFE_HUMAN]	Protein binding; metal ion binding	Extracellular; cytoplasm; mitochondrion; endosome; membrane; cell surface; organelle lumen	Transport; cellular homeostasis; coagulation; response to stimulus
30	P02790	Hemopexin OS = Homo sapiens GN = HPX PE = 1 SV = 2—[HEMO_HUMAN]	Catalytic activity; protein binding; metal ion binding; transporter activity	Extracellular; cytoplasm; organelle lumen	Regulation of biological process; response to stimulus; metabolic process; transport; cellular homeostasis; cell communication; defense response
31	P04004	Vitronectin OS = Homo sapiens GN = VTN PE = 1 SV = 1—[VTNC_HUMAN]	Receptor activity; protein binding	Extracellular; membrane	Transport; response to stimulus; regulation of biological process; metabolic process; cellular component movement; coagulation; cell organization and biogenesis; defense response; cell communication
32	P04217	Alpha-1B-glycoprotein OS = Homo sapiens GN = A1BG PE = 1 SV = 4—[A1BG_HUMAN]	Protein binding	Extracellular	

Table 2 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
33	P05090	Apolipoprotein D OS = Homo sapiens GN = APOD PE = 1 SV = 1—[APOD_HUMAN]	Transporter activity; protein binding	Extracellular; cytoplasm; endoplasmic reticulum; cytosol; ribosome	Response to stimulus; development; metabolic process; transport; cell communication; regulation of biological process; cell differentiation; cell organization and biogenesis; cell proliferation; defense response; cellular component movement
34	P10745	Retinol-binding protein 3 OS = Homo sapiens GN = RBP3 PE = 1 SV = 2—[RET3_HUMAN]	Catalytic activity	Extracellular	Metabolic process; transport; cell communication; regulation of biological process; response to stimulus
35	P10909	Clusterin OS = Homo sapiens GN = CLU PE = 1 SV = 1—[CLUS_HUMAN]	Protein binding; catalytic activity	Extracellular; nucleus; cytoplasm; mitochondrion; endoplasmic reticulum; cytosol; membrane; organelle lumen	Cell organization and biogenesis; cell communication; cell death; regulation of biological process; response to stimulus; transport; metabolic process; coagulation; cell proliferation; development; cell differentiation; defense response
36	P13645	Keratin, type I cytoskeletal 10 OS = Homo sapiens GN = KRT10 PE = 1 SV = 6—[K1C10_HUMAN]	Structural molecule activity	Extracellular; nucleus; cytoplasm; cytoskeleton	Development; cell differentiation; response to stimulus
37	P19652	Alpha-1-acid glycoprotein 2 OS = Homo sapiens GN = ORM2 PE = 1 SV = 2—[A1AG2_HUMAN]		Extracellular	Regulation of biological process; transport; defense response; response to stimulus
38	P22352	Glutathione peroxidase 3 OS = Homo sapiens GN = GPX3 PE = 1 SV = 2—[GPX3_HUMAN]	Antioxidant activity; catalytic activity; protein binding	Extracellular	Response to stimulus; metabolic process; reproduction; cell organization and biogenesis
39	P36222	Chitinase-3-like protein 1 OS = Homo sapiens GN = CHI3L1 PE = 1 SV = 2—[CH3L1_HUMAN]	Catalytic activity; structural molecule activity	Extracellular; cytoplasm; endoplasmic reticulum	Metabolic process; cell death; defense response; response to stimulus; cell communication; regulation of biological process; development; transport
40	P36955	Pigment epithelium-derived factor OS = Homo sapiens GN = SERPINF1 PE = 1 SV = 4—[PEDF_HUMAN]	Enzyme regulator activity	Extracellular; cytoplasm	Development; response to stimulus; cell proliferation; metabolic process; regulation of biological process; defense response; cell differentiation; reproduction
41	P41222	Prostaglandin-H2 D-isomerase OS = Homo sapiens GN = PTGDS PE = 1 SV = 1—[PTGDS_HUMAN]	Catalytic activity; transporter activity	Extracellular; nucleus; cytoplasm; endoplasmic reticulum; membrane; Golgi	Metabolic process; transport; regulation of biological process; response to stimulus
42	P43652	Afamin OS = Homo sapiens GN = AFM PE = 1 SV = 1—[AFAM_HUMAN]		Extracellular	Transport

Table 2 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
43	P51884	Lumican OS = Homo sapiens GN = LUM PE = 1 SV = 2—[LUM_HUMAN]	Structural molecule activity; protein binding	Extracellular; cytoplasm; Golgi; organelle lumen; vacuole	Metabolic process; response to stimulus; cell organization and biogenesis; regulation of biological process; development
44	P55083	Microfibril-associated glycoprotein 4 OS = Homo sapiens GN = MFAP4 PE = 1 SV = 2—[MFAP4_HUMAN]	Protein binding	Extracellular	Cell communication; regulation of biological process; response to stimulus; metabolic process; cell organization and biogenesis
45	Q0KKI6	Immunoblobulin light chain (Fragment) OS = Homo sapiens PE = 2 SV = 1—[Q0KKI6_HUMAN]	Protein binding	Extracellular; membrane	Response to stimulus; metabolic process; regulation of biological process; cell communication; transport; defense response
46	Q16270	Insulin-like growth factor-binding protein 7 OS = Homo sapiens GN = IGFBP7 PE = 1 SV = 1—[IBP7_HUMAN]	Protein binding	Extracellular	Cell growth; cell organization and biogenesis; regulation of biological process; development; reproduction; cell proliferation; response to stimulus; metabolic process
47	Q5UGI6	Serine/cysteine proteinase inhibitor clade G member 1 splice variant 2 (Fragment) OS = Homo sapiens GN = SERPING1 PE = 2 SV = 1—[Q5UGI6_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; cytoplasm; organelle lumen	Defense response; metabolic process; regulation of biological process; response to stimulus; transport; coagulation
48	Q5VY30	Plasma retinol-binding protein(1-182) OS = Homo sapiens GN = RBP4 PE = 2 SV = 1—[Q5VY30_HUMAN]	Transporter activity; protein binding	Extracellular; cytoplasm; cytosol	Metabolic process; development; transport; reproduction; cell communication; regulation of biological process; response to stimulus; cell proliferation
49	Q6FHW3	DF protein OS = Homo sapiens GN = DF PE = 2 SV = 1—[Q6FHW3_HUMAN]	Catalytic activity	Extracellular; cytoplasm; organelle lumen	Transport; metabolic process; regulation of biological process; response to stimulus; defense response; cell communication; coagulation
50	Q8IZF3	Probable G-protein-coupled receptor 115 OS = Homo sapiens GN = GPR115 PE = 2 SV = 3—[GP115_HUMAN]	Signal transducer activity; receptor activity	Membrane	Cell communication; regulation of biological process; response to stimulus
51	Q8IZJ3	C3 and PZP-like alpha-2-macroglobulin domain-containing protein 8 OS = Homo sapiens GN = CPAMD8 PE = 1 SV = 2—[CPMD8_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; membrane	Metabolic process; regulation of biological process

