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Data Article

Comprehensive characterization of human aqueous humor proteomes from primary open-angle glaucoma using data-independent acquisition dataset on a TimsTOF pro mass spectrometer

Hu Xiao[a,b], Da Qian Lu[b,c,d], Jimmy Ka-Wai Cheung[c], Ying Hon Sze[b,c], Jing Fang Bian[b,c,d], Chi-wai Do[b,c,d,e], Lei Zhou[b,c,d,f], Xiao Li Xing[g,*], Thomas Chuen Lam[a,b,c,d,e,*]

[a] The Hong Kong Polytechnic University Shenzhen Research Institute, Shenzhen, Guangdong, PR China
[b] Centre for Myopia Research, School of Optometry, The Hong Kong Polytechnic University, Hong Kong
[c] Centre for Eye and Vision Research (CEVR), 17 W Hong Kong Science Park, Hong Kong
[d] Research Centre for SHARP Vision (RCSV), The Hong Kong Polytechnic University, Hong Kong
[e] Research Centre for Chinese Medicine Innovation (RCMI), The Hong Kong Polytechnic University, Hong Kong
[f] Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong
[g] Eye Institute and School of Optometry, Tianjin Medical University Eye Hospital, Tianjin, China

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ABSTRACT

Primary open-angle glaucoma (POAG) is the foremost cause of irreversible visual impairment, attributed to a complex array of ocular diseases. The lack of reliable prognostic indicators necessitates the development of biomarkers that can be used to detect and monitor glaucoma early in its course. In this study, human aqueous humor databases dedicated to advanced and not advanced POAG from Chinese were compiled to facilitate research on POAG, using advanced label-free quantitative proteomics technology by a TimsTOF Pro mass spectrometer. Proteins and peptides were identified and quantified by DIA-NN bioinformatics. All mass spectrometry data were deposited in the ProteomeXchange Consortium

* Corresponding authors.
E-mail addresses: xxing04@tmu.edu.cn (X.L. Xing), thomas.c.lam@polyu.edu.hk (T.C. Lam).

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via the PRIDE partner (https://www.ebi.ac.uk/pride) with the dataset identifier PRIDE: PXD060484.

Specifications Table

Subject	Health Sciences, Medical Sciences & Pharmacology
Specific subject area	Human aqueous humor in primary open-angle glaucoma
Type of data	Raw, Analyzed, Processed.
Data collection	Proteins were digested by trypsin, chromatographically separated using the Bruker nanoElute system, and analyzed using the Bruker TimsTOF pro mass spectrometer. Data acquisitions were conducted in the data-independent acquisition (DIA). Raw data (DIA) was identified and quantified by DIA-NN (Version 1.9.2).
Data source location	Centre for Myopia Research, School of Optometry, The Hong Kong Polytechnic University, Kowloon, Hong Kong
Data accessibility	Repository name: PRoteomics IDEntifications Database (PRIDE) Data identification number: PXD060484 Direct URL to data: https://www.ebi.ac.uk/pride Username: reviewer_pxd060484@ebi.ac.uk Password: KaGeAykpS288
Related research article	None

1. Value of the Data

- There were 66 specimens of human aqueous humor (one eye per patient) from Chinese patients suffering from glaucoma used for this experiment.
- High quality and the most comprehensive proteome of the human glaucoma aqueous humor.
- The comprehensive human dataset would support molecular understanding of the mechanism of glaucoma and support investigations into other anterior ocular diseases.

2. Background

Globally, primary open-angle glaucoma (POAG) constitutes the foremost cause of irreversible visual impairment, attributed to a complex array of ocular diseases [1–4]. Given the absence of reliable prognostic indicators, there is a pressing need to develop biomarkers that enable monitoring of glaucoma progression [5–7]. In this regard, the aqueous humor of the anterior segment is of paramount importance. This study aims to establish a comprehensive aqueous humor proteomics database for research on primary open-angle glaucoma (POAG), encompassing two groups dedicated to advanced and not advanced POAG, respectively.

