Structure and composition of exfoliation aggregates

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INTRODUCTION

Exfoliation syndrome (XFS) is a condition characterized by the production of insoluble fibrillar aggregates (exfoliation material; XFM) in the eye and elsewhere. Many patients with XFS progress to exfoliation glaucoma (XFG), a significant cause of global blindness. We used quantitative mass spectrometry to analyze the composition of XFM in lens capsule specimens and in aqueous humor (AH) samples from patients with XFS, patients with XFG, and unaffected individuals.

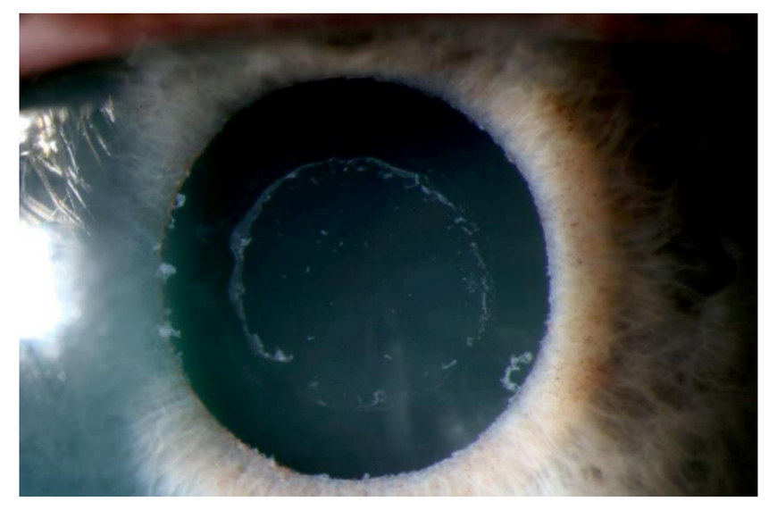


Figure 1. Dust-like aggregates accumulate on the anterior surface of the lens in a patient with exfoliation syndrome

DESIGN & METHODS

Pieces of lens capsule and samples of AH were obtained with consent from patients undergoing cataract surgery. Tryptic digests of capsule or AH were analyzed by high-performance liquid chromatography–mass spectrometry and relative differences between samples were quantified using the tandem mass tag technique. The distribution of XFM on the capsular surface was visualized by SEM and super-resolution light microscopy.