Table 2 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
52	Q9HCB6	Spondin-1 OS = Homo sapiens GN = SPON1 PE = 1 SV = 2—[SPON1_HUMAN]	Protein binding	Extracellular	
53	Q9UBM4	Opticin OS = Homo sapiens GN = OPTC PE = 1 SV = 1—[OPT_HUMAN]	Structural molecule activity; protein binding	Extracellular	Development; regulation of biological process

pathogenesis of Glaucoma. Sacca et al. [16] have described Apolipoprotein B & E to be the up-regulated protein markers of endothelial damage in AH of POAG patients by antibody microarray analysis, while our study found Apolipoprotein A-4 and C-3 to be up-regulated in both glaucoma subtypes. Apolipoprotein E did not show significant up-regulation in our analysis. This study did not find significantly down-regulated proteins common to both PACG and POAG AH, as compared to cataract. Some of the down-regulated proteins were included in the "blood coagulation" pathway, F5, FGG and FGB. Our results indicate that the vascular remodeling, blood coagulation and immune response pathways are majorly affected by glaucoma.

On analysis, our study found that some proteins unique to PACG aqueous were seen to be part of the actin cytoskeleton pathway. In POAG and steroid-induced glaucoma, Clark et al. [17] had earlier found that changes in the actin cytoskeleton contribute to increased intraocular pressure especially by the formation of cross-linked actin networks (CLANs) which are thought to alter trabecular meshwork contractility, making the cells unresponsive to pressure change thus increasing IOP. In POAG aqueous, some unique proteins were a part of the gap junction pathway. It is assumed that in glaucoma, under conditions of elevated IOP, astrocytes (which are required for ionic and metabolic homeostasis of RGCs axon by connection and communication in the gap junctions) may lose their gap junction intracellular communication, thus contributing to glaucomatous neuropathy by altering homeostasis of RGC axons [18]. In cataract cases, a number of unique proteins were found to be part of the complement and coagulation cascade, but the precise interplay is still not completely understood.

Significant increase in oxidative stress is also known to play a role in the pathogenesis of PACG and POAG where oxidative stress leads to an increase in antioxidant enzymes and causes a decrease in total antioxidant potential (TRAP). Thus, superoxide dismutase (SOD), glutathione peroxidase (GPx) activities and TRAP may be useful oxidative stress markers in the aqueous humor of glaucoma patients [19]. Our study also reported few proteins in all the nine PACG and POAG AH samples having antioxidant activity. Antioxidant activity was observed in cataract AH samples also. Moreover, Levkovitch [20] reported apoptosis of retinal ganglion cells (RGCs) in

Table 3 List of common proteins present in all nine samples of POAG AH

Number	Accession no.	Description	Molecular function	Cellular component	Biological process
1	A8K050	cDNA FLJ75376, highly similar to Homo sapiens peptidoglycan recognition protein L (PGLYRP) mRNA OS = Homo sapiens PE = 2 SV = 1—[A8K050_HUMAN]	Metal ion binding; catalytic activity; receptor activity; signal transducer activity	Extracellular; membrane	Metabolic process; cell communication; defense response; regulation of biological process; response to stimulus; cell differentiation; development
2	A8KAJ3	cDNA FLJ77823, highly similar to Homo sapiens EGF-containing fibulin-like extracellular matrix protein 1, transcript variant 3, mRNA OS = Homo sapiens PE = 2 SV = 1—[A8KAJ3_HUMAN]	Catalytic activity; receptor activity; signal transducer activity; protein binding; metal ion binding	Extracellular; cell surface; membrane	Metabolic process; regulation of biological process; cell communication; response to stimulus; cell organization and biogenesis; cell differentiation; development
3	B2R8I2	cDNA, FLJ93914, highly similar to Homo sapiens histidine-rich glycoprotein (HRG), mRNA OS = Homo sapiens PE = 2 SV = 1—[B2R8I2_HUMAN]	Enzyme regulator activity; protein binding; metal ion binding	Extracellular; membrane; cytoplasm; organelle lumen	Development; transport; regulation of biological process; response to stimulus; coagulation; cell proliferation; metabolic process; cell organization and biogenesis; cell growth; cell death; cellular component movement; defense response; cell communication
4	B3KXD3	cDNA FLJ45230 fis, clone BRCAN2021325, highly similar to Carboxypeptidase E (EC 3.4.17.10) OS = Homo sapiens PE = 2 SV = 1—[B3KXD3_HUMAN]	Catalytic activity; metal ion binding; protein binding	Extracellular; nucleus; cytoplasm; Golgi; membrane	Development; metabolic process; cell communication; regulation of biological process; response to stimulus; cell organization and biogenesis
5	B4DQI1	Complement C2b Fragment OS = Homo sapiens GN = C2 PE = 2 SV = 1—[B4DQI1_HUMAN]	Catalytic activity; protein binding	Extracellular	Metabolic process; regulation of biological process; response to stimulus; defense response; cell organization and biogenesis; transport
6	B4E1B3	cDNA FLJ53950, highly similar to Angiotensinogen OS = Homo sapiens PE = 2 SV = 1—[B4E1B3_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; cytoplasm	Development; reproduction; cell growth; cell organization and biogenesis; regulation of biological process; metabolic process; response to stimulus; transport; cellular homeostasis; cell communication; cell differentiation; cell proliferation; cellular component movement; cell death; defense response
7	B7Z544	cDNA FLJ51742, highly similar to Inter-alpha-trypsin inhibitor heavy chain H4 OS = Homo sapiens PE = 2 SV = 1—[B7Z544_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; cytoplasm; membrane	Defense response; response to stimulus; metabolic process; regulation of biological process

