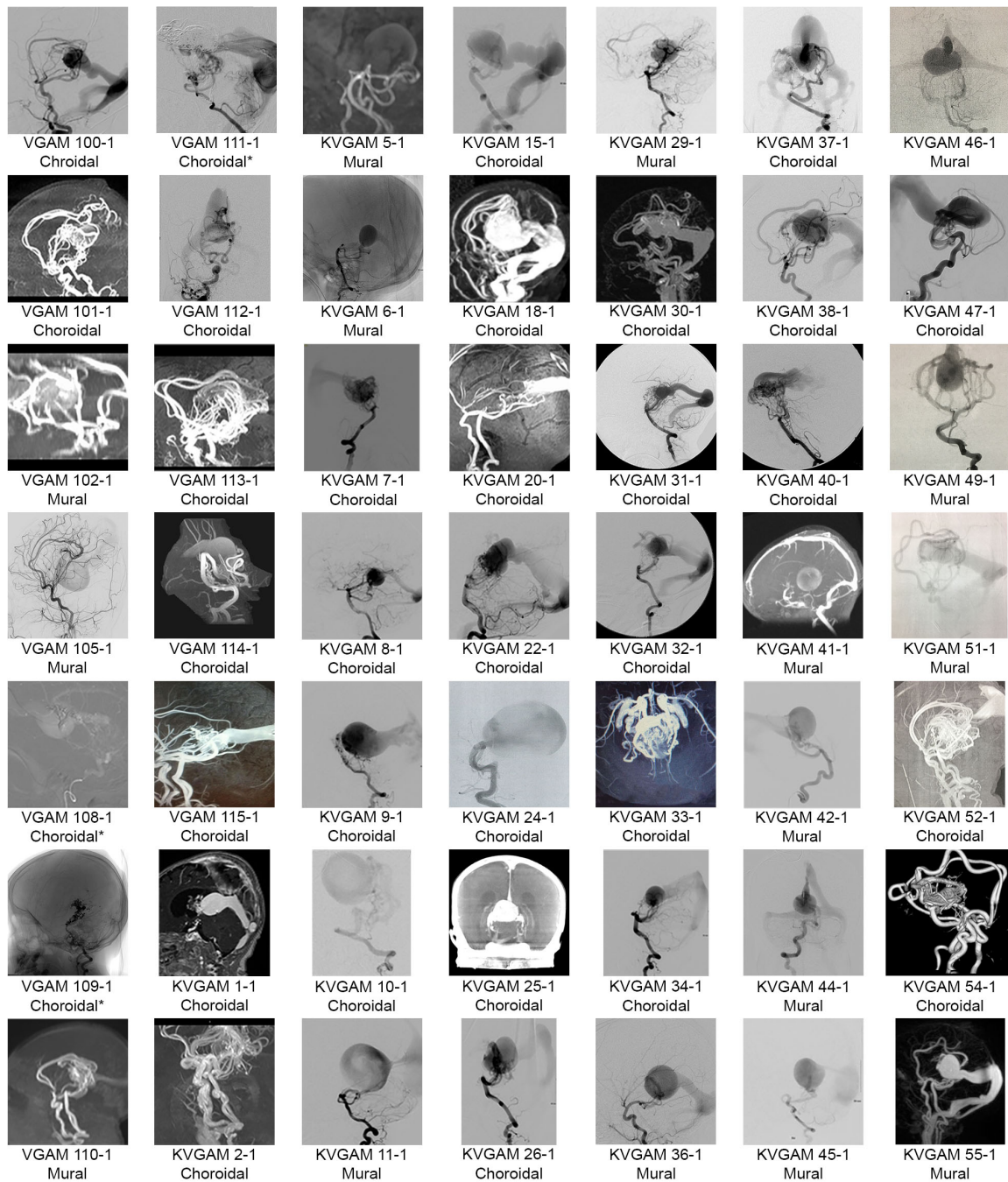


SUPPLEMENTARY FIGURES

Supplementary figure 1. VOGM type and representative imaging for probands, Related to Table 2

