The American Journal of Human Genetics, Volume 93

Supplemental Data

Mutations in IMPG1 Cause Vitelliform Macular Dystrophies

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А		Leu154	Leu238	Ile625
/ \	SPACR Human	FSNSQ <mark>EHLDLLQQRIKQRSF</mark> P	AVLE <mark>EORVELSVSL</mark> VNOKEK	SNLT <mark>GFKQLEILNFRNG</mark> SVIV
	SPACR Horse	F <mark>SNSQ<mark>EHLDLLQQRIKQR</mark>NF</mark> P	RGPL <mark>E</mark> QK <mark>VE</mark> LSISL <mark>ANRRE</mark> K	SNLT <mark>GFKQL<mark>E</mark>ILNFRNG<mark>SVIV</mark></mark>
	SPACR Dog	<mark>e</mark> snsq <mark>ehldllqqrlkqr</mark> hep	EGPLEQK <mark>VE</mark> LSITLTNQREK	SNLT <mark>CEKQLETLNERNG</mark> SVTV
	SPACR Marmoset	<mark>F</mark> SNSQ <mark>EHLDLLQQRIKE</mark> RS <mark>E</mark> P	SVSE <mark>EQKVEL</mark> SISL <mark>V</mark> NQR <mark>E</mark> K	SNLT <mark>CEKQLEILNERNG</mark> SVI <mark>V</mark>
	SPACR Cow	FSNSQ <mark>EHLDLLQQR<mark>M</mark>KQRNEL</mark>	EDALEQKVELSISLANQKEK	SNLT <mark>GEKQLEILNERNG</mark> STIV
	SPACR Elephant	ESSSQEHLELLQQRIKQRNLP	EDPLEOKVELSVTLANOR <mark>E</mark> K	SNLT <mark>GE</mark> KQL <mark>ETLNERNG</mark> STTV
	SPACR Mouse	FSNSQ <mark>ehldllqqrikqrsf</mark> p	EFSS <mark>EEKVE</mark> FSTSLPRH <mark>REK</mark>	SNLT <mark>CE</mark> KQL <mark>E</mark> ILS <mark>E</mark> RNGSVIV
	SPACR Rat	<mark>F</mark> SNSQ <mark>EHLDLLQQRT</mark> GQRS <mark>F</mark> S	EISSEEKVE SISLPHREK	SNLT <mark>CE</mark> KQL <mark>E</mark> ILS <mark>E</mark> RNGSVIV
	SPACR Chicken	<mark>F</mark> SNSQ <mark>EHLEIIQRRUKH</mark> RT <mark>F</mark> Q	ELPAEQM <mark>VE</mark> SVTLTDQE <mark>V</mark> T	SNLT <mark>CE</mark> KQL <mark>E</mark> ILNERNGSVIV
	SPACR Platypus	<mark>F</mark> SNSQ <mark>EHLELIQQRVKQRNF</mark> P	EVIEDUV <mark>VE SV</mark> ILSNEE <mark>F</mark> T	SNLT <mark>CE</mark> KQL <mark>E</mark> ILNERNGSVIV
	SPACR Xenopus	<mark>E</mark> SSSQ <mark>EHS</mark> EIIQQRVKYKTET	E0PV00IVE TVTL TNOEFT	SNLT <mark>CEKE</mark> IEILNE <mark>K</mark> KGSVIV
	SPACR Zebrafish	ESSTQEHLDIVAS <mark>RV</mark> NQLDEQ	ŨRPVEHIVRESVIV <mark>7</mark> 0PV <mark>v</mark> s	SNLT <mark>GEKELEILNE</mark> KN <mark>C</mark> SVVV
В	SPACR SEA1	230VLEEQRVE <mark>L</mark> SVSLVNQK	KAELADSQSPYYQELAGKSQLQM-QK	IFKKLPGFKKIHVLGFR 389
	SPACRCAN SEA1	234 ATKPAGEQIAEFSIHLLGKQ		The second se
		2853GPLLVPFTLNFTITNLQ		
	SPACR SEA2	567 -APKGRELVVFFS RVANMA	a second and the second and the second	
	SPACRCAN SEA2 MUC1 SEA	892 YTQTSGALVVFFSLRVTNMM 1034 PQLSTGVSFFFLSFHISNLQ	the state of the s	
	HUCI SEA			
	SPACR SEA1	390 PKKEKDGSSSTEMC		
	SPACRCAN SEA1	397 SPKENDSGVDVYY		
		2916 SEKDGAATGVDAICTH		
	SPACR SEA2 SPACRCAN SEA2	630 NGSVIVNSKMKFAKSVPYNLTKA 956 NGSIVVNSRMKFANSVPPNVNNA		
		1096 PGSVVVOLTLAFREGTINVH		
	HOUL SEA	1050 P <mark>ODVV</mark> QBIDAFREGTIWM	VEIQUATINA ASKINGTISS VOV	WITTING

