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ORIGINAL PAPER

Aqueous humor protein dysregulation in primary angle-closure glaucoma

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Abstract

Purpose Primary angle-closure glaucoma (PACG) is associated with increased intraocular pressure, optic nerve damage, and progressive vision loss, but the molecular mechanism that underpins retinal ganglion neuropathy in PACG remains poorly understood. To better understand the pathogenesis of human PACG, we performed the first comprehensive proteomic analysis of aqueous humor (AH) samples from PACG patients and matched control donors to study pathogenic alteration in AH composition in disease.

Methods High-resolution, label-free, liquid chromatography–tandem mass spectrometry-based quantitative proteomic analyses were performed in AH samples collected from PACG patients and a matched control cohort of patients with cataracts.

Results The AH proteome comprised of 1363 distinct proteins, of which more than 50% were differentially expressed in PACG (773 total; 501 up-regulated, 272 down-regulated). AH from PACG patients was enriched in atypical collagens and fibronectins, suggesting that the composition of the trabecular matrix is significantly altered in disease. Pathway and cluster analyses revealed that AH protein modulation in PACG is closely associated with biological processes including platelet degranulation, cellular import/export mechanisms, and control of protease activity. In addition, critical mediators of oxygen homeostasis and neuronal function in AH were significantly dysregulated in disease, strongly implicating oxidative stress responses in PACG-associated nerve damage.

Conclusions Altered AH proteome in human PACG indicated oxidative stress in the neuronal damage that preceded vision loss. Identifying key mediators of PACG pathology will yield new prognostic biomarkers and novel targets for future therapeutic interventions.

Keywords Glaucoma · Aqueous humor · Proteomics · Primary angle-closure glaucoma · ApoE

Sunil S. Adav and Jin Wei have contributed equally to this work.

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Introduction

Glaucoma is a neurodegenerative eye condition and the leading cause of blindness worldwide, with the number of affected patients over 40 years old projected to exceed 75 million by the year 2020, and surpass 100 million cases globally by 2040 [1, 2]. PACG is characterized by appositional or synechial closure of the anterior chamber angle of the eye and will likely account for half of all new glaucoma diagnoses by 2020 [3]. The outflow of AH from the eye is reduced in PACG, leading to elevated intraocular pressure (IOP) and damage to retinal ganglion cells and the optic nerve. Accordingly, high IOP is a major risk factor for glaucoma, and therapeutic pressure reduction can significantly delay disease progression [4], but the molecular mechanisms that underpin these key features of PACG pathogenesis remain largely unknown.

In almost all types of glaucoma, the permeability and morphology of the trabecular meshwork (TM) are substantially altered which causes damage to retinal ganglion cells (RCGs) [5]. Dysregulation of AH homeostasis likely represents a critical component of this process and has therefore been implicated in the pathogenesis of glaucoma [6]. AH remains in close proximity to the site of pathogenesis in the glaucomatous eye; hence, proteomic evaluation of AH may provide insights into understanding disease pathogenesis. However, to date, detailed AH protein composition in PACG patients has not been assessed, in part due to the difficulties associated with acquiring clinical samples from the anterior chamber of the human eye. Indeed, sample collection from this site may confer a high risk of damaging the cornea and lens and only small volumes of AH can be withdrawn for analysis [7]. The key protein mediators of PACG pathogenesis have not yet been identified, and the therapeutic opportunities for affected patients are unclear.

Recent technological advances in mass spectrometry-based proteomics and bioinformatic methods have enabled the quantitative analysis of proteins in complex biological samples with a high level of resolution and accuracy. Applying this technology to a range of different disorders has revolutionized our understanding of the cellular and physiological processes that promote human pathology [8–11], and uncovered new disease biomarkers [8]. Some investigators have previously sought to assess AH protein profile in glaucoma, but their studies were limited to identifying small numbers of proteins (6-448) in patients with the open-angle disease or animal models [12–15]. Indeed, while proteome analysis has also been applied to other ocular diseases including age-related macular degeneration, diabetic retinopathy, and cataract formation [9–11, 16], AH proteomic profiling has not yet been used to elucidate glaucoma pathogenesis in PACG. We, therefore, used high-resolution, liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based quantitative proteomics to identify PACG-associated alterations in the protein composition of human AH. The differentially expressed proteins may provide novel insight into understanding underlying disease mechanism.

