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Supplemental Data

Biallelic Mutations in *PLA2G5,* Encoding Group V Phospholipase A_2, Cause Benign Fleck Retina

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Figure S1. Multiple Alignment of Nine Mammalian *PLA2G5* Orthologs

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Homo sapiens                MKGLLPLAWF LACSVPAVQG GLLDLKSMIE KVTGKNALTN YGFYGCYCGW GGRGTPKDGT
Pan troglodytes             MKGLLPLAWF LACSVPAVQG GLLDLKSMIE KVTGKNALTN YGFYGCYCGW GGRGTPKDGT
Callithrix jacchus          MKGLLPLAWF LACSVPAVQG GLLDLKSMIE KVTGKNALKN YGFYGCYCGW GGHGTPKDGT
Bos taurus                  MKGLLMLAWF LACSVPAVPG SLLDLKSMIE KVTGKPALKY YGFYGCYCGW GGHGTPMDGT
Canis familiaris            MNGLLTLAWL LACCVRAVPG GLLDLKSMIE KVTGKSALTN YGFYGCYCGW GGRGTPKDGT
Mus musculus                MKGLLTLAWF LACSVPAVPG GLLELKSMIE KVTRKNAFKN YGFYGCYCGW GGRGTPKDGT
Rattus norvegicus           MKRLLTLAWF LACSVPAVPG GLLELKSMIE KVTGKNAVKN YGFYGCYCGW GGHGTPKDGT
Oryctolagus cuniculus       MKGLLTLAWF LACGVPAVPG SLLDLKSMIE KVTGKNALTN YGFYGCYCGW GGRGTPMDGT
Pteropus vampyrus           MKSLLTLAWF LACSVPTVPG GLLELKSMIK TVTGKNALIN YGFYGCYCGW GGHGTPKDGT

                            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                65         75         85         95        105        115
Homo sapiens                DWCCWAHDHC YGRLEEKGCN IRTQSYKYRF AWGVVTCEPG PFCHVNLCAC DRKLVYCLKR
Pan troglodytes             DWCCWAHDHC YGRLEEKGCN IRTQSYKYRF AWGVVTCEPG PFCHVKLCAC DRKLVYCLKR
Callithrix jacchus          DWCCWVHDHC YGRLEEKGCN IWTQSYKYRF AWGLVTCEPG SFCRVQLCAC DRKLVYCLKR
Bos taurus                  DWCCWKHDHC YAQMETQDCD VLTQAYRYRV AWGFIICEHG SRCQQQLCAC DQKFVYCLKR
Canis familiaris            DWCCWVHDRC YGRLEEKGCH IRTQSYKYRF AQGLVTCEYG PLCQMQLCTC DRKLVYCLKR
Mus musculus                DWCCQMHDRC YGQLEEKDCA IRTQSYDYRY TNGLVICEHD SFCPMRLCAC DRKLVYCLRR
Rattus norvegicus           DWCCRMHDRC YGLLEEKHCA IRTQSYDYRF TQDLVICEHD SFCPVRLCAC DRKLVYCLRR
Oryctolagus cuniculus       DWCCWVHDKC YGRLEERACN IRTQSYKYRF ARGLVTCELG SLCQMLLCTC DRKFVYCLKR
Pteropus vampyrus           DWCCWVHDRC YEWLEEKGCY YRTQSYKYRV TRGLVTCELG PLCQVELCAC DRKLVYCLNR

