Experimental Eye Research 197 (2020) 108077

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

The aqueous humor proteome of primary open angle glaucoma: An extensive review

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A B S T R A C T

Background: We reviewed the literature on the aqueous humor (AH) proteome of primary open angle glaucoma (POAG) patients in order to obtain deeper insight into the pathophysiology of POAG.

Methods: We searched Pubmed and Embase up to May 2019 for studies that compared AH protein composition between POAG (cases) and cataract (controls). Untargeted studies (measuring the whole proteome, by LC-MS/MS) were divided into two subgroups depending on the type of surgery during which POAG AH was collected: glaucoma filtration surgery (subgroup 1) or cataract surgery (subgroup 2). We reanalyzed the raw data (subgroup 1) or combined the reported data (subgroup 2) to perform GO enrichment (GOrilla) and pathway analysis (Pathvisio).

Results: Out of 93 eligible proteomic studies, seven were untargeted studies that identified 863 AH proteins. We observed 73 differentially expressed proteins in subgroup 1 and 87 differentially expressed proteins in subgroup 2. Both subgroups were characterized by activation of the acute immune response, dysregulation of folate metabolism and dysregulation of the selenium micronutrient network. For subgroup 1 but not for subgroup 2, proteins of the complement system were significantly enriched.

Conclusion: AH proteome of POAG patients shows strong activation of the immune system. In addition, analysis suggests dysregulation of folate metabolism and dysregulation of selenium as underlying contributors. In view of their glaucoma surgery, POAG patients of subgroup 1 most likely are progressive whereas POAG patients in subgroup 2 most likely have stable POAG. The proteome difference between these subgroups suggests that the complement system plays a role in POAG progression.

1. Introduction

Glaucoma is a neurodegenerative disorder characterized by progressive loss of retinal ganglion cells and their axons, resulting in visual field loss (Quigley and Broman, 2006; Tham et al., 2014). For primary open-angle glaucoma (POAG), which is the most common subtype of glaucoma, the underlying disease mechanism is not exactly known (Gupta and Weinreb, 1997). A major risk factor is intraocular pressure (IOP), which is determined by the balance in production and drainage of the aqueous humor (AH). For every 1-mmHg increase in IOP, there is a 10% increase in relative risk of POAG (Weinreb, 2005). Since IOP and AH play such an important role in POAG, it is assumed that AH composition changes during POAG development and progression. Identifying these changes could give more insight in the underlying disease mechanism and could be used as a biomarker or risk factor for POAG.

Proteins are a valuable source of potential biomarkers as they are key players in the physiological processes. AH protein concentration is much lower than in blood. Several studies estimate that AH on average contains between 10 and 100 mg/dl protein whereas blood contains approximately 6000 mg/dl (Chowdhury et al., 2010; Kuchle et al., 1994; Rosenfeld et al., 2015; Tripathi et al., 1989). In addition, protein composition differs due to filtration and active secretion from ciliary body (Chowdhury et al., 2010). As AH sampling is invasive, AH is usually only obtained from patients that undergo ocular surgery. As such, most studies investigating the AH use the most common ocular disease, cataract, as their control group (Adav et al., 2018; Chowdhury et al., 2010; Murthy et al., 2015).

Several studies have analyzed proteins and protein composition of

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https://doi.org/10.1016/j.exer.2020.108077
Received 29 December 2019; Received in revised form 26 March 2020; Accepted 18 May 2020
Available online 27 May 2020

AH of POAG patients. These include studies that quantified the protein expression of one or a few target proteins, i.e. targeted studies. With techniques such as microarrays several hundred of proteins can be investigated simultaneously (Duan et al., 2010; Grus et al., 2008; Izzotti et al., 2010; Sacca et al., 2012). Clearly, this approach yields a lot of information on many proteins. While the number of proteins on the array can be large, it still is a subset of the total proteome and the choice of the subset depends on the researcher. Therefore, these studies can be viewed as semi-targeted. With liquid chromatography tandem-mass spectrometry (LC-MS/MS), it is in principle possible to detect any protein present in a sample. Studies utilizing this technique can be considered as untargeted studies giving unbiased information on AH proteome (Adav et al., 2018; Ji et al., 2015; Kaeslin et al., 2016; Kaur et al., 2018; Kliuchnikova et al., 2016; Salamanca et al., 2018; Sharma et al., 2018).

Together, all untargeted and targeted studies performed until now, have created a large amount of information on the proteins in AH of glaucoma patients. To our knowledge there has not been an extensive review that compared and/or combined the outcome of these proteomic studies to gain more insight on the role of the AH in POAG pathogenesis. We therefore performed a systematic review of the literature on the AH proteome of POAG patients. We mainly focused on untargeted studies since these are unbiased and in principle cover the whole proteome. In addition, these studies deliver lists of differentially expressed proteins that can be used for pathway analyses aimed at identifying POAG pathophysiology.

2. Methods

2.1. Literature search

We conducted a PubMed and Embase database search for articles published prior to May 1st, 2019 using the following search terms: “primary open angle glaucoma” (all fields) and “aqueous humor” (all fields). Additionally, filters were used to ensure that all entries were written in English, had an abstract available, and that the studies were performed on human aqueous humor. Titles and abstracts were scanned to select articles in which the aqueous humor protein composition was compared between POAG and a cataract control group.

2.2. Group definitions

The studies were classified according to the analysis method used (Table 1). We additionally divided the untargeted studies into 2 subgroups based on the type of surgery the POAG patients underwent at time of AH collection. These were glaucoma filtration surgery (subgroup 1) and cataract extraction surgery (subgroup 2). The controls in all studies were cataract patients without glaucoma.

2.3. Data extraction and analysis

2.3.1. Untargeted subgroup 1 (POAG @ GFS)

Raw data were retrieved from the ProteomeXchange database (PXD007624, PXD002623 and PXD004928, (Adav et al., 2018; Kaur et al., 2018; Kliuchnikova et al., 2016). For PXD004928 this data was not yet made public and access was kindly provided by the authors. Raw data of each study were re-analyzed separately with MaxQuant software (Max Planck Institute (Tyanova et al., 2016)) using the default settings. These settings include Oxidation [M] and acetyl [protein N-term] as variable modification, carbamidomethyl [C] as fixed modification and peptide discovery with a false discovery rate (FDR) of 0.01.

Label free quantification (LFQ) results were further analyzed with Perseus software (Max Planck Institute)(Tyanova et al., 2016). Usual suspects of contamination were excluded for further analysis with the exception of keratins. Keratins are naturally present in ocular tissue such as the lens and cornea. For combining the result files, the data of each study were scaled so that per protein the average ($\bar{\chi}$) LFQ intensity was the same between each study:

$$\left(\frac{LFQ\ intensity\ [sample]}{\bar{\chi}\ LFQ\ intensity\ [study]}\right) \times \bar{\chi}\ LFQ\ intensity\ [combined\ studies]$$

To handle missing values we adopted the data processing strategy from Bijlsma et al. (2006) In short, for each duplicate or triplicate an average was calculated based on the samples with non-zero values. If all duplicates had a zero value the protein was considered not detected. In the final processed dataset we only included proteins that were detected in more than 70% of the POAG or cataract group in at least one study. These were statistically compared using the built-in multiple sample ANOVA function. Conservatively the threshold for differentially expressed proteins is FDR-adjusted p (q) $<$ 0.05. We however used less stringent criteria and considered proteins differentially expressed with p $<$ 0.05.