Figure S1: Amino-acid alignments

(A) Multiple amino-acid sequence alignment of SPACR for a region surrounding the p.Leu154Pro, p.Leu238Arg and p.Ile625Met missense changes. The site of the mutation is indicated by an arrowhead. (B) Multiple amino-acid sequence alignment of SPACR and SPACRCAN SEA1 and SEA2 domains with the non-proteolytic SEA6 domain of Mucin 16 (Muc16) and the proteolytic SEA domain of Mucin 1. Conserved amino-acids are in grey. Two missense *IMPG1* mutations found in this study are in blue and the missense *IMPG1*

mutation found in BCAMD condition is in red. The consensus sequence for autoproteolysis is in yellow.

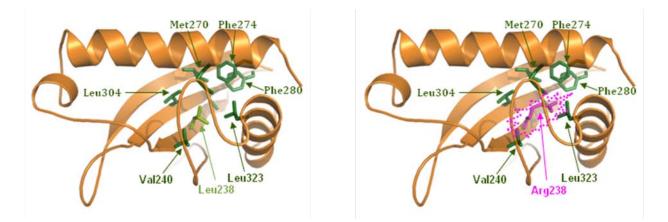
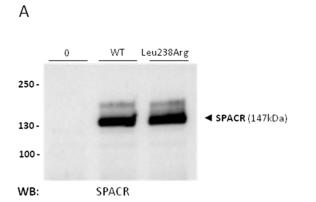
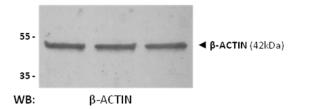
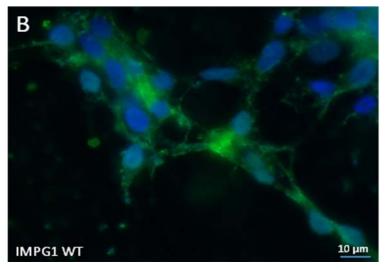


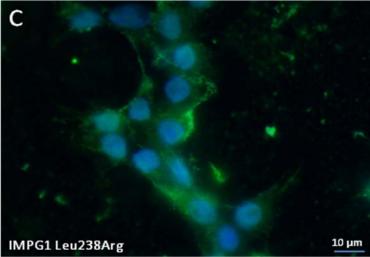
Figure S2: SPACR SEA1 domain structure

Three-dimensional model of the N-terminal SEA1 domain of SPACR using Pymol showing α -helices (helix shape) and β -sheets (ribbon shape). Interactions of wild-type Leu238 (pale green) and mutated Arg238 (flashy pink) with the hydrophobic residues (Val240, Met270, Phe274, Phe280, Leu304, and Leu323) surrounding the position 238 are shown. The highly unfavourable contacts between the polar and charged side-chain of the arginine residue are depicted by the dotted pink line.









<u>Figure S3</u>: Characterization of wild-type and p.Leu238Arg mutant SPACR proteins by Western blot analysis and immunofluorescence.

Cos7 cells were transiently transfected with pRK5 fused in-frame with wild-type (WT) or p.Leu238Arg mutant IMPG1. (**A**) Protein expression levels of wild-type (WT) and p.Leu238Arg mutant were examined by Western blot. Lane 0, untransfected lysate. SPACR was detected with a rabbit anti-SPACR and an anti-rabbit-HRP antibodies. Beta-Actin was detected with mouse β -Actin and anti-mouse-AP antibodies. Both WT and mutant proteins migrate at the predicted molecular weight of 147 kDa. (**B**, **C**) SPACR is stained in green (rabbit anti-SPACR and donkey anti-rabbit Alexa A488), and nuclei are stained in blue (DAPI). Both wild-type (**B**) and mutant (**C**) proteins localize to the cytoplasm with the same pattern.

<u>Table S1</u> : Heterozygous variants common to affected patients on the mapped locus on
chromosome 6p12.1-q24.3

Position	Gene	Туре	Exon	Variation	EVS
57 398 187	PRIM2	indel	10	-/A	0/13006
71 212 388	FAM135A	missense	10	p.Ala308Val	0/13006
76 728 529	IMPG1	missense	7	p.Leu238Arg	0/13006
84 798 955	MRAP2	missense	4	p.Arg125Cys	6/13006
88 265 144	RARS2	missense	5	p.Lys126Arg	0/13006
128 134 516	THEMIS	missense	4	p.Ala389Thr	117/13006
148 789 688	SASH1	missense	5	p.Leu132Val	12/13006

EVS: Exome Variant Server