                            ....|....| ....|...
                               125        135
Homo sapiens                NLRSYNPQYQ YFPNILCS
Pan troglodytes             NLRSYNPQYQ YFPNILCS
Callithrix jacchus          NLWSYNPRYQ YFPNILCF
Bos taurus                  NMRSYNPLYQ YFPNFLCT
Canis familiaris            NLRSYNPHYQ YFPNILCS
Mus musculus                NLWTYNPLYQ YYPNFLC-
Rattus norvegicus           NLWSYNRLYQ YYPNFLC-
Oryctolagus cuniculus       NLWSYNPHYR YYPNFFCT
Pteropus vampyrus           NLRSYNPGYR FFPNIFCT
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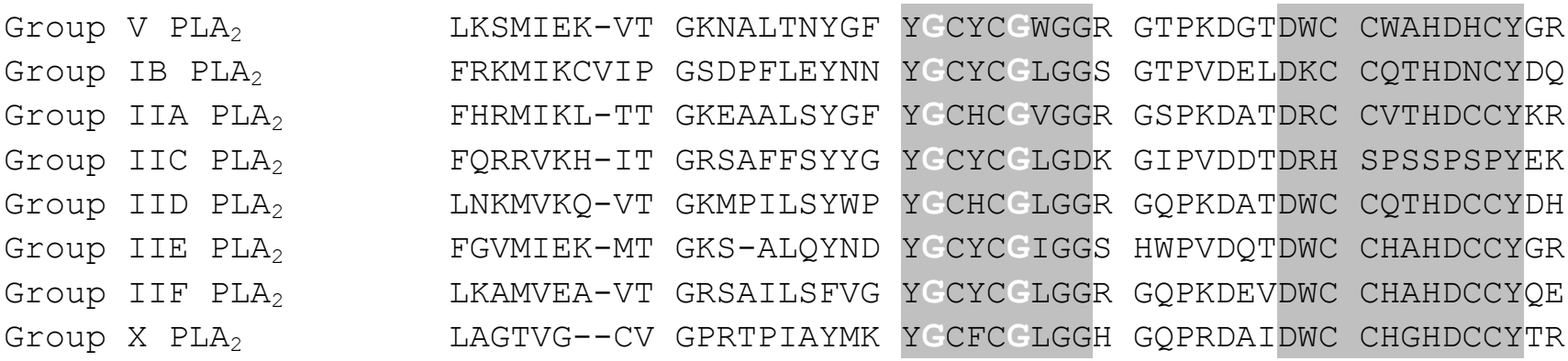
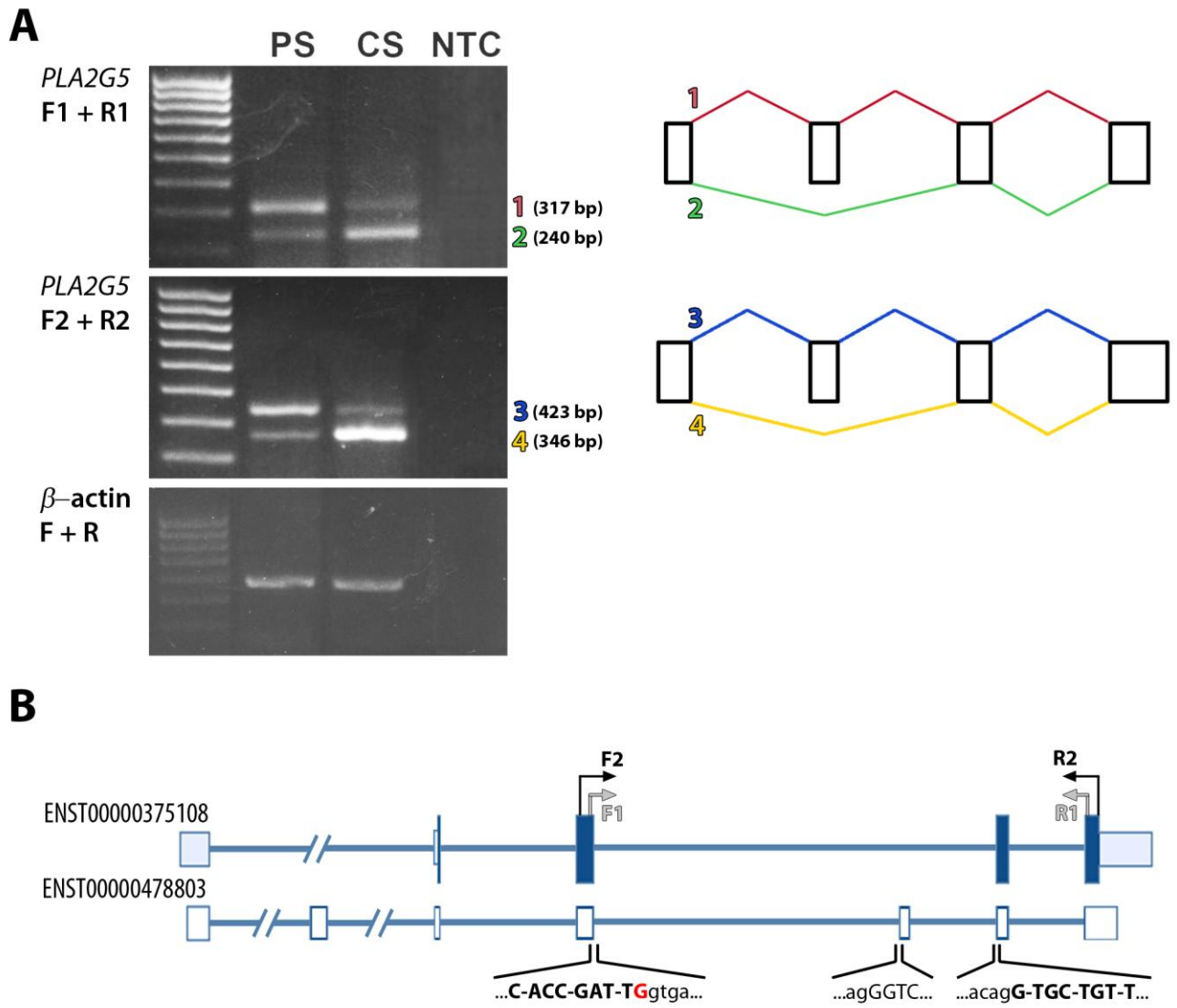
The alignment is numbered in accordance with *Homo sapiens* group V phospholipase A_2 sequence (ENST00000375108). The positions of mutated residues, Gly45 (c.133 G>T, p.Gly45Cys in families J and M) and Gly49 (c.145 G>A, p.Gly49Ser in family L), are highlighted in bold white font. The position of the Ca^{2+} binding loop and active site are highlighted in grey consecutively. The PLA2G5 protein is evolutionarily the newest secreted phospholipase A_2 family member; *PLA2G5* orthologs have only been identified in mammals.[11] The alignment was performed with ClustalW using the following Ensembl transcripts: *Homo sapiens*, ENST00000375108; *Pan troglodytes*, ENSPTRT00000000558; *Callithrix jacchus*, ENSCJAT00000054887; *Bos Taurus*, ENSBTAT00000052261; *Canis familiaris*, ENSCAFT00000023974; *Mus musculus*, ENSMUST00000102511; *Rattus norvegicus*, ENSRNOT00000022716; Oryctolagus cuniculus, ENSOCUT00000008354; *Pteropus vampyrus*, ENSPVAT00000011947.