2.3.2. Untargeted subgroup 2 (POAG @ cataract)

We extracted all data available in the manuscripts. The type of data presented varied considerably between the studies. This consisted of a list of proteins with corresponding fold change and p-value ((Kaeslin et al., 2016)), a list of proteins with fold change $<$ 0.5 or higher than 1.5 without corresponding p-value ((Ji et al., 2015)), a list of proteins with significant fold change (p $<$ 0.05 (Sharma et al., 2018);) and a list of proteins detected in each group without fold change or p-value ((Salamanca et al., 2018). Given the diverse nature of these data, we defined a set of arbitrary criteria to enable us to make lists of upregulated and downregulated proteins per study. Upregulated proteins either had a fold change $>$ 1.5, were detected in POAG but not in controls, or were reported as significantly upregulated by the authors. Downregulated proteins either had fold change $<$ 0.6 or were detected in control patients but not in POAG. For combined analysis of the different studies, we considered proteins upregulated or downregulated if they matched our arbitrary criteria in at least two studies.

2.3.3. Semi-targeted

We extracted all data available in the manuscripts. For all studies this entailed a list of significantly differentially expressed proteins between glaucoma and control.

2.3.4. Targeted

We provided the reported biomarkers, group sizes (n), and to enable comparison between different studies, the cohen's d effect size. To

Table 1
Criteria for defining proteomic study groups. For untargeted studies the type of surgery for AH collection of POAG patients was additionally used as a criterion. AH of the control group was collected during cataract surgery. GFS: Glaucoma filtration surgery.

Groups	Detectable proteins	Techniques	Type of POAG surgery
Untargeted subgroup 1	Virtually full proteome	e.g. LC-MS/MS	GFS
Untargeted subgroup 2	Virtually full proteome	e.g. LC-MS/MS	Cataract extraction
Semi-targeted	Several hundred	e.g. microarray	
Targeted	One or a few	e.g. ELISA	

calculate the effect size the mean and/or the standard deviation (SD) had to be estimated for some studies. Estimations were done based on the data available and according to formulas described before by Hozo et al. and the Cochrane handbook (Higgins et al., 2019; Hozo et al., 2005). In short:

2.3.4.1. Median and the interquartile range (IQR) available

$$estimated\ mean = median$$

$$estimated\ SD = \frac{IQR}{1.35}$$

2.3.4.2. Median, minimum (min) and maximum (max) available

$$estimated\ mean = \frac{2 \times median + min + max}{4}$$

$$estimated\ SD = \frac{max - min}{4}$$

2.3.4.3. Bar graph only. Missing data was estimated from the graph using scale measurements.

2.4. Comparison of data from subgroup 1 and 2

For both subgroup 1 and 2 we were able to combine data and generate lists of regulated proteins. Yet, these two lists were derived in a different manner. In subgroup 1 they were the result from an ANOVA with stringency $P < 0.05$ or 0.1). In subgroup 2, we applied arbitrary criteria to obtain this list (see above). In order to compare between subgroups, we decided to compare the list of subgroup 2 with the list of subgroup1 with p level of 0.1 since in this way both subgroups have similar numbers of regulated proteins, allowing for a fair comparison.

2.5. Pathway analyses

For the two subgroups of untargeted studies, biological processes were annotated using Gorilla (Eden et al., 2009). Per subgroup, upregulated and downregulated proteins were assessed for GO enrichment separately using a two unranked lists. The total amount of proteins identified across these LC-MS/MS studies was considered the background set. Pathway overrepresentation analysis was performed using Pathvisio (Pathvisio 3.3.0; http://www.pathvisio.org, (Kutmon et al., 2015; van Iersel et al., 2008). The required curated *Homo sapiens* pathways were obtained from Wikipathways (http://www.wikipathways.org, (Kelder et al., 2012; Kutmon et al., 2016). Pathways were considered significantly enriched if they had a Z-score ≥ 1.96, a permuted p-value < 0.05, and > 1 significant gene in the pathway.

3. Results

3.1. Literature search results

A schematic representation of our literature search is depicted in Fig. 1. Of the eligible studies, 80 were hypothesis-driven (Supplemental Table 1), and four were semi-targeted (Supplemental Table 2 (Duan et al., 2010; Grus et al., 2008; Izzotti et al., 2010; Sacca et al., 2012),). The remaining nine studies were untargeted proteome studies using LC-MS/MS that were divided into a group of POAG patients that underwent GFS (subgroup 1 (Adav et al., 2018; Kaur et al., 2018; Kliuchnikova et al., 2016), and a group that underwent cataract extraction surgery (subgroup 2 (Ji et al., 2015; Kaeslin et al., 2016; Salamanca et al., 2018; Sharma et al., 2018),). Two studies were excluded as they included POAG patients that suffered from ocular comorbidities such as cornea decompensation (Anshu et al., 2011; Rosenfeld et al., 2015). An overview of the studies and the proteomics information the authors published is presented in Table 2. For subgroup 1 and subgroup 2 we additionally summarized the clinical characteristics of the included patients, as reported by the authors (Supplemental Tables 3 and 4).

3.2. AH proteome of POAG patient subgroup 1 (POAG @ GFS)

The three studies of subgroup 1 consist of 21 POAG and 25 cataract patients. Reanalyzing the RAW data and combining the outcome yielded 592 unique proteins (Fig. 2A). Based on our detection criteria (i.e. detected in more than 70% of the POAG samples or more than 70% of the cataract samples in at least 1 study), 248 proteins were included for further analysis (Fig. 2B). Of these, 57 proteins met the detection criteria in all three studies (Figs. 2B) and 53 proteins were detected in only one study (Fig. 2C).

Multiple sample ANOVA of the combined dataset of 248 proteins showed 30 significantly upregulated and 23 significantly downregulated proteins ($p < 0.05$) (Table 3 and Table 4). With less stringent criteria ($p < 0.1$) we found an additional 10 upregulated and 10 downregulated proteins, possibly associated with this POAG population undergoing GFS surgery (Table 5 and Table 6). Only 14 proteins would be considered significantly regulated when correcting for multiple testing ($q < 0.05$).

3.3. AH proteome of POAG patient subgroup 2 (AH @ cataract)

The four studies of subgroup 2 included in total 30 POAG and 51 cataract patients and reported 639 proteins (Fig. 3A).(Ji et al., 2015; Kaeslin et al., 2016; Salamanca et al., 2018; Sharma et al., 2018) The number of proteins that were reported in more than 1 study was quite low. More than 50% (338 of 639) lacked replication (Fig. 3A). Based on the arbitrary criteria we made (see methods), 30 proteins were upregulated (Fig. 3B, Tables 5 and Table 7 proteins downregulated in at least 2 studies (Fig. 3C and Table 8).

3.4. Comparison of the POAG proteome between subgroups

We compared the 73 regulated proteins of subgroup 1 with the 87 regulated proteins of subgroup 2. Combined this yielded 136 regulated proteins of which 24 proteins were identified in both subgroups. Remarkably only 13 of these 24 proteins had the same direction of expression (Table 9) whereas 11 proteins had significant regulations in the opposite direction (Table 9).

