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Unveiling the levels and significance of different serpin family proteins in aqueous humor dynamics

Eliza Williams[1], Jeremy Altman[1], Saleh Ahmed[1], Garrett Jones[1], Anshuman Khadanga[1], Danny Alevy[1], Kathryn Bollinger[2], Amy Estes[2], Steven Safille[1], Tae Jin Lee[1], Shruti Sharma[1] and Ashok Sharma[1,2,3*]

Abstract

Background Alterations in the constituents of the aqueous humor (AH) are associated with various ocular pathologies, including primary open-angle glaucoma (POAG). AH contains a variety of immunomodulatory molecules, including serine protease inhibitors (serpins), which regulate several proteolytic cascades such as coagulation, angiogenesis, and inflammation. The purpose of this study was to examine the levels of different serpins in human AH and their association with POAG pathology.

Methods The abundance of all 37 serpins was determined using LC-MS/MS analysis in 289 human AH samples (cataract: $n=209$; POAG: $n=80$). The potential involvement of these serpins in POAG was examined by correlating their levels with clinical parameters such as intraocular pressure (IOP) and optic nerve damage.

Results Among the 37 serpins present in the human proteome, 26 were detected in aqueous humor. The thirteen most abundant serpins in AH include SERPINA1, SERPINF1, SERPINC1, SERPINA3, SERPING1, AGT, SERPINF2, SERPINA4, SERPINA6, SERPIND1, SERPINI1, SERPINA7, and SERPINA5. Seven serpins were downregulated in subjects with POAG, including SERPINI1 (FC = 0.26), SERPINA4 (FC = 0.40), SERPINA6 (FC = 0.42), SERPINA7 (FC = 0.46), SERPINC1 (FC = 0.74), AGT (FC = 0.76), and SERPING1 (FC = 0.78).

Conclusion This study highlights significant alterations in serpin levels within the AH of individuals with POAG. Sex-specific and race-specific differences in the levels of several serpins were also observed. Further studies are needed to clarify the specific mechanisms by which these serpins may contribute to POAG progression and to investigate their potential clinical relevance.

Keywords Serpins, Aqueous humor, Glaucoma, Inflammation, Mass spectrometry

*Correspondence:
Ashok Sharma
assharma@augusta.edu
[1]Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta University, 1120 15th Street, CA4143, Augusta, GA 30912, USA
[2]Department of Ophthalmology, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA
[3]Department of Population Health Sciences, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA

Introduction

Glaucoma is the leading cause of irreversible blindness and is characterized by the progressive loss of retinal ganglion cells (RGCs), optic nerve atrophy, and visual field deterioration [1]. Primary open-angle glaucoma (POAG), the most common subtype, affects over 50 million adults worldwide [2]. Current treatments primarily focus on lowering intraocular pressure (IOP), which is the most significant risk factor for developing POAG [3–7]. IOP is regulated by the inflow and drainage of aqueous humor (AH), a clear, water-like fluid in the anterior chamber of the eye. In addition to regulating IOP, AH plays critical roles in nutrient delivery and waste removal [8, 9]. Alterations in the composition of AH have been associated with various ocular diseases, including POAG and age-related macular degeneration [10–13]. Furthermore, markers of inflammation, metabolic dysfunction, and oxidative stress are frequently detected in the AH of glaucoma patients [14–16].

AH contains significant concentrations of immunomodulatory molecules, including serine protease inhibitors (serpins) [17, 18]. The human serpin family consists of 37 proteins that regulate a variety of proteolytic cascades, such as coagulation, angiogenesis, and inflammation [19–22]. Altered serpin expression, whether due to deficiency, overproduction, or misfolding, has been implicated in the pathogenesis of several diseases [23–25], including changes observed in both the aqueous and vitreous humor of patients with POAG [11, 18, 22]. Given the critical role of AH homeostasis in POAG, these immunomodulatory molecules may be important to understanding the disease's underlying mechanisms. However, the specific impact of AH serpin levels on the development and progression of POAG remains largely unknown.

In this study, using a large sample size ($n = 289$), we investigated the abundance of all 37 known serpins in human AH. We also examined the correlation between serpin levels in the AH and key clinical parameters of POAG, including IOP, optic nerve morphology, and retinal nerve fiber layer thickness. This comprehensive analysis aims to uncover novel insights into the role of serpins in the pathophysiology of POAG and to identify potential therapeutic targets for its management.

Table 1 Demographic information of subjects

Demographic Characteristics	Cataract ($n = 209$)	POAG ($n = 80$)	*P*-value
Age (years, mean ± SD)	66.95 ± 9.47	69.24 ± 10.84	0.10[a]
IOP (mmHg, mean ± SD)	15.32 ± 3.43	14.97 ± 4.29	0.54[a]
Sex (F/M)	130/79	54/26	0.48[b]
Race (African American/White/Other)	111/93/5	48/29/3	0.34[b]

a: two-sample t-test b: chi-square test

Materials and methods

Subjects

All AH samples used in this study were collected from 289 subjects undergoing either cataract or glaucoma surgery at Augusta University Medical Center. AH is typically discarded during standard surgical procedures; however, in this study, it was collected for molecular analysis. The study was approved by the Institutional Review Board of Augusta University (IRB #611480), and written informed consent was obtained from all participants prior to sample collection. The cohort included 80 AH samples from patients with POAG undergoing glaucoma surgery and 209 samples from patients undergoing cataract surgery. Only patients with POAG were included; individuals with other glaucoma subtypes were excluded. There were no significant differences between the two groups in terms of age, race, sex, or IOP levels (Table 1).

LC-MS/MS analysis

The AH samples (60 µL) were lyophilized and reconstituted in 30 µL of 8 M urea in 50 mM Tris-HCl (pH 8.0). Cysteine residues were reduced using 20 mM dithiothreitol, followed by alkylation with 55 mM iodoacetamide. The urea concentration was subsequently reduced to less than 1 mM by adding 240 µL of 50 mM ammonium bicarbonate buffer. Protein concentration from each sample was measured using the Bradford assay kit (Thermo Fisher Scientific, Rockford, IL) to ensure equal concentration before proceeding to the digestion step. Protein digestion was performed by adding trypsin at a 1:20 (w/w) ratio and incubating overnight at 37 °C.