Materials and methods

Subjects and sample preparation

The experimental protocol was approved by the Institutional Review Board at the Tan Tock Seng Hospital (TTSH) and complied with the Declaration of Helsinki's ethical principles for human experimentation. Five patients were recruited via the glaucoma department of TTSH between January 2012 and January 2013 ($n = 2$ patients with PACG and cataracts, $n = 3$ patients cataracts only), and informed consent was obtained from all subjects prior to donation of AH samples. Each study participant underwent a thorough ophthalmic evaluation including measurement of IOP, best corrected visual acuity, preoperative cup-disk ratio, and gonioscopy testing, as well as fundus examination. PACG patients additionally underwent a Humphrey visual field analysis. Further ophthalmological and medical tests included a preoperative physical examination and measurements of blood pressure and glucose level. Inclusion criteria for PACG patients included optic disk changes characteristic of glaucoma, IOP > 20 mmHg, and ≥ 180 degrees of closed angle. Cataract-only (control patients) originated from the same geographical regions as the PACG patients and were age and gender matched.

Glaucoma patients had visually significant cataracts and glaucoma refractory to medical treatment alone. AH samples were obtained from these

glaucoma patients immediately prior to combined trabeculectomy and cataract surgery. Control patients had visually significant cataracts, and AH samples were similarly obtained immediately prior to undergoing cataract surgery. The clinical characteristics of patients are tabulated in Table 1. A volume of 100–120 µl AH was aspirated from each eye via anterior chamber limbal paracentesis using a 30-gauge needle attached to an insulin syringe. Contact with other intraocular structures such as the iris and the anterior lens capsule was carefully avoided to prevent contamination with non-AH proteins. All samples were stored at − 80 °C until further processing.

Peptide extraction and mass spectrometry

In-gel protein digestion and peptide extractions were performed as described previously [17, 18]. Peptides were reconstituted in 0.1% formic acid (FA) and analyzed on a Dionex Ultimate 3000 RSLC nanoLC system coupled to a Q-Exactive instrument (Thermo Fisher, MA). Approximately 2 µg of peptides was injected into an acclaim peptide trap column via the autosampler of the Dionex RSLC nanoLC system at a temperature of 35 °C and flow rate of 300 nl/min. Mobile phase A (0.1% FA in 5% acetonitrile) and mobile phase B (0.1% FA in acetonitrile) were used to establish a 60-min gradient. Peptides were then analyzed on a Dionex EASY-spray column (PepMap® C18, 3um, 100A) using an EASY nanospray source at an electrospray potential of 1.7 kV. A full MS scan (350–1600 m/z range) was acquired at a resolution of 70,000 at m/z 200, with a maximum ion accumulation time of 100 ms. Dynamic exclusion was set to 30 s. Resolution for MS/MS spectra was set to 35,000 at m/z 200. The AGC setting was 1E6 for the full MS scan and 2E5 for the MS2 scan. The 10 most intense ions above a 1000 count threshold were selected for high-energy collision dissociation (HCD) fragmentation, with a maximum ion accumulation time of 120 ms. An isolation width of 2 Da was used for the MS2 scan. Single and unassigned charged ions were excluded from MS/MS. For HCD, normalized collision energy was set to 28. The underfill ratio was defined as 0.1%.

Data analysis

MS data analyses were performed using Thermo Scientific™ ProteomeDiscoverer™ (PD) 1.4 software connected to an in-house Mascot server (V 2.4.1, Matrix Science, Boston, MA). The MS/MS spectra were deisotoped and deconvoluted using the MS2 spectrum processor. Protein identifications were carried out by using Sequest HT and Mascot to compare MS/MS spectra against the UniProt Human database (Released on 7/25/2016, 70,849 sequences, 23,964,784 residues). An automatic target-decoy search strategy was used in combination with percolator to score peptide spectral matches for estimation of false discovery rate (FDR). Percolator parameters were set to maximum delta $Cn = 0.05$; FDR < 1%, validation based on q-value [19]. The search was restricted to a maximum of 2 missed trypsin cleavages, peptide precursor mass tolerances of 10 ppm, and 0.02 Da mass tolerances for fragment ions. Static peptide modification was carbamidomethylation of cysteine residues (+ 57.021 Da), and dynamic peptide modifications were oxidation of methionine residues (+ 15.995 Da) and deamidation of asparagine and glutamine residues (+ 0.984 Da). Calculation of area under the curve (AUC) for each precursor ion peak was conducted using the event detector and precursor ion area detector algorithm (embedded in PD 1.4) with a mass precision setting of 2 ppm. Search results were exported from ProteomeDiscoverer software as protein groups into an Excel file for further analyses (Supplementary Table S1). The reported PACG/control ratio for each protein was calculated by dividing the AUC in PACG by the corresponding AUC in controls. Differentially expressed proteins

Table 1 Clinical characteristics of the subjects involved in the study

	ID	Age (year)	Gender	Race	IOP (mmHg)	VFD	CCT (µm)
Patients	1	76	Female	Chinese	20	− 19.96	538
	2	66	Female	Chinese	45	− 19.81	NA
Controls	1	66	Male	Chinese	18	NA	NA
	2	76	Male	Chinese	10	NA	NA
	3	70	Female	Chinese	10	NA	NA

were defined as up-regulated where abundance was ≥ twofold increased, or as down-regulated where abundance was ≥ 0.5-fold reduced relative to controls (Supplementary Table S1). Hierarchical clustering of AH proteins was performed using Gene Pattern [20]. Bioinformatic analyses were performed using PANTHER tools [21, 22].

