Molecular Pharmacology

CYP4V2 in Bietti's Crystalline Dystrophy: Ocular Localization, Metabolism of ω-3 PUFAs and Functional Deficit of the H331P Variant

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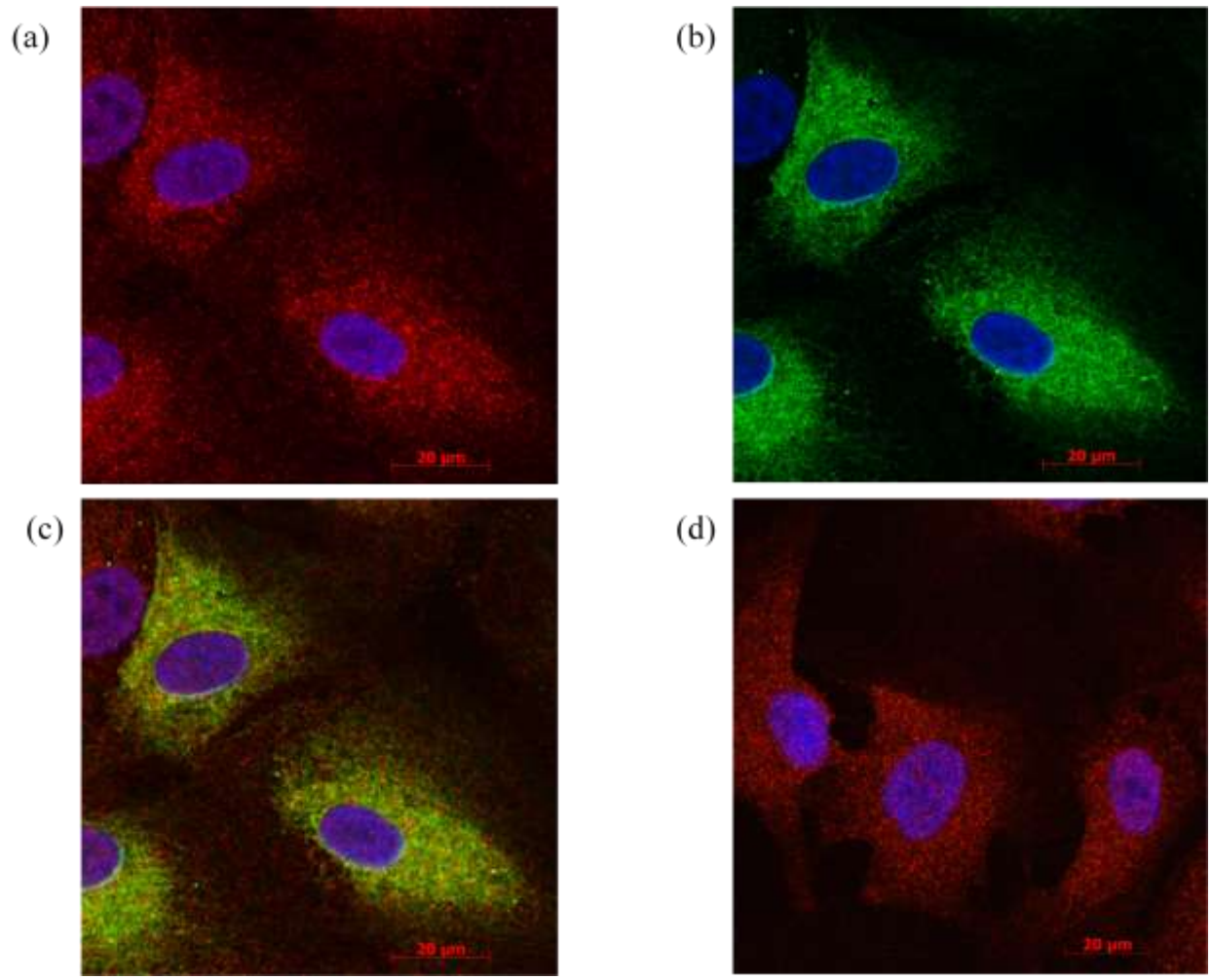
Supplemental Table 1. GC-EI/MS analysis of lipid profiling.