3.5. Comparison of the POAG proteomes of untargeted studies with targeted studies

Next, we investigated whether the 136 significantly differentially expressed proteins have been previously reported. The targeted studies combined investigated 105 proteins (Supplemental Table 1). The four semi-targeted studies identified 46 significantly upregulated and 3 significantly downregulated proteins of which 47 were unique to their respective studies (Supplemental Table 2). A large proportion of the significant regulated proteins found by LC-MS/MS were novel findings. Only 12 of the regulated proteins have been investigated previously (Table 10). For several of these 12 proteins the results differ between untargeted and targeted studies. Lastly, the untargeted studies did not cover all known AH proteins. Of the 136 proteins previously found significantly regulated in POAG AH, approximately 2/3rd (74 of 105 proteins and 14 of 49 proteins) were not identified in our dataset.

3.6. GO enrichment and pathway analysis

The LC-MS/MS studies analyzed the AH of in total 51 POAG and 76 cataract patients and identified 863 AH proteins (Supplemental Table 5). These proteins were considered as the detectable aqueous

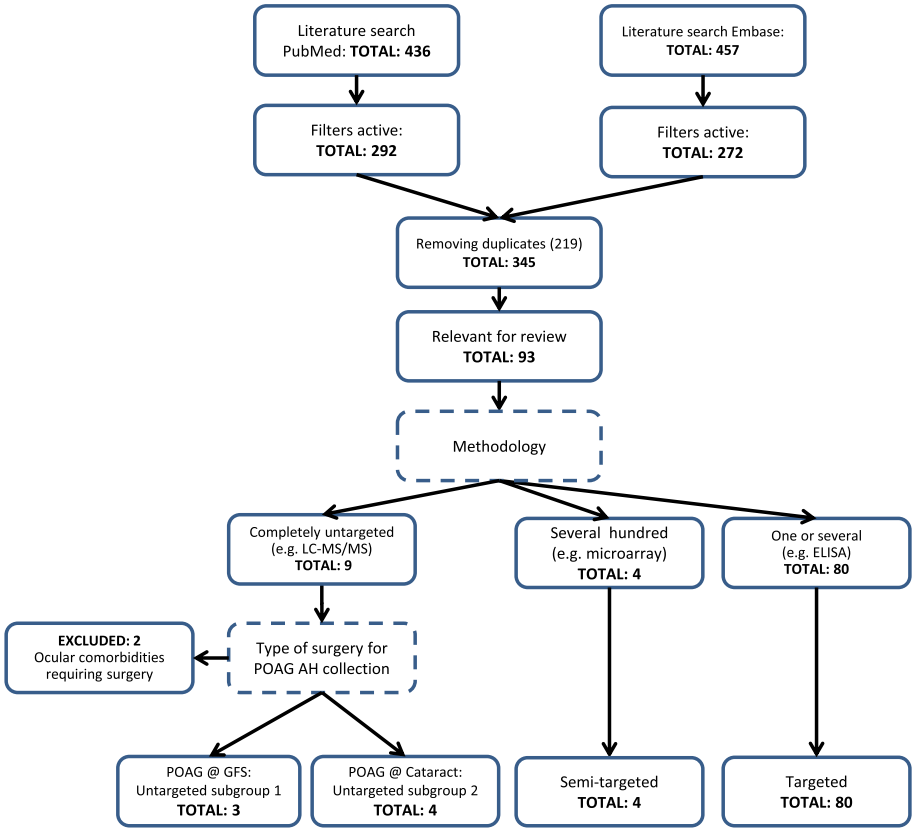

Fig. 1. Workflow for literature search, selection and categorization. GFS: glaucoma filtration surgery.

humor "proteome" and used as background for gene ontology enrichment and pathway analysis. Upregulated and downregulated proteins were analyzed separately. For GO enrichment 827 of the 863 proteins were associated with GO terms.

GO analysis indicated that upregulated proteins of subgroup 1 were part of processes such as acute inflammatory response and platelet degranulation ($p < 0.001$; Supplemental Fig. 1a). Downregulated proteins were mainly related to immune response and complement activation ($p < 0.001$; Supplemental Fig. 1b). Upregulated proteins in subgroup 2 were also related to acute inflammatory response ($p < 0.001$). In addition, the proteins were related to fatty acid related metabolism and blood coagulation ($p < 0.001$; Supplemental Fig. 2a). In contrast to subgroup 1, complement proteins were not significantly enriched. Instead the downregulated proteins were related to IL-12 mediated signaling ($p < 0.001$; Supplemental Fig. 2b).

Pathway overrepresentation analysis showed 11 pathways significantly overrepresented in subgroup 1 (Supplemental Tables 6) and 7 pathways in subgroup 2 (Supplemental Table 7). The findings were similar to the GO enrichment. Both subgroups had regulated proteins that enriched the folate metabolism and selenium micronutrient network. Proteins of subgroup 1 additionally enriched several pathways related to complement system whereas proteins of subgroup 2 enriched vitamin b12 metabolism.

4. Discussion

We reviewed the studies on the AH proteome of glaucoma patients. The focus was on untargeted proteomic studies, using LC-MS/MS, which are unbiased and in principle cover the whole proteome. We compared and combined the data of 7 untargeted proteomic studies that measured the AH proteome of a total of 51 POAG and 76 cataract patients. A total of 863 proteins were identified, which illustrates the potential of LC-MS/MS. Of these 863 proteins, 136 proteins were differentially regulated in AH of POAG patients and may represent clues for glaucoma pathways.

4.1. Variability

The outcomes of the LC-MS/MS studies varied substantially. This might be the result of biological differences (e.g. study population, medication, and reason for surgery) and methodological differences (e.g. AH collection, sample preparation, type of mass spectrometer and data analysis). Considering that most previously investigated proteins were small peptides e.g. cytokines it is no surprise that LC-MS/MS did not identify the majority of these proteins. LC-MS/MS is less sensitive for detection of small peptides and requires specific sample preparation techniques (reviewed by (Finoulst et al., 2011). When we consider only those proteins that were identified with LC-MS/MS and also in targeted studies, the level of agreement in study outcomes was quite low (Table 10). The reason is not clear.

4.2. Limitations and strengths

A limitation of our study was that not all data were available for analysis. We had to resort to arbitrary criteria to enable proper comparison of the study outcomes. Most likely we failed to detect some regulated proteins due to lack of reported expression data. In addition, we decided to manually combine isoforms to a single protein since the analysis depth differed between studies, with several studies not reporting proteins at the isoform level.

A strength of our study was that we divided the LC-MS/MS studies into two subgroups based on the type of surgery during which AH was collected. The subgroups are probably more homogeneous, since different types of surgery may introduce different technical artifacts or confounders. In addition, the type of surgery also relates to the type or stage of glaucoma of these patients. Patients undergoing GFS (subgroup

Table 2
Study characteristics of the proteome studies included in this extensive review. Studies are indicated by the name of the first author.