Figure S2. Multiple Alignment of Conventional Secreted Phospholipase A_2 Proteins (Groups I/II/V/X) around Residues Mutated in Patients with Benign Fleck Retina



Mutated group V phospholipase A_2 (PLA_2) amino acid residues, Gly45 (c.133 G>T, p.Gly45Cys in families J and M) and Gly49 (c.145 G>A, p.Gly49Ser in family L), are highlighted in bold white font. The position of the Ca^{2+} binding loop and active site are highlighted in grey consecutively. The alignment was performed with ClustalW using the following Ensembl transcripts: group IB PLA_2, ENST00000308366; group IIA PLA_2, ENST00000375111; group IIC PLA_2, ENST00000429261; group IID PLA_2, ENST00000375105; group IIE PLA_2, ENST00000375116; group IIF PLA_2, ENST00000375102; group V PLA_2, ENST00000375108; group X PLA_2, ENST00000438167.

Figure S3. Reverse Transcriptase Polymerase Chain Reactions (RT-PCR) Analysis of *PLA2G5* Expression



A series of reverse transcriptase polymerase chain reactions (RT-PCR) reactions were performed to assess whether the variant identified in subject K-2, c.185G>A, affects pre-mRNA splicing of the *PLA2G5* transcript *in vivo.* Leukocyte RNA was extracted from whole blood samples collected from patient K-2 and a control sample using a QIAamp RNA blood extraction kit (Qiagen, Crawley, UK) according to the manufacturer's guidelines. cDNA was reverse-transcribed using a cDNA synthesis kit (BioLine, London, UK) with random

hexamer primer mix. RT-PCR reactions were performed using primers and condition listed in Table S4.

