Supplementary Information

Differential protein expression in tears of patients with primary open angle and

pseudoexfoliative glaucoma

Damiana Pieragostino,^{a,b1} Sonia Bucci,^{b,c1} Luca Agnifili,^d Vincenzo Fasanella,^d Simona

D'Aguanno,^{f,g} Alessandra Mastropasqua,^d Marco Ciancaglini,^e Leonardo Mastropasqua,^d Carmine

Di Ilio,^{a,b} Paolo Sacchetta,^{a,b} Andrea Urbani,^{b,f,g} and Piero Del Boccio^{a,b}

^a Department of Biomedical Sciences, University "G. d'Annunzio" of Chieti-Pescara, Chieti. Italy.

^bResearch Centre on Aging (Ce.S.I), University "G. d'Annunzio" of Chieti-Pescara, Chieti. Italy.

^c Department of Neuroscience and Imaging, University "G. d'Annunzio" of Chieti-Pescara, Chieti. Italy.

^d Ophthalmic Clinic, Department of Medicine and Ageing Science. University "G. d'Annunzio" of Chieti-Pescara, Chieti. Italy.

^e Department of surgical Science, Ophthalmology Clinic, University of L'Aquila. L'Aquila. Italy

^f Laboratory of Proteomics, IRCCS-Santa Lucia Foundation, Rome, Italy.

^g Department of Internal Medicine, University of Tor Vergata, Rome. Italy

¹DP and SB contributed equally to this work

Presented, in part, at the 6th Annual National Conference of the Italian Proteomics Association held

in Turin 21st-24th June 2011.

*Corresponding Author:

Dott.ssa Damiana Pieragostino, PhD

Department of Biomedical Sciences, University "G. d'Annunzio" of Chieti-Pescara, Italy

Via dei Vestini, 66100 Chieti - Italy

Tel.+39 0871 541593/(3554516)

Fax.+39 0871 541598

e-mail: dpieragostino@unich.it

Keywords: Mass Spectrometry, Proteomics, Tears, Primary Open Angle Glaucoma, Pseudo-Exfoliative

Glaucoma, Anti-glaucoma therapy, Prostaglandin analogues.

Figure 1S

12% SDS-PAGE of 8 different CTRL tear samples.

Figure 2S

Analytical performances of expression analysis. Figure 2S, panel A for CTRL, E for PXG and I for POAG, shows paired peptide exact masses associated to their retention time, of two injections of the same condition distributed along a diagonal line. Figure 2S, panels B for CTRL, F for PXG, L for POAG, shows distribution of mass error. Figure 2S, panel C for CTRL, G for PXG, M for POAG retention time coefficient of variation expressed as percentage (% CV RT) and, finally, the intensity coefficient of variation expressed as percentage (% CV intensity) has a Gaussian distribution in panels D for CTRL, H for PXG, N for POAG.

Table 1S

Clinical characteristics and demographics of enrolled subjects

Table 2S

Details of proteins identified by LC-ESI-MS^E, reported in a separated excel file.

Table 3S

Most important networks involved in each comparison.

Table 4 S

Details of MS\MS ion search of digested Cystatin S, isolated by fraction collection experiment. Table shows b- and y-ions from **<u>IIPGGIYDADLNDEWVQR</u>** peptide fragmentation. Match to Query 50: 2073.094048 from(1037.554300,2+) intensity(21732.9902) by monoisotopic mass of neutral peptide Mr(calc): 2073.0167, obtaining ions score of 88 and Expect value of 5e-08. Matches ions are shown in red: 21/160 fragment ions using 42 most intense peaks Electronic Supplementary Material (ESI) for Molecular BioSystems This journal is The Royal Society of Chemistry 2011

Figure 1S



Figure 2S









Table 1S

	POAG	PXG	CTRL	
Number of eyes	9	7	8	
Mean Age (years ±SD)	57.8±5.03	58.71±3.59	55.87±3.18	p=n.s.*
Gender M/F	4/5	4/3	4/4	p=n.s.*
Mean IOP (mmHg±SD)	14.7 ±2.4	15.4±3.21	13.8±2.3	p=n.s.*
Mean time on therapy (years±SD)	3.4±1.6	3±1.15	NA	p=n.s.*
MD (db±SD)	$-4.2\pm0.8^{\$\dagger}$	-3.6 ±0.9 ^{§†}	$+0.8\pm0.4^{\dagger}$	p= n.s. [§] p<0.05 [†]