Results

Trabecular meshwork composition in human PACG

Proteomic analysis of the human eye has previously been used to shed light on the pathology of POAG [23]. We assessed AH protein profiles in PACG patients (mean age 71.0 ± 7 years) and matched control patients with cataracts only (70.7 ± 5 years) using a label-free, quantitative mass spectrometric method. Using this label-free quantitative approach, and protein identified with more than two peptides, we detected a total of 1363 distinct proteins (Supplementary Table S1) in human AH using a percolator false discovery rate (FDR) ≤ 1%, together with validation based on q value (maximum delta *Cn* = 0.05 [19]), representing a significant improvement in number of proteins identified compared with previous published studies of AH proteome.

When we assessed AH protein fold change in PACG patients (AUC of PACG/AUG of control) (Fig. 1a, b), 773 proteins were found differentially expressed in PACG, with 501 being up-regulated and 272 down-regulated (Fig. 2 and Supplementary Table S2). AH from PACG patients displayed significant down-regulation of a 72 kDa type IV collagenase (metalloproteinase family) that has potential to degrade extracellular matrix (ECM) components including gelatin type I and collagen types IV, V, VII, and X (Fig. 3a). The down-regulation of collagenase may accumulate ECM components and alter its physiological composition. Altered protein turnover of the trabecular meshwork could promote outflow resistance and increase IOP in patients with PACG. This finding suggested that PACG is associated with altered activity of ocular matrix metalloproteinases (MMPs), which regulate the balance of ECM synthesis and proteolysis to control AH outflow from the eye. The proteomics data revealed significant up-regulation of MMP16 in PACG patients. AH proteomics may have enriched with corresponding substrates including collagen chains type alpha-1, 2 and 3 (Fig. 3a), suggesting that enzymatic processing of critical ECM components is defective in disease. Our findings revealed AH enrichment with atypical collagens and fibronectins (Fig. 3b), due to the depleted level of collagenase, suggesting that the composition of the trabecular meshwork is significantly altered in PACG.

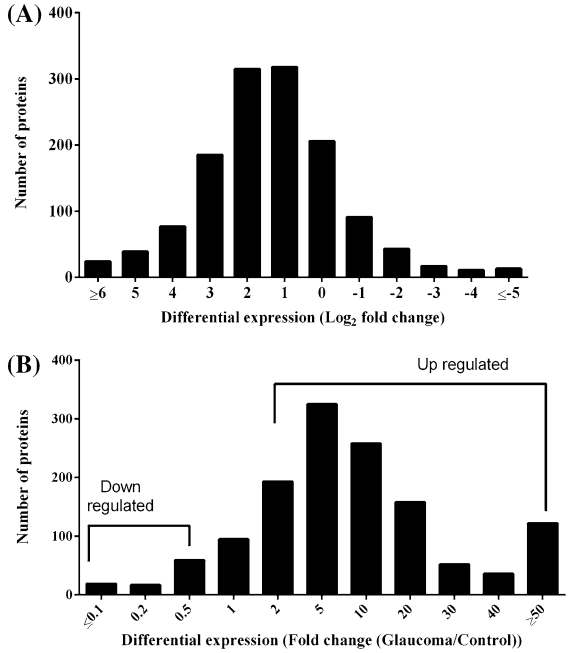

Fig. 1 Aqueous humor (AH) proteins differentially expressed in human PACG. **a** Frequency distribution of AH proteins displayed in $\log_2$ format. **b** Number of AH proteins plotted against fold change (PACG/control)

AH protein regulators of nerve function and oxygen homeostasis in PACG

AH is secreted by cells of the non-pigmented ciliary body and is therefore thought to be enriched in extracellular secretory proteins. Consistent with this concept, we observed that AH of PACG patients contained abundant components of secretory vesicles and granules, platelet granules, endocytic vesicles, and extracellular exosomes (Fig. 4). In particular, among the AH proteins up-regulated in PACG were voltage-dependent anion-selective channel protein 1 (VDAC1; fold-change $\log_2$ (FCL2) = 7.46 ± 1.96), and AP-3