The digested AH samples were purified using a C18 spin plate (Nest Group, Southborough, MA, USA), subsequently separated with an Ultimate 3000 nano-UPLC system (Thermo Fisher Scientific) and further analyzed using an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). The reconstituted peptide mixture (4 µL) was washed and trapped on a PepMap100 C18 trap column (5 µm, 0.3 × 5 mm) at a flow rate of 20 µL/min for 10 min using 2% acetonitrile in water containing 0.1% formic acid. Peptide separation was achieved on a PepMap100 RSLC C18 column (2.0 µm, 75 µm x 150 mm) with a gradient of 2–40% acetonitrile in 0.1% formic acid over 160 min (flow rate: 300 nL/min; column temperature: 40 °C). The eluted peptides were analyzed using data-dependent acquisition in positive mode with the following parameters: precursor scanning by the Orbitrap MS analyzer at 120,000 FWHM from 300 to 1500 m/z; MS/MS scanning by the ion-trap MS analyzer in top-speed mode (2-second cycle time) with dynamic exclusion settings (repeat count: 1; repeat duration: 15 s; exclusion duration: 30 s). Fragmentation was performed

using higher-energy collisional dissociation (HCD) with a normalized collision energy of 30%.

Protein identification and quantification

To quantify and identify the proteins, raw mass spectrometry (MS) data were processed using Proteome Discoverer software (v1.4; Thermo Fisher Scientific) and searched against the Uniprot-SwissProt human database, which contains 20,385 entries, using the SequestHT algorithm. The search parameters included a precursor ion tolerance of 10 ppm, a product ion tolerance of 0.6 Da, and a false discovery rate of < 0.01, with static carbamidomethylation (+ 57.021 Da) for cysteine residues and dynamic modifications including oxidation (+ 15.995 Da) for methionine, and phosphorylation (+ 76.966 Da) for serine, threonine, and tyrosine. Peptide-spectrum matches (PSMs) were validated using the Percolator PSM validator integrated within the Proteome Discoverer software. Proteins that could not be distinguished by MS/MS analysis due to similar peptide compositions were grouped using parsimony principles. The resulting report provided a semi-quantitative measure of relative protein levels in the AH samples, including identities and spectrum counts (number of PSMs) for each protein.

Optical coherence tomography (OCT) measurements

SPECTRALIS Tracking Laser Tomography (Heidelberg Engineering, Franklin, MA, USA) was used to calculate the retinal nerve fiber layer (RNFL) thickness, which generated a 24-line high-definition radial scan of the optic nerve head centered on the Bruch's membrane opening (BMO). The neuroretinal rim was defined based on these images as the region between the BMO and the closest point along the internal limiting membrane. RNFL thickness was determined using three circular scans centered on the optic nerve head delineated by the BMO. These measurements were compared to a healthy eye reference database, adjusted for BMO size and age, and presented using the Garway-Heath sector format to allow for structure-function correlation. Multi-layer segmentation software was utilized to evaluate the macula and ganglion cell layer (GCL), with results displayed through a GCL thickness map.

Heidelberg retinal tomograph (HRT) measurements

The Heidelberg Retinal Tomograph (HRT; Heidelberg Engineering, Franklin, MA, USA) was used to obtain high-resolution imaging of the optic nerve head. This confocal scanning laser ophthalmoscopy system captures images at multiple focal planes, which are then compiled to generate a three-dimensional topographic representation of the optic nerve. Key stereometric parameters were calculated, including the cup shape measure, cup-to-disc area ratio, and rim volume. The cup-to-disc area ratio, an important indicator that tends to increase in patients with progressive glaucoma, was evaluated to quantify the extent of optic disc cupping. This metric reflects changes in the steepness and depth of the optic cup. In eyes without glaucomatous damage, the cup shape measure is typically negative, while in glaucomatous eyes it becomes less negative or even positive, indicating structural changes in the optic nerve head [26, 27]. Additionally, the rim area was assessed, representing the neuroretinal rim that forms the border between the cup and disc. In glaucoma patients, this value is generally reduced, reflecting the loss of retinal nerve fibers characteristic of the disease.

Statistical analysis

All statistical analyses were conducted using the R project for statistical computing (version 4.2.0). Clinical characteristics of POAG and cataract subjects were compared using chi-squared tests for categorical variables and two-tailed t-tests for continuous variables. A trimmed mean of M values (TMM) normalization of peptide-spectrum match (PSM) values from LC-MS/MS analysis was performed prior to data analysis, and the proportion of samples with detected proteins was determined. Mean PSM values less than 2 were considered not detected. To test for differences in protein abundance between the two groups, the edgeR package in R was used to fit a negative binomial model with empirical Bayes estimation. Correlations between serpins and clinical parameters (e.g., OCT, IOP, and HRT measures) were evaluated using Pearson's correlation. The Benjamini-Hochberg approach was used to adjust the p-value with the false discovery rate. Statistical significance was defined as $p < 0.05$.

Results

The levels of Serpins in human aqueous humor

A total of 37 serine protease inhibitors (serpins) are present in the human proteome [24, 28], of which 26 were detected in the AH samples analyzed (Fig. 1). In total, 13 serpins were highly abundant in the AH samples, including SERPINA1 (21,204 ± 347), SERPINF1 (13,112 ± 239), SERPINC1 (4,993 ± 108), SERPINA3 (4,118 ± 80), SERPING1 (2,816 ± 46), AGT (2,176 ± 47), SERPINF2 (653 ± 32), SERPINA4 (396 ± 21), SERPINA6 (340 ± 15), SERPIND1 (285 ± 21), SERPINI1 (217 ± 11), SERPINA7 (181 ± 12), and SERPINA5 (88 ± 6). All 13 of these serpins were detected in more than 50% of the AH samples.