Group	Study	Technique	POAG (n)	CAT (n)	Population	Centri-fugation	Immuno Depletion	Total Proteins	Proteins listed	Expression	↑	↓	POAG unique	CAT unique
Untargeted	Kliuchnikova	LC-MS/MS	7	11	Russia	No	No	269	All (RAW)	per sample	0	0	12	26
Subgroup 1	Kaur	LC-MS/MS	9	9	India	No	No	814	All (RAW)	per sample	x/97	x/97	206	221
POAG @ GFS	Adav	LC-MS/MS	5	5	Singapore	No	No	865	All (RAW)	per sample	43 ± 18	105 ± 45	265	165
Untargeted	Kaeslin	HRM/MS	5	5	Switzerland	No	No	448	All	FC	34	53	0	0
Subgroup 2	Salamanca	LC-MS/MS	4	8	Spain	Yes	No	309	All	name	N/A	N/A	27	108
POAG @ Cataract	Sharma	LC-MS/MS	15	32	US	No	No	401	33 (sig)	FC (sig)	33	0	N/A	N/A
	Ji	iTRAC LC-MS/MS	6	6	China	Yes	No	445	All	FC (sig)*	138*	124*	N/A	N/A
Semi-targeted	Grus	Seldi-tof MS	22	24	Germany	No	No	N/A	1 (sig)	FC (sig)	1	N/A	N/A	N/A
	Sacca	Antibody array	14	11	Italy	No	No	N/A	13 (sig)	FC (sig)	13	N/A	N/A	N/A
	Izotti	Antibody array	10	14	Italy	No	No	N/A	31 (sig)	FC (sig)	29	2	N/A	N/A
	Duan	gel spots LC-MS/MS	5	5	China	No	No	N/A	7 (sig)	FC (sig)	7	N/A	N/A	N/A

POAG: primary open-angle glaucoma; CAT: cataract extraction; n: amount of patients; GFS: glaucoma filtration surgery; (RAW): data obtained from raw data files; (sig): significantly differentially expressed; ↑amount of proteins upregulated in POAG; ↓ amount of proteins downregulated in POAG; FC: fold change. N/A: no data available. *based on fold change not p-values.

1) are likely progressive POAG patients, while POAG patients undergoing cataract surgery (subgroup 2), probable have a medically controlled, stable POAG. Dividing the POAG patients over two subgroups not only reduces experimental variation but also enables characterization of the AH proteomes of different glaucoma stages.

4.3. AH protein profile of POAG at GFS (subgroup 1)

Upregulated proteins of subgroup 1 suggest a strong acute inflammatory response and platelet degranulation. Recently, a study showed an association between blood platelet activation and POAG severity (Ma et al., 2019). In line with the results, the DBA/2 J glaucoma mouse model has increased ocular infiltration of platelet-monocyte complexes (Williams et al., 2019). These progressive POAG patients additionally have complex dysregulation of the complement system (Supplemental Fig. 3). As evident from our GO enrichment analysis, several complement proteins were downregulated. In line with this, a study on plasma found a negative association between plasma C3 levels and POAG disease severity (Li et al., 2018). However, other complement proteins such as C1q were significantly upregulated. In glaucoma animal models, similar upregulation was observed and inhibition of the complement system by targeting C1q was neuroprotective (Howell et al., 2011; Williams et al., 2016). The complement system was also one of the enriched pathways of upregulated proteins in a study on post-mortem vitreous humor and retina of POAG patients (Mirzaei et al., 2017).

Pathway enrichment analysis indicated that the regulated proteins are also involved in the selenium micronutrient network and folate metabolism. Ramdas et al. recently published a systematic review to determine the association between vitamins in the blood and POAG and found no correlation between blood folic acid concentration and POAG (Ramdas et al., 2018). However, this does not exclude the possibility of a local dysregulation of folate metabolism in AH. For instance, homocysteine, a neurotoxic metabolite of the folate pathway, is increased in POAG AH (Ghanem et al., 2012; Roedl et al., 2007; You et al., 2018). Additionally, some studies report an increased POAG risk with mutations in *MTFHR*, a crucial enzyme in this cycle (Al-Shahrani et al., 2016; Gupta et al., 2014; Junemann et al., 2005). In respect to the selenium micronutrient studies suggest a link between selenium AH concentration and glaucoma but the results are still inconclusive (Bruhn et al., 2009; Hohberger et al., 2018; Junemann et al., 2018; Najafi et al., 2014). An analysis of the effect of selenium on cultured trabecular meshwork cells showed an increased resistance to outflow due to elevated selenium levels (Conley et al., 2006). Also, a high intake of selenium may increase the risk of glaucoma (Ramdas, 2018).

4.4. AH protein profile of POAG patients at cataract surgery (subgroup 2)

Similar to subgroup 1, the upregulated proteins in subgroup 2 indicate an acute inflammatory response. In addition, GO terms related to lipid metabolism such as negative regulation of fatty acid biosynthesis and regulation of lipoprotein lipase (LPL) activity were enriched. One study reported a correlation between serum lipoprotein LPL and retinal nerve fiber layer thickness suggesting lipid metabolism may play a role in the development of POAG (Shiba et al., 2015). GO enrichment was also found for blood coagulation. Hypercoagulability has been previously reported in POAG patients (Matsumoto et al., 2001; O'Brien et al., 1997).

Several significantly enriched pathways were mainly the result of the same subset of regulated proteins (Supplemental Table 7). The pathway that contained the most significantly regulated proteins was the vitamin B12 metabolism pathway. A recent review found no relation between vitamin B12 and POAG (Ramdas et al., 2018). Yet, a correlation between plasma vitamin B12 and retinal nerve fiber layer thickness was found in patients with vitamin B12 deficiency (Turkyilmaz et al., 2013). This warrants further studies on vitamin B12

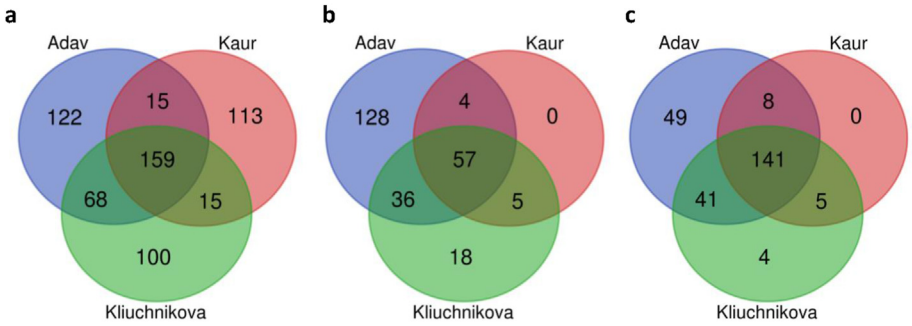

Fig. 2. Venn diagram of the proteins identified in studies of subgroup 1 (POAG @ GFS) (a) and the proteins identified that met our detection criteria (i.e. identified in more than 70% of either cataract or POAG patients in at least 1 study (b). For the 248 proteins that met our detection criteria we additionally visualized their expression without detection criteria (i.e. identified in at least 1 patient) (c). Studies are indicated by the name of the first author.

Table 3
Proteins upregulated in POAG subgroup 1 (POAG @ GFS) ($p < 0.05$). Values are represented as Log2 transformed mean LFQ intensity.