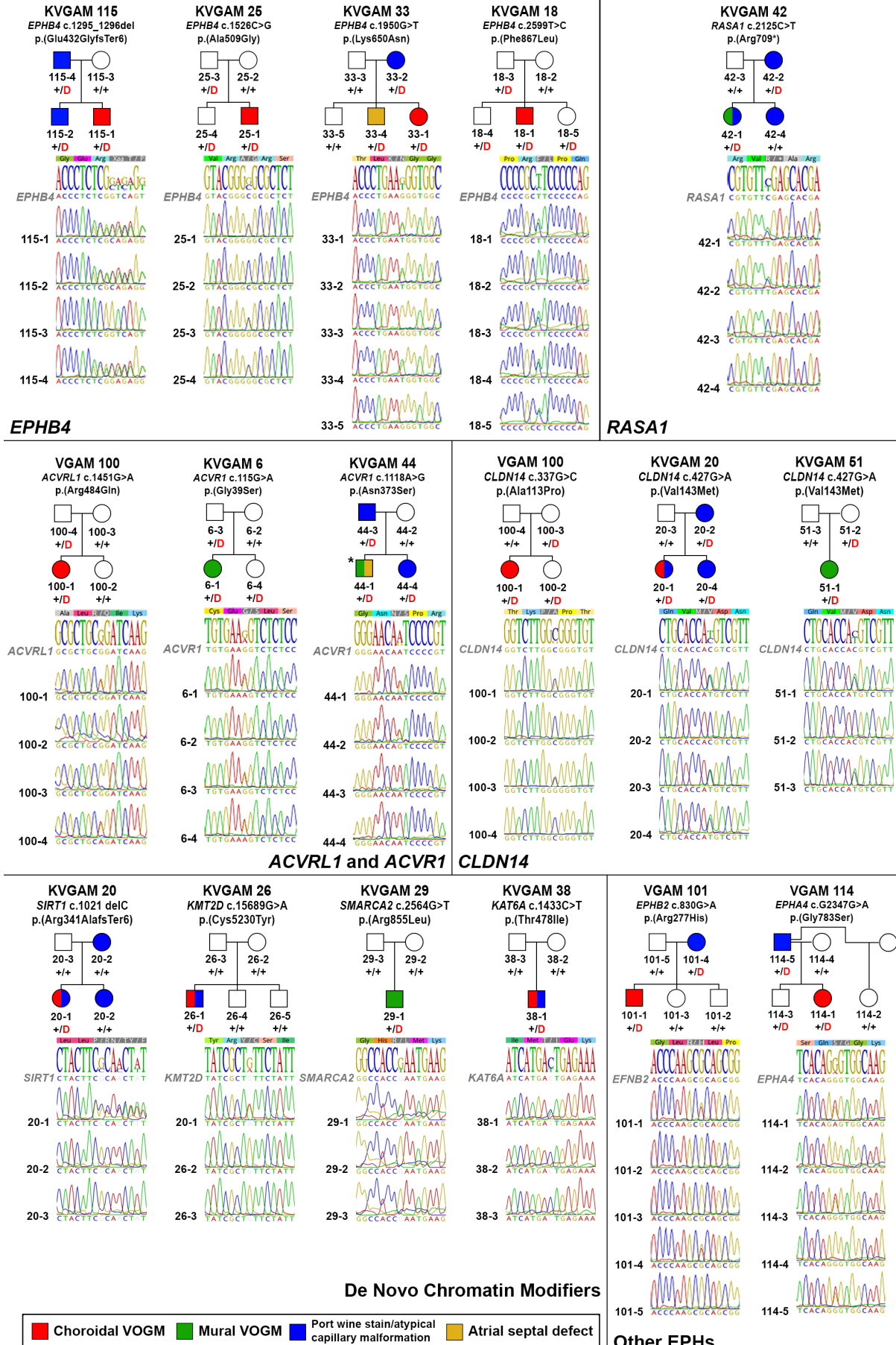


* = Post Embolization

Per Detailed Angiogram Report: VGAM 104-1: Choroidal; VGAM 107-1: Choroidal; KVGAM 3-1: Mural; KVGAM 4-1: Choroidal; KVGAM 35-1: Choroidal; KVGAM 43-1: Mural.

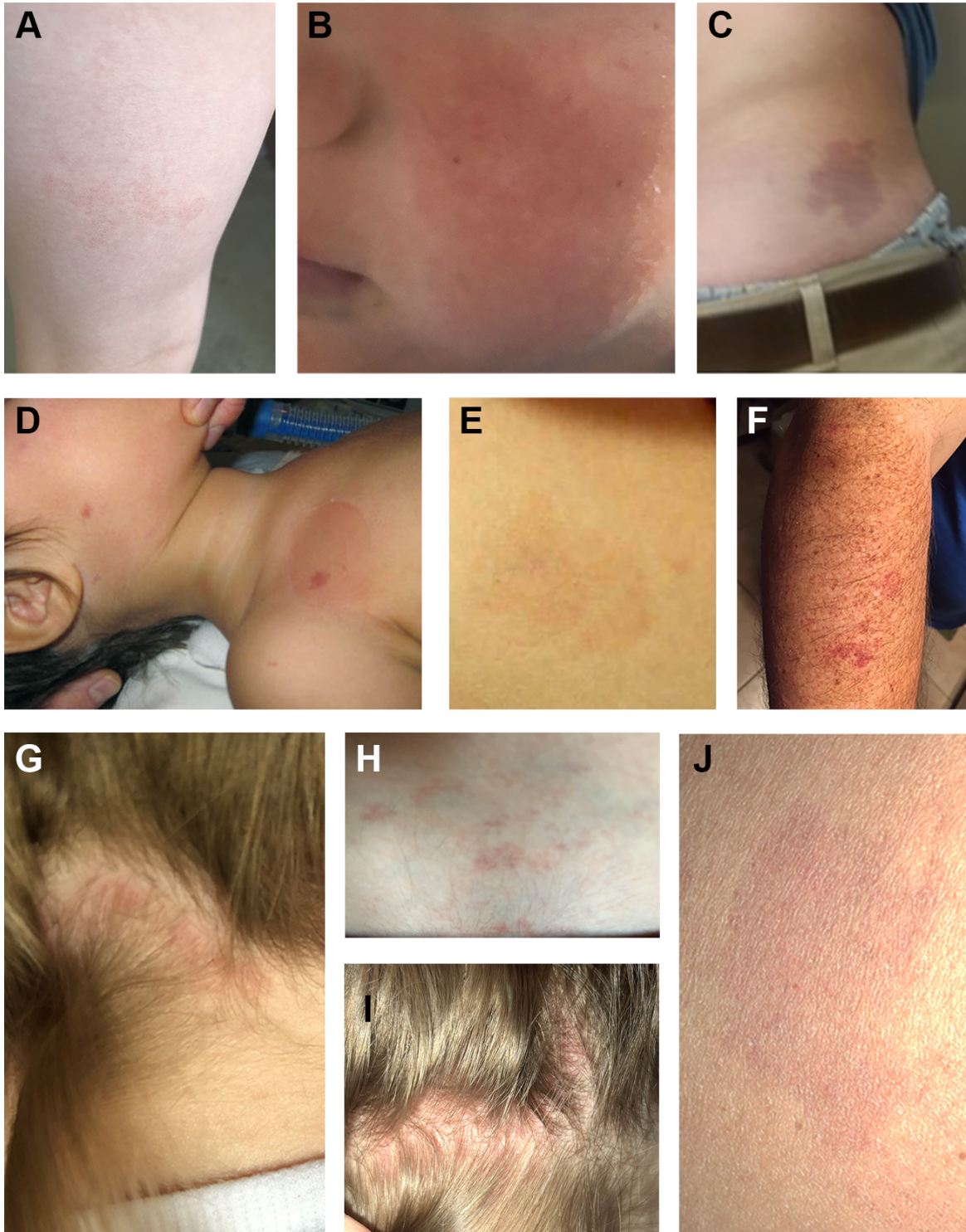
Representative images of 3-Tesla time-of-flight magnetic resonance angiography or digital subtraction angiography for all patients with available imaging, with patient codes and VOGM subtype. In cases for which imaging was not available, VOGM subtype was ascertained from detailed transcripts of angiographies during or before endovascular treatment of VOGM.