Sex- and race-specific differences in Serpins in human aqueous humor

To evaluate baseline (without glaucoma) sex- and race-specific differences in serpin levels, the 13 most frequently detected serpins were further analyzed among subjects with cataracts (Table 2). The levels of five serpins were significantly lower in females than in males:

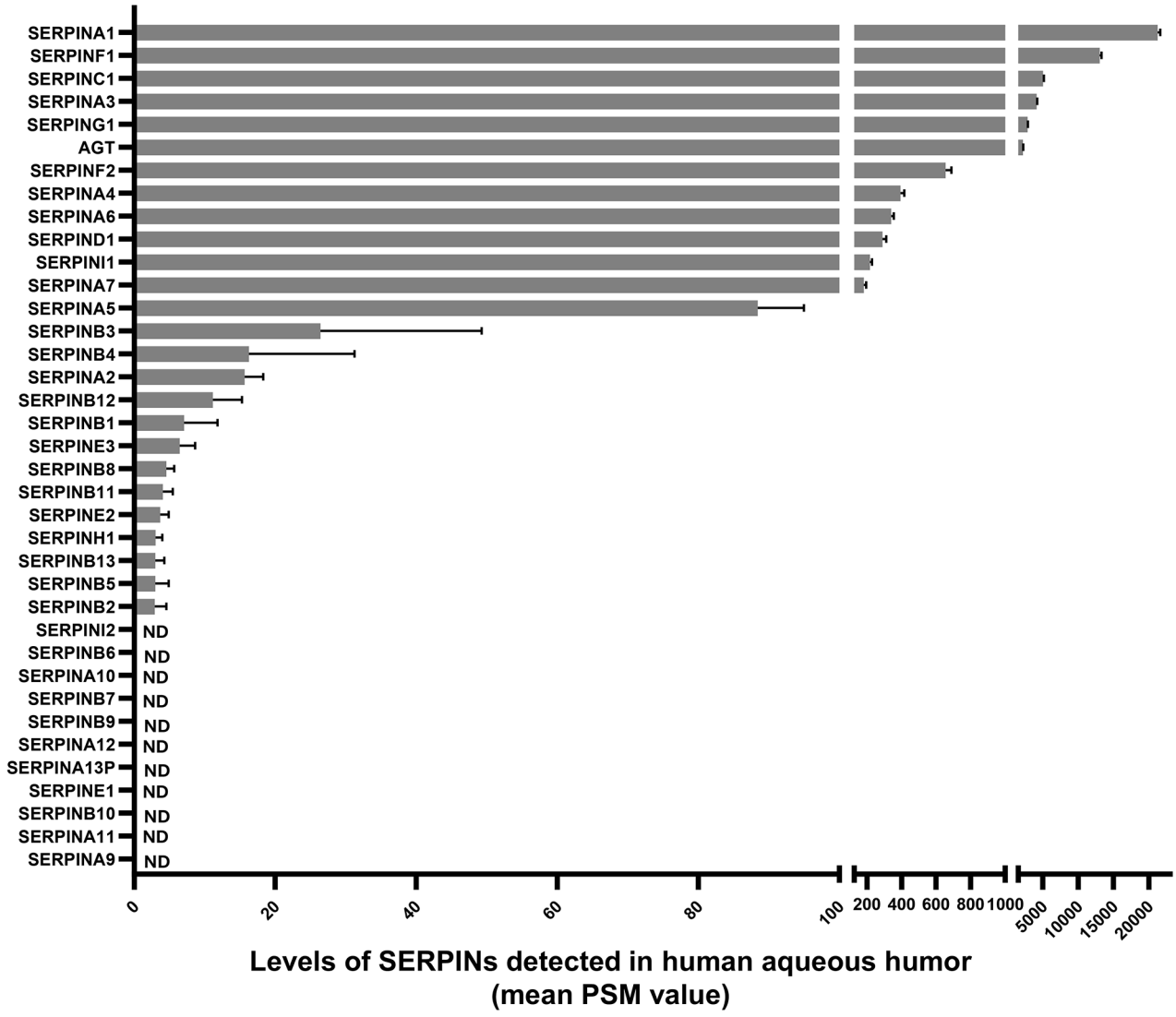

Fig. 1 Levels of all 37 serpin family proteins in human aqueous humor samples. SERPINA1 showed the highest overall abundance. Peptide-spectrum match (PSM) values below 2 were classified as not detected (ND). Error bars represent the standard error of the mean (SEM)

SERPINA1 (fold change [FC] = 0.87; $p < 0.01$), SERPINC1 (FC = 0.89; $p = 0.01$), SERPINF2 (FC = 0.73; $p = 0.01$), SERPINA4 (FC = 0.77; $p = 0.02$), and SERPIND1 (FC = 0.64; $p = 0.01$). One serpin, SERPINF1, was significantly upregulated in females (FC = 1.15; $p < 0.01$). Additionally, three serpins were significantly downregulated in African American compared to Caucasian subjects: SERPINA1 (FC = 0.80; $p < 0.01$), SERPINF1 (FC = 0.89; $p < 0.01$), and SERPINC1 (FC = 0.86; $p < 0.01$). In contrast, SERPIND1 was significantly upregulated in African American subjects (FC = 1.68; $p < 0.01$).

POAG-specific alterations in aqueous humor Serpins

Seven serpins were differentially expressed between subjects with POAG and those with cataracts, with adjusted p-values < 0.05 (Fig. 2). All seven serpins were significantly downregulated in POAG subjects compared to cataract controls, as shown in Table 3. The specific fold changes (FC) and adjusted p-values are as follows: SERPINI1 (FC = 0.26; $p < 0.01$), SERPINA4 (FC = 0.40; $p = 0.01$), SERPINA6 (FC = 0.42; $p < 0.01$), SERPINA7 (FC = 0.46; $p = 0.03$), SERPINC1 (FC = 0.74; $p < 0.01$), AGT (FC = 0.76; $p < 0.01$), and SERPING1 (FC = 0.78; $p < 0.01$).