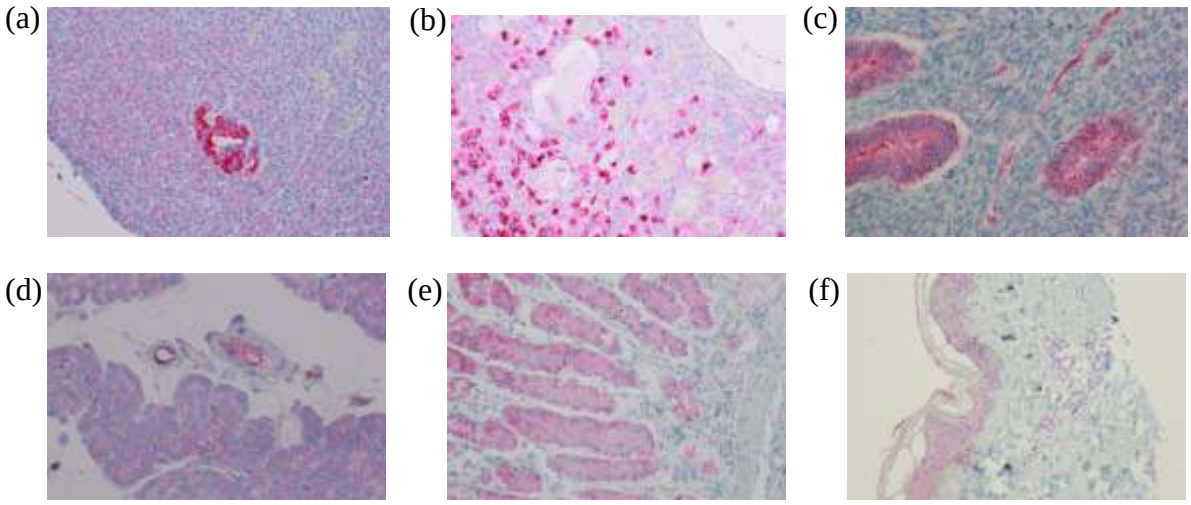
	Name of compounds	Monitored ion (m/z)	Retention time (min)
d2-C18:0	2,2-d2-Stearic acid	300	21.30
C14:0	Tetradecanoic acid/Myristic acid	199	13.86
C16:0	Hexadecanoic acid/Palmitic acid	239	17.77
C16:1	9-cis-Hexadecenoic acid/cis-9 Palmitoleic acid	237	17.36
C18:0	Octadecanoic acid/Stearic acid	255	21.30
C18:1 cis-9	9-cis-Octadecenoic acid/cis-9 Oleic acid	265	20.91
C18:1 cis-11	cis-11 Vaccenic acid	265	21.01
C20:4	cis-5, 8, 11, 14-eicosatetraenoic acid/arachidonic acid	79	23.66
C20:5	Eicosapentaenoic acid/timnodonic acid	79	23.57
C22:6	Docosahexaenoic acid/cervonic acid	79	27.65

Supplemental Table 2. CYP4V2 expression in normal human tissues.