3. Data Description

In this study, we divided the samples into two groups, advanced and not advanced POAG (grouping information was included in “Group.xlsx”). In the advanced POAG group, samples were obtained from 44 patients with visual field mean deviation (VFMD) less than -12 dB. Not advanced POAG ($n = 22$) is characterized by VFMD more than or equal to -12 dB. Utilizing advanced label-free quantitative proteomics technology, comprehensive proteomes of advanced and not advanced glaucoma in human aqueous humor can now be identified. Employ-

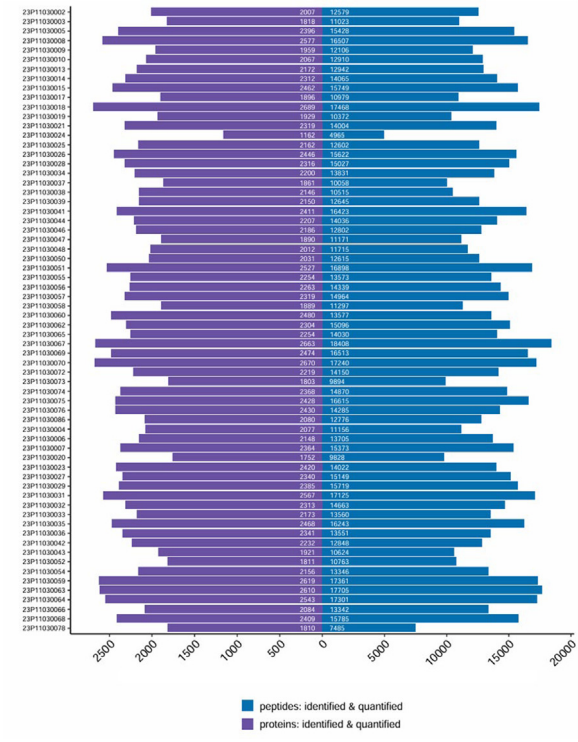

Fig. 1. The MS-DAP quality control report indicated the count of peptides or proteins identified in each sample at 1 % FDR.

ing the TimsTOF Pro Bruker mass spectrometer and DIA-NN bioinformatics [8–11], we successfully quantified non-redundant proteins without the need for pre-constructed libraries. A total of 2853 proteins and 26,815 peptides were identified by DIA-NN at 1 % FDR. Quality control was conducted using the Mass Spectrometry Downstream Analysis Pipeline (MS-DAP, version 1.2.1) [12,13]. Figure 1, 2, 3 and 4 show the results of human aqueous humor quality control for advanced and not advanced glaucoma. Also, this advancement provides researchers with access to the most comprehensive proteome of human aqueous humor to date. Beyond advancing the study of glaucoma and aqueous humor proteomes, this database will also support investigations into proteomes associated with other ocular diseases with the involvement of the anterior chamber.

4. Experimental Design, Materials and Methods

4.1. Clinical characteristics between advanced and non-advanced POAG groups

The clinical characteristics of patients in the advanced and non-advanced POAG groups are presented in Table 1. A total of 66 patients were enrolled in this study, comprising 44 (67 %) with advanced POAG and 22 (33 %) with non-advanced POAG. No statistically significant differences were observed between the two groups in terms of age, gender, disease duration, or intraocular pressure (IOP). The overall median age was 64.50 years (52.00, 69.00), with median ages of 63.50 years (51.00, 69.25) and 65.00 years (52.50, 68.75) in the advanced and non-advanced groups, respectively ($p = 0.844$). Of the total cohort, 26 patients were female

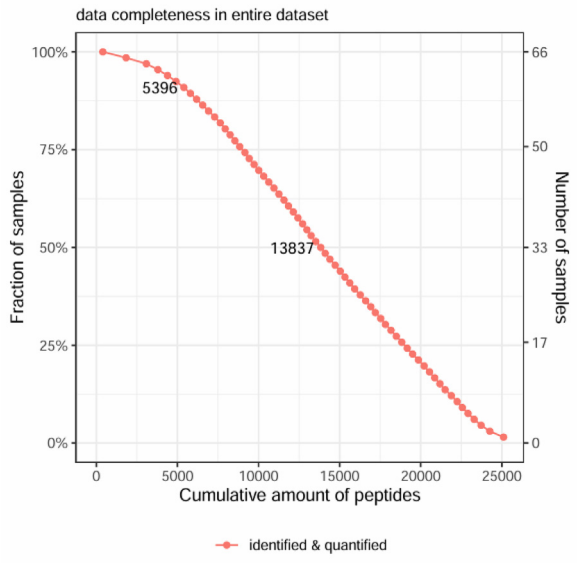

Fig. 2. A cumulative distribution illustrating the consistent identification of peptides across various samples.

Table 1
Clinical characteristics of the advanced and non-advanced POAG groups.