POAG: primary open angle glaucoma PXG: pseudo-exfoliative glaucoma CTRL: controls SD: standard deviation M=male; F=female; IOP= intra-ocular pressure; MD= mean defect db= decibel *t-test for independent samples † t-test for independent samples; CTRL versus POAG, CTRL versus PXG § t-test for independent samples; POAG versus PXG NA: not applicable NS: not significant

Table 3S

	CTRL vs PXG comparison						
Network	Associated Network Functions	score					
	Dermatological Diseases and Conditions, Genetic						
1	Disorder, Inflammatory Disease	49					
	Protein Synthesis, Cell Cycle, Inflammatory						
2	Response	49					
	CTRL vs POAG comparison						
Network	Associated Network Functions	score					
	Inflammatory Response, Cellular Function and						
1	Maintenance, Antigen Presentation	60					
	Cell-To-Cell Signaling and Interaction, Tissue						
2	Development, Cell Morphology	19					
PXG vs POAG comparison							
Network	Associated Network Functions	score					
1	Cellular Movement, Lipid Metabolism, Molecular Transport	40					

Table 4S

#	b	b ⁺⁺	b*	b*++	b ⁰	b ⁰⁺⁺	Seq.	у	y ⁺⁺	y*	y*++	y ⁰	y ⁰⁺⁺	#
1	114.0913	57.5493					Ι							18
2	227.1754	114.0913					Ι	1960.9399	980.9736	1943.9134	972.4603	1942.9294	971.9683	17
3	324.2282	162.6177					P	1847.8559	924.4316	1830.8293	915.9183	1829.8453	915.4263	16
4	381.2496	191.1285					G	1750.8031	875.9052	1733.7766	867.3919	1732.7925	866.8999	15
5	438.2711	219.6392					G	1693.7816	847.3945	1676.7551	838.8812	1675.7711	838.3892	14
6	551.3552	276.1812					Ι	1636.7602	818.8837	1619.7336	810.3705	1618.7496	809.8784	13
7	714.4185	357.7129					Y	1523.6761	762.3417	1506.6496	753.8284	1505.6655	753.3364	12
8	829.4454	415.2264			811.4349	406.2211	D	1360.6128	680.8100	1343.5862	672.2968	1342.6022	671.8047	11
9	900.4825	450.7449			882.4720	441.7396	A	1245.5858	623.2966	1228.5593	614.7833	1227.5753	614.2913	10
10	1015.5095	508.2584			997.4989	499.2531	D	1174.5487	587.7780	1157.5222	579.2647	1156.5382	578.7727	9
11	1128.5936	564.8004			1110.5830	555.7951	L	1059.5218	530.2645	1042.4952	521.7513	1041.5112	521.2592	8
12	1242.6365	621.8219	1225.6099	613.3086	1224.6259	612.8166	N	946.4377	473.7225	929.4112	465.2092	928.4272	464.7172	7
13	1357.6634	679.3353	1340.6369	670.8221	1339.6529	670.3301	D	832.3948	416.7010	815.3682	408.1878	814.3842	407.6958	6
14	1486.7060	743.8566	1469.6795	735.3434	1468.6955	734.8514	E	717.3679	359.1876	700.3413	350.6743	699.3573	350.1823	5
15	1672.7853	836.8963	1655.7588	828.3830	1654.7748	827.8910	W	588.3253	294.6663	571.2987	286.1530			4
16	1771.8537	886.4305	1754.8272	877.9172	1753.8432	877.4252	V	402.2459	201.6266	385.2194	193.1133			3
17	1899.9123	950.4598	1882.8858	941.9465	1881.9018	941.4545	Q	303.1775	152.0924	286.1510	143.5791			2
18							R	175.1190	88.0631	158.0924	79.5498			1