A. *PLA2G5* was amplified between exons 3 and 5 using primers RT_F1 and RT_R1 (top gel) and primers RT_F2 and RT_R2 (middle gel). For both reactions two alternative *PLA2G5* amplimers were detected. The lower bands for each reaction represent the expected segment of protein coding *PLA2G5* transcript (240 bp and 346 bp respectively; Ensembl transcript ENST00000375108). The upper bands represent a segment of a transcript containing an additional alternatively spliced 77 bp exon between exons 3 and 4, previously detected in a non-coding *PLA2G5* transcript (317 bp and 423 bp respectively; Ensembl transcript ENST00000478803). The relative abundance of the two amplimers between PS and CS are consistently different in both reactions; the alternatively spliced exon is detected at a higher level in the PS. A schematic representation of the alternatively spliced products detected is given on the right. The house keeping gene, *β-actin* was also amplified as a positive loading control (bottom gel).

B. Schematic representation of *PLA2G5* protein coding transcript ENST00000375108 and non-coding transcript ENST00000478803. The positions of the *PLA2G5* RT-PCR primers used are highlighted. Nucelotide sequence surounding the alternatively spliced *PLA2G5* intron-exon boundaries are shown (transcribed sequence in capital, translated sequence in bold font). The position of the mutated nucleotide identified in patient K-2 (c.185 G>A, p.Trp62X) is highlighted in red.

PS, patient sample; CS, control sample; NTC, no template control.

Table S1. Segments of Homozygosity Yielded from Homozygosity Mapping in Families J and K

Chromosome	From	To	Genetic Distance (Marshfield linkage map)	Reference
chr1	54,742,471 (rs590041)	64,361,979 (rs855824)	19 cM	Family J, regions > 1 cM
chr2	232,025,284 (rs6437002)	236,731,624 (rs952608)	14 cM	
chr1	20,238,860 (rs3738122)	23,266,939 (rs1832047)	5 cM	
chr7	pter	8,546,068 (rs2189903)	15 cM	Family K, regions > 10 cM
chr18	pter	4,925,739 (rs9961128)	12 cM	
chr1	18,477,450 (rs10796459)	24,578,011 (rs12407356)	12 cM	
chr1	110,701,174 (rs12118197)	144,989,739 (rs2590125)	11 cM	

Genotypes of subjects J-1, J-2, J-3, J-4, J-5, and J-6 were generated using the Genechip Human Mapping 50K Xba Array (Affymetrix, Santa Clara, CA, USA). Genotypes of subject K-2 were generated using the Affymetrix Genome-Wide Human SNP Array 6.0.

Table S2. Primer Sequences and Conditions used for *PLA2G5* Mutation Screening

Primer	Sequence (5'-3')	Optimised annealing temperature (ºC)[a]	Amplicon size (bp)
exon 2F	TGACGGGGAGTGGAATAGATGGG	68	203
exon 2R	TCTCTACGACCTCAATGGCTGGTGT		
exon 3F	TTGCACCTCCCTTCCCACTAAT	68	343
exon 3R	TACTCATCTTCCAGAACTGATATGG		
exon 4F	ACACTACCAGATCCTCCCTGCCA	68	319
exon 4R	TTCTGCACCCAACTCCTCCTC		
exon 5F	AGTCCATGGGGTCTCTGCTG	68	681
exon 5R	AAAGAGGCACCAGCGATCCC		

[a]Polymerase chain reactions (PCRs) were performed in a 12.5 µl volume containing 1 Unit Taq polymerase (BIOTAQ DNA Polymerase, Bioline, London, UK). The thermal cycling profile for all reactions was: initial denaturation (94°C for 2 minutes), amplification (35 cycles of: 94°C denaturation for 30 seconds, ** °C annealing for 30 seconds, 72°C extension for 30 seconds) and final extension (72 °C for 7 minutes).

Table S3. Summary of Coding *PLA2G5* Sequence Variants Identified Here and Elsewhere