Variable	Overall, $N = 66$[a]	Groups		p-value
		Advanced POAG $N = 44$ (67 %)[1]	Not advanced POAG $N = 22$ (33 %)[1]	
Age	64.50 [52.00, 69.00]	63.50 [51.00, 69.25]	65.00 [52.50, 68.75]	0.844[b]
Gender				0.130[c]
Female	26 (39.39 %)	14 (31.82 %)	12 (54.55 %)	
Male	40 (60.61 %)	30 (68.18 %)	10 (45.45 %)	
Duration of disease (months)	12.00 [6.00, 24.00]	12.00 [6.00, 24.00]	12.00 [4.50, 24.00]	0.789[b]
IOP (mmHg)	19.68 [16.85, 23.78]	19.90 [18.16, 23.99]	18.40 [16.13, 23.09]	0.362[b]

[a] Median [IQR]; n (%).
[b] p value for the Mann-Whitney U test.
[c] p value for the Chi-square test.

(39.39 %), including 14 females (31.82 %) in the advanced group and 12 females (54.55 %) in the non-advanced group ($p = 0.130$). Median disease duration was 12.00 months (6.00, 24.00) overall, 12.00 months (6.00, 24.00) in the advanced group, and 12.00 months (4.50, 24.00) in the non-advanced group ($p = 0.789$). Mean IOP was 19.68 mmHg (16.85, 23.78 mmHg) overall, 19.90 mmHg (18.16, 23.99 mmHg) in the advanced group, and 18.40 mmHg (16.13, 23.09 mmHg) in the non-advanced group ($p = 0.362$). Therefore, no significant differences were found in these baseline demographic and clinical variables between the two groups.

The statistical analysis of clinical characteristics was conducted using R software (http://www.R-project.org; Version 4.2.1). A p-value < 0.05 was established as the threshold for statistical significance. The Mann-Whitney U test, a non-parametric method, was employed to assess differences between two independent groups. Categorical variables were analysed using the Chi-square test.

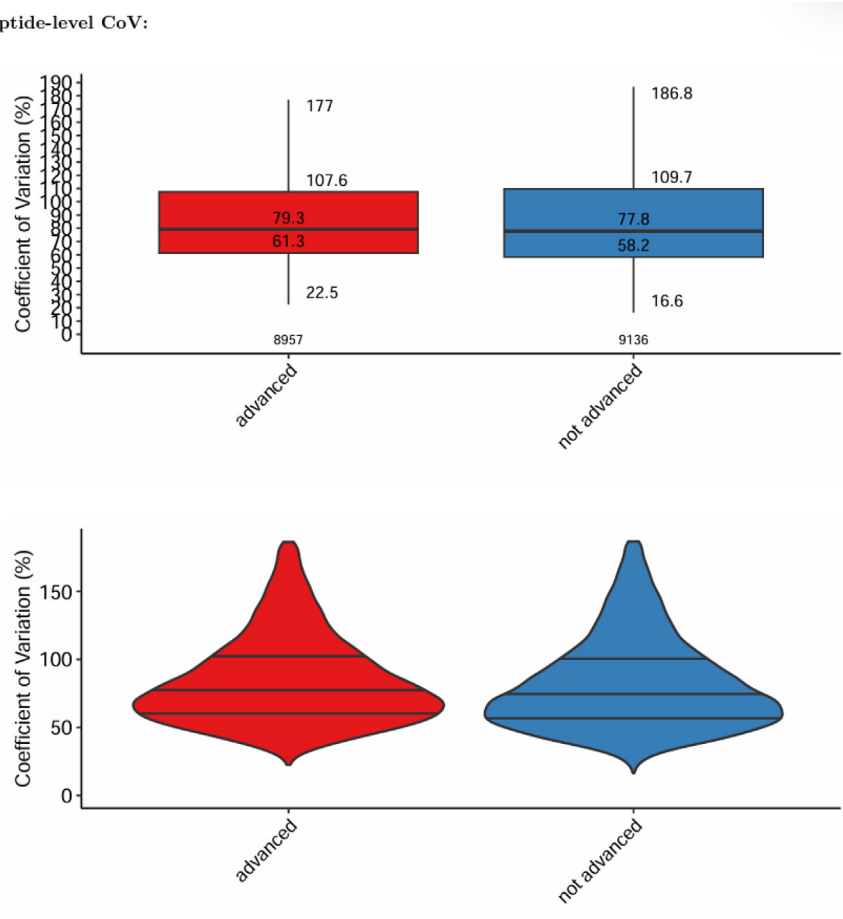

Fig. 3. The Coefficient of Variation (CoV) for peptides in both advanced and not advanced POAG groups.