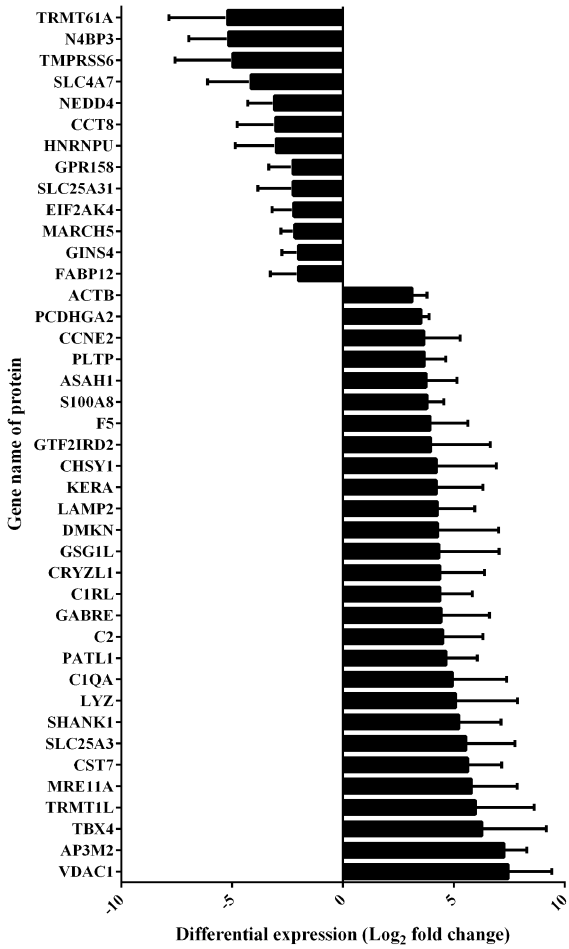

Fig. 2 Relative abundances of proteins in the AH of PACG patients. Protein fold changes (ratio of glaucoma/control) are shown in $\log_2$ format. Shown are the most significantly changed proteins in the AH of PACG

complex subunit mu-2 (AP3M2; FCL: 7.27 ± 1.02), which regulate vesicular cargo delivery to neurites and nerve terminals. Other AH proteins substantially enriched in PACG patients included the DNA double-strand break repair protein MRE11A (FCL: 5.80 ± 2.06), acetylcholine receptor subunit alpha (CHRNA1; FCL: 4.39 ± 3.86), and other proteins typically associated with the cornea/crystalline lens, such as gamma-crystallin D (CRYGD; FCL: 2.62 ± 3.83), alpha-crystallin A (CRYAA; FCL: 2.88 ± 1.85), and keratocan (KERA; FCL: 4.22 ± 2.10). In addition, AH from PACG patients displayed marked accumulation of the developmental regulator T-box transcription factor 4 (TBX4; FCL: 6.28 ± 2.91), putative modulator of neuronal function TRMT1-like protein (TRMT1L; FCL: 5.97 ± 2.67), protease inhibitor cystatin-F (CST7; FCL: 5.63 ± 1.54), mitochondrial phosphate carrier protein isoform B (SLC25A3; FCL: 5.55 ± 2.21), organizer of the synaptic junction SHANK1 (FCL: 5.24 ± 1.90), and anti-microbial enzyme lysozyme C (LYZ; FCL: 5.09 ± 2.79).

While a wide-range of AH proteins was significantly up-regulated in PACG, we also detected substantial deficits in key regulators of neuronal development, axon guidance, and dendrite extension/branching processes (Fig. 2). Distinguished among these were neuronal death-associated ubiquitin-protein ligase NEDD4 (FCL: − 3.09 ± 1.20), NEDD4-binding protein 3 (N4BP3; FCL: − 5.16 ± 1.80), and protein plexin-B2 (PLXNB2; FCL: − 1.19 ± 2.05). Other AH proteins significantly down-regulated in PACG included T-complex protein 1 subunit theta (CCT8; FCL: − 3.04 ± 1.73), which assists folding of actin and tubulin, as well as a number of apolipoproteins that have already been implicated in glaucoma pathology via effects on cholesterol uptake/redistribution within the neuronal network (Supplementary Table S2).

Intriguingly, the proteomic analysis also revealed enrichment of AH of PACG patients with critical mediators of oxygen homeostasis including hemoglobins (HBB, HBA1, and HBD), suggesting increased oxygen demand among activated glial cells, as well as a marginal deficit in the hypoxia-associated protein kallikrein (Supplementary Table S2). This study also detected increased levels of AH proteins known to mediate oxidative stress responses including clusterin (FCL: 3.57 ± 2.46), ferritin light chain (FCL: 4.81 ± 2.66), ferritin heavy chain (FCL: 2.75 ± 1.90), and peroxiredoxin 2 (PRDX2; FCL: 4.78 ± 0.99), which plays a major role in tissue protection against reactive oxygen species (ROS) via elimination of peroxides. Proteomics data of AH also revealed significant increase in SOD and catalase in PACG.