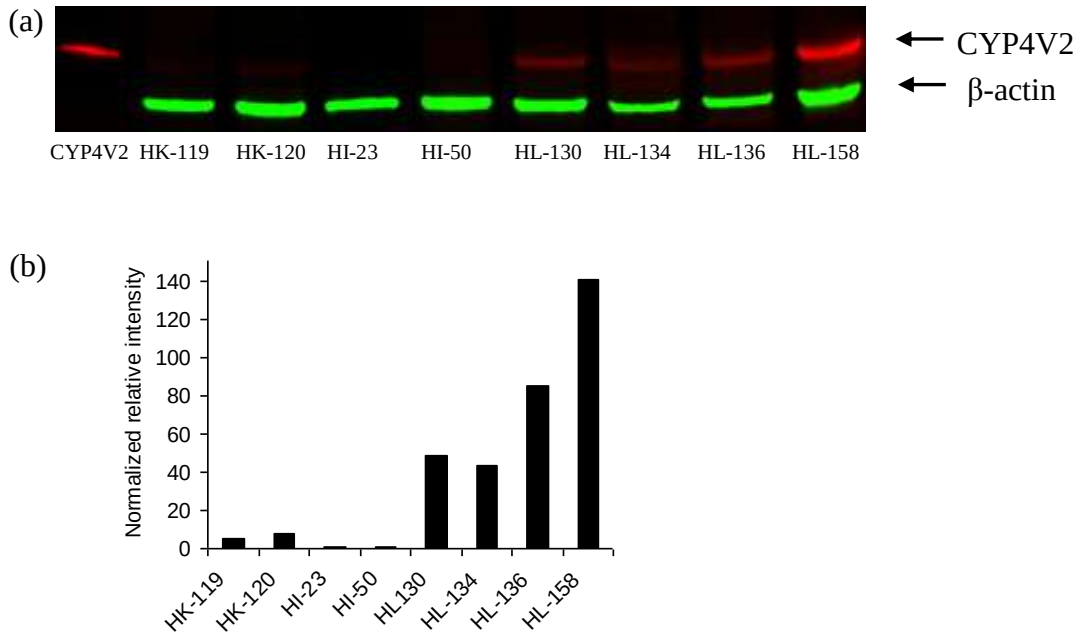
Staining intensity	Tissue	Staining pattern	Supplemental figure 3
Strong positive	Liver	hepatocytes (2-3+)	
	Pancreas	islet cells(4+); acinar cells(1-2+)	a
	Hypophysis	endothelium(3+), small vessels(3+)	b
	Uterus	glandular epithelium(2-4+)	c
	Prostate	endothelium(4+), gland cells(3+)	
Positive	Adrenal gland	endothelial cells(2-3+), interstitial capillaries(3+)	
	Testis	endothelium(3+), seminiferous tubules(2+)	
	Parathyroid	endothelium(3+), small vessels(3+)	d
	Breast	duct(2+), endothelium, small vessels(4+)	
	Tonsil	endothelium(2+), capsule(2+)	
	Esophagus	muscle(2+)	
	Stomach	glandular cells(3+), endothelium(2+)	e
	Skin	Granulisum layer(2+), endothelium(3+)	f
Weak positive	Cerebrum (Cerebral gray matter)	endothelium(2+), vessels(2+)	
	Cerebrum (Cerebral white matter)	endothelium(2+), vessels(2+)	
	Cerebellum	endothelium(2+), vessels(2+)	
	Spleen	overall staining (no specific pattern)	
	Bone	overall staining (no specific pattern)	
	Lung	overall staining (no specific pattern)	
	Heart	endothelium(2+)	
	Kidney	glomeruli (-), proximal tubules(2+)	
	Salivary	glandular cells(2-3+)	
	Uterine cervix	endothelium(2+)	
	Striated muscle	overall staining (no specific pattern)	
	Larynx	epithelium(2+), glands(2+), endothelium(2+)	
Negative	Thyroid		
	Ovary		
	Thymus gland		
	Intestine		
	Colon		
	Nerve (Peripheral nerve)		
	Lung		



Supplemental Figure 1. Immunocytochemical analysis of CYP4V2 expressed in ARPE-19 cells. Immunofluorescence staining revealed that CYP4V2 protein was co-localized in the ER with calreticulin. DAP1 (blue) was used for counter-staining. (a) Calreticulin expression is shown in red. (b) CYP4V2 expression is shown in green. (c) Overlay of (a) and (b). (d) Overlay of preimmune IgG-treated samples.



Supplemental Figure 2. CYP4V2 expression in the normal human tissues. Immunohistochemical staining corresponds to Supplemental Table 2.



Supplemental Figure 3. CYP4V2 expression in drug metabolizing organs. (a) CYP4V2 expression level in 50 μg of kidney (HK), small intestine (HI) and liver (HL) microsomes was determined by Western blot. The red color indicates CYP4V2, and the green color indicates β-actin. The band at the far right is purified CYP4V2. (b) The relative CYP4V2 expression level normalized to β-actin. This demonstrates that human liver expresses CYP4V2 at a moderate level with pronounced inter-individual differences.

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CYP4A11   287 RHLDFLDILLLAKMENGSILSDKDLRAEVDTFMFEGHDTTASGISWILYALATHPKHQERCREEIHSLL 355
CYP4B1    282 RHLDFLDILLGARDEDDIKLSDADLRAEVDTFMFEGHDTTTSGISWFLYCMALYPEHQHRCREEVREIL 350
CYP4F2    294 KILDFIDVLLLSKDEDGKKLSDEDIRAEADTFMFEGHDTTASGLSWVLYHLAKHPEYQERCRQEVQELL 362
CYP4F3B   294 KILDFIDVLLLSKDEDGKKLSDEDIRAEADTFMFEGHDTTASGLSWVLYHLAKHPEYQERCRQEVQELL 362
CYP4F12   294 KILDFIDVLLLSKDEDGKALSDEDIRAEADTFMFGGHDTTASGLSWVLYNLARHPEYQERCRQEVQELL 362
CYP4V2    295 KRRAFLDLLLSVTDDEGNRLSHEDIREEVDTFMFEGHDTTAAAINWSLYLLGSNPEVQKKVDHELDDVF 363
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Supplemental Figure 4. Highly conserved region in the I-helix of CYP4 enzymes.