Table 3 continued

Number	Accession no.	Description	Molecular function	Cellular component	Biological process
8	D9IWP9	Beta-2-glycoprotein I (Fragment) OS = Homo sapiens PE = 2 SV = 1—[D9IWP9_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cell surface	Cell proliferation; regulation of biological process; metabolic process; coagulation; response to stimulus; cellular component movement; development; cell death; transport
9	F6KPG5	Albumin (Fragment) OS = Homo sapiens PE = 2 SV = 1—[F6KPG5_HUMAN]	DNA binding; metal ion binding; protein binding; antioxidant activity; catalytic activity	Extracellular; nucleus; membrane; cytoplasm; organelle lumen	Transport; metabolic process; response to stimulus; coagulation; cell communication; cell death; regulation of biological process
10	G3V2V8	Epididymal secretory protein E1 (Fragment) OS = Homo sapiens GN = NPC2 PE = 2 SV = 1—[G3V2V8_HUMAN]			
11	G3V357	Ribonuclease pancreatic OS = Homo sapiens GN = RNASE1 PE = 2 SV = 1—[G3V357_HUMAN]	Catalytic activity		Metabolic process
12	G3XAM2	Complement factor I light chain OS = Homo sapiens GN = CFI PE = 3 SV = 1—[G3XAM2_HUMAN]	Catalytic activity; receptor activity; protein binding; metal ion binding	Extracellular; membrane	Metabolic process; transport; regulation of biological process; response to stimulus; defense response
13	G8JLA8	Transforming growth factor-beta-induced protein ig-h3 OS = Homo sapiens GN = TGFBI PE = 2 SV = 1—[G8JLA8_HUMAN]	Protein binding		
14	M0R3H1	B-cell receptor CD22 (Fragment) OS = Homo sapiens GN = CD22 PE = 4 SV = 1—[M0R3H1_HUMAN]	Protein binding	Membrane; cell surface	
15	O60888	Protein CutA OS = Homo sapiens GN = CUTA PE = 1 SV = 2—[CUTA_HUMAN]	Protein binding	Membrane; extracellular	Response to stimulus; cell organization and biogenesis
16	P00747	Plasminogen OS = Homo sapiens GN = PLG PE = 1 SV = 2—[PLMN_HUMAN]	Catalytic activity; metal ion binding; protein binding	Extracellular; membrane; cell surface; cytoplasm; organelle lumen	Transport; metabolic process; cell death; coagulation; response to stimulus; cell proliferation; regulation of biological process; cell organization and biogenesis; cell differentiation; cellular homeostasis; development; cellular component movement

Table 3 continued

Number	Accession no.	Description	Molecular function	Cellular component	Biological process
17	P00748	Coagulation factor XII OS = Homo sapiens GN = F12 PE = 1 SV = 3—[FA12_HUMAN]	Catalytic activity; protein binding	Extracellular; membrane	Defense response; metabolic process; response to stimulus; regulation of biological process; coagulation
18	P01008	Antithrombin-III OS = Homo sapiens GN = SERPINC1 PE = 1 SV = 1—[ANT3_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; membrane	Response to stimulus; coagulation; metabolic process; regulation of biological process; defense response
19	P01009	Alpha-1-antitrypsin OS = Homo sapiens GN = SERPINA1 PE = 1 SV = 3—[A1AT_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; endoplasmic reticulum; Golgi; organelle lumen	Response to stimulus; transport; defense response; coagulation; metabolic process; regulation of biological process
20	P01011	Alpha-1-antichymotrypsin OS = Homo sapiens GN = SERPINA3 PE = 1 SV = 2—[AACT_HUMAN]	DNA binding; enzyme regulator activity; protein binding	Extracellular; nucleus	Defense response; response to stimulus; metabolic process; regulation of biological process
21	P01023	Alpha-2-macroglobulin OS = Homo sapiens GN = A2 M PE = 1 SV = 3—[A2MG_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; cytosol; organelle lumen	Defense response; metabolic process; regulation of biological process; response to stimulus; transport; cell communication; coagulation; cell organization and biogenesis; cell differentiation
22	P01024	Complement C3 OS = Homo sapiens GN = C3 PE = 1 SV = 2—[CO3_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; membrane	Defense response; regulation of biological process; response to stimulus; metabolic process; cell communication; transport; development; cell organization and biogenesis
23	P01034	Cystatin-C OS = Homo sapiens GN = CST3 PE = 1 SV = 1—[CYTC_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; vacuole; endosome; endoplasmic reticulum; membrane; nucleus	Development; response to stimulus; cell death; defense response; reproduction; cell proliferation; regulation of biological process; metabolic process; cell organization and biogenesis; cell differentiation
24	P01860	Ig gamma-3 chain C region OS = Homo sapiens GN = IGHG3 PE = 1 SV = 2—[IGHG3_HUMAN]	Protein binding	Extracellular; membrane	Response to stimulus; metabolic process; regulation of biological process; cell communication; transport; defense response
25	P01861	Ig gamma-4 chain C region OS = Homo sapiens GN = IGHG4 PE = 1 SV = 1—[IGHG4_HUMAN]	Protein binding	Extracellular; membrane	Response to stimulus; metabolic process; regulation of biological process; cell communication; transport; defense response