Cluster/pathway analysis of the AH proteome implicates hypoxia responses in PACG

To obtain insight into the cellular pathways most likely impacted by changes in the AH proteome, we performed gene ontology (GO) analysis and hierarchical clustering of the differentially expressed

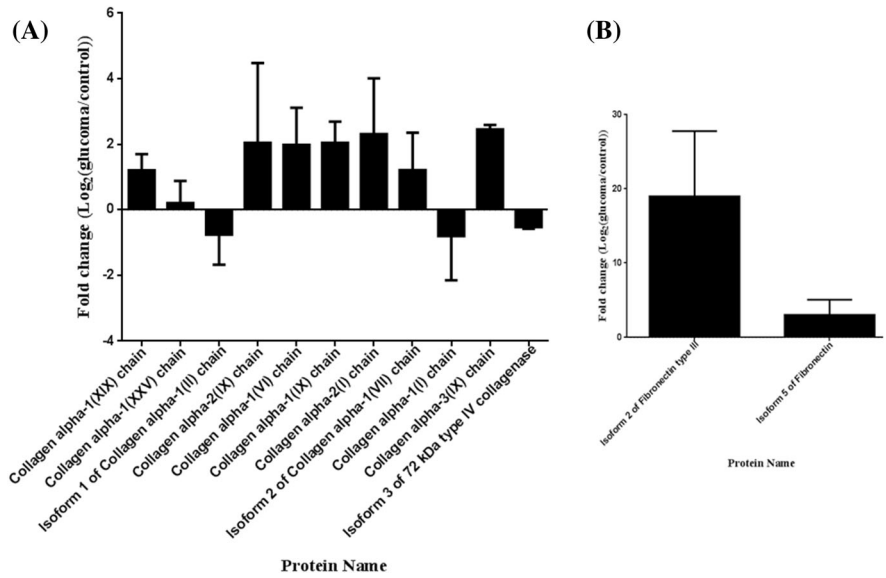

Fig. 3 Accumulation of different collagens and fibronectin in AH of PACG. Protein fold changes (ratio of peak area of glaucoma/peak area of control) are shown in $\log_2$ format. **a** Fold change in different collagens. **b** Fold change in fibronectins

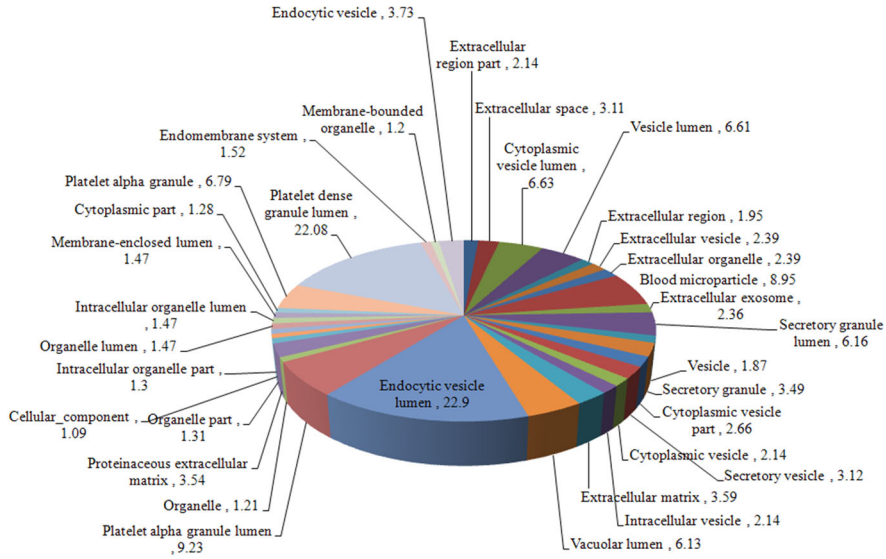

Fig. 4 Classification of up-regulated proteins in the AH of PACG patients. Platelet granules endolytic vesicles and secretory vesicles were enriched in the AH proteome of PACG. Proteins were classified based on their cellular functions using panther gene ontology bioinformatics tool. Shown are significantly enriched GO terms ($p \leq 0.001$) with the Bonferroni correction for multiple testing. The numbers after the protein class indicate the fold enrichment of the proteins

proteins. The GO analysis indicated key features of PACG, including platelet degranulation, dysregulation of endocytic/exocytosis/secretion mechanisms, and effects on multiple immune system components (Fig. 5). Hierarchical clustering identified several distinct groups of AH proteins that were similarly dysregulated in the PACG patient group (Fig. 6 and Fig. S1). AH proteomics of PACG suggested enrichment of endopeptidase inhibitor SERPIND1, histone acetylator KAT6A, G-protein-signaling modulator 2 (GPSM2), haptoglobin (HP), haptoglobin-related protein (HPR), histidine-rich glycoprotein (HRG), unconventional myosin-Vc nucleolar protein 14 (NOP14), antioxidant haptoglobin (HP), gamma-aminobutyric acid receptor subunit epsilon (GABRE), catalase (CAT), and complement C1r subcomponent-like protein (C1RL). Together, these data indicated that PACG is associated with disruption of oxygen homeostasis and ECM dynamics in the AH, likely leading to impaired neuronal function and eventual vision loss.