Gene	Uniprot ID	Protein names	Studies	Cataract	n	POAG	n	Difference	p-value	q-value
CPB2	Q96IY4	Carboxypeptidase B2	3	22.83	3	24.84	6	2.01	0.0007	0.0200
ABI3BP	Q7Z7G0	Target of Nesh-SH3	2	24.03	6	25.57	2	1.54	0.0015	0.0324
TIMP2	P16035	Metalloproteinase inhibitor 2	2	25.39	4	26.98	6	1.59	0.0004	0.0333
C1QB	P02746	Complement C1q subcomponent subunit B	1	25.75	5	26.91	4	1.16	0.0020	0.0335
A1BG	P04217	Alpha-1B-glycoprotein	3	26.93	24	27.75	21	0.82	0.0015	0.0360
ORM2	P19652	Alpha-1-acid glycoprotein 2	3	26.39	22	27.18	21	0.79	0.0029	0.0428
CFI	P05156	Complement factor I	3	25.02	20	25.53	11	0.51	0.0036	0.0477
AGT	P01019	Angiotensinogen	3	26.27	21	26.88	20	0.61	0.0041	0.0523
IGKV3D-15	P01624	Ig kappa chain V-III region POM	1	28.78	5	30.16	5	1.38	0.0055	0.0688
TPP1	O14773	Tripeptidyl-peptidase 1	3	23.52	10	24.50	8	0.98	0.0076	0.0743
SERPINA3	P01011	Alpha-1-antichymotrypsin	3	29.54	25	30.18	21	0.64	0.0070	0.0777
HPR	P00739	Haptoglobin-related protein	1	24.58	5	26.27	3	1.69	0.0067	0.0780
COL9A2	Q14055	Collagen alpha-2(IX) chain	2	23.70	5	24.69	5	0.99	0.0094	0.0843
ORM1	P02763	Alpha-1-acid glycoprotein 1	3	29.55	25	30.06	21	0.51	0.0120	0.0966
TPI1	P60174	Triosephosphate isomerase	2	24.30	5	25.09	5	0.79	0.0151	0.0985
LUM	P51884	Lumican	3	24.35	13	25.18	11	0.83	0.0147	0.0999
SPARCL1	Q14515	SPARC-like protein 1	3	24.25	10	24.74	8	0.49	0.0137	0.1006
C9	P02748	Complement component C9	3	25.06	18	25.71	15	0.65	0.0144	0.1011
GC	P02774	Vitamin D-binding protein	3	29.16	25	29.59	21	0.43	0.0186	0.1155
SERPINC1	P01008	Antithrombin-III	3	28.16	22	28.52	21	0.36	0.0198	0.1189
B2M	P61769	Beta-2-microglobulin	3	25.85	20	26.46	18	0.61	0.0213	0.1214
HRG	P04196	Histidine-rich glycoprotein	3	26.53	23	26.95	21	0.42	0.0258	0.1248
AHSG	P02765	Alpha-2-HS-glycoprotein	3	26.98	25	27.57	21	0.59	0.0246	0.1268
IGKV3D-11	A0A0A0MRZ8	Ig kappa chain V-III region VG	3	27.60	5	28.88	9	1.28	0.0258	0.1278
ALDH3A1	P30838	Aldehyde dehydrogenase, dimeric NADP-preferring	3	24.45	15	25.24	7	0.79	0.0230	0.1283
HBD	P02042	Hemoglobin subunit delta	3	24.40	6	29.06	6	4.66	0.0240	0.1301
CST3	P01034	Cystatin-C	3	30.85	21	31.42	20	0.57	0.0343	0.1651
VTN	P04004	Vitronectin	3	25.95	17	26.42	18	0.47	0.0371	0.1708
ALB	P02768	Serum albumin	3	37.10	25	37.47	21	0.37	0.0391	0.1734
C8A	P07357	Complement component C8 alpha chain	3	23.87	7	25.45	3	1.58	0.0410	0.1737

in POAG. These patients also had enrichment of regulated proteins related to the selenium micronutrient network and folate metabolism pathway just like subgroup 1.

4.5. Overlap between stable and progressive POAG

The proteins with overlapping expression provide information on general processes involved in glaucoma pathogenesis. For instance, the overlapping proteins C1QB, SERPINC1, SERPINA3, SAA4, A1BG, and C1S suggest acute inflammatory response. Activation of the immune system in POAG is extensively reviewed elsewhere (Bell et al., 2013; Rieck, 2013; Tezel, 2011). Recently, it was shown that inflammation related T-cell infiltration can lead to prolonged cell death of retinal ganglion cells even after IOP elevation was restored, highlighting the need for additional IOP independent treatments (Chen et al., 2018). In addition, the present pathway analysis suggests that both subgroups have dysregulation of folate metabolism and selenium micronutrient pathway. Several of the significantly regulated AH proteins overlapping between both subgroups (Table 9) were not represented in any of the enriched pathways. Their role in the pathophysiology of glaucoma is unclear.

4.6. Differences between stable and progressive POAG

Obviously, there are differences in surgical procedure, and perhaps other differences such as type of glaucoma medication, between the two subgroups (Supplemental Tables 3 and 4). Nonetheless, comparison could give insight into the mechanism that drives POAG progression. A major difference was the strong dysregulation of the complement system in progressive POAG (subgroup 1) which was not observed in stable POAG (subgroup 2) (Supplemental Fig. 3). Dysregulation of the complement system in AH of progressive POAG patients may reflect changes in complement activity in the retina during rapid progressive retinal ganglion cell death. Whether the observed changes in AH composition are cause or consequence of changes in complement activity in the retina is uncertain. It would be valuable to know if complement activity in the retina can be modulated via the AH. Interestingly, a study that quantified complement factor C3 in POAG sera found that C3 concentration was negatively correlated with POAG severity as assessed by mean deviation of the visual field (Li et al., 2018).

On protein level there were also some remarkable differences (Table 9). COL9A2 and SPARCL1 are related to extracellular matrix organization and increased expression in subgroup 1 could contribute to uncontrolled IOP often observed in these patients. The other proteins

Table 4
Proteins downregulated in POAG subgroup 1 (POAG @ GFS) ($p < 0.05$). Values are presented as Log2 transformed mean LFQ intensity.