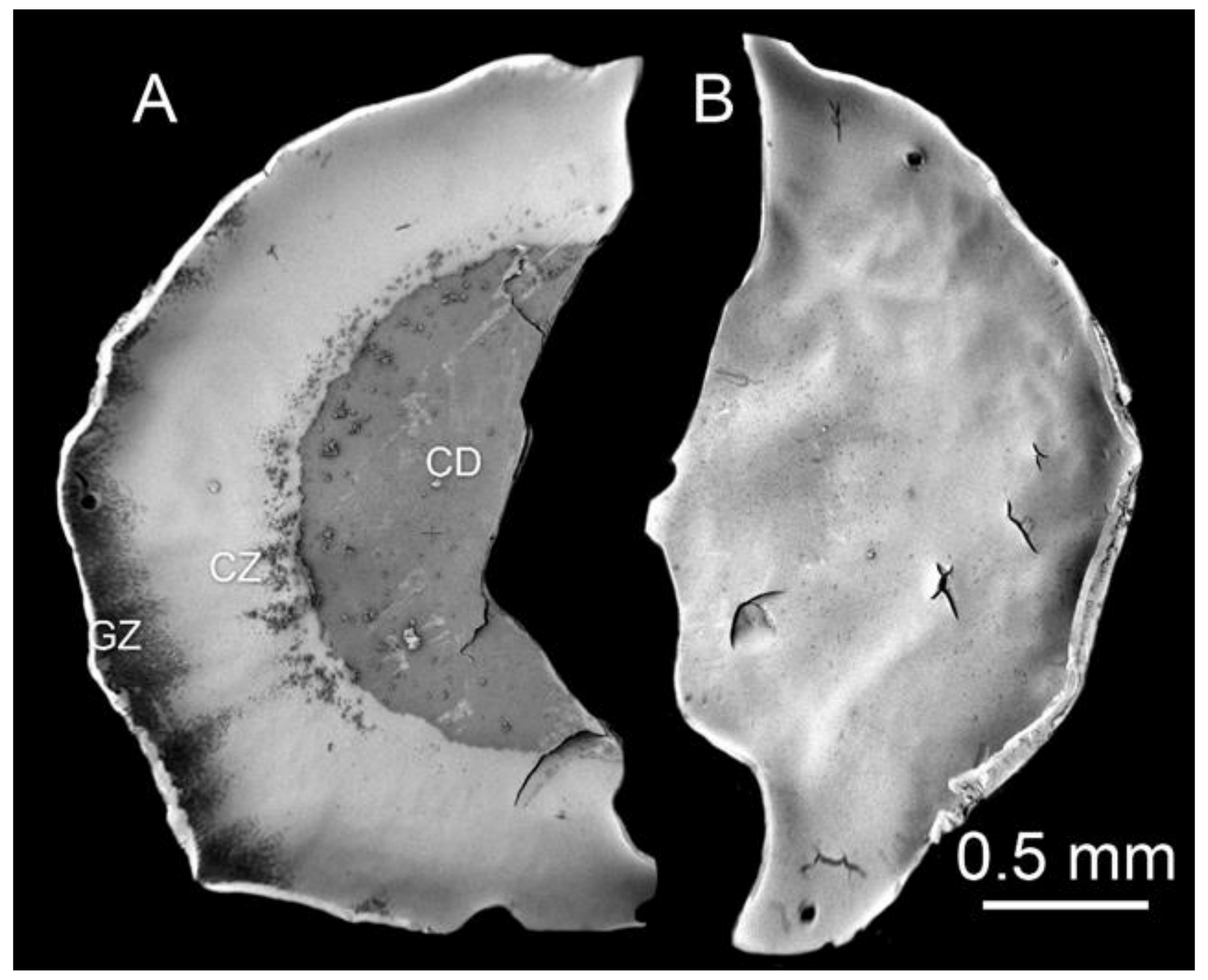


Figure 2. Scanning electron micrograph showing the presence of exfoliation material on a capsular specimen from an XFS patient (A) or a control sample (B, from a patient without XFS). Comparative proteomic analysis was used to identify proteins present in XFS samples and absent in controls. CD, central disk; CZ, clear zone; GZ, granular zone.