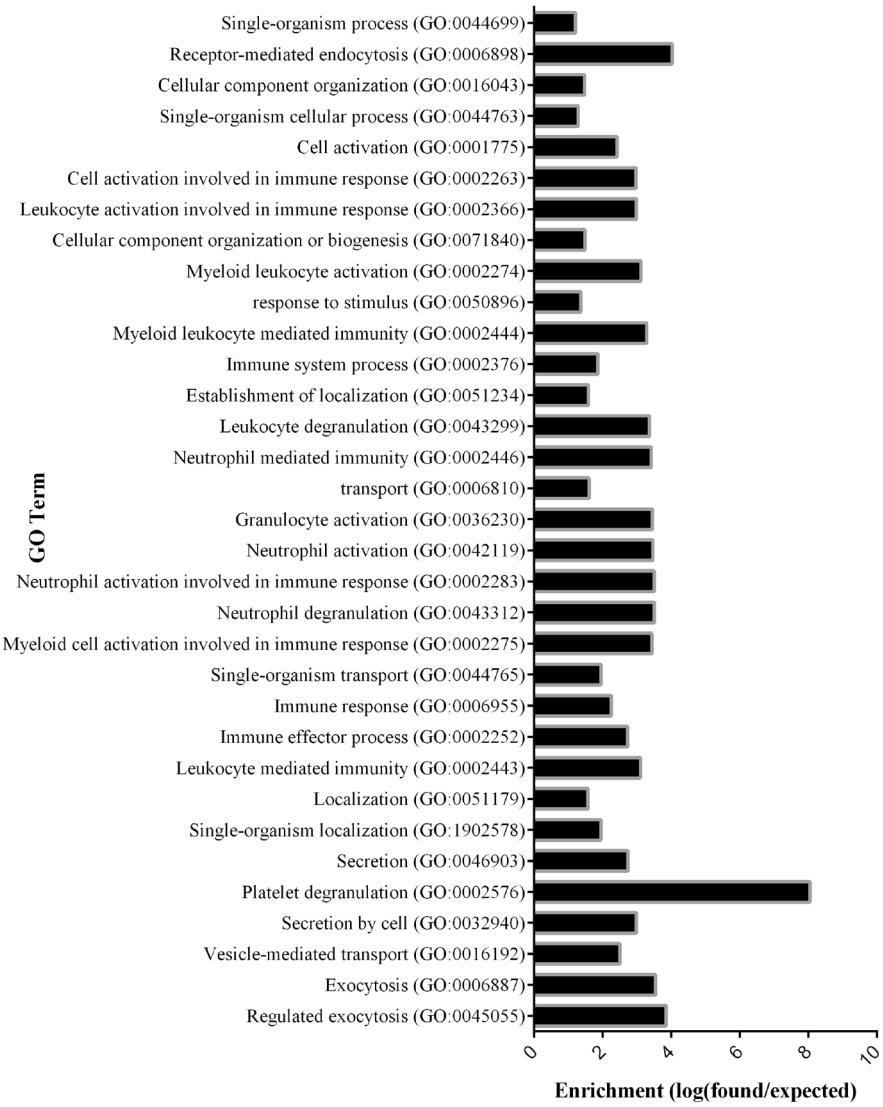

Fig. 5 Proteins up-regulated in the AH of PACG patients were analyzed for GO term enrichment for biological processes (PANTHER^{TM} Version 13.0). Shown are significantly enriched GO terms ($p \leq 0.001$) with the Bonferroni correction for multiple testing

Discussion

To our knowledge, this study represents the first comprehensive analysis of the AH proteome in PACG patients. Previous attempts to characterize AH protein composition in glaucoma identified ~ 700 proteins across a range of different disease types [24–26], while in the present report we successfully identified 1376 distinct AH proteins in AH of PACG, representing a significant advance over earlier studies of the human glaucoma proteome. Our data revealed differential expression of 773 proteins in PACG (up-regulated 501 and 272 down-regulated proteins). Gene ontology analysis of up-regulated protein suggested that these proteins are primarily associated with vesicle-mediated shuttling, signal transduction, and oxidative stress responses. This study also detected substantial down-regulation of proteins known to regulate neuromuscular junction strength, synaptic growth, and immune mediators. This indicates that several dysfunction pathways in human PACG and may be more complex than previously appreciated.