Supplementary figure 2. Chromatogram alignments and segregation of select candidate variants, Related to Table 2 and Figure 2, 4, and 5



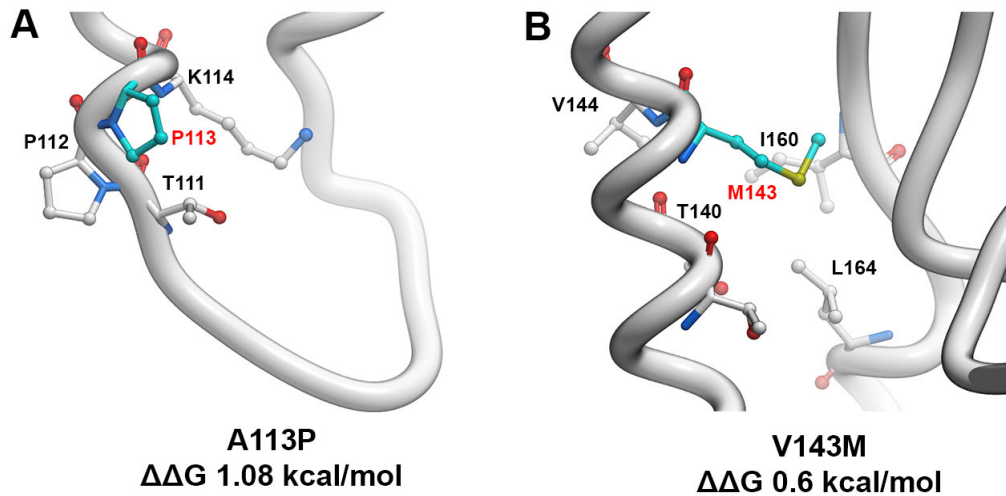
Mutations identified by exome sequencing were confirmed by direct PCR amplification with custom primers followed by Sanger sequencing.

Supplementary figure 3. Cutaneous manifestations in VOGM probands and family members, Related to Figure 4 and 5