DIAGRAM OR EXAMPLE

Sample ID #	Gender	Age	Ethnicity	Clinical Diagnosis	Category	IOP mmHg	Cup/disk	NFL (µm)	Visual Field loss (dB)	AH proteome	Capsule proteome	AH ELISA	Confocal Microscopy	SEM
6	Male	63	Black	No Glaucoma	CAT	20	0.65						X	X
T6	Female	76	White	No Glaucoma	CAT	17	0.20						X	X
T7	Female	64	Black	Glaucoma Sus	CAT	18	0.50		-2.92					X
T8	Female	84	White	No Glaucoma	CAT	18	0.35						X	X
T9	Male	75	White	No Glaucoma	CAT	12	0.30						X	X
T10	Female	69	White	No Glaucoma	CAT	20	0.40						X	X
T11	Female	64	White	No Glaucoma	CAT	19	0.40							X
T12	Male	78	White	No Glaucoma	CAT	12	0.25							X
T13	Female	83	White	No Glaucoma	CAT	14	0.30							X
T14	Male	78	White	No Glaucoma	CAT	16	0.55							X
T19	Male	72	White	No Glaucoma	CAT	15	0.1			X	X			
T20	Female	75	White	No Glaucoma	CAT	12	0.1				X			
T21	Female	74	White	No Glaucoma	CAT	16	0.4					X		
T22	Female	71	White	No Glaucoma	CAT	18	0.2			X	X			
T23	Male	77	White	No Glaucoma	CAT	12	0.4			X	X			
T24	Male	58	White	No Glaucoma	CAT	16	0.25			X	X			
T25	Female	76	Black	No Glaucoma	CAT	12	0.50				X			
T26	Male	67	White	No Glaucoma	CAT	19	0.40			X	X			
T32	Female	81	Black	Glaucoma Sus	CAT	17	0.55	*	-3.37			X		
T36	Female	75	White	No Glaucoma	CAT	14	0.3					X		
T39	Male	85	White	No Glaucoma	CAT	15	0.4					X		
S12	Female	73	White	No Glaucoma	CAT/GL	20	0.70							X
S13	Female	70	Black	CAG	CAT/GL	14	0.65	63	-7.71					X
S28	Female	66	White	UG	CAT/GL	21	0.35	70	-9.35					X
S63	Male	66	Black	POAG	CAT/GL	18	0.8	67	-14.58			X	X	
S65	Male	71	Black	POAG	CAT/GL	13	0.95	57	-29.28			X		
S66	Male	65	Black	POAG	CAT/GL	09	0.85	61	-19.27			X		
S68	Female	66	Black	POAG	CAT/GL	22	0.55	97	-2.29			X		
S71	Female	69	Black	SOAG	CAT/GL	18	0.70	59	-23.37	X				
S78	Female	59	Black	POAG	CAT/GL	11	0.85	68	-4.40	X	X			
S79	Male	66	White	PG	CAT/GL	21	0.40	83	-2.18	X	X			
S80	Male	66	Black	POAG	CAT/GL	11	0.90	54	-27.81	X	X			
S83	Male	82	White	POAG	CAT/GL	12	0.90	*	-4.10	X	X			
K1	Female	68	Asian		XFS	15	0.65				X			
GH1	Female	78	White		XFS	14	0.45				X			
S48	Female	81	White		XFG	12	0.4						X	
S69	Female	72	White		XFS	17	0.5			X				
S85	Female	72	White		XFS	15	0.5					X		
T1	Female	83	*		XFS	16	0.6				X			
SK48	Male	82	White		XFS	12	0.4						X	X
SI1	Female	81	*	OAG	XFG	18	0.75	70	-7.14				X	X
SI2	Female	71	Black	OAG	XFG	24	0.95	58	-28.92		X			
SI3	Female	93	White	OAG	XFG	6	0.80	60	-16.35		X			
S26	Female	80	White	OAG	XFG	18	0.55	88	1.80					X
S73	Female	80	White	OAG	XFG	30	0.8	*	-0.78	X	X			
S74	Female	78	White	OAG	XFG	15	0.9	65	*	X	X			
S75	Male	74	White	OAG	XFG	15	0.9	50	-22.50	X				
S76	Male	70	White	OAG	XFG	29	0.3	89	-0.11	X				
S77	Male	71	White	OAG	XFG	15	0.5	68	-22.50	X				
S84	Male	73	Asian	CAG	XFG	10	0.85	49	-14.42			X		
S86	Female	79	White	OAG	XFG	20	0.2	78	1.87			X		
S87	Male	73	Asian	CAG	XFG	12	0.6	72	-4.23			X		