Earlier studies have reported that hemoglobin (HB) levels are reduced in glaucoma, particularly in the optic nerve [27], whereas, in this proteomics study, we observed a significant increase in AH levels of HBB, HBD, and HBA1. In fact, oxygen supply is vital for neuronal viability and function, but the mechanism of

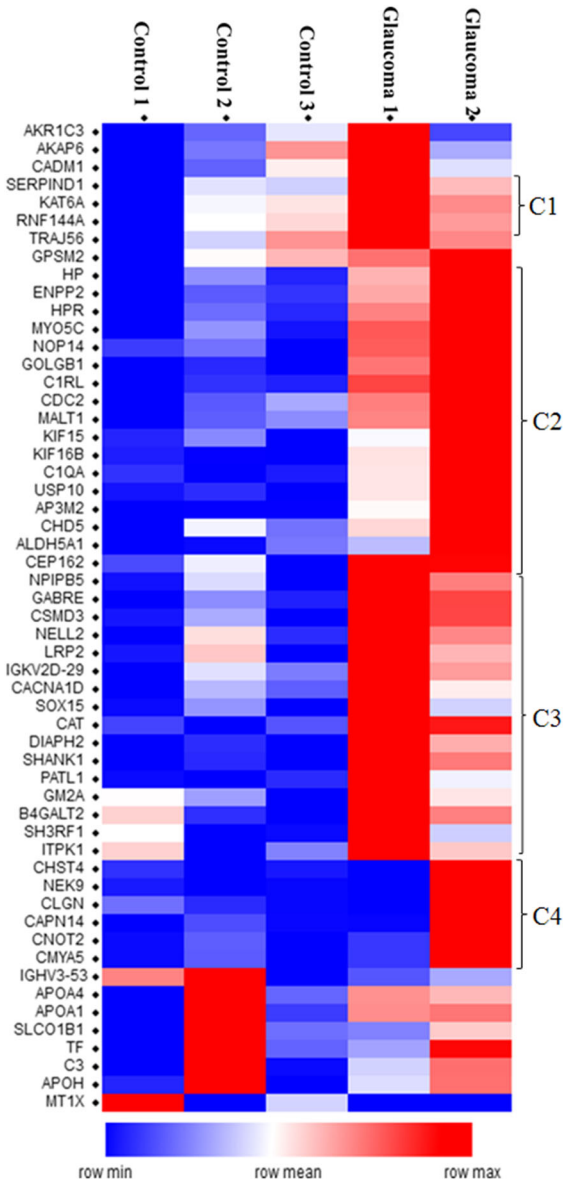

Fig. 6 Hierarchical cluster analysis of the differentially expressed proteins identified in AH proteome of individual PACG patients and controls. Up-regulated protein expression values are displayed in *red*, the down-regulation values are in *blue*, and the intermediate values are in shades of *red* and *blue*

retinal ganglion cell (RGC) oxygenation in the human eye is not yet fully understood. Recent data have indicated that HB is present at steady state in the inner retina, optical nerve head, and in RGCs [28], but hypoxic conditions (low oxygen supply) can induce EPO signaling to further upregulate HB expression in glaucomatous tissues [29]. Induction of ocular HBs observed in AH of PACG, indicates a mechanism of increasing oxygen availability and reduces cellular stress in the affected eye [30]. Indeed, earlier studies have already reported that low oxygen availability in the optic nerve and retina leads to up-regulation of the transcriptional regulator hypoxia-inducible factor (HIF)-1α [31], which activates a complex program of gene transcription to increase oxygen delivery under tissue stress conditions [32]. Together, these observations support the concept of increased HB levels to provide some protection against hypoxic/oxidative injury in human PACG. Indeed, glaucoma is associated not only with dysregulation of blood flow and tissue hypoxia [31] but also with RGC death which contributes to optic nerve degeneration [33]. Consistent with these data, this study observed that AH of PACG patients was enriched in proteins clusterin and ferritin heavy and light chains, which are known to mediate apoptosis and clearance of cellular debris, as well as mediators of the immune response to tissue damage [34]. These findings suggest that the blood-aqueous barrier may be breached in PACG, allowing a passive influx of proteins into the anterior chamber of the eye.

The oxidative agents like peroxide and superoxide anions are present in AH [35]. Goyal et al. [36] noted a significant increase in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in AH of POAG and PACG patients. Consistent with studies by Goyal et al. [36], our proteomics data of AH also showed significant increase in SOD and catalase in PACG. This study also found increased abundance of peroxide-eliminating protein (PRDX2) in PACG. According to Miric et al. [37], cataract significantly alters AH homeostasis, where lipid peroxidation markers and antioxidants depend on the cataract maturity stage and age. In this study, control patients had visually significant cataracts, and their average age was 70.6 years, while it was 71 in PACG group, and protein abundances discussed are relative ratios with control. The significant increase in the level of neopterin in AH of mature cataract patients with pseudoexfoliation indicated increased degree of oxidative stress [38]. Consistent to studies, our proteomics analysis of AH revealed significant increase in SOD, catalase and PRDX2 in PACG, which indicate higher oxidative stress in PACG than cataract patients. Proteins including pro-interleukin-16 (IL16), interleukin-6 receptor subunit (IL6ST),