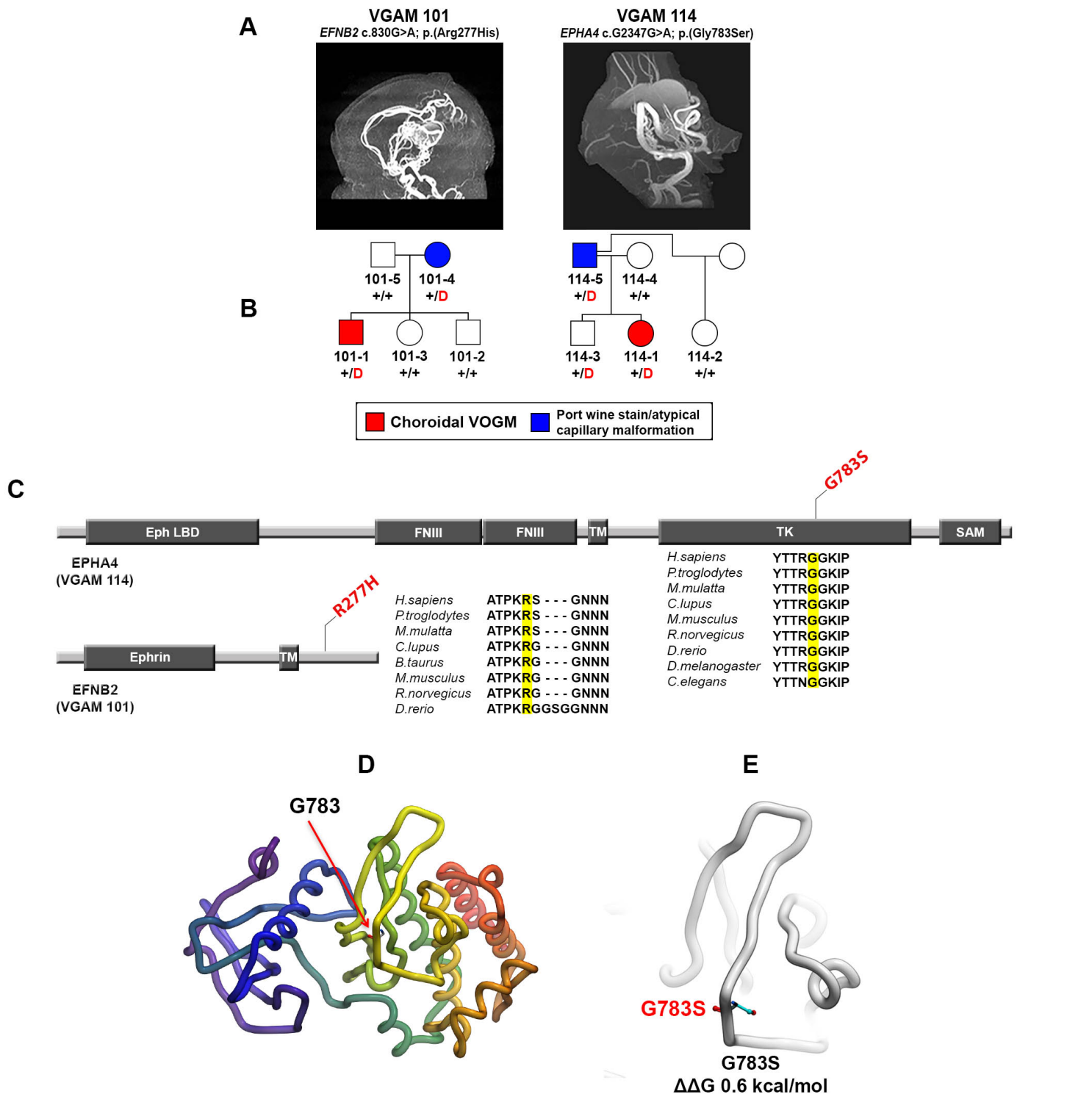
Atypical capillary malformation on the posterolateral aspect of the distal third of the thigh (A) and left cheek (B) of VGAM115-2. Panel C demonstrates an atypical capillary malformation on left flank of VGAM115-4 – both individuals depicted in panels A-C carry *EPHB4* c.1295_1296del; p.Glu432fs. Atypical capillary malformations on the angle of the jaw and superior right thorax of KVGAM42-1 (D) and KVGAM42-2 (E). Both individuals depicted in panels D-E carry *RASAI* c.2125C>T; p.Arg708*. (F). Atypical capillary malformation on the posterolateral aspect of the forearm in VGAM114-5, carrier of *EPHA4* c.2347G>A; p.Gly783Ser. (G). Nuccal atypical capillary malformation in a proband harboring *CLDN14* p.(Val143Met) (H). Lumbar atypical capillary malformation in the same proband depicted in panel G. (I). Nuccal atypical capillary malformation in the sister of the proband depicted in panels G and H, also harboring *CLDN14* p.Val143Met. (J). Atypical capillary malformation in the thigh of the mother of individuals depicted in panels G-I, carrying and transmitting *CLDN14* p.Val143Met.

Supplementary figure 4. *In silico* modeling of *CLDN14* mutations, Related to Figure 5