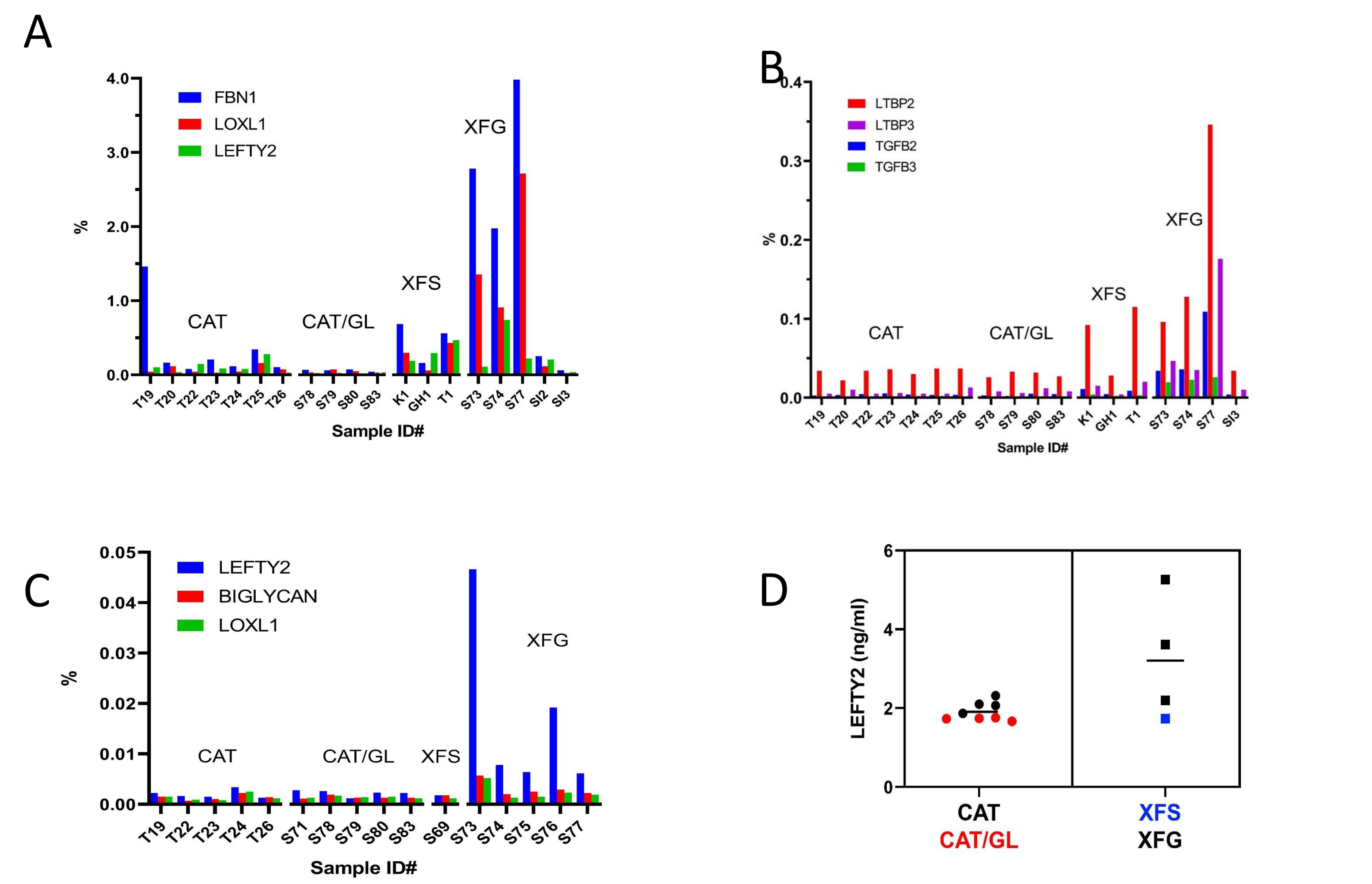
Table 1. Patient demographics and clinical details

