

Supplementary information to

DMD-AR-2022-000993 entitled "Activity and expression of carboxylesterases and arylacetamide deacetylase in human ocular tissues"

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Supplementary Table 1. Details of samples obtained from postmortem donors.

DONOR	SEX/AGE^A	EYE/TISSUE^B	CAUSE OF DEATH	REMARKS
B1	M 63	LE conjunctiva RE conjunctiva	Brain injury	--
B2	M 54	LE conjunctiva RE conjunctiva	Cardiac arrest	Rosacea
B3	F 76	RE conjunctiva	Brain stroke	Diabetic
B4	F 82	RE conjunctiva RE globe LE globe	Brain stroke	RE cataract surgery RE w/o cornea LE w/o cornea
B5	M 52	RE conjunctiva RE globe LE globe	Brain injury	RE w/o cornea LE w/o cornea
B6	M 64	RE conjunctiva RE globe	Cardiac arrest	RE w/o cornea
B7	M 64	RE conjunctiva LE conjunctiva RE globe LE globe	Choking	RE w/o cornea LE w/o cornea
B8	M 64	RE conjunctiva LE conjunctiva RE globe LE globe	Cardiac arrest	RE w/o cornea LE w/o cornea
B9	M 74	RE conjunctiva LE conjunctiva RE globe	Brain hemorrhage	Diabetic retinopathy RE cataract surgery RE vitrectomy
B10	F 73	RE conjunctiva LE conjunctiva RE globe LE globe	Brain stroke	RE w/o cornea LE w/o cornea
B 11	M 74	RE conjunctiva LE conjunctiva RE globe LE globe	Cardiac arrest	RE w/o cornea LE w/o cornea
B 12	F 68	RE conjunctiva RE globe LE globe	Brain stroke	RE w/o cornea LE w/o cornea
B13	M 83	RE conjunctiva	Brain stroke	--
B14	M 68	RE conjunctiva LE conjunctiva	Brain stroke	RE: conjunctival degeneration LE: conjunctival degeneration

^A, F/M, female/male; age in years

^B, LE = left eye, RE = right eye; LE/RE globe = the entire eye globe was removed

Supplementary Table 2. Details of samples obtained from patient donors.

DONOR	SEX/AGE^A	EYE/TISSUE^B	CAUSE OF EVISCERATION	REMARKS
A1	F 85	LE Cornea	Terminal glaucoma	--
A2	M 62	LE Cornea	Terminal glaucoma	--
A3	F 16	RE Cornea	Phthisis	--
A4	M 79	RE Vitreous	Terminal glaucoma	--
A5	M 72	LE Cornea LE Retina	Painful blind eye	Diabetic retinopathy
A6	F 85	LE Cornea LE Vitreous	Terminal glaucoma	--
A7	F 86	RE Cornea RE Vitreous RE Retina	Painful blind eye	Corneal neovascularization
A8	F 79	RE Cornea RE Retina	Painful blind eye, phthisis	--
A9	F 88	RE Vitreous RE Retina	Terminal glaucoma	--

^A, F/M, female/male; age in years

^B, LE = left eye, RE = right eye

Supplementary Table 3. MRM parameters used for the analysis of CESs and AADAC selected peptides are shown. Labeled and unlabeled MRMs, three in total are presented while for the concentration (peak area ratio) determination, only the two giving the highest signals were used.

<i>Enzyme</i>	<i>Peptide sequence</i>	<i>CE (V)</i>	<i>Hydrophobicity from SSRCalc</i>	<i>RT (min)</i>	<i>Precursor ion (m/z)</i>	<i>Product ion (m/z) (y or b)</i>
Na⁺/K⁺ ATPase	AAVPDAVGK (light)	18.8	10.62	9.1	414.23	586.32 (y6)
		15.8			414.23	242.15 (b3)
		22.8			414.23	293.66 (y6+2)
	AAVPDAVGK (heavy)	18.8			418.25	594.34 (y6)
		15.8			418.25	242.15 (b3)
		22.8			418.25	297.68 (y6+2)
CES1	FWANFAR (light)	23.3	27.42	14.9	456.23	578.30 (y5)
		23.3			456.23	507.27 (y4)
		24.3			456.23	764.38 (y6)
	FWANFAR (heavy)	23.3			461.23	588.31 (y5)
		23.3			461.23	517.28 (y4)
		24.3			461.23	774.39 (y6)
CES2	ADHGDELPFVFR (light)	17.1	31.09	16.6	468.23	333.19 (y5+2)
		18.1			468.23	738.31 (b7)
		22.1			468.23	665.38 (y5)
	ADHGDELPFVFR (heavy)	17.1			471.57	338.20 (y5+2)
		18.1			471.57	738.31 (b7)
		22.1			471.57	675.39 (y5)
CES2	AGVHTFLGIPFA K (light)	19.2	36.1	17.3	453.26	462.27 (y4)
		19.2			453.26	231.64 (y4+2)
		19.2			453.26	783.41 (b8)
	AGVHTFLGIPFA K (heavy)	19.2			455.93	470.30 (y4)
		19.2			455.93	235.65 (y4+2)
		19.2			455.93	783.41 (b8)
CES3	LAFPEATEEEK (light)	19.5	20.58	10.8	421.87	575.77
		24.5			421.87	(y10+2)
		28.5			421.87	559.26
	LAFPEATEEEK (heavy)	19.5			424.55	(b10+2)
		24.5			424.55	430.21 (b8+2)
		28.5			424.55	579.78 (y10+2) 559.26 (b10+2) 430.21 (b8+2)
CES3	NTIYPLTVDGTV FPK (light)	37.8	36.04	17.4	832.95	1173.65 (y11)
		28.8			832.95	492.25 (b4)
		24.8			555.63	763.40 (y7)
		37.8			836.96	1181.68 (y11)