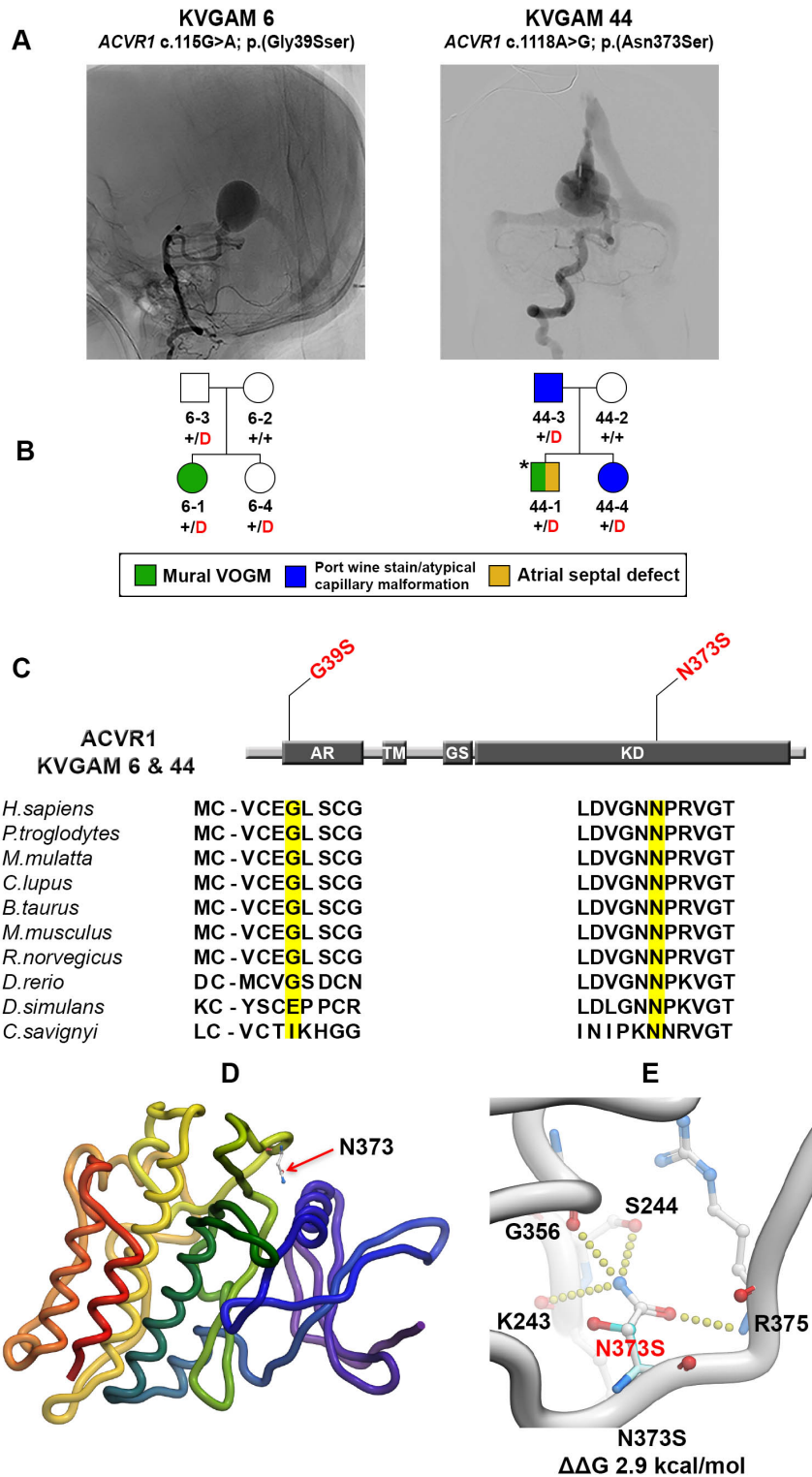
- (A) *In silico* modeling of p.Ala113Pro. Ala113 is the first residue of Helix-3 and lies between P112 and K114 at the interface of Claudin-14 and the cytoplasmic side of the lipid bilayer. Just preceding helix-3 is the loop that connects helix-2 and -3, on which posttranslational modification sites are present, including for palmitoylation. The presence of Proline in an alpha helix can result in helix distortion since it lacks the backbone NH to establish the hydrogen bonding necessary to complete the H-bond chain. Additionally the steric or rotamer effects prevent Proline from adopting helical geometry. The two adjacent Proline residues 112 and 113 likely influence the conformation of the helix 2-3 connector loop and destabilize the initial portion of helix-3. Calculated $\Delta\Delta G$ of A113P is 1.08 Kcal/mol.
- (B) *In silico* modeling of p.Val143Met located towards the end of helix-3 at the outer leaflet of the lipid bilayer. The Valine side chain is tightly packed against β sheets, being surrounded by the hydrophobic side chains of L164, I160 and V144. The V143M mutation ($\Delta\Delta G = 0.6$ Kcal/mol) results in steric clash of the larger Methionine side chain with the beta sheets.

Supplementary figure 5. Mutations affecting other members of the Ephrin family in VOGM patients displaying CM-AVM-like cutaneous manifestations, Related to Figure 3



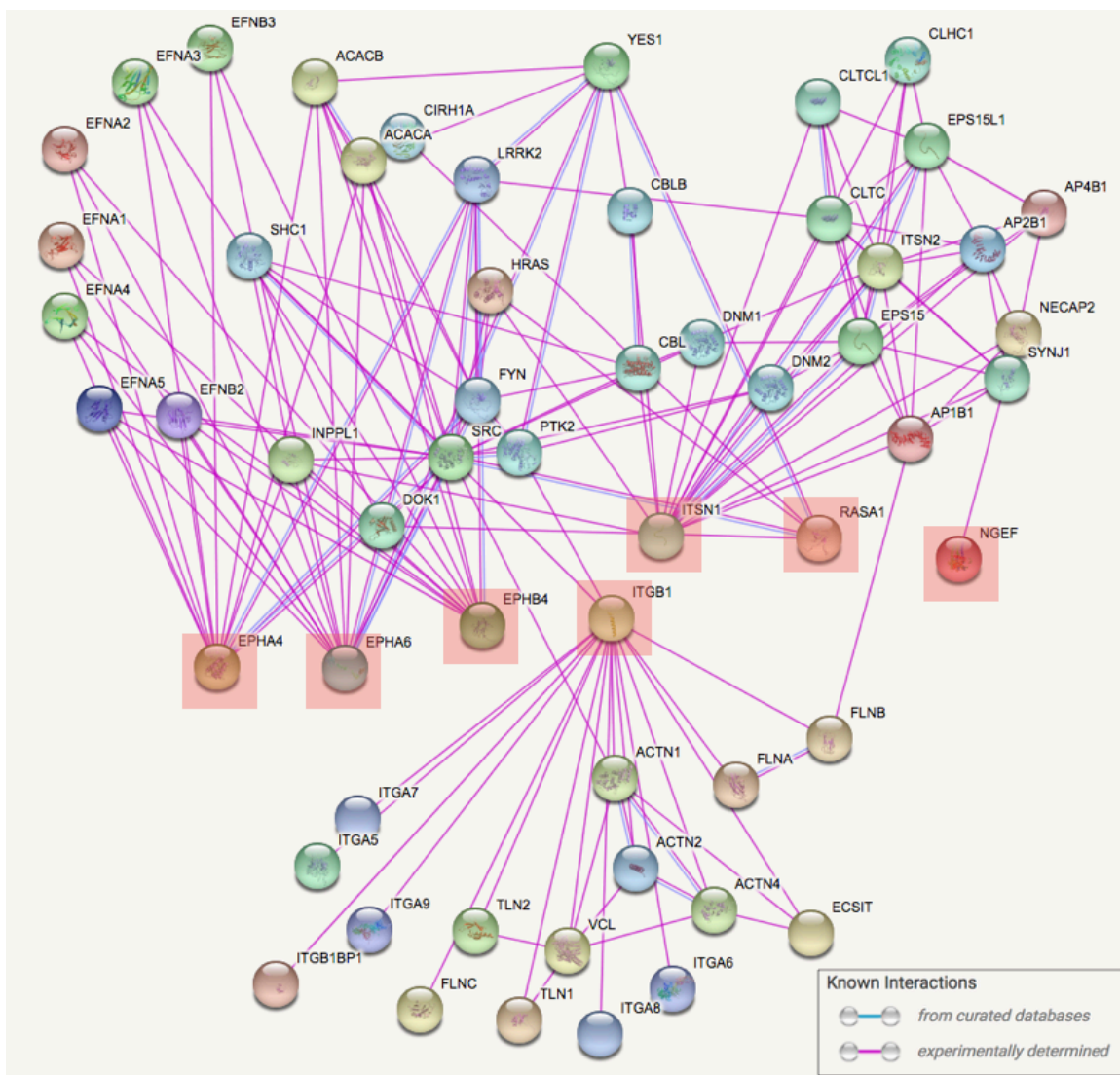
- (A) Representative magnetic resonance angiography and digital subtraction angiography images demonstrating choroidal Vein of Galen malformations in probands.
- (B) Pedigrees depicting kindred structure and phenotypic variance. Note the presence of atypical capillary malformations represented by blue symbols in both parents transmitting *EFNB2* and *EPHA4* mutations in families VGAM101 and VGAM114. Red 'D' denotes damaging mutation, '+' denotes wild type sequence.
- (C) Linear representation of affected Ephrin polypeptides. Functional domains are represented by dark rectangles. Amino acid modifications are mapped on the protein structure in red lettering. Conservation of the wild-type amino acid substituted by the four deleterious missense mutations is depicted on the right of each molecule schematic. LB = ligand binding domain; CRD = Cysteine-rich domain; FNIII = Fibronectin III domain; TM = Transmembrane domain; TK = Tyrosine kinase domain; SAM = Sterile alpha motif.
- (D) *In silico* modelling of the repercussion of Eph-A4 p.Gly783Ser. Gly783 lies at the base of a flexible loop in Eph-A4.
- (E) The Gly-to-Ser substitution at this position ($\Delta\Delta G$ 0.6Kcal/mol) is predicted to affect loop stability.