Table 3 continued

Number	Accession no.	Description	Molecular function	Cellular component	Biological process
26	P02647	Apolipoprotein A-I OS = Homo sapiens GN = APOA1 PE = 1 SV = 1—[APOA1_HUMAN]	Transporter activity; protein binding; enzyme regulator activity	Extracellular; nucleus; cytoplasm; endosome; endoplasmic reticulum; organelle lumen; cytosol; membrane	Metabolic process; regulation of biological process; cell proliferation; transport; response to stimulus; cell communication; coagulation; cell organization and biogenesis; cell differentiation; development; cellular component movement; defense response
27	P02649	Apolipoprotein E OS = Homo sapiens GN = APOE PE = 1 SV = 1—[APOE_HUMAN]	Transporter activity; protein binding; antioxidant activity; catalytic activity; metal ion binding; enzyme regulator activity	Extracellular; nucleus; cytoplasm; endosome; Golgi; cytoskeleton; membrane; cell surface; organelle lumen	Response to stimulus; metabolic process; cell proliferation; regulation of biological process; transport; cellular homeostasis; cell organization and biogenesis; cell communication; cell death; coagulation; cell differentiation; development; cell growth; cellular component movement; defense response
28	P02652	Apolipoprotein A-II OS = Homo sapiens GN = APOA2 PE = 1 SV = 1—[APOA2_HUMAN]	Transporter activity; protein binding; enzyme regulator activity	Extracellular; cytoplasm; endosome; endoplasmic reticulum; organelle lumen; cytosol	Metabolic process; defense response; response to stimulus; regulation of biological process; transport; cell communication; cell organization and biogenesis; development
29	P02748	Complement component C9 OS = Homo sapiens GN = C9 PE = 1 SV = 2—[CO9_HUMAN]	Protein binding	Extracellular; membrane; cytoplasm	Cell death; metabolic process; regulation of biological process; response to stimulus; defense response; coagulation
30	P02763	Alpha-1-acid glycoprotein 1 OS = Homo sapiens GN = ORM1 PE = 1 SV = 1—[A1AG1_HUMAN]	Protein binding	Extracellular	Regulation of biological process; transport; defense response; response to stimulus
31	P02765	Alpha-2-HS-glycoprotein OS = Homo sapiens GN = AHSG PE = 1 SV = 1—[FETUA_HUMAN]	Enzyme regulator activity	Extracellular	Development; transport; defense response; response to stimulus; reproduction; metabolic process; regulation of biological process; cell growth; cell organization and biogenesis; cell communication
32	P02766	Transthyretin OS = Homo sapiens GN = TTR PE = 1 SV = 1—[TTHY_HUMAN]	Protein binding	Extracellular; cytoplasm	Metabolic process; transport; cell communication; regulation of biological process; response to stimulus; cell organization and biogenesis

Table 3 continued

Number	Accession no.	Description	Molecular function	Cellular component	Biological process
33	P02768	Serum albumin OS = Homo sapiens GN = ALB PE = 1 SV = 2—[ALBU_HUMAN]	DNA binding; metal ion binding; protein binding; antioxidant activity; catalytic activity	Extracellular; nucleus; cytoplasm; organelle lumen	Transport; metabolic process; response to stimulus; coagulation; cell communication; cell death; regulation of biological process
34	P02774	Vitamin D-binding protein OS = Homo sapiens GN = GC PE = 1 SV = 1—[VTDB_HUMAN]	Protein binding; transporter activity	Extracellular; cytoplasm; cytosol; organelle lumen; vacuole	Transport; metabolic process
35	P02790	Hemopexin OS = Homo sapiens GN = HPX PE = 1 SV = 2—[HEMO_HUMAN]	Catalytic activity; protein binding; metal ion binding; transporter activity	Extracellular; cytoplasm; organelle lumen	Regulation of biological process; response to stimulus; metabolic process; transport; cellular homeostasis; cell communication; defense response
36	P04004	Vitronectin OS = Homo sapiens GN = VTN PE = 1 SV = 1—[VTNC_HUMAN]	Receptor activity; protein binding	Extracellular; membrane	Transport; response to stimulus; regulation of biological process; metabolic process; cellular component movement; coagulation; cell organization and biogenesis; defense response; cell communication
37	P04217	Alpha-1B-glycoprotein OS = Homo sapiens GN = A1BG PE = 1 SV = 4—[A1BG_HUMAN]	Protein binding	Extracellular	
38	P05090	Apolipoprotein D OS = Homo sapiens GN = APOD PE = 1 SV = 1—[APOD_HUMAN]	Transporter activity; protein binding	Extracellular; cytoplasm; endoplasmic reticulum; cytosol; ribosome	Response to stimulus; development; metabolic process; transport; cell communication; regulation of biological process; cell differentiation; cell organization and biogenesis; cell proliferation; defense response; cellular component movement
39	P10745	Retinol-binding protein 3 OS = Homo sapiens GN = RBP3 PE = 1 SV = 2—[RET3_HUMAN]	Catalytic activity	Extracellular	Metabolic process; transport; cell communication; regulation of biological process; response to stimulus
40	P10909	Clusterin OS = Homo sapiens GN = CLU PE = 1 SV = 1—[CLUS_HUMAN]	Protein binding; catalytic activity	Extracellular; nucleus; cytoplasm; mitochondrion; endoplasmic reticulum; cytosol; membrane; organelle lumen	Cell organization and biogenesis; cell communication; cell death; regulation of biological process; response to stimulus; transport; metabolic process; coagulation; cell proliferation; development; cell differentiation; defense response