Gene	Uniprot ID	Protein names	Studies	Cataract	n	POAG	n	Difference	p-value	q-value
FN1	P02751	Fibronectin	1	28.76	5	26.14	5	−2.62	0.0005	0.0216
ATP5F1 A	P25705	ATP synthase subunit alpha, mitochondrial	1	28.52	5	26.44	2	−2.08	0.0010	0.0251
CAPN10	Q9HC96	Calpain-1	1	27.49	5	25.72	4	−1.77	0.0005	0.0260
KRT2	P35908	Keratin, type II cytoskeletal 2 epidermal	3	31.78	24	30.15	20	−1.63	0.0017	0.0336
FGA	P02671	Fibrinogen alpha chain	3	31.27	7	29.53	6	−1.74	0.0002	0.0360
C4BPA	P04003	C4b-binding protein alpha chain	1	28.73	5	27.4	5	−1.29	0.0003	0.0380
PROS1	P07225	Vitamin K-dependent protein S	1	25.74	5	24.98	2	−0.76	0.0026	0.0390
KRT10	P13645	Keratin, type I cytoskeletal 1	3	32.50	25	30.62	20	−1.88	0.0058	0.0718
CFH	P08603	Complement factor H	3	28.88	7	27.18	8	−1.70	0.0074	0.0770
C7	P10643	Complement component C7	3	26.12	6	25.41	6	−0.71	0.0085	0.0795
ACTB	P60709	Actin, cytoplasmic 1	2	26.82	6	25.57	5	−1.25	0.0103	0.0883
IGHV1-3	P01743	Ig heavy chain V–I region HG3	2	24.67	2	21.63	5	−3.04	0.0132	0.1003
FGG	P02679	Fibrinogen gamma chain	3	31.50	6	30.76	6	−0.74	0.0161	0.1045
APLP2	Q06481	Amyloid-like protein 2	3	24.71	21	23.66	15	−1.05	0.0192	0.1171
CLSTN1	O94985	Calsyntenin-1	3	26.50	22	26.03	16	−0.47	0.0206	0.1201
C6	P13671	Complement component C6	2	25.22	5	24.35	6	−0.87	0.0242	0.1280
AZGP1	P25311	Zinc-alpha-2-glycoprotein	3	27.58	19	27.03	17	−0.55	0.0371	0.1671
C5	P01031	Complement C5	2	25.98	6	25.35	6	−0.63	0.0342	0.1685
HP	P00738	Haptoglobin	1	33.09	5	32.01	5	−1.08	0.0360	0.1709
IGKV2D-28	A0A075B6P5	Ig kappa chain V-II region FR	2	28.34	5	24.81	7	−3.53	0.0407	0.1730
PIKFYVE	Q9Y2I7	1-phosphatidylinositol 3-phosphate 5-kinase	1	31.15	5	29.63	5	−1.53	0.0406	0.1762
WIF1	Q9Y5W5	Wnt inhibitory factor 1	3	24.33	17	23.66	14	−0.67	0.0440	0.1832
KPRP	Q5T749	Keratinocyte proline-rich protein	2	26.10	11	25.17	7	−0.93	0.0483	0.1954

Table 5
Proteins likely upregulated in POAG subgroup 1 (POAG @ GFS)($0.05 < p < 0.1$). Values are presented as Log2 transformed mean LFQ intensity.

Gene	Uniprot ID	Protein names	Studies	Cataract	n	POAG	n	Difference	p-value	q-value
LYZ	P61626	Lysozyme C	3	26.18	22	26.84	15	0.66	0.0518	0.2061
CAT	P04040	Catalase	2	21.98	3	23.77	6	1.79	0.0637	0.2560
SERPINA4	P29622	Kallistatin	2	24.00	6	24.30	8	0.31	0.0680	0.2639
APOC3	P02656	Apolipoprotein C-III	2	23.10	4	24.22	6	1.12	0.0758	0.2790
LGALS3BP	Q08380	Galectin-3-binding protein	3	24.57	15	24.87	12	0.30	0.0763	0.2757
OPTC	Q9UBM4	Opticin	3	28.13	25	28.89	18	0.76	0.0765	0.2717
BTD	P43251	Biotinidase	3	24.55	8	25.71	8	1.17	0.0787	0.2743
SAA4	P35542	Serum amyloid A-4 protein	1	25.57	5	26.06	5	0.48	0.0835	0.2818
IGHV3-9	P01782	Ig heavy chain V-III region DOB	1	25.51	5	26.75	4	1.24	0.0854	0.2847
HBA1	P69905	Hemoglobin subunit alpha	3	31.04	18	32.57	9	1.52	0.0856	0.2810

Table 6
Proteins likely downregulated in POAG subgroup 1 (POAG @ GFS) ($0.05 < p < 0.1$). Values are presented as Log2 transformed mean LFQ intensity.

Gene	Uniprot ID	Protein names	Studies	Cataract	n	POAG	n	Difference	p-value	q-value
C1QC	P02747	Complement C1q subcomponent subunit C	1	26.22	5	25.49	5	−0.73	0.0652	0.2571
C8B	P07358	Complement component C8 beta chain	3	23.67	6	22.72	3	−0.96	0.0695	0.2671
GAPDH	P04406	Glyceraldehyde-3-phosphate dehydrogenase	3	25.40	15	24.88	6	−0.52	0.0728	0.2736
KRT1	P04264	Keratin. type II cytoskeletal 1	3	32.85	25	31.98	19	−0.87	0.0811	0.2779
A2M	P01023	Alpha-2-macroglobulin	3	30.93	25	30.45	21	−0.47	0.0868	0.2808
HPX	P02790	Hemopexin	3	29.79	25	28.17	21	−1.62	0.0877	0.2784
IGHG3	P01860	Ig gamma-3 chain C region	3	28.66	23	28.28	20	−0.38	0.0917	0.2874
PTGDS	P41222	Prostaglandin-H2 D-isomerase	3	31.31	25	30.98	21	−0.33	0.0963	0.2989
IGHM	P01871	Ig mu chain C region	3	29.76	6	28.82	4	−0.94	0.0970	0.2962
C1S	P09871	Complement C1s subcomponent	3	24.97	7	24.55	7	−0.42	0.0971	0.2923

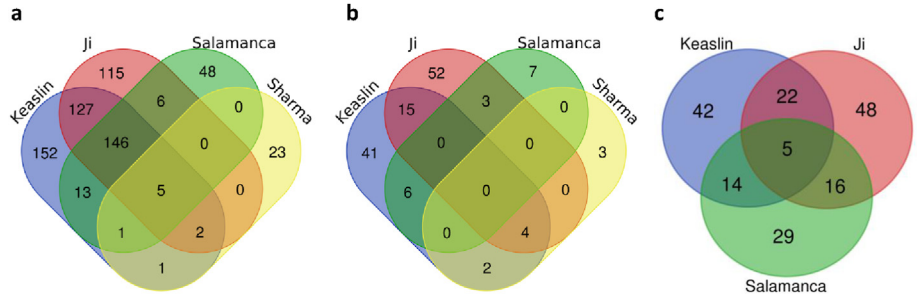

Fig. 3. Venn diagram of total number of proteins reported by the studies included in subgroup 2 (POAG patients @ Cataract) (a) and the number of upregulated proteins (b) and downregulated proteins (c), according to our arbitrary criteria.

Table 7
Proteins upregulated in POAG subgroup 2 (POAG @ Cataract). Values are mean Log2 (fold change). "?" denotes proteins that were detected in both POAG and cataract patients but fold change was not provided in the manuscript. ND: Not Detected.