Supplementary figure 6. Damaging ACVR1 mutations in mural Vein of Galen malformation, Related to Figure 3



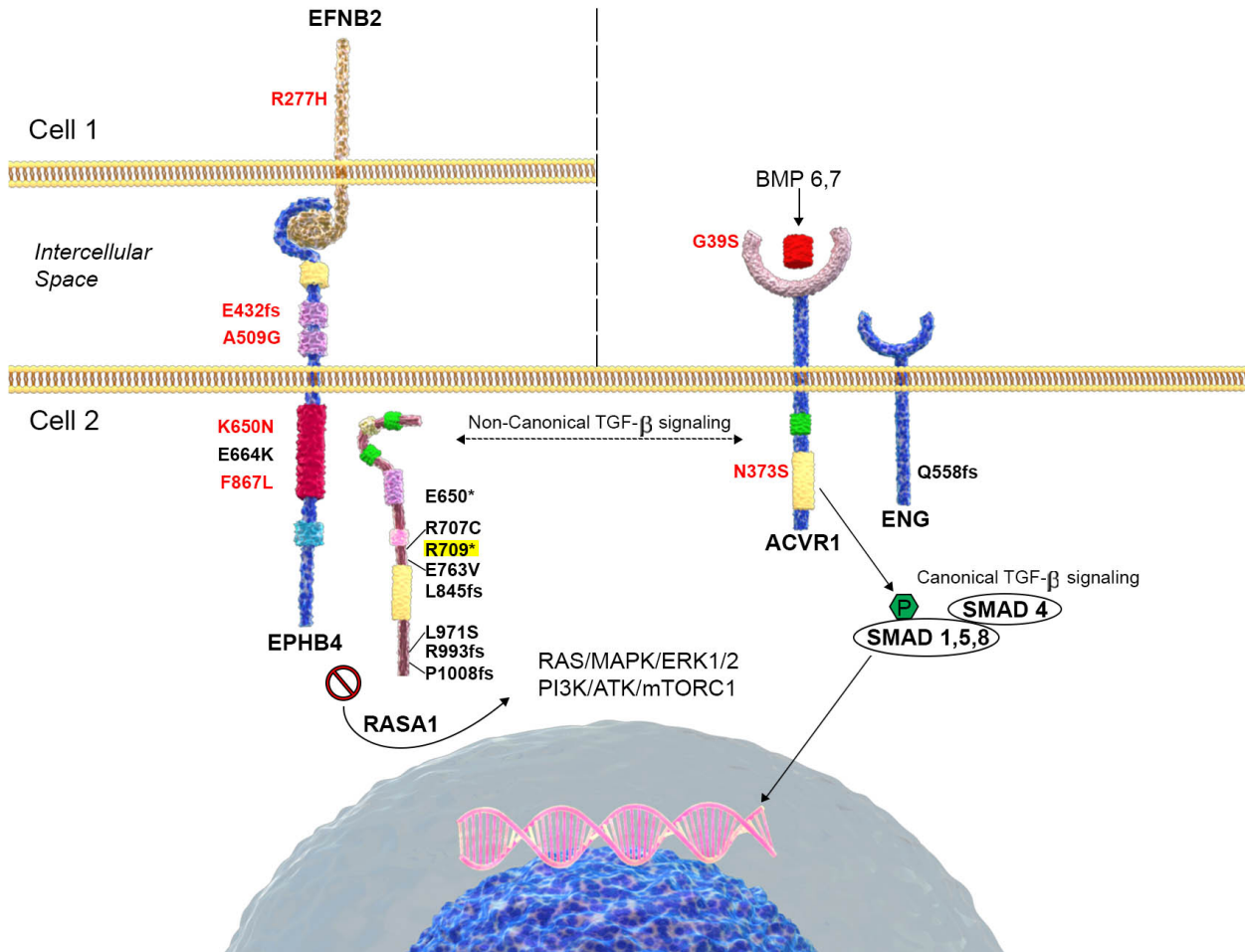
- (A) Representative images from digital subtraction angiographies demonstrating mural Vein of Galen Malformations in probands.
- (B) Pedigrees depicting kindred structure and phenotypic variance. Blue symbols represent atypical capillary malformations in individuals harboring the c.1118A>G; p.Asn373Ser mutation. Yellow coloring of half the symbol denotes concomitant presence of an atrial septal defect and (asterisk) partial anomalous pulmonary venous return in the proband of family KVGAM44. Red 'D' denotes damaging mutation, '+' denotes wild type sequence.
- (C) Linear representation of the ACVR1 polypeptide, with functional domains represented as dark rectangles. Amino acid modifications are mapped (in red) on the protein structure. Conservation of the wild-type amino acid substituted by the two deleterious missense mutations is depicted below each. AR = activin receptor; TM = Transmembrane domain; KD = Kinase domain.
- (D) *In silico* modelling of p.Asn373Ser. Asn373 in a 3D model of ACVR1.
- (E) Predicted energetically costly ($\Delta\Delta G$ 2.9Kcal/mol) loss of hydrogen bond formation between the Asn373 side chain and backbone atoms of Gly356, Lys243, Arg375 and the side chain of Ser244 caused by Asn373 substitution to serine.