Table 3 continued

Number	Accession no.	Description	Molecular function	Cellular component	Biological process
41	P19652	Alpha-1-acid glycoprotein 2 OS = Homo sapiens GN = ORM2 PE = 1 SV = 2—[A1AG2_HUMAN]		Extracellular	Regulation of biological process; transport; defense response; response to stimulus
42	P22352	Glutathione peroxidase 3 OS = Homo sapiens GN = GPX3 PE = 1 SV = 2—[GPX3_HUMAN]	Antioxidant activity; catalytic activity; protein binding	Extracellular	Response to stimulus; metabolic process; reproduction; cell organization and biogenesis
43	P35527	Keratin, type I cytoskeletal 9 OS = Homo sapiens GN = KRT9 PE = 1 SV = 3—[K1C9_HUMAN]	Structural molecule activity	Extracellular; nucleus; cytoskeleton; cytoplasm	Reproduction; development; cell organization and biogenesis
44	P36222	Chitinase-3-like protein 1 OS = Homo sapiens GN = CHI3L1 PE = 1 SV = 2—[CH3L1_HUMAN]	Catalytic activity; structural molecule activity	Extracellular; cytoplasm; endoplasmic reticulum	Metabolic process; cell death; defense response; response to stimulus; cell communication; regulation of biological process; development; transport
45	P36955	Pigment epithelium-derived factor OS = Homo sapiens GN = SERPINF1 PE = 1 SV = 4—[PEDF_HUMAN]	Enzyme regulator activity	Extracellular; cytoplasm	Development; response to stimulus; cell proliferation; metabolic process; regulation of biological process; defense response; cell differentiation; reproduction
46	P41222	Prostaglandin-H2 D-isomerase OS = Homo sapiens GN = PTGDS PE = 1 SV = 1—[PTGDS_HUMAN]	Catalytic activity; transporter activity	Extracellular; nucleus; cytoplasm; endoplasmic reticulum; membrane; Golgi	Metabolic process; transport; regulation of biological process; response to stimulus
47	P43652	Afamin OS = Homo sapiens GN = AFM PE = 1 SV = 1—[AFAM_HUMAN]		Extracellular	Transport
48	P51884	Lumican OS = Homo sapiens GN = LUM PE = 1 SV = 2—[LUM_HUMAN]	Structural molecule activity; protein binding	Extracellular; cytoplasm; Golgi; organelle lumen; vacuole	Metabolic process; response to stimulus; cell organization and biogenesis; regulation of biological process; development
49	P55083	Microfibril-associated glycoprotein 4 OS = Homo sapiens GN = MFAP4 PE = 1 SV = 2—[MFAP4_HUMAN]	Protein binding	Extracellular	Cell communication; regulation of biological process; response to stimulus; metabolic process; cell organization and biogenesis
50	Q16270	Insulin-like growth factor-binding protein 7 OS = Homo sapiens GN = IGFBP7 PE = 1 SV = 1—[IBP7_HUMAN]	Protein binding	Extracellular	Cell growth; cell organization and biogenesis; regulation of biological process; development; reproduction; cell proliferation; response to stimulus; metabolic process

Table 3 continued

Number	Accession no.	Description	Molecular function	Cellular component	Biological process
51	Q5T4F6	Cartilage acidic protein 1 (Fragment) OS = Homo sapiens GN = CRTAC1 PE = 2 SV = 1—[Q5T4F6_HUMAN]	Metal ion binding	Extracellular	
52	Q5UGI6	Serine/cysteine proteinase inhibitor clade G member 1 splice variant 2 (Fragment) OS = Homo sapiens GN = SERPING1 PE = 2 SV = 1—[Q5UGI6_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; cytoplasm; organelle lumen	Defense response; metabolic process; regulation of biological process; response to stimulus; transport; coagulation
53	Q5VY30	Plasma retinol-binding protein(1-182) OS = Homo sapiens GN = RBP4 PE = 2 SV = 1—[Q5VY30_HUMAN]	Transporter activity; protein binding	Extracellular; cytoplasm; cytosol	Metabolic process; development; transport; reproduction; cell communication; regulation of biological process; response to stimulus; cell proliferation
54	Q65ZC9	Single-chain Fv (Fragment) OS = Homo sapiens GN = scFv PE = 1 SV = 1—[Q65ZC9_HUMAN]	Protein binding		
55	Q6FHW3	DF protein OS = Homo sapiens GN = DF PE = 2 SV = 1—[Q6FHW3_HUMAN]	Catalytic activity	Extracellular; cytoplasm; organelle lumen	Transport; metabolic process; regulation of biological process; response to stimulus; defense response; cell communication; coagulation
56	Q8IZF3	Probable G-protein-coupled receptor 115 OS = Homo sapiens GN = GPR115 PE = 2 SV = 3—[GP115_HUMAN]	Signal transducer activity; receptor activity	Membrane	Cell communication; regulation of biological process; response to stimulus
57	Q8IZJ3	C3 and PZP-like alpha-2-macroglobulin domain-containing protein 8 OS = Homo sapiens GN = CPAMD8 PE = 1 SV = 2—[CPMD8_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; membrane	Metabolic process; regulation of biological process
58	Q99969	Retinoic acid receptor responder protein 2 OS = Homo sapiens GN = RARRES2 PE = 1 SV = 1—[RARR2_HUMAN]	Protein binding	Extracellular	Metabolic process; development; regulation of biological process; response to stimulus; defense response; cellular component movement; cell differentiation; transport
59	Q9UBM4	Opticin OS = Homo sapiens GN = OPTC PE = 1 SV = 1—[OPT_HUMAN]	Structural molecule activity; protein binding	Extracellular	Development; regulation of biological process