gene	Uniprot	Protein name	Kaeslin	Ji	Salamanca	Sharma
C1QB	P02746	Complement C1q subcomponent subunit B	1.67	1.14	ND	ND
APOC3	P02656	Apolipoprotein C-III	1.85	2.21	Cataract only	1.75
A1BG	P04217	Alpha-1B-glycoprotein	0.60	0.90	?	ND
SERPINF2	P08697	Alpha-2-antiplasmin	1.00	0.80	?	1.56
SERPINA3	P01011	Alpha-1-antichymotrypsin	1.19	1.24	?	ND
APOA4	P06727	Apolipoprotein A-IV	1.12	1.08	?	ND
SAA4	P35542	Serum amyloid A-4 protein	1.17	2.33	ND	ND
SERPINC1	P01008	Antithrombin-III	0.87	1.50	?	ND
KRT16	P08779	Keratin, type I cytoskeletal 16	1.52	4.15	ND	ND
LRG1	P02750	Leucine-rich alpha-2-glycoprotein	0.94	0.94	ND	ND
PGLYRP2	Q96PD5	N-acetylmuramoyl-L-alanine amidase	1.05	0.64	?	ND
PLG	P00747	Plasminogen	0.88	0.63	?	ND
FCGBP	Q9Y6R7	IgGFc-binding protein	1.07	0.83	?	0.94
ITIH1	P19827	Inter-alpha-trypsin inhibitor heavy chain H1	1.43	?	POAG only	ND
SERPING1	P05155	Plasma protease C1 inhibitor	0.64	−2.33	POAG only	ND
ITIH4	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	1.49	1.24	?	2.04
AZGP1	P25311	Zinc-alpha-2-glycoprotein	0.71	0.98	?	ND
KRT1	P04264	Keratin, type II cytoskeletal 1	−2.74	2.72	POAG only	ND
APOC1	P02654	Apolipoprotein C–I	1.68	0.96	ND	ND
ECM1	Q16610	Extracellular matrix protein 1	0.65	−0.86	POAG only	ND
FETUB	Q9UGM5	Fetuin-B	2.20	N/A	Cataract only	0.90
HSPA1A	P08107	Heat shock 70 kDa protein 1A/1 B	1.32	1.18	ND	ND
IGFBP2	P18065	Insulin-like growth factor-binding protein 2	1.01	N/A	POAG only	ND
NBL1	P41271	Neuroblastoma suppressor of tumorigenicity 1	2.67	−1.02	POAG only	ND
NTM	Q9P121	Neurotrimin	−2.34	1.19	POAG only	ND
RELN	P78509	Reelin	−0.29	1.26	POAG only	ND
SHBG	P04278	Sex hormone-binding globulin	1.18	N/A	POAG only	ND
IGKC	P01834	Ig K chain C region	0.58	N/A	ND	3.76
IGHG4	P01861	Ig gamma-4 chain C region	0.76	2.64	ND	ND
IGHM	P01871	Ig mu chain C region	0.82	2.26	ND	ND

are related to energy metabolism (TPI1, AZGP1) or inflammatory processes. Whether and how these changes in expression are related to POAG progression is uncertain.

5. Recommendations for future studies

The untargeted LC-MS/MS approach clearly has great potential and already revealed many AH proteins with altered expression in POAG. While writing this review we encountered several difficulties in combining the data from the various studies. Improving on these issues, enabling stringent meta-analysis of these valuable data, would further exploit the power of this technique.

A first, obvious recommendation would be that authors, but also journals, ensure that the raw data of LC-MS/MS studies are uploaded to a public depository. This was not the case for all studies included in this review. In addition, reported datasets were sometimes not complete or used different (older) annotations. If authors would provide, in addition to the raw data, also a processed output file (e.g. Excel) containing UniprotID, protein name, gene symbol and expression per sample, the valuable proteomics data can be combined and used for meta-analysis more easily, even by authors that don't have mass spectrometry software available.

Second, there is an unmet need to standardize the LC-MS/MS protocols. It is important to report the total AH protein concentration since total AH protein concentrations may differ between glaucoma patients and controls (Prata et al., 2007; Rosenfeld et al., 2015). A noticeable difference between study protocols was sample centrifugation i.e. two studies performed centrifugation. We are unable to provide a clear recommendation if samples should be centrifuged. Surely a fraction of cells that are present can be considered as irrelevant debris from e.g. aqueous tap or iris (Stamer and Clark, 2017). On the other hand, some cells in AH might be biologically relevant. For instance, an ongoing study shows that certain immune cells are present in AH and differ between glaucoma patients and controls (ARVO abstract 2019; (Nair et al., 2019). Centrifugation may not be practically feasible in most operating theaters. As centrifugation will affect the detected proteome, authors should clearly state this in their methods. In addition, to increase sensitivity of the LC-MS/MS, we suggest depleting AH of albumin and immunoglobulins as more than half of the total LC-MS/MS peak intensity was caused by these proteins.

Third, the clinical data provided in the publications are often scarce and incomplete. Detailed clinical data of the patients are important for proper interpretation, to account for variation and to compare and combine data from different studies. In addition, this allows for correlation analysis between proteins and clinical data that can be useful for therapy or for biomarker research. Glaucoma experts could agree on a data template for authors to report relevant clinical data, such as age, gender, ethnicity, BMI, IOP (current and at diagnosis), disease severity, rate of disease progression, current medication (especially ocular medication) and ocular surgical history.

Lastly, we like to suggest that authors replicate key findings of LC-MS/MS using targeted techniques e.g. qPCR, ELISA or Western blot. Only a few of the proteins identified in the LC-MS/MS studies discussed in our review, had been measured before using other techniques. The outcome of these techniques often differed considerably from the outcome of the LC-MS/MS studies. In part, this may be due to differences in clinical data across the different studies. Replication with targeted techniques in patients with the same, extensively documented clinical background will significantly strengthen study results.

6. Conclusion

The results of our review indicate an involvement of the immune system in POAG. In addition, selenium and folate pathways appear to be involved. Especially intriguing were the differences in AH composition between POAG patients with GFS and POAG patients with cataract surgery. These patients probably differ in POAG stage i.e. progressive vs. stable. It is certainly valuable to distinguish these subgroups

Table 8
Proteins downregulated in POAG subgroup 2 (POAG @ Cataract). Values are mean Log2 (fold change). "?" denotes proteins that were detected in both POAG and cataract patients but fold change was not provided in the manuscript. ND: Not Detected.