4.2. Sample collection and ethical considerations

This prospective study enrolled patients diagnosed with POAG who were scheduled to undergo combined glaucoma and cataract surgery at the glaucoma department of Tianjin Medical University Eye Hospital between October 2020 and August 2021. All participants provided written informed consent. The study protocol and procedures were approved by the Ethics Committee of Tianjin Medical University Eye Hospital (Approval No.: 2020KY-13) and the Ethics Committee of The Hong Kong Polytechnic University (Approval No.: HSEARS20230320005). The research adhered to the principles of the Declaration of Helsinki.

All surgical procedures were performed by a single, experienced glaucoma surgeon (Dr. XiaoLi Xing) to minimize technical variability. The specific surgical technique was selected based on each patient's ocular condition and clinical indications. Procedures included trabeculectomy, phacoemulsification with intraocular lens implantation combined with trabeculectomy, or combined with goniosynechialysis. Surgical decisions were made following established clinical decision-making protocols.

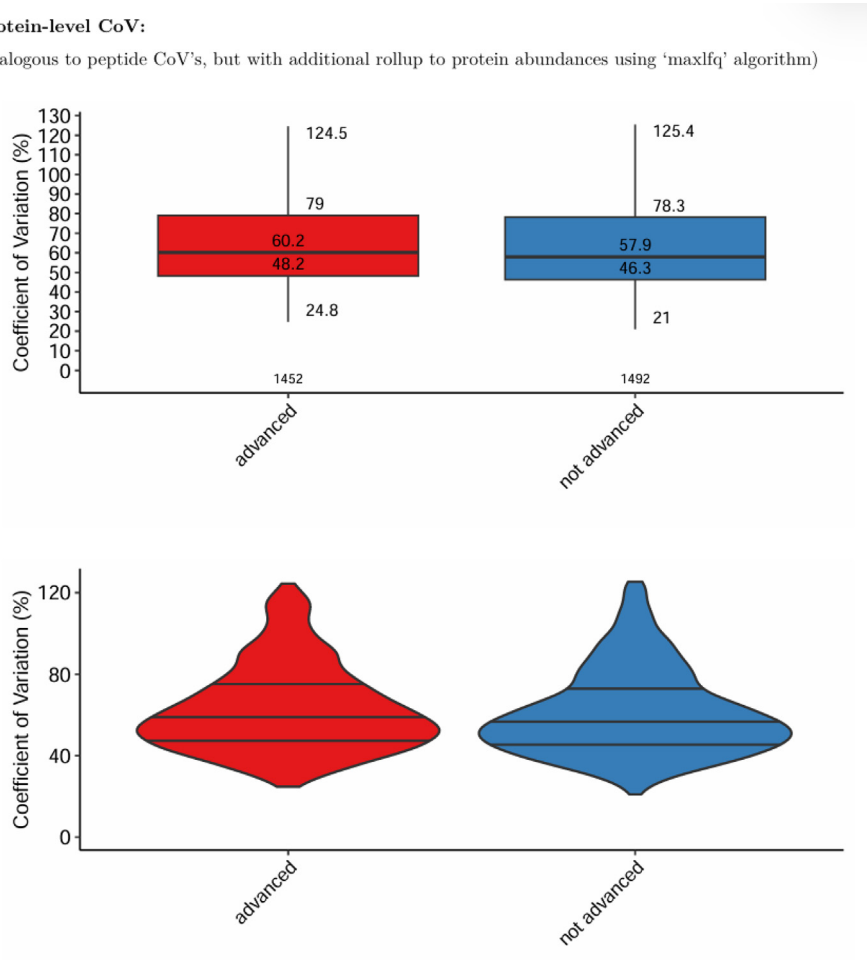

Fig. 4. The Coefficient of Variation (CoV) for proteins in both advanced and not advanced POAG groups.

Intraoperative aqueous humor samples were collected following a standardized protocol. During each procedure, a corneal side incision was made at the 2 o'clock position, and approximately 50 –100 µL of aqueous humor was extracted. The aqueous humor samples were immediately transferred into 1.5 mL Eppendorf tubes and rapidly frozen by immersion in liquid nitrogen.