vascular endothelial growth factor (VEGF), etc., were identified and up-regulated in AH of PACG. Similarly, earlier reports [39, 40] also noted significant elevation of these interleukins in acute primary angle-closure glaucoma, which indicates that immune activation occurs during glaucoma. Proteomics of AH also revealed elevated levels of complement C1q, C1r, C2, C6 and C8, indicating severity of glaucomatous damage. When iris touches the TM, it leads inflammation and stimulates the production of cytokines\interleukin because the outflow is hampered [41]. The expression of VEGF gets induced in response to hypoxia or hypoglycemic stress [42]. Hypoxic condition also induces EPO signaling and up-regulation of HB [28]. The up-regulation of SOD, catalase, PRDX2 etc.; enhanced level of interleukins, and elevated expressions of HBs play major role in PACG pathogenies and also suggest complex interactions between enzymes (SOD, catalase, PRDX2), inflammatory proteins (interleukins\cytokines), and hypoxic condition during PACG pathogenies.

ApoD gene expression is up-regulated in anterior segment tissues from patients with pseudoexfoliation syndrome [43], and ApoE expression levels have been identified as a potential biomarker of POAG [44], which is further associated with increased AH levels of ApoA1 and ApoC3 [45]. Proteomic analysis of AH of PACG revealed up-regulation of multiple apoproteins, suggesting that mechanisms of cholesterol distribution within the neuronal network may be disrupted in this pathology. While an earlier report has suggested that ApoE alleles may influence the risk of POAG but not PACG in a Saudi population [46], AH protein expression levels were not assessed in the earlier study. An increased level of secretory protein RS1 was noted in the AH of PACG and was consistent with previous study [47, 48]. RS1is expressed in retinal neurons during development and has recently been shown to influence retinal synapse formation in a mouse model of retinoschisis [49]. Together, these data indicate that critical protein mediators of cholesterol transport and neuronal function in human AH were altered in PACG and are likely contribute to disease progression.

Limitations of this study

AH is in direct contact with the critical site of pathogenesis in glaucoma and therefore exhibits considerable potential to provide vital information on the pathophysiology of this disease. While AH composition is thought to play a major role in eye drainage defects in PACG, sampling this material requires the use of highly invasive procedures (e.g., aqueous tap, trabeculectomy or phacoemulsification [50]) which confer a significant risk of damaging the patient cornea and/or lens. Sampling human AH is further complicated by the shallow geometry of the anterior chamber of the eye; hence, needle insertion is an extremely difficult task and only small sample volumes are available for study ($\sim$ 15 µl total). The frequency of successful AH sampling using these methods is correspondingly low, and the recruitment of age- and gender-matched control donors is similarly challenging. However, very little is known about the pathophysiology of PACG, and no single proteomic analysis has defined AH composition in this disease (unlike the numerous proteomic studies already performed in POAG cohorts). The current study is the first to successfully use high-throughput, LC–MS/MS-based proteomics technology to define AH composition in human PACG, and achieved sufficient resolution to detect significant differential expression of even low-abundance ocular proteins. A major limitation of our study was the low number of patients enrolled in the study. The small volumes of AH obtained, precluded the use of further validation methods such as western blotting or multiple reactions monitoring (MRM). However, given the current paucity of data in PACG, this report provides proteomic composition of AH which could be useful for another researcher for a targeted approach. Further, AH of PACG revealed alterations in different pathways which may add to current scientific knowledge and will guide future efforts to develop novel developments in understanding the disease mechanism and design therapeutic strategies.

In summary, this study provides the first comprehensive analysis of AH protein composition in PACG, identified 773 proteins that were differentially expressed in PACG patients, and uncovered a number of critical cellular processes that impact on neuronal function and oxidative stress which are likely to influence disease progression. In particular, proteomic analysis of AH of PACG implicates HB biology and mechanisms of oxygen homeostasis in the neuronal damage in PACG. This information will guide future

efforts to define the roles of these pathways in disease pathogenesis.

Availability of data and material

The data supporting the conclusions of this article is included in the present article. Table S1. Protein groups identified by SequestHT and Mascot using Proteome Discoverer software (version 1.4). Table S2. Differential Expressed Proteins. The mass spectrometry proteomics data have been deposited to the Proteome Xchange Consortium via the PRIDE partner repository [51] with the dataset identifier PXD006202 and the following submission details.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate This study was in agreement with the Declaration of Helsinki and approved by the Institutional Review Board at the Tan Tock Seng Hospital (TTSH). Informed consent was obtained from each patient.

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