Uniprot accession|protein ID	fold change	p-value	corrected p-value	XFS and XFG (Avg. intensity)	CONTROL (Avg. intensity)	confidence
EXPT 1 (XFS and XFG Vs CAT)						
Q08397|LOXL1	18.69	4.39E-08	2.21E-05	4.62E+06	2.47E+05	high
O00292|LFTY2	4.39	1.54E-06	3.88E-04	3.14E+06	7.17E+05	high
P10600|TGFB3	7.61	6.03E-06	9.60E-04	9.21E+04	1.21E+04	high
Q92954|PRG4	4.06	1.48E-05	1.49E-03	2.55E+05	6.29E+04	high
P61812|TGFB2	5.89	4.90E-05	4.12E-03	1.58E+05	2.68E+04	high
P25067|CO8A2	2.74	3.38E-04	2.43E-02	4.62E+04	1.68E+04	med
Q9NS15|LTBP3	4.43	3.99E-04	2.51E-02	1.54E+05	3.47E+04	med
P55001|MFAP2	3.33	4.72E-04	2.64E-02	4.38E+05	1.32E+05	med
Q14767|LTBP2	2.89	6.11E-04	3.08E-02	6.29E+05	2.18E+05	med
EXPT 2 (XFS and XFG Vs CAT/GL)						
Q9H3Y0|CRSPL	18.74	2.61E-09	1.47E-06	6.33E+04	3.38E+03	high
P10600|TGFB3	13.74	1.93E-07	5.41E-05	3.17E+04	2.31E+03	high
P35555|FBN1	16.15	6.67E-07	1.30E-04	4.75E+06	2.94E+05	high
O00292|LFTY2	7.65	4.21E-06	4.7E-04	9.82E+05	1.28E+05	high
Q08397|LOXL1	13.82	1.50E-05	1.06E-03	3.19E+06	2.31E+05	high
Q14767|LTBP2	3.42	3.80E-05	2.08E-03	4.87E+05	1.42E+05	high
Q9NS15|LTBP3	3.73	3.71E-04	6.72E-03	1.53E+05	4.10E+04	high
P61812|TGFB2	4.05	4.28E-04	7.08E-03	7.31E+04	1.80E+04	high

Table 2. Selected upregulated proteins in experiment 1 (XFS vs CAT) and 2 (XFS vs CAT/GL). High confidence indicates a corrected p-value ≤0.01. Medium confidence indicates ≤0.05.

Table 2. Differentially expressed proteins in XFS and XFG samples vs. controls.