Coding DNA variants		SIFT		Polyphen 2		Blosum 62 score (-4 to 11)	Reference *(observed allele count)*
Nucleotide	**Protein**	**Prediction**	**Tolerance index (0 to 1)**	**Prediction**	**HumVar Score (0 to 1)**		
c.9 C>T	p.(=)	not applicable				6	1000G [rs2020887], EVS *(1244/3632)*, this study
c.15 C>G	p.(=)	not applicable				4	EVS *(1/4877)*
c.48 T>C	p.(=)	not applicable				7	EVS *(1/4877)*
c.88 G>A	p.Glu30Lys	tolerated	0.78	Benign	0.03	1	EVS *(1/4877)*
c.102 G>T	p.(=)	not applicable				6	Bushman_pop [rs111762734]
c.108 C>T	p.(=)	not applicable				6	internal db *(1/448)*, EVS *(3/4875)*
c.110 C>G	p.Ala37Gly	tolerated	0.35	PRD	0.97	0	EVS *(1/4877)*
c.133 G>T	**p.Gly45Cys**	intolerant	0.00	PRD	1.00	-3	this study [families J and M]
c.144 C>T	p.(=)	not applicable				9	1000G [rs11573265], EVS *(77/4801)*, this study
c.145 G>A	**p.Gly49Ser**	intolerant	0.02	PRD	1.00	0	this study [family L]
c.157 C>T	**p.Arg53X**	not applicable				-4	this study [family L]
c.181 G>A	p.Asp61Asn	intolerant	0.01	PRD	1.00	1	EVS *(1/4877)*
c.185 G>A	**p.Trp62X**	not applicable				-4	this study [family K]
c.292 G>A	p.Glu98Lys	intolerant	0.04	Benign	0.47	1	EVS *(1/4855)*
c.297 C>T	p.(=)	not applicable				6	EVS *(2/4876)*
c.311 A>C	p.His104Pro	tolerated	0.23	Benign	0.00	-2	EVS *(1/4877)*
c.312 T>C	p.(=)	not applicable				8	internal db *(1/448)*, EVS *(7/4871)*
c.368 G>A	p.Arg123Gln	tolerated	0.53	Benign	0.10	1	EVS *(2/4876)*
c.383delA	**p.Gln128ArgfsX88**	not applicable				-4	this study [family M]
c.402 C>T	p.(=)	not applicable				6	Bushman_pop [rs112000348]
c.406 C>T	p.Leu136Phe	tolerated	0.71	Benign	0.00	0	EVS *(1/4877)*

SIFT (v4.0.4) results are reported to be tolerant if tolerance index ≥ 0.05 or intolerant if tolerance index < 0.05. Polyphen-2 (v2.1.0r367) appraises mutations qualitatively as Benign, Possibly Damaging or Probably Damaging (PRD) based on the model's false positive rate. Blosum62 substitution matrix score positive numbers indicate a substitution more likely to be tolerated evolutionarily and negative numbers suggest the opposite. The cDNA is numbered according to Ensembl transcript ID ENST00000375108, in which +1 is the A of the translation start codon. Novel changes are in bold font.

EVS denotes variants in the Exome Variant Server, NHLBI Exome Sequencing Project, Seattle, WA, USA [accessed 13/09/2011]. 1000G represents variants identified in the 20101123 sequence and alignment release of the 1000 genomes project (1094 genomes). Bushman_pop denotes variants identified only among the four Bushmen genomes sequenced by Schuster *et al*.[46] Our internal db contains data from 224 exomes (cases with neurodegenerative disease).

Table S4. Primer Sequences and Conditions Used for Reverse Transcriptase Polymerase Chain Reactions (RT-PCRs)

Primer	**Sequence (5'-3')**	**Optimised annealing temperature (ºC)[a]**	**Amplicon size (bp)**
PLA2G5_RT_F1	CCCTGACAAACTACGGCTTC	62	240[b]/317[c]
PLA2G5_RT_R1	AGTAGACGAGCTTCCGGTCA		
PLA2G5_RT_F2	GAGGCTTGCTGGACCTAAAA	62	346[b]/423[c]
PLA2G5_RT_R2	GAGGCCTAGGAGCAGAGGAT		
β-actin_RT_F	CTGGGACGACATGGAGAAAA	60	564
β-actin_RT_R	AAGGAAGGCTGGAAGAGTGC		

[a]Reverse transcriptase polymerase chain reactions (RT-PCRs) were performed in a 25 µl volume containing 2 Units Taq polymerase (BIOTAQ DNA Polymerase, Bioline, London, UK). The thermal cycling profile for all reactions was: initial denaturation (94°C for 2 minutes), amplification (up to 40 cycles of: 94°C denaturation for 30 seconds, ** °C annealing for 30 seconds, 72°C extension for 45 seconds) and final extension (72 °C for 7 minutes).
[b]size of PCR amplimer when amplifying Ensembl transcript ENST00000375108.
[c]size of PCR amplimer when amplifying Ensembl transcript ENST00000478803 containing an alternatively spliced 77 bp exon.