Supplementary figure 7. Interactome of mutated Ephrin receptor signaling genes in VOGM, Related to Table 2 and Figure 3



All 7 genes (highlighted) contributing to the significant result of the Ephrin receptor signaling pathway in IPA pathway analysis were input into String. These 7 genes are mapped into a single experimentally supported STRING interactome.

Supplementary figure 8. The genomic landscape of VOGM, Related to Figure 3



Schematic representation of signaling between two primitive endothelial cells showing contact-mediated interaction between the membrane-bound ligand Ephrin-B2 in the upper cell and its receptor, Eph-B4, in the lower cell. The figure depicts crosstalk between Eph-B4, its effector and binding partner RASA1, and activin type 1 receptors mediated by non-canonical TGF-β signaling. Mutations discovered in our cohort affecting *EPHB4*, *EFNB2*, and *ACVR1* are shown in red, and previously reported mutations in *RASA1*, *EPHB4*, and *ENG* in VOGM patients are indicated in black. *RASA1* p.Arg709* (highlighted in yellow) is the sole *RASA1* mutation detected in our cohort.

SUPPLEMENTARY TABLES

Supplementary Table 1. VOGM patient clinical and demographic characteristics, Related to STAR Methods			
Variable	Type of VOGM		Grand Total (%)
	<i>Choroidal (%)</i>	<i>Mural (%)</i>	
	37 (67.27)	18 (32.73)	55 (100.00)
Gender			
Female	11 (29.73)	8 (44.44)	19 (34.55)
Male	26 (70.27)	10 (55.56)	36 (65.45)
Total	37 (100.00)	18 (100.00)	55 (100.00)
Age at diagnosis			
Prenatal	15 (40.54)	7 (38.89)	22 (40.00)
Neonate (0-30 days)	12 (32.43)	0 (0.00)	12 (21.82)
Infant (1 month-2 years)	9 (24.32)	10 (55.56)	19 (34.55)
Postnatal	0 (0.00)	0 (0.00)	0 (0.00)
Young child (3-6 years)	0 (0.00)	0 (0.00)	0 (0.00)
Child (7-12 years)	1 (2.70)	0 (0.00)	1 (1.82)
Adolescent (13-17 years)	0 (0.00)	0 (0.00)	0 (0.00)
Unknown	0 (0.00)	1 (5.56)	1 (1.82)
Total	37 (100.00)	18 (100.00)	55 (100.00)
Term vs. preterm delivery			
Term (\geq 37 wks)	32 (86.49)	13 (72.22)	45 (81.82)
Preterm	4 (10.81)	4 (22.22)	8 (14.55)
Unknown	1 (2.70)	1 (5.56)	2 (3.64)
Total	37 (100.00)	18 (100.00)	55 (100.00)
Family history of cutaneous vascular abnormalities*			
Yes	23 (62.16)	8 (44.44)	31 (56.36)
No	7 (18.92)	7 (38.89)	14 (25.45)
Unknown	7 (18.92)	3 (16.67)	10 (18.18)
Total	37 (100.00)	18 (100.00)	55 (100.00)
Self-reported ethnicity			
European	29 (78.38)	13 (72.22)	42 (76.36)
European/Asian	3 (8.11)	1 (5.56)	4 (7.27)
Mixed	1 (2.70)	1 (5.56)	2 (3.64)
European/Hispanic	1 (2.70)	0 (0.00)	1 (1.82)
Latin American/African American	1 (2.70)	0 (0.00)	1 (1.82)
Asian	0 (0.00)	2 (11.11)	2 (3.64)
African	0 (0.00)	1 (5.56)	1 (1.82)
Ashkenazi Jewish	2 (5.41)	0 (0.00)	2 (3.64)
Unknown	2 (5.41)	0 (0.00)	2 (3.64)
Total	37 (100.00)	18 (100.00)	55 (100.00)
Associated conditions			Total
High output cardiac failure	23 (62.16)	1 (5.56)	24 (43.64)
Progressive macrocephaly	22 (59.46)	13 (72.22)	35 (63.64)
Hydrocephalus	24 (64.86)	9 (50.00)	33 (60.00)
Prominent facial or scalp vasculature	18 (48.65)	9 (50.00)	27 (49.09)
Structural cardiac abnormalities†	4 (10.81)	1 (5.56)	5 (9.09)