Table 4 List of common proteins present in all nine samples of Cataract AH

Number	Accession	Description	Molecular function	Cellular component	Biological process
1	B2R8I2	cDNA, FLJ93914, highly similar to Homo sapiens histidine-rich glycoprotein (HRG), mRNA OS = Homo sapiens PE = 2 SV = 1—[B2R8I2_HUMAN]	Enzyme regulator activity; protein binding; metal ion binding	Extracellular; membrane; cytoplasm; organelle lumen	Development; transport; regulation of biological process; response to stimulus; coagulation; cell proliferation; metabolic process; cell organization and biogenesis; cell growth; cell death; cellular component movement; defense response; cell communication
2	B3KXD3	cDNA FLJ45230 fis, clone BRCAN2021325, highly similar to Carboxypeptidase E (EC 3.4.17.10) OS = Homo sapiens PE = 2 SV = 1—[B3KXD3_HUMAN]	Catalytic activity; metal ion binding; protein binding	Extracellular; nucleus; cytoplasm; Golgi; membrane	Development; metabolic process; cell communication; regulation of biological process; response to stimulus; cell organization and biogenesis
3	B4DPP8	cDNA FLJ53075, highly similar to Kininogen-1 OS = Homo sapiens PE = 2 SV = 1—[B4DPP8_HUMAN]	Enzyme regulator activity; protein binding; metal ion binding	Extracellular; membrane; cytoplasm; organelle lumen	Transport; defense response; response to stimulus; regulation of biological process; cellular homeostasis; coagulation; metabolic process; cell death
4	B4E1B2	cDNA FLJ53691, highly similar to Serotransferrin OS = Homo sapiens PE = 2 SV = 1—[B4E1B2_HUMAN]	Protein binding; metal ion binding	Extracellular; cytoplasm; mitochondrion; endosome; membrane; cell surface; organelle lumen	Transport; cellular homeostasis; coagulation; response to stimulus
5	F2YQ21	Osteopontin-D OS = Homo sapiens PE = 2 SV = 1—[F2YQ21_HUMAN]	Protein binding	Extracellular; cytoplasm	Cell differentiation; defense response; response to stimulus; development; reproduction; regulation of biological process; cell organization and biogenesis; cellular component movement; cell growth
6	F6KPG5	Albumin (Fragment) OS = homo sapiens PE = 2 SV = 1—[F6KPG5_HUMAN]	DNA binding; metal ion binding; protein binding; antioxidant activity; catalytic activity	Extracellular; nucleus; membrane; cytoplasm; organelle lumen	Transport; metabolic process; response to stimulus; coagulation; cell communication; cell death; regulation of biological process
7	G3V357	Ribonuclease pancreatic OS = Homo sapiens GN = RNASE1 PE = 2 SV = 1—[G3V357_HUMAN]	Catalytic activity		Metabolic process
8	H6VRF8	Keratin 1 OS = Homo sapiens GN = KRT1 PE = 3 SV = 1—[H6VRF8_HUMAN]	Catalytic activity; motor activity; receptor activity; structural molecule activity; protein binding; transporter activity	Extracellular; nucleus; cytoskeleton; membrane	Defense response; metabolic process; regulation of biological process; response to stimulus; development; transport; coagulation

Table 4 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
9	P00751	Complement factor B OS = Homo sapiens GN = CFB PE = 1 SV = 2—[CFAB_HUMAN]	Protein binding; catalytic activity	Extracellular; membrane	Metabolic process; regulation of biological process; response to stimulus; defense response
10	P01009	Alpha-1-antitrypsin OS = Homo sapiens GN = SERPINA1 PE = 1 SV = 3—[A1AT_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; endoplasmic reticulum; Golgi; organelle lumen	Response to stimulus; transport; defense response; coagulation; metabolic process; regulation of biological process
11	P01023	Alpha-2-macroglobulin OS = Homo sapiens GN = A2 M PE = 1 SV = 3—[A2MG_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; cytosol; organelle lumen	Defense response; metabolic process; regulation of biological process; response to stimulus; transport; cell communication; coagulation; cell organization and biogenesis; cell differentiation
12	P01024	Complement C3 OS = Homo sapiens GN = C3 PE = 1 SV = 2—[CO3_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; membrane	Defense response; regulation of biological process; response to stimulus; metabolic process; cell communication; transport; development; cell organization and biogenesis
13	P01034	Cystatin-C OS = Homo sapiens GN = CST3 PE = 1 SV = 1—[CYTC_HUMAN]	Protein binding; enzyme regulator activity	Extracellular; cytoplasm; vacuole; endosome; endoplasmic reticulum; membrane; nucleus	Development; response to stimulus; cell death; defense response; reproduction; cell proliferation; regulation of biological process; metabolic process; cell organization and biogenesis; cell differentiation
14	P01860	Ig gamma-3 chain C region OS = Homo sapiens GN = IGHG3 PE = 1 SV = 2—[IGHG3_HUMAN]	Protein binding	Extracellular; membrane	Response to stimulus; metabolic process; regulation of biological process; cell communication; transport; defense response
15	P01861	Ig gamma-4 chain C region OS = Homo sapiens GN = IGHG4 PE = 1 SV = 1—[IGHG4_HUMAN]	Protein binding	Extracellular; membrane	Response to stimulus; metabolic process; regulation of biological process; cell communication; transport; defense response
16	P02647	Apolipoprotein A-I OS = Homo sapiens GN = APOA1 PE = 1 SV = 1—[APOA1_HUMAN]	Transporter activity; protein binding; enzyme regulator activity	Extracellular; nucleus; cytoplasm; endosome; endoplasmic reticulum; organelle lumen; cytosol; membrane	Metabolic process; regulation of biological process; cell proliferation; transport; response to stimulus; cell communication; coagulation; cell organization and biogenesis; cell differentiation; development; cellular component movement; defense response