Table 2 Baseline sex- and race- specific differences in the levels of SERPINs in subjects with cataracts

Gene symbol	Female PSM ± SEM	Male PSM ± SEM	Female vs. Male FC	*P*-value	African American PSM ± SEM	Caucasian PSM ± SEM	African American vs. Caucasian FC	*P*-value
SERPINA1	20,032.34 ± 488.82	23,020.18 ± 609.20	0.87	< 0.01*	19,070.11 ± 488.16	23,961.17 ± 528.56	0.80	< 0.01*
SERPINF1	13,949.09 ± 327.15	12,180.97 ± 415.89	1.15	< 0.01*	12,593.88 ± 406.01	14,084.66 ± 313.06	0.89	< 0.01*
SERPINC1	4,953.03 ± 142.24	5,570.34 ± 184.12	0.89	0.01*	4,863.35 ± 152.06	5,642.39 ± 168.95	0.86	< 0.01*
SERPINA3	3,974.53 ± 110.39	4,262.68 ± 165.20	0.93	0.15	4,097.94 ± 138.72	4,091.37 ± 127.71	1.00	0.97
SERPING1	2,965.06 ± 62.59	2,814.05 ± 73.37	1.05	0.12	2,997.38 ± 68.39	2,840.34 ± 66.38	1.06	0.10
AGT	2,215.49 ± 63.00	2,346.00 ± 84.78	0.94	0.22	2,221.69 ± 68.62	2,319.16 ± 78.91	0.96	0.35
SERPINF2	575.96 ± 45.09	787.33 ± 63.25	0.73	0.01*	656.00 ± 55.12	666.74 ± 52.05	0.98	0.89
SERPINA4	377.05 ± 31.42	491.17 ± 39.32	0.77	0.02*	421.73 ± 37.34	413.80 ± 32.90	1.02	0.87
SERPINA6	341.68 ± 20.64	382.89 ± 29.90	0.89	0.26	346.45 ± 25.42	366.60 ± 23.60	0.95	0.56
SERPIND1	242.31 ± 27.15	381.55 ± 47.55	0.64	0.01*	366.31 ± 41.07	218.43 ± 25.07	1.68	< 0.01*
SERPINI1	242.88 ± 16.49	228.71 ± 19.32	1.06	0.58	251.65 ± 18.54	218.62 ± 17.10	1.15	0.19
SERPINA7	172.40 ± 17.38	215.93 ± 22.72	0.80	0.13	199.18 ± 20.88	179.27 ± 18.53	1.11	0.48
SERPINA5	92.76 ± 9.98	93.76 ± 12.13	0.99	0.95	102.82 ± 12.01	82.84 ± 9.30	1.24	0.19

FC: Fold Change; * p-value < 0.05

Further investigation into demographic variations was conducted by stratifying subjects based on sex and race. Sex-specific differences were assessed by comparing serpin levels between males and females. Among female subjects, those with POAG exhibited significantly lower levels of several serpins compared to females with cataracts, including SERPINI1 (fold change [FC] = 0.28; $p = 0.01$), SERPINA5 (FC = 0.37; $p = 0.03$), SERPING1 (FC = 0.68; $p < 0.01$), SERPINA7 (FC = 0.37; $p = 0.03$), SERPINA6 (FC = 0.37; $p < 0.01$), SERPINC1 (FC = 0.64; $p < 0.01$), AGT (FC = 0.72; $p < 0.01$), and SERPINF1 (FC = 0.75; $p = 0.02$) (Fig. 3). Although SERPINA4 also showed a notable decrease (FC = 0.43), the difference was not statistically significant.

In the male subset, SERPINI1 (FC = 0.25) and SERPINA4 (FC = 0.36) levels trended lower in POAG patients, similar to the female subset, but these reductions were not statistically significant. The other seven serpins with significant decreases in females showed no substantial reduction in males.

Within the African American subset, four serpins were significantly downregulated in subjects with POAG compared to those with cataracts: SERPINI1 (FC = 0.27; $p = 0.01$), SERPINC1 (FC = 0.72; $p = 0.02$), SERPING1 (FC = 0.73; $p < 0.01$), and AGT (FC = 0.75; $p = 0.02$) (Fig. 4). In the Caucasian subset, three of these serpins, including SERPINI1 (FC = 0.28), SERPINC1 (FC = 0.76), and AGT (FC = 0.76), showed similar trends, but the differences