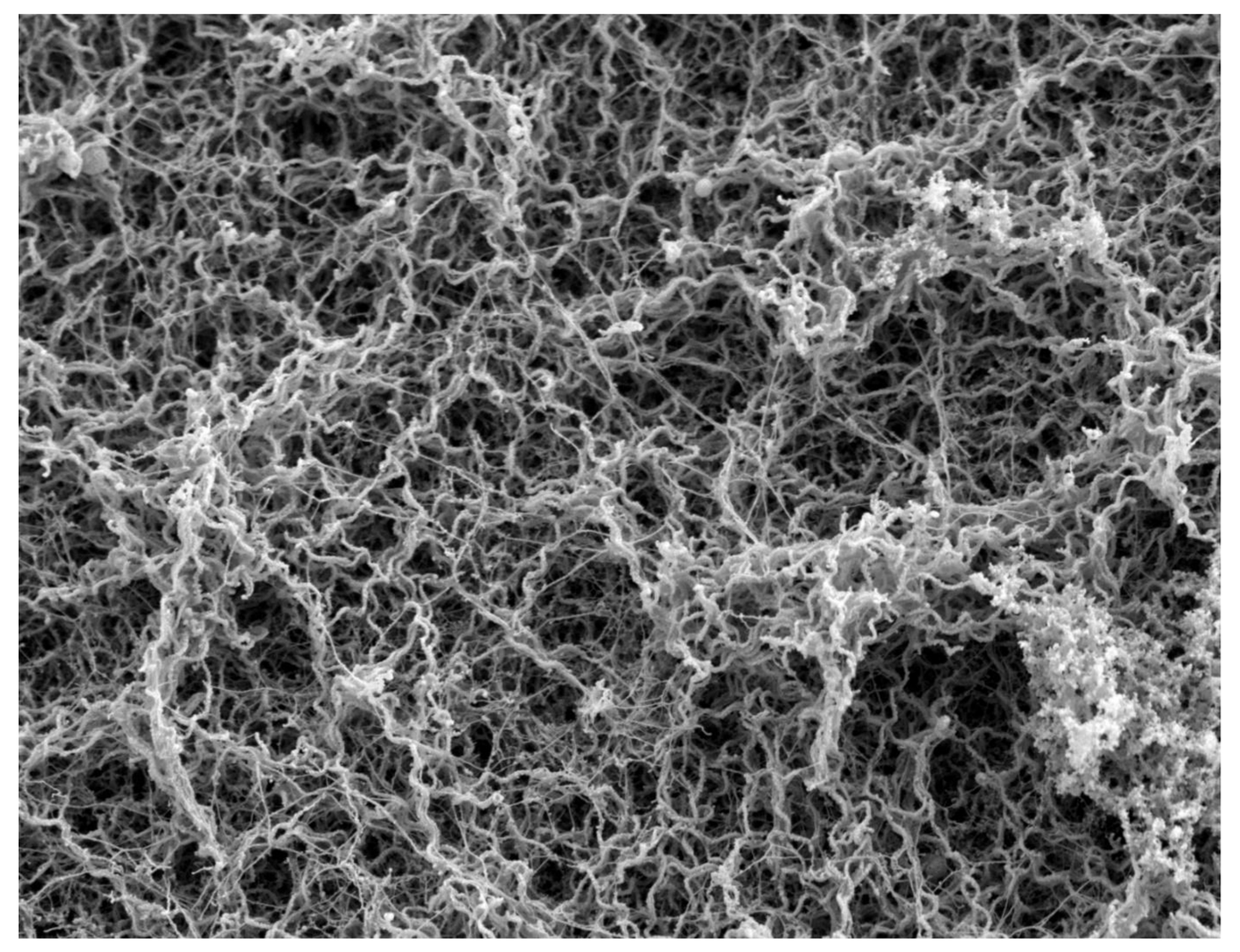
RESULTS



Differentially expressed proteins in individual patient samples (T19, T20, etc.). A. XFS aggregates are characterized by strong expression of fibrillin 1 (FBN1), Lysyl oxidase-like 1 (LOXL1), and left-right differentiation factor-2 (LEFTY2). B. Aggregates also contain elevated levels of TGFbeta and latent-TGFbeta binding proteins (LTBP's). C. In aqueous humor samples LOXL1 is not elevated but biglycan and, especially, LEFTY2, are increased. D. ELISA analysis showing that LEFTY levels are elevated in samples from XFG patients. CAT, cataract; CAT/GL, cataract with glaucoma; XFS exfoliation syndrome patient, XFS; and XFG, exfoliation glaucoma.

CONCLUSIONS

This quantitative study provides new insights into the composition of pseudoexfoliation aggregates. Three proteins (FBN1, LOXL1 and LEFTY2) were especially abundant in the aggregates, although other members of the TGFbeta signaling pathway (TGFbeta2, TGFbeta3, LTBP2 (Latent TGFbeta binding protein 2) LTBP3) were also prominent.



Scanning electron microscopy detected two types of fibers in the XFM aggregates: thin (10 nm) straight fibers and thick (30 nm) rough surfaced, helical fibers.

NEXT STEPS

This project provided information on the ultrastructure and composition of pseudoexfoliation aggregates. Moving forward, we would like to localize the proteins identified by mass spec analysis on the fibrils that comprise the material. Specifically, we hope to understand the ultrastructural relationship between fibrillin-1 and LOXL1.

LEFTY2, a novel member of the TGFbeta growth factor family, was identified in both the capsule and aqueous humor samples from pseudoexfoliation patients. The level of LEFTY2 was particularly elevated in samples from pseudoexfoliation patients who had developed glaucoma. Based on this observation, we are currently organizing a larger study to determine whether LEFTY2 can serve as a biomarker for glaucoma progression. We are also interested in determining the cellular source of LEFTY2 and exploring its potential role in disease progression.

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