Table 4 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
17	P02649	Apolipoprotein E OS = Homo sapiens GN = APOE PE = 1 SV = 1—[APOE_HUMAN]	Transporter activity; protein binding; antioxidant activity; catalytic activity; metal ion binding; enzyme regulator activity	Extracellular; nucleus; cytoplasm; endosome; Golgi; cytoskeleton; membrane; cell surface; organelle lumen	Response to stimulus; metabolic process; cell proliferation; regulation of biological process; transport; cellular homeostasis; cell organization and biogenesis; cell communication; cell death; coagulation; cell differentiation; development; cell growth; cellular component movement; defense response
18	P02652	Apolipoprotein A-II OS = Homo sapiens GN = APOA2 PE = 1 SV = 1—[APOA2_HUMAN]	Transporter activity; protein binding; enzyme regulator activity	Extracellular; cytoplasm; endosome; endoplasmic reticulum; organelle lumen; cytosol	Metabolic process; defense response; response to stimulus; regulation of biological process; transport; cell communication; cell organization and biogenesis; development
19	P02763	Alpha-1-acid glycoprotein 1 OS = Homo sapiens GN = ORM1 PE = 1 SV = 1—[A1AG1_HUMAN]	Protein binding	Extracellular	Regulation of biological process; transport; defense response; response to stimulus
20	P02765	Alpha-2-HS-glycoprotein OS = Homo sapiens GN = AHSG PE = 1 SV = 1—[FETUA_HUMAN]	enzyme regulator activity	extracellular	development; transport; defense response; response to stimulus; reproduction; metabolic process; regulation of biological process; cell growth; cell organization and biogenesis; cell communication
21	P02766	Transthyretin OS = Homo sapiens GN = TTR PE = 1 SV = 1—[TTHY_HUMAN]	Protein binding	Extracellular; cytoplasm	Metabolic process; transport; cell communication; regulation of biological process; response to stimulus; cell organization and biogenesis
22	P02768	Serum albumin OS = Homo sapiens GN = ALB PE = 1 SV = 2—[ALBU_HUMAN]	DNA binding; metal ion binding; protein binding; antioxidant activity; catalytic activity	Extracellular; nucleus; cytoplasm; organelle lumen	Transport; metabolic process; response to stimulus; coagulation; cell communication; cell death; regulation of biological process
23	P02774	Vitamin D-binding protein OS = Homo sapiens GN = GC PE = 1 SV = 1—[VTDB_HUMAN]	Protein binding; transporter activity	Extracellular; cytoplasm; cytosol; organelle lumen; vacuole	Transport; metabolic process
24	P02790	Hemopexin OS = Homo sapiens GN = HPX PE = 1 SV = 2—[HEMO_HUMAN]	Catalytic activity; protein binding; metal ion binding; transporter activity	Extracellular; cytoplasm; organelle lumen	Regulation of biological process; response to stimulus; metabolic process; transport; cellular homeostasis; cell communication; defense response
25	P04217	Alpha-1B-glycoprotein OS = Homo sapiens GN = A1BG PE = 1 SV = 4—[A1BG_HUMAN]	Protein binding	Extracellular	

Table 4 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
26	P10745	Retinol-binding protein 3 OS = Homo sapiens GN = RBP3 PE = 1 SV = 2—[RET3_HUMAN]	Catalytic activity	Extracellular	Metabolic process; transport; cell communication; regulation of biological process; response to stimulus
27	P10909	Clusterin OS = Homo sapiens GN = CLU PE = 1 SV = 1—[CLUS_HUMAN]	Protein binding; catalytic activity	Extracellular; nucleus; cytoplasm; mitochondrion; endoplasmic reticulum; cytosol; membrane; organelle lumen	Cell organization and biogenesis; cell communication; cell death; regulation of biological process; response to stimulus; transport; metabolic process; coagulation; cell proliferation; development; cell differentiation; defense response
28	P13645	Keratin, type I cytoskeletal 10 OS = Homo sapiens GN = KRT10 PE = 1 SV = 6—[K1C10_HUMAN]	Structural molecule activity	Extracellular; nucleus; cytoplasm; cytoskeleton	Development; cell differentiation; response to stimulus
29	P19652	Alpha-1-acid glycoprotein 2 OS = Homo sapiens GN = ORM2 PE = 1 SV = 2—[A1AG2_HUMAN]		Extracellular	Regulation of biological process; transport; defense response; response to stimulus
30	P22352	Glutathione peroxidase 3 OS = Homo sapiens GN = GPX3 PE = 1 SV = 2—[GPX3_HUMAN]	Antioxidant activity; catalytic activity; protein binding	Extracellular	Response to stimulus; metabolic process; reproduction; cell organization and biogenesis
31	P35527	Keratin, type I cytoskeletal 9 OS = Homo sapiens GN = KRT9 PE = 1 SV = 3—[K1C9_HUMAN]	Structural molecule activity	Extracellular; nucleus; cytoskeleton; cytoplasm	Reproduction; development; cell organization and biogenesis
32	P35908	Keratin, type II cytoskeletal 2 epidermal OS = Homo sapiens GN = KRT2 PE = 1 SV = 2—[K22E_HUMAN]	structural molecule activity	extracellular; nucleus; cytoplasm; Golgi; cytoskeleton	development; cell differentiation; cell proliferation; cellular component movement
33	P36222	Chitinase-3-like protein 1 OS = Homo sapiens GN = CHI3L1 PE = 1 SV = 2—[CH3L1_HUMAN]	Catalytic activity; structural molecule activity	Extracellular; cytoplasm; endoplasmic reticulum	Metabolic process; cell death; defense response; response to stimulus; cell communication; regulation of biological process; development; transport
34	P36955	Pigment epithelium-derived factor OS = Homo sapiens GN = SERPINF1 PE = 1 SV = 4—[PEDF_HUMAN]	Enzyme regulator activity	Extracellular; cytoplasm	Development; response to stimulus; cell proliferation; metabolic process; regulation of biological process; defense response; cell differentiation; reproduction
35	P41222	Prostaglandin-H2 D-isomerase OS = Homo sapiens GN = PTGDS PE = 1 SV = 1—[PTGDS_HUMAN]	Catalytic activity; transporter activity	Extracellular; nucleus; cytoplasm; endoplasmic reticulum; membrane; Golgi	Metabolic process; transport; regulation of biological process; response to stimulus