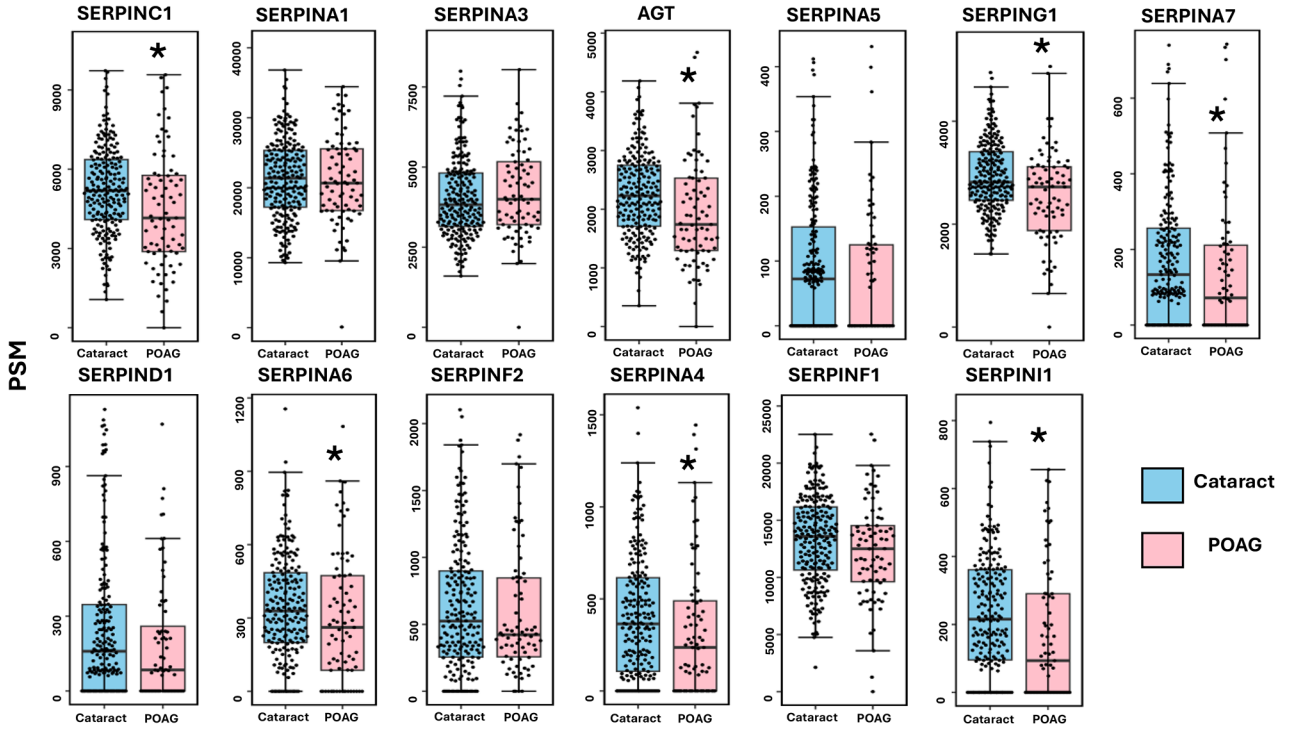

Fig. 2 Levels of the 13 most abundantly detected serpins in aqueous humor (AH) samples, shown for subjects with cataracts ($n = 209$) and primary open-angle glaucoma (POAG; $n = 80$). Significant reductions were observed in the levels of SERPINC1, AGT, SERPING1, SERPINA7, SERPINA6, SERPINA4, and SERPINI1 in the POAG group. Box plots represent the first quartile (Q1), median, and third quartile (Q3)

were not statistically significant. SERPING1 did not exhibit a notable change in the Caucasian subset.

Correlation of aqueous humor Serpins with OCT parameters

A correlation analysis was performed to explore potential associations between serpin levels in aqueous humor and clinical parameters, including retinal nerve fiber layer (RNFL) thickness. SERPINA4 levels were significantly correlated with multiple RNFL measurements: average RNFL thickness ($\rho = 0.21$; $p < 0.01$), inferior thickness ($\rho = 0.22$; $p < 0.01$), nasal thickness ($\rho = 0.15$; $p = 0.02$), superior thickness ($\rho = 0.14$; $p = 0.04$), and temporal thickness ($\rho = 0.20$; $p < 0.01$) (Table 4). SERPINI1 levels also showed strong positive correlations with RNFL parameters: average thickness ($\rho = 0.33$; $p < 0.01$), inferior ($\rho = 0.33$; $p < 0.01$), nasal ($\rho = 0.25$; $p < 0.01$), superior ($\rho = 0.32$; $p < 0.01$), and temporal thickness ($\rho = 0.13$; $p = 0.049$). Additionally, SERPINA6 levels were significantly correlated with inferior ($\rho = 0.14$; $p = 0.04$) and temporal thickness ($\rho = 0.19$; $p < 0.01$). Temporal RNFL thickness was also significantly correlated with levels of SERPINA7 ($\rho = 0.15$; $p = 0.02$) and SERPINC1 ($\rho = 0.14$; $p = 0.04$). Additional significant correlations between serpins and OCT parameters in both the cataract and POAG subsets are summarized in Table 4.

Correlation of aqueous humor Serpins with HRT parameters and IOP levels

To assess the relationship between serpin levels and structural indicators of glaucoma, we analyzed correlations between the 13 most abundant serpins and HRT parameters, including linear cup-to-disc ratio, maximum cup depth, cup-to-disc area ratio, rim area, rim volume, disc area, and cup area. SERPINI1 was positively correlated with rim area ($\rho = 0.17$; $p = 0.02$) and rim volume ($\rho = 0.18$; $p = 0.02$) and negatively correlated with the cup-to-disc area ratio ($\rho = -0.16$; $p = 0.04$), suggesting a

Table 3 Changes in the levels of SERPINs in subjects with POAG compared to subjects with cataracts

	Overall		Males		Females		Caucasians		African Americans	
Gene Symbol	FC	Adj. p-value	FC	Adj. p-value	FC	Adj. p-value	FC	Adj. p-value	FC	Adj. p-value
SERPINI1	0.26	< 0.01*	0.25	0.13	0.28	< 0.01*	0.28	0.07	0.27	0.01*
SERPINA4	0.40	0.01*	0.36	0.45	0.43	0.06	0.28	0.07	0.50	0.19
SERPINA6	0.42	< 0.01*	0.64	0.89	0.37	< 0.01*	0.38	0.08	0.50	0.10
SERPINA7	0.46	0.03*	0.61	0.89	0.37	0.03*	0.26	0.07	0.58	0.29
SERPINA5	0.58	0.13	1.18	0.95	0.37	0.03*	0.49	0.26	0.58	0.29
SERPINC1	0.74	< 0.01*	0.95	0.95	0.64	< 0.01*	0.76	0.09	0.72	0.02*
AGT	0.76	< 0.01*	0.84	0.81	0.72	< 0.01*	0.76	0.08	0.75	0.02*
SERPING1	0.78	< 0.01*	1.00	0.98	0.68	< 0.01*	0.84	0.23	0.73	< 0.01*
SERPINF1	0.84	0.06	0.96	0.95	0.75	0.02*	0.92	0.68	0.78	0.07

FC: Fold Change (POAG vs. Cataracts); * adj. p-value < 0.05

potential protective role in optic nerve head structure. SERPINA5 showed a positive correlation with cup area ($\rho = 0.16$; $p = 0.04$) and a negative correlation with the Reinhard O. W. Burk (RB) discriminant function ($\rho = -0.19$; $p = 0.01$), a composite HRT metric used to distinguish glaucomatous from non-glaucomatous optic nerve heads. In addition, SERPINA7 levels were negatively correlated with IOP levels ($\rho = -0.14$; $p = 0.02$). Further significant correlations between other serpins and HRT parameters in both cataract and POAG subsets are summarized in Table 4.