Gene	Uniprot ID	Name	Kaeslin	Ji	Salamanca	Sharma
APLP2	Q06481	Amyloid-like protein 2	−1.36	−2.53	Cataract only	ND
FGG	P02679	Fibrinogen gamma chain	−0.81	?	Cataract only	ND
WIF1	Q9Y5W5	Wnt inhibitory factor 1	−0.80	−0.99	?	ND
KRT5	P13647	Keratin, type II cytoskeletal 5	−1.38	1.10	Cataract only	ND
A2M	P01023	Alpha-2-macroglobulin	−0.95	−0.96	?	ND
CLSTN1	O94985	Calsyntenin-1	−0.82	−3.76	?	ND
C1S	P09871	Complement C1s subcomponent	−2.20	?	Cataract only	ND
ENPP2	Q13822	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	−0.89	−1.91	?	ND
PSAP	P07602	Proactivator polypeptide	−0.15	−3.11	Cataract only	ND
IGFBP6	P24592	Insulin-like growth factor-binding protein 6	−0.93	−2.24	Cataract only	ND
HBB	P68871	Hemoglobin subunit beta	−1.44	−1.85	Cataract only	ND
FBLN1	P23142	Fibulin-1	0.02	−1.42	Cataract only	ND
SERPINI1	Q99574	Neuroserpin	−2.45	−1.76	Cataract only	ND
CFHR1	Q03591	Complement factor H-related protein 1	0.07	−0.81	Cataract only	ND
DKK3	Q9UBP4	Dickkopf-related protein 3	−0.25	−2.81	Cataract only	ND
LCN1	P31025	Lipocalin-1	1.21	−3.24	Cataract only	ND
FBN1	P35555	Fibrillin-1	−1.17	−2.02	?	ND
LGALS3BP	Q08380	Galectin-3-binding protein	−0.89	−2.56	?	ND
CRYGS	P22914	Beta-crystallin S	−2.75	1.36	Cataract only	ND
SPARCL1	Q14515	SPARC-like protein 1	−0.54	−0.96	Cataract only	ND
ALDH3A1	P30838	Aldehyde dehydrogenase, dimeric NADP-preferring	−4.99	−2.22	ND	ND
TPI1	P60174	Triosephosphate isomerase	−2.46	?	Cataract only	ND
TPP1	O14773	Tripeptidyl-peptidase 1	−1.46	−0.82	?	ND
COL9A2	Q14055	Collagen alpha-2(IX) chain	−1.55	?	Cataract only	ND
HPR	P00739	Haptoglobin-related protein; Haptoglobin	−2.18	−1.81	ND	ND
CTSL	P07711	Cathepsin L1	−0.80	−1.79	Cataract only	ND
CA1	P00915	Carbonic anhydrase 1	−1.39	0.61	Cataract only	ND
CADM1	Q9BY67	Cell adhesion molecule 1	−0.50	−1.73	Cataract only	ND
CDH2	P19022	Cadherin-2	0.40	−0.77	Cataract only	ND
CTBS	Q01459	Di-N-acetylchitobiase	−1.63	−1.18	ND	ND
FAM3C	Q92520	Protein FAM3C	−0.36	−1.09	Cataract only	ND
GNS	P15586	N-acetylglucosamine-6-sulfatase	−1.02	−2.15	ND	ND
IGLC7	A0M8Q6	Immunoglobulin lambda constant 7	ND	−1.71	Cataract only	ND
IGLL5	B9A064	Immunoglobulin lambda-like polypeptide 5	−1.57	−2.24	ND	ND
IGKV1D-33	P01608	Immunoglobulin kappa variable 1D-33	ND	−1.41	Cataract only	ND
IGKV3-20	P01622	Ig kappa chain V-III region Ti	−1.42	−1.98	ND	ND
IGHG1	P01857	Ig gamma-1 chain C region	−1.42	−1.34	ND	ND
IGKV3D-11	P04433	Ig kappa chain V-III region VG	−0.88	−0.95	ND	ND
IGKV2D-28	P06309	Immunoglobulin kappa variable 2D-28	−0.94	−1.04	ND	ND
IGHV4-34	P06331	Immunoglobulin heavy variable 4-34	ND	−1.2	Cataract only	ND
IGLV3-21	P80748	Ig lambda chain V-III region LOI	−0.74	ND	Cataract only	ND
IMPG2	Q9BZV3	Interphotoreceptor matrix proteoglycan 2	−1.43	−1.02	?	ND
ITIH5	Q86UX2	Inter-alpha-trypsin inhibitor heavy chain H5	−0.60	−0.78	Cataract only	ND
LMAN2	Q12907	Vesicular integral-membrane protein VIP36	−0.98	ND	Cataract only	ND
LRP2	P98164	Low-density lipoprotein receptor-related protein 2	−0.60	−3.68	Cataract only	ND
LSAMP	Q13449	Limbic system-associated membrane protein	−0.80	0.63	Cataract only	ND
MFAP4	P55083	Microfibril-associated glycoprotein 4	−1.13	−1.56	?	ND
MIF	P14174	Macrophage migration inhibitory factor	−2.51	ND	Cataract only	ND
MMP2	P08253	72 kDa type IV collagenase;Matrilysin	0.50	−1.46	Cataract only	ND
OAF	Q86UD1	Out at first protein homolog	−1.24	−1.57	ND	ND
PKM	P14618	Pyruvate kinase isozymes M1/M2	−2.39	?	Cataract only	ND
PPIA	P62937	Peptidyl-prolyl cis-trans isomerase A	−1.27	1.45	Cataract only	ND
SCG3	Q8WXD2	Secretogranin-3	−1.16	−0.99	ND	ND
SCG5	P05408	Neuroendocrine protein 7B2	−0.55	−0.90	Cataract only	ND
SOD1	P00441	Superoxide dismutase [Cu-Zn]	−1.52	?	Cataract only	ND
SPOCK1	Q08629	Testican-1	−1.49	−2.48	ND	ND
VGF	O15240	Neurosecretory protein VGF	−1.51	−1.12	ND	ND

of patients in future studies, considering the clinical relevance. These data revealed that progressive POAG patients have strong dysregulation of the complement system, which may provide a target for therapy. While these results need further confirmation, we are confident that studying the AH proteome will add to our understanding of the molecular pathophysiology of POAG and reveal new targets for intervention.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exer.2020.108077.

Table 9
Proteins significantly differentially regulated in opposite direction between the POAG subgroups. ↑: significantly upregulated; ↓: significantly downregulated.

Gene	Uniprot ID	Protein name	Subgroup 1	Subgroup 2
TPP1	O14773	Tripeptidyl-peptidase 1	↑	↓
COL9A2	Q14055	Collagen alpha-2(IX) chain	↑	↓
TPI1	P60174	Triosephosphate isomerase	↑	↓
LGALS3BP	Q08380	Galectin-3-binding protein	↑	↓
SPARCL1	Q14515	SPARC-like protein 1	↑	↓
ALDH3A1	P30838	Aldehyde dehydrogenase, dimeric NADP-preferring	↑	↓
HPR	P00739	Haptoglobin-related protein	↑	↓
IGKV3D-11	P04433	Ig kappa chain V-III region VG	↑	↓
AZGP1	P25311	Zinc-alpha-2-glycoprotein	↓	↑
KRT1	P04264	Keratin, type II cytoskeletal 1	↓	↑
IGHM	P01871	Ig mu chain C region	↓	↑

Table 10
Proteins identified as significant in glaucoma with untargeted proteomics (subgroup 1 and subgroup 2) that have been studied using semi-targeted or targeted approaches. Uncertain confirmation indicates that the findings were not significant or that multiple studies report conflicting results.

Gene	Uniprot ID	Protein name	Subgroup 1	Subgroup 2	semi-targeted	Targeted	Confirmation
ALB	P02768	Serum albumin	↑	N/A	↑		Yes
APOC3	P02656	Apolipoprotein C-III	↑	↑		↑	Yes
CST3	P01034	Cystatin-C	↑	N/A	↑		Yes
TIMP2	P16035	Metalloproteinase inhibitor 2	↑	N/A		↑	Yes
A2M	P01023	Alpha-2-macroglobulin	↓	↓		↑	No
CFH	P08603	Complement factor H	↓	0.09		NS	Uncertain
FN1	P02751	Fibronectin	↓	0.46; 2.33		NS	Uncertain
PTGDS	P41222	Prostaglandin-H2 D-isomerase	↓	−0.44;-3.66	↑	↑	No
ENPP2	Q13822	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	−0.38	↓		↑	No
MIF	P14174	Macrophage migration inhibitory factor	N/A	↓		NS	Uncertain
MMP2	P08253	Matrix metalloproteinase-2	N/A	↓		NS; ↓;↑	Uncertain
SOD1	P00441	Superoxide dismutase [Cu–Zn]	N/A	↓	↓		Yes

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