	NTIYPLTVDGTV	28.8			836.96	492.25 (b4)
	FPK (heavy)	24.8			558.31	771.42 (y7)
CES3	TPEEILAEK (light)	27.4	17.74	11.6	515.28	464.75 (y8+2)
		27.4			515.28	831.45 (y7)
		30.4			515.28	702.40 (y6)
	TPEEILAEK (heavy)	27.4			519.29	468.77 (y8+2)
		27.4			519.29	839.47 (y7)
		30.4			519.29	710.43 (y6)
CES3	FAPPQPAEPWNF VK (light)	36.2	33.14	17.0	814.42	705.36
		21.1			543.28	(y12+2)
		21.1			543.28	395.72 (y6+2)
		36.2			818.43	790.42 (y6)
	FAPPQPAEPWNF VK (heavy)	21.1			545.96	709.38
		21.1			545.96	(y12+2)
						399.73 (y6+2)
						798.45 (y6)
AADAC	FWSEYFTTDR (light)	30.2	32.45	16.2	676.30	1018.45 (y8)
		30.2			676.30	802.37 (y6)
		32.2			676.30	931.42 (y7)
	FWSEYFTTDR (heavy)	30.2			681.31	1028.46 (y8)
		30.2			681.31	812.38 (y6)
		32.2			681.31	941.42 (y7)
AADAC	YPGFLDVR (light)	24.3	28.07	15.5	483.76	402.22 (y7+2)
		27.3			483.76	706.34 (y6)
		20.3			483.76	693.32 (b6)
	YPGFLDVR (heavy)	24.3			488.76	407.23 (y7+2)
		27.3			488.76	716.40 (y6)
		20.3			488.76	693.32 (b6)
AADAC	TPTPGSLELAQK (light)	32.2	21.72	12.1	621.34	942.53 (y9)
		34.2			621.34	471.77 (y9+2)
		33.2			621.34	300.16 (b3)
	TPTPGSLELAQK (heavy)	32.2			625.35	950.55 (y9)
		34.2			625.35	475.78 (y9+2)
		33.2			625.35	300.17 (b3)

*CE, collision energy; RT, retention time

Supplementary Table 4. Correlation between the hydrolytic activities and CES1 content.

Activities	NPA	DME	FDA	Procaine	CES1
NPA	1				
DME	0.05	1			
FDA	0.21	0.73	1		
Procaine	0.06	0.75	0.8	1	
CES1	0.06	0.48	0.41	0.13	1

Pearson correlation coefficients (r^2) are shown.

Supplementary Figure 1. Western blotting of human ocular samples with the anti-CES2 antibody