Discussion

Using high-resolution mass spectrometry, this study represents the most comprehensive profiling to date of serpin family proteins in the AH of individuals with and without POAG. We quantified all 37 known human serpins and identified 26 in the AH, with 13 consistently detected across the cohort. Notably, seven serpins were significantly downregulated in subjects with POAG, and several showed correlations with key clinical parameters, including RNFL thickness, and HRT metrics. Together with previous studies, these findings suggest a potential role for serpins in the molecular pathology of POAG and their possible utility as disease biomarkers or therapeutic targets [14, 29, 30].

Among the seven serpins significantly downregulated in POAG (SERPINI1, SERPINA4, SERPINA6, SERPINA7, SERPINC1, AGT, and SERPING1), SERPINI1 showed the greatest reduction (~4-fold). SERPINI1, also known as neuroserpin, is a neuronal serpin that regulates proteolysis in the nervous system and has established neuroprotective functions [19, 31–33]. Its positive correlation with RNFL thickness, a marker of glaucoma severity [34, 35], supports the hypothesis that decreased neuroserpin is linked to RGC degeneration in POAG. These findings are further supported by previous animal studies showing that neuroserpin gene therapy preserves RGC survival in models of optic nerve injury [36].

SERPINA4, another significantly downregulated serpin, also correlated with RNFL thickness. SERPINA4 inhibits tissue kallikrein, a protease involved in generating kinins [19, 37, 38] that regulate blood pressure and inflammation [39–43]. Its decrease in POAG suggests that reduced vascular protective mechanisms may contribute to disease pathogenesis [44–47]. Other serpins such as SERPINC1 (antithrombin III), AGT (angiotensinogen), and SERPING1 (C1 esterase inhibitor) also play roles in vascular homeostasis and immune regulation, processes known to be altered in glaucoma [48–50].

Sex- and race-stratified analyses revealed demographic-specific differences in serpin expression. Females with POAG exhibited more pronounced downregulation of several serpins, including SERPINA5, SERPINA6, and

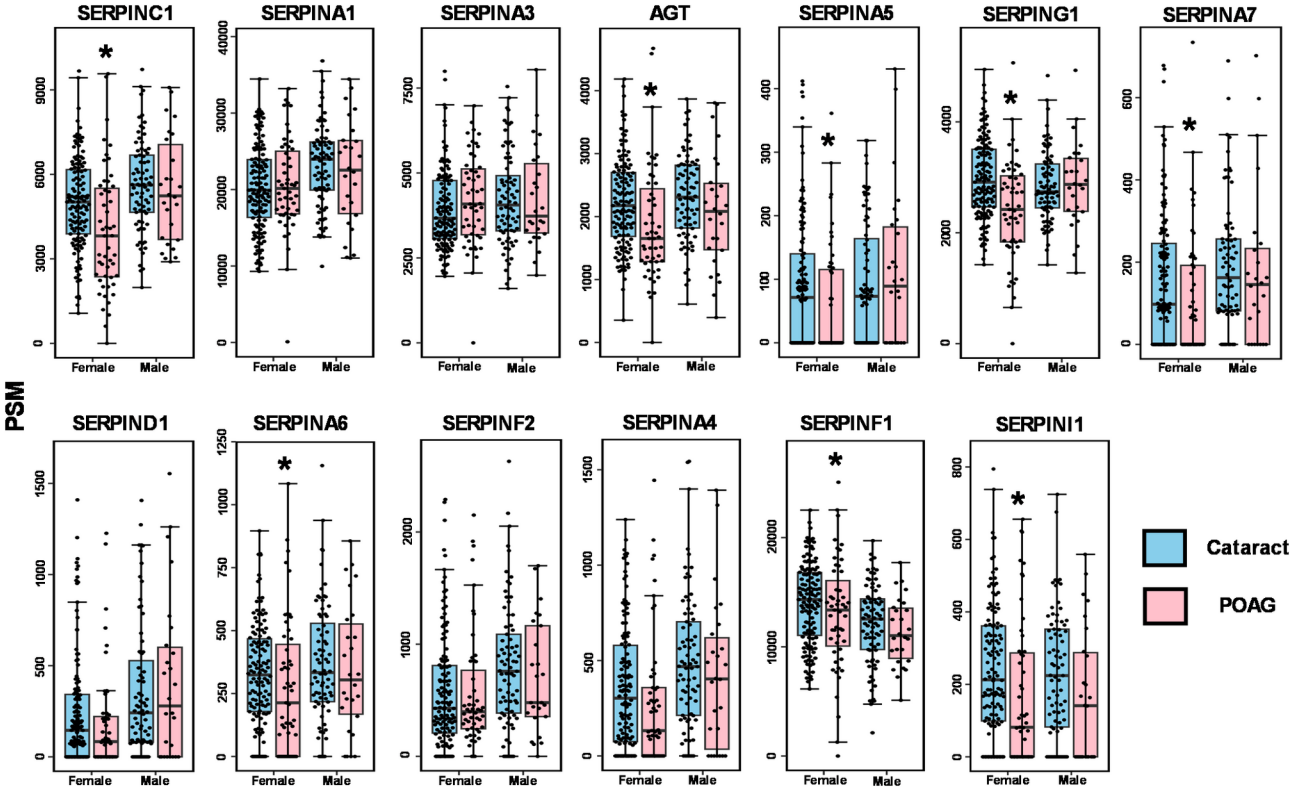

Fig. 3 Sex-specific differences in serpin levels in aqueous humor (AH) samples. Data are stratified by sex and further categorized into cataract (male: $n=79$; female: $n=130$) and primary open-angle glaucoma (POAG; male: $n=26$; female: $n=54$) groups. Among female subjects, significant reductions in the levels of SERPINC1, AGT, SERPINA5, SERPING1, SERPINA6, SERPINF1, and SERPINI1 were observed in the POAG group. Box plots represent the first quartile (Q1), median, and third quartile (Q3)