4.3. Sample preparation

A protein sample (50 µg) of each sample was reduced with 10 mM dithiothreitol (DTT) for 30 min at 37 °C. Following reduction, the sample was cooled to room temperature and subsequently alkylated in the dark for 45 min at room temperature using 55 mM iodoacetamide (IAA). Ammonium bicarbonate (50 mM) was added to each sample to adjust the volume to 100 µl and mixed thoroughly. Subsequently, the solution was passed through a 10 KDa ultrafiltration tube and centrifuged at 20 °C with a speed of 12,000 g for 20 min. The trapped proteins were

then digested with trypsin at a ratio of 40:1 (protein: enzyme) overnight at 37 °C. The digested peptides were eluted with 100 µl of 50 mM ammonium bicarbonate. The solution was vacuum dried and reconstituted by adding 2 % Acetonitrile (ACN) and 0.1 % Formic acid (FA) to an equal peptide concentration.

4.4. LC-MS/MS settings

The separation process was conducted using a nano-liquid chromatography system. Initially, an enrichment and desalting step was executed using a trap column where the sample underwent separation in a tandem self-packed C18 column (150 µm inner diameter, 1.8 µm particle size, 32 cm length), employing an effective gradient flow rate of 500 nL/min: 0–5 min, 5 % mobile phase B (98 % ACN, 0.1 % FA); 5–45 min, mobile phase B linearly increased from 5 % to 25 %; 45–50 min, mobile phase B increased from 25 % to 35 %; 50–52 min, mobile phase B increased from 35 % to 80 %; 52–54 min, 80 % mobile phase B; 54.5–65 min, 5 % mobile phase B.

Eluting peptides were ionized via a CSI nano-source and subsequently introduced into the tandem mass spectrometer, TimsTOF Pro (Bruker Corporation, Billerica, MA), for detection in Data-Dependent Acquisition (DIA) mode. The principal parameter settings were set as follows: the voltage of the ion source was set at 1.6 kV; the range of ion mobility was 0.75 - 1.40 $V{\cdot}s/cm^2$, and the scan range of the primary mass spectrometry was between 100 - 1700 m/*z*. The range of 395 - 1195 m/*z* was divided into 4 steps, and each step was further subdivided into 8 windows, amounting to 32 windows for continuous window fragmentation and information acquisition. The fragmentation mode was CID, with a fragmentation energy of 10 eV. The mass width of each window was 25 Da, with the cycle time for each DIA scan set at 0.95 s.

4.5. Data analysis

In this study, raw data files from the spectral library were processed utilizing DIA-NN (version 1.9.2) in library-free mode, adhering to the default settings specified under "robust LC (high precision)." The precursor ions were analyzed within an *m/z* range of 300 to 1800 and a charge state range of 1 to 4. Protein identification was achieved through in-silico tryptic digestion at 1 % FDR, employing the UniProt database for reference (UP000005640, 82,685 proteins, Aug 2024). The Mass Spectrometry Downstream Analysis Pipeline (MS-DAP) (Version 1.2.1) was used for quality control.

Limitations

None.

Ethics statement

Ethical clearance certificate with number HSEARS20230320005 was granted by The Hong Kong Polytechnic University, and number 2020KY-13 was provided by Tianjin Medical University Eye Hospital

CRediT author statement

Hu Xiao: Conceptualization, Data curation, Formal analysis, Software, Writing – Original draft preparation, Writing – Reviewing and Editing. **Da Qian Lu:** Conceptualization, Data curation.

Jimmy Ka-Wai Cheung: Conceptualization, Formal analysis, Data curation, Writing – Reviewing and Editing. **Ying Hon Sze:** Formal analysis, Software, Data curation. **Jing Fang Bian:** Conceptualization, Writing – Reviewing and Editing. **Chi-wai Do:** Conceptualization, Writing – Reviewing and Editing. **Lei Zhou:** Conceptualization, Software, Formal analysis, Writing – Reviewing and Editing. **Xiao Li Xing:** Conceptualization, Data curation, Funding acquisition, Writing – Reviewing and Editing, Supervision, Project administration. **Thomas Chuen Lam:** Conceptualization, Data curation, Writing – Reviewing and Editing, Supervision, Project administration, Funding acquisition.

Data Availability

PXD060484 (Original data) (PRIDE)

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Declaration of competing interest

In this article, the authors declare that they have no competing financial interests or personal relationships that may have affected the work reported.

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