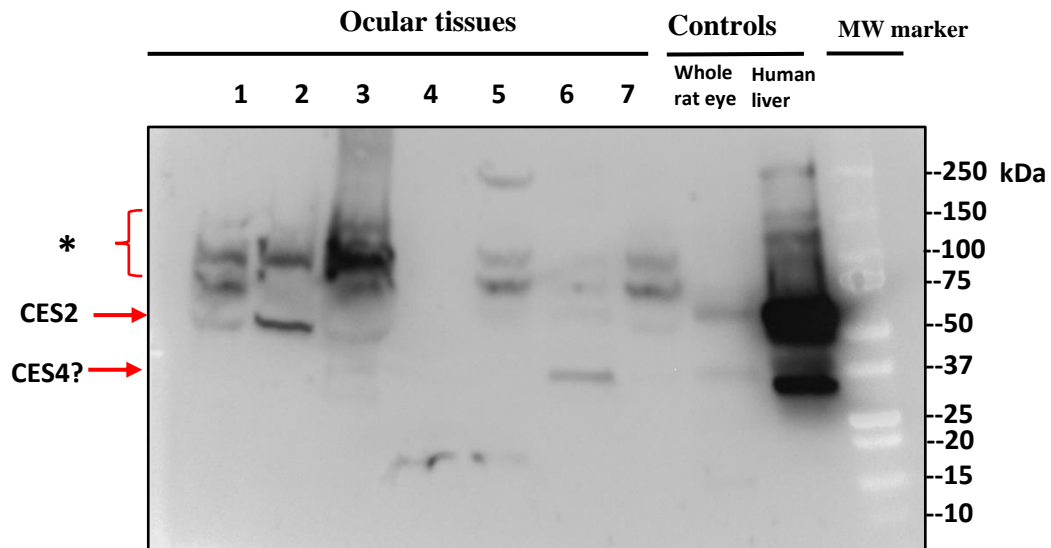


Figure S1. Western blot image of ocular tissues with the anti-CES2 antibody. Protein samples (40 μ g) were loaded on lanes as follows: 1 = conjunctiva, 2 = cornea, 3 = vitreous, 4 = lens, 5 = iris-ciliary body, 6 = retina, and 7 = RPE/choroid, followed by positive control samples and the MW marker. The relative migration of the sample and the MW marker bands were calculated using ImageJ (Abràmoff et al., 2004). Estimation of the protein sizes was done from a linear line of log MW versus distance migrated. Arrows indicate the position of CES2 and tentatively identified CES4/CES1P1 and the bracket shows the larger immunoreactive bands (*), putatively due to non-specific binding.

Supplementary Figure 2. Western blotting of human vitreous, retina and RPE/choroid samples with the anti-BDNF (A) and anti-bestrophin-1 antibodies (B)