SERPINA7 compared to males. SERPINA5 modulates coagulation and fibrinolysis [19], while SERPINA6 and SERPINA7 are transport proteins for cortisol and thyroid hormones, respectively. The functional relevance of these hormonal transporters in POAG remains to be elucidated but suggests a possible endocrine contribution to disease progression [51, 52]. African American patients with POAG also showed more significant downregulation of SERPINI1, SERPING1, SERPINC1, and AGT, aligning with known racial disparities in glaucoma prevalence and severity [53–57].

Correlational analyses further demonstrated associations between specific serpins and structural markers of glaucomatous damage. For example, SERPINI1 was positively correlated with rim area and rim volume and negatively correlated with cup-to-disc area ratio, indicating a potential protective effect on optic nerve head integrity. SERPINA5 was associated with cup area and inversely related to the Reinhard Burk discriminant function, a metric used in HRT to differentiate glaucomatous from healthy eyes. Additionally, SERPINA7 showed a modest but significant negative correlation with IOP.

Taken together, these findings suggest that serpins, particularly neuroserpin and those involved in vascular and inflammatory regulation, are altered in POAG and may contribute to disease mechanisms through effects

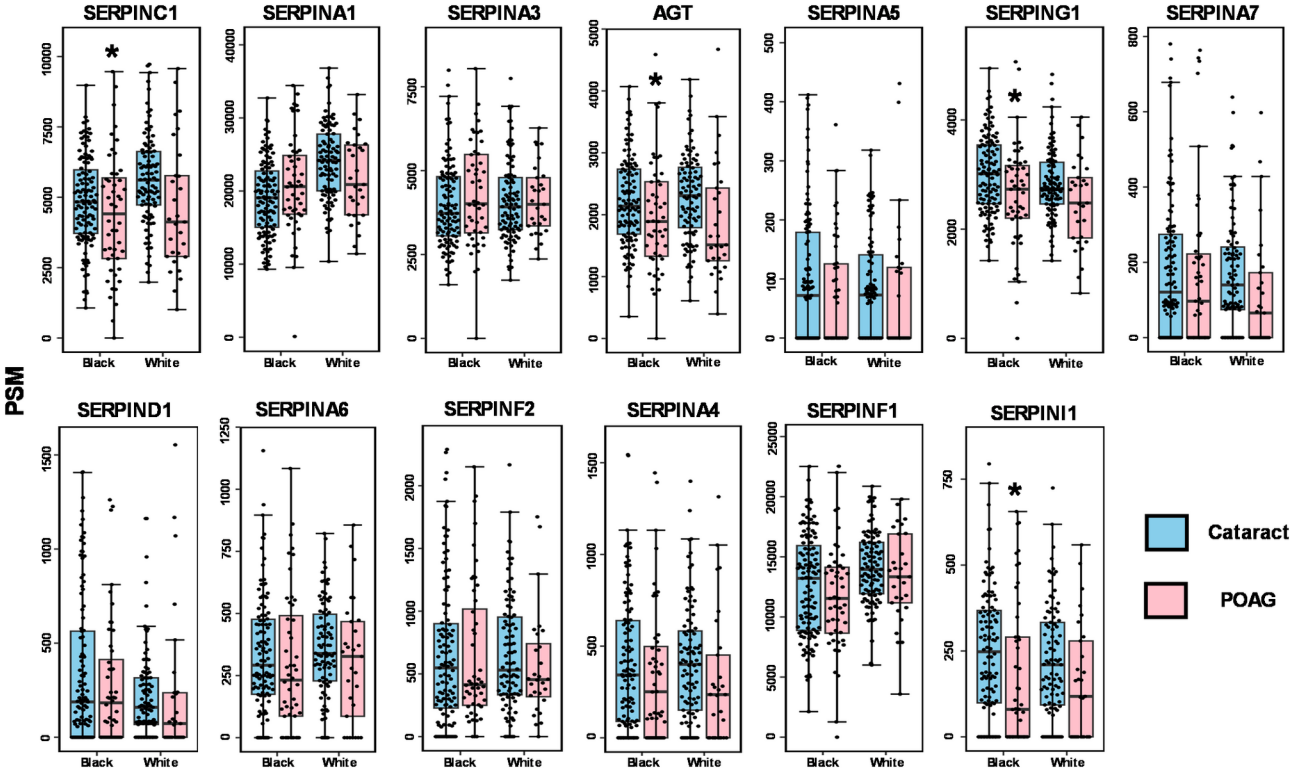

Fig. 4 Race-specific changes in the levels of the 13 most abundantly detected SERPINs in aqueous humor (AH) samples. Data is stratified by race (Black and White) and further categorized into cataract (Black: $n=111$; White: $n=93$) and POAG (Black: $n=48$; White: $n=29$) groups. In the Black population, significant decreases were observed in the levels of SERPINC1, AGT, SERPING1, and SERPINI1 in the POAG group. Box plots represent the first quartile (Q1), median, and third quartile (Q3)

on neuroprotection, vascular stability, or immune modulation. While causality cannot be established from this cross-sectional analysis, the observed associations highlight the need for further investigation.

Limitations of this study include the relatively small number of POAG subjects within demographic subgroups, which may limit statistical power for sex- and race-specific analyses. Furthermore, systemic comorbidities were not controlled for, which could confound protein expression profiles. Only POAG and cataract patients were included, excluding other glaucoma subtypes that may exhibit distinct serpin patterns.