Table 4 continued

Number	Accession	Description	Molecular function	Cellular component	Biological process
36	P61626	Lysozyme C OS = Homo sapiens GN = LYZ PE = 1 SV = 1—[LYSC_HUMAN]	Catalytic activity; protein binding	Extracellular	Defense response; response to stimulus; metabolic process
37	Q13822	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2 OS = Homo sapiens GN = ENPP2 PE = 1 SV = 3—[ENPP2_HUMAN]	Catalytic activity; receptor activity; metal ion binding; protein binding	Extracellular; membrane	Metabolic process; transport; cellular component movement; response to stimulus; cell communication; regulation of biological process
38	Q16270	Insulin-like growth factor-binding protein 7 OS = Homo sapiens GN = IGFBP7 PE = 1 SV = 1—[IBP7_HUMAN]	Protein binding	Extracellular	Cell growth; cell organization and biogenesis; regulation of biological process; development; reproduction; cell proliferation; response to stimulus; metabolic process
39	Q5T4F6	Cartilage acidic protein 1 (Fragment) OS = Homo sapiens GN = CRTAC1 PE = 2 SV = 1—[Q5T4F6_HUMAN]	Metal ion binding	Extracellular	
40	Q5UGI6	Serine/cysteine proteinase inhibitor clade G member 1 splice variant 2 (Fragment) OS = Homo sapiens GN = SERPING1 PE = 2 SV = 1—[Q5UGI6_HUMAN]	Enzyme regulator activity; protein binding	Extracellular; cytoplasm; organelle lumen	Defense response; metabolic process; regulation of biological process; response to stimulus; transport; coagulation
41	Q8IZF3	Probable G-protein-coupled receptor 115 OS = Homo sapiens GN = GPR115 PE = 2 SV = 3—[GP115_HUMAN]	Signal transducer activity; receptor activity	Membrane	Cell communication; regulation of biological process; response to stimulus
42	Q9UBM4	Opticin OS = homo sapiens GN = OPTC PE = 1 SV = 1—[OPT_HUMAN]	Structural molecule activity; protein binding	Extracellular	Development; regulation of biological process

Table 5 List of proteins identified by individual analysis in all the samples analyzed representing the "Constitutive AH proteome"

No.	Description
1	Carboxypeptidase E (EC 3.4.17.10)
2	Albumin (Fragment)
3	Ribonuclease pancreatic
4	Alpha-1-antitrypsin
5	Alpha-2-macroglobulin
6	Complement C3
7	Cystatin-C
8	Ig gamma-4 chain C region
9	Apolipoprotein A-I
10	Apolipoprotein E
11	Apolipoprotein A-II
12	Alpha-1-acid glycoprotein 1
13	Alpha-2-HS-glycoprotein
14	Transthyretin
15	Serum albumin
16	Hemopexin
17	Alpha-1B-glycoprotein
18	Retinol-binding protein 3
19	Clusterin
20	Alpha-1-acid glycoprotein 2
21	Glutathione peroxidase 3
22	Chitinase-3-like protein 1
23	Pigment epithelium-derived factor
24	Prostaglandin-H2 D-isomerase
25	Insulin-like growth factor-binding protein 7
26	Serine/cysteine proteinase inhibitor clade G member 1 splice variant 2 (Fragment)
27	Probable G-protein-coupled receptor 115
28	Opticin

glaucoma to be associated with increased IOP. Furthermore, it is seen that excitotoxicity also has a role in apoptotic retinal ganglion cell death in glaucoma [21]. Other than this oxidative damage to the trabecular meshwork also leads to mitochondrial damage and apoptosis, affecting the AH outflow of glaucomatous patients as compared to controls [1, 22].

Since aqueous humor is a plasma ultrafiltrate, it is expected to exhibit a very broad dynamic range of protein abundance, similar to plasma (mg/ml–pg/ml) [23]. In this study, since we have not pre-depleted the samples for albumin and immunoglobulins, we found that highly abundant proteins such as albumin, immunoglobulins, serotransferrin, transthyretin, apolipoproteins and alpha-2-macroglobulin were among the majorly represented AH proteins identified in all analyzed samples. However, with optimized chromatographic separation of peptide mixtures, we were able to resolve and detect very low abundant proteins in our study. An example is the identification of transforming growth factor-β2 (TGF-β2) protein in AH from all three diseases in our study. TGF-β2 is one of the most important immunosuppressive cytokines present in pg/ml concentration in AH [24]. Elevated TGF-β2 and elevated IOP causes extracellular matrix (ECM) component deposition which is assumed to be the likely cause of glaucoma [25].

Most previous studies describing human AH proteome have pooled samples from different patients at the protein level itself, before analyzing on LC–MS. Escoffier et al. [26] analyzed the AH proteome with 2D-LC–MS/MS from individual samples, as well as

by pooling the samples, both at protein and peptide level and discussed the results obtained by three approaches pointing out that pooling samples at the protein level to increase sensitivity is an inappropriate strategy. The authors concluded that analyzing samples individually and as peptide pools are complementary approaches, and should be combined for biomarker analysis in complex samples such as AH. In our study, we have analyzed our samples as described by Escoffier et al. [26] with some modifications. In brief, the LC–MS/MS data of individual samples were acquired separately and all the spectra from a disease type were combined at the analysis level to achieve the sensitivity of detecting as many low abundant/scarce proteins as possible. This strategy proved useful for detecting proteins which could have been left undetected in individual searches, due to their low quantity or low spectral counts. The peptides from these low abundance proteins could pass the stringent analysis criteria when searched together in Proteome Discoverer 1.4, which they may otherwise have failed to pass due to possible poor spectral quality, etc. A limitation of this study was the small number of eyes studied; however, 75 glaucoma patients were screened to final age and treatment matched with patients without any systemic disease.

In conclusion, this study has highlighted significant differences in the AH of PACG eyes as compared to POAG eyes and those having a cataract. A large number of the proteins specific to PACG eyes are reported for the first time and suggest a different pathophysiology for the rise in IOP in such eyes. This study may help enhance our understanding of their pathophysiology and aid in the identification of specific biomarkers for each of these glaucomas.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of Institutional Ethics committee, AIIMS, New Delhi.

Informed consent Informed consent was obtained from all individual participants included in the study.

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