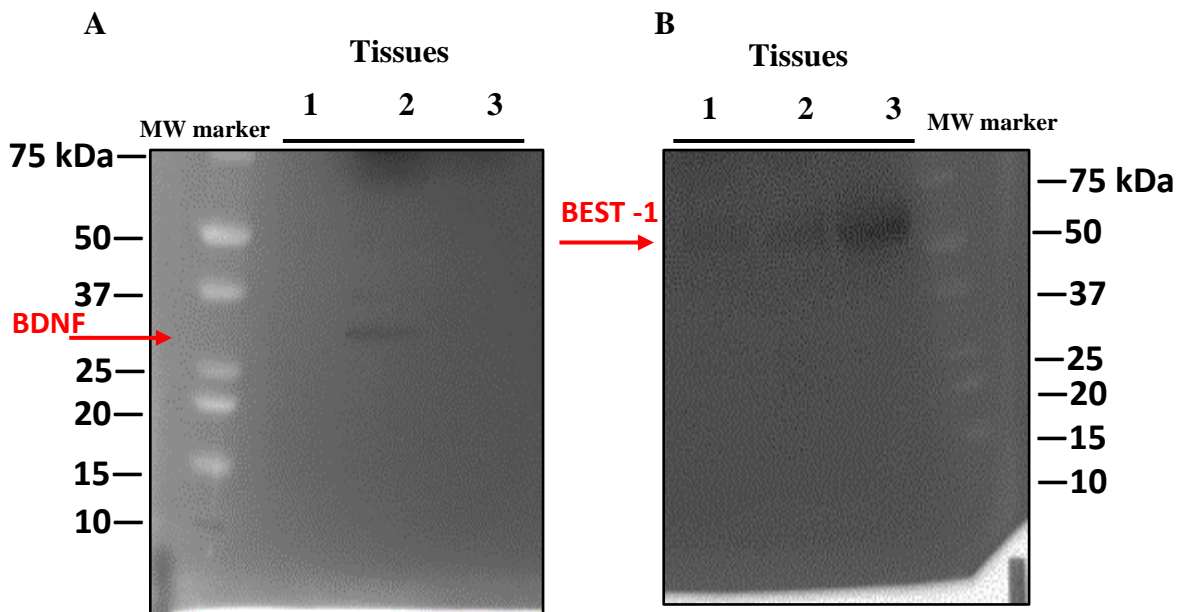
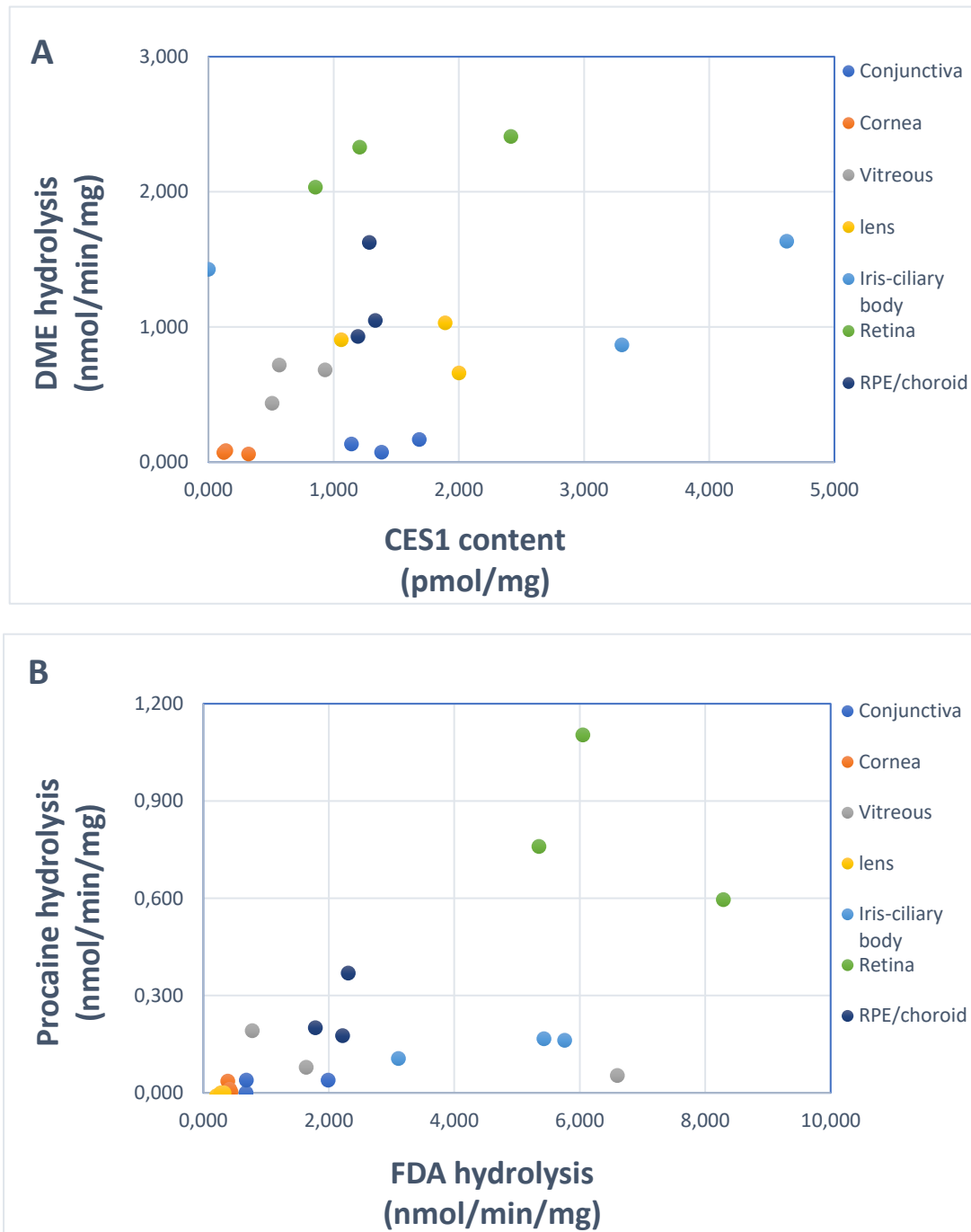


Figure S2. Western blot image of ocular tissues with anti-BDNF (panel A) and anti-bestrophin-1 (panel B) antibodies. Protein samples (20 μ g) were loaded on as follows: 1 = vitreous, 2 = retina, and 3 = RPE/choroid, and the MW marker. The relative migration of the sample and the MW marker bands were calculated using ImageJ (Abràmoff et al., 2004). Estimation of the protein sizes was done from a linear line of log MW versus distance migrated. Arrows indicate the positions of BDNF and bestrophin-1.

Reference

Abràmoff MD, Magalhães, PJ and Ram, SJ (2004) Image processing with ImageJ. *Biophotonics International* **11**: 36-42

Supplementary Figure 3. Correlation between CES1 content and hydrolysis activities of the three human ocular tissue pools



Correlation between the human CES1 content and hydrolysis activities. Correlation between the CES1 content and DME hydrolysis (**A**) and between fluorescein diacetate (FDA) and procaine hydrolysis (**B**) in human ocular tissues are shown. Individual pool, each as a data point is shown, and each tissue is represented with a different colour.