Table 4 Serpins significantly correlated with clinical parameters

		Overall		Cataract		POAG	
Serpins	**Clinical Variables**	**Corr.**	**P-value**	**Corr.**	**P-value**	**Corr.**	**P-value**
OCT Parameters							
SERPINA4	Avg. RNFL Thickness	0.21	< 0.01*	0.12	0.12	0.07	0.61
SERPINA4	Inferior Thickness	0.22	< 0.01*	0.14	0.08	0.11	0.42
SERPINA4	Nasal Thickness	0.15	0.02*	0.06	0.47	0.11	0.41
SERPINA4	Superior Thickness	0.14	0.04*	0.03	0.74	0.02	0.91
SERPINA4	Temporal Thickness	0.20	< 0.01*	0.18	0.02*	-0.02	0.88
SERPINI1	Avg. RNFL Thickness	0.33	< 0.01*	0.12	0.12	0.35	0.01*
SERPINI1	Inferior Thickness	0.33	< 0.01*	0.14	0.07	0.33	0.01*
SERPINI1	Nasal Thickness	0.25	< 0.01*	0.11	0.17	0.27	0.04*
SERPINI1	Superior Thickness	0.32	< 0.01*	0.13	0.10	0.36	< 0.01*
SERPINI1	Temporal Thickness	0.13	0.049*	-0.09	0.26	0.19	0.14
SERPINA6	Inferior Thickness	0.14	0.04*	-0.05	0.53	-0.02	0.90
SERPINA6	Temporal Thickness	0.19	< 0.01*	0.17	0.03*	-0.04	0.77
SERPINA3	Temporal Thickness	0.06	0.36	0.20	0.01*	-0.07	0.59
SERPINA7	Temporal Thickness	0.15	0.02*	0.11	0.15	0.09	0.51
SERPINC1	Temporal Thickness	0.14	0.04*	0.12	0.14	0.01	0.95
SERPINF2	Temporal Thickness	0.08	0.23	0.18	0.02*	-0.16	0.23
HRT Parameters							
SERPINA4	Height Variability of Contour (mm)	0.05	0.53	-0.20	0.02*	0.21	0.18
SERPINA4	Linear Cup to Disk Ratio	0.06	0.38	0.21	0.01*	0.03	0.84
SERPINA4	Max Cup Depth (mm)	-0.09	0.24	-0.20	0.03*	0.14	0.43
SERPINI1	Cup to Disc Area Ratio	-0.16	0.04*	-0.06	0.51	-0.28	0.08
SERPINI1	Linear Cup to Disk Ratio	0.00	0.99	0.26	< 0.01*	-0.17	0.19
SERPINI1	Rim Area (mm^2)	0.17	0.02*	0.05	0.59	0.29	0.06
SERPINI1	Rim Volume (mm^3)	0.18	0.02*	0.02	0.87	0.35	0.02*
SERPINC1	Disc Area (mm^2)	-0.10	0.21	-0.22	0.01*	0.05	0.78
SERPINC1	Rim Area (mm^2)	-0.06	0.43	-0.21	0.02*	-0.08	0.61
SERPINA5	Cup Area (mm^2)	0.16	0.04*	0.10	0.28	0.22	0.22
SERPINA6	Linear Cup to Disk Ratio	0.09	0.16	0.25	< 0.01*	0.14	0.28
SERPINF2	Cup Area (mm^2)	-0.10	0.20	-0.18	0.04*	0.03	0.87
SERPINA4	Reinhard O. W. Burk (RB) discriminant function value	0.06	0.44	-0.04	0.62	0.35	0.04*
SERPINA5	Reinhard O. W. Burk (RB) discriminant function value.	-0.19	0.01*	-0.12	0.19	-0.34	0.05
SERPING1	Reinhard O. W. Burk (RB) discriminant function value	-0.02	0.85	-0.25	< 0.01*	0.00	0.98
Other Parameters							
SERPINA7	IOP	-0.14	0.02*	-0.16	0.03*	-0.14	0.24

Corr.: Correlation coefficient; * adj. p-value < 0.05

Conclusions

In conclusion, this study provides a detailed map of serpin expression in human AH and highlights multiple serpins with potential relevance to POAG. These proteins may serve as biomarkers for disease detection or progression and offer insight into novel molecular pathways underlying glaucomatous neurodegeneration. Future longitudinal and mechanistic studies are needed to validate these findings and explore the therapeutic potential of targeting serpins in glaucoma.

Abbreviations

AGT	Angiotensinogen
AH	Aqueous Humor
BMO	Bruch's membrane opening
CBG	Corticosteroid-binding globulin
CTGF	Connective tissue growth factor
FC	Fold change
GCL	Ganglion cell layer
HRT	Heidelberg Retinal Tomograph
IOP	Intraocular pressure
MS	Mass spectrometry
OCT	Optical Coherence Tomography
POAG	Primary open-angle glaucoma
PSM	Peptide spectrum match
RGC	Retinal ganglion cell
RNFL	Retinal nerve fiber layer
TBG	Thyroxine-binding globulin
TMM	Trimmed mean of M values
VFI	Visual Field Index

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12886-025-04119-3.

Supplementary Material 1

Supplementary Material 2

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Not applicable.

Author contributions
A.S., E.W., and S.Sh. designed the project; S.A., A.K., D.A., G.J., and J.A. developed the methodology; A.S. and T.J.L. conducted software usage; T.J.L., S.Sa. and E.W. formally analyzed the results; A.E., K.B., A.S., and S.Sh. provided resources for sample collection; E.W., T.J.L., S.A., G.J., and J.A. curated data; E.W., A.K., S.Sa., D.A., and J.A. drafted the manuscript; A.S. and S.Sh. revised the manuscript; T.J.L., D.A. and E.W. created visual aids and figures; A.S. supervised the project and acquired funding. All authors have read and agreed to the published version of the manuscript.

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Data availability
Data is provided in supplementary information files.

Declarations

Ethics approval and consent to participate
This study was approved by the Institutional Review Board of Augusta University (IRB #611480). Written informed consent was obtained by all participants prior to sample collection.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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