*History of vascular birthmarks was self-reported in most cases, including first and second-degree relatives.

†Structural cardiac abnormalities include atrial septal defects, congenital valvular insufficiency, partial anomalous pulmonary venous return, patent foramen ovale, patent ductus arteriosus, and congenital pulmonary artery stenosis.

Category	Cases*	Cases*	Controls
	(xGen; N=88)	(MedExome; N=71)	(Roche V2; N=5,367)
Read length (bp)	99	99	50-94
# of reads per sample (M)	48.9	53.7	99.7
Median coverage at each targeted base (X)	54.4	37.2	67
Mean coverage at each targeted base (X)	59.4	43.8	79.1
% of all reads that map to target	60.70%	48.30%	57.60%
% of all bases that map to target	46.10%	39.10%	49.00%
% of targeted bases read at least 8x	98.40%	96.30%	94.60%
% of targeted bases read at least 10x	98.00%	95.00%	93.40%
% of targeted bases read at least 15x	96.30%	89.80%	89.90%
% Mean error rate	0.30%	0.30%	0.40%

*88 VOGM samples were sequenced using the xGen Exome Research Panel v1.0 capture reagent (IDT). The remaining cases were sequenced using the MedExome capture reagent. 8X, 10X and 15X were comparable across the platforms.

Cases, N = 52							Controls, N = 1,789						
	Observed		Expected		Enrichment	p-value		Observed		Expected		Enrichment	p-value
	N	Rate	N	Rate				N	Rate				
	All Genes							All Genes					
Total	63	1.21	58.10	1.12	1.08	0.28	Total	1830	1.02	1949.9	1.09	0.94	1.00
Syn	15	0.29	16.50	0.32	0.91	0.68	Syn	484	0.27	549.6	0.31	0.88	1.00
D-Mis	14	0.27	6.80	0.13	2.05	0.01	D-Mis	222	0.12	232.8	0.13	0.95	0.77
T-Mis	29	0.56	29.70	0.57	0.98	0.58	T-Mis	974	0.54	993.3	0.56	0.98	0.73
LoF	5	0.10	5.10	0.10	0.98	0.58	LoF	150	0.08	174.3	0.10	0.86	0.97
Damaging	19	0.37	12.00	0.23	1.59	0.04	Damaging	372	0.21	407.1	0.23	0.91	0.96

Syn = Synonymous single nucleotide variant; D-Mis = Missense variants with “D” annotation per MetaSVM; T-Mis = Missense variants with “T” or “.” annotation per MetaSVM; LoF = Predicted loss-of-function variants (canonical splice site, frameshift indels, stop gains, and stop losses); Damaging = D-mis and/or LoF.

Supplementary Table 4. <i>De novo</i> burden analysis comparing cases to controls, Related to Table 1						
	# of <i>de novo</i> mutations		# of <i>de novo</i> mutations per subject		Binomial P-value*	Odds ratio (95% CI)#
	Cases N=52	Controls ^{&} N=1,789	Cases N=52	Controls ^{&} N=1,789		
LoF-intolerant genes (n = 3,230)						
Total	14	597.9	0.27	0.33	0.5	NA
Syn	2	150.7	0.04	0.08	0.38	NA
Mis	11	402.1	0.21	0.22	0.96	2.06 (0.45 - 9.41)
D-Mis	6	75.9	0.12	0.04	0.03	5.96 (1.17 - 30.22)
LoF	1	45.1	0.02	0.03	1	1.67 (0.15 - 18.85)
Damaging	7	121.0	0.13	0.07	0.12	4.36 (0.89 - 21.37)
All chromatin genes (n = 547)						
Total	4	77.1	0.08	0.04	0.42	NA
Syn	0	14.2	0.00	0.01	1	NA
Mis	3	55.8	0.06	0.03	0.51	NA
D-Mis	3	14.2	0.06	0.01	3.4x10 ⁻³	NA
LoF	1	7.1	0.02	0.00	0.57	NA
Damaging	4	21.4	0.08	0.01	8.6x10 ⁻⁴	NA
LoF-intolerant chromatin genes (n = 272)						
Total	4	55.8	0.08	0.03	0.16	NA
Syn	0	10.7	0.00	0.01	1	NA
Mis	3	41.5	0.06	0.02	0.26	NA
D-Mis	3	8.3	0.06	0.00	9.0x10 ⁻⁵	NA
LoF	1	3.6	0.02	0.00	0.3	NA
Damaging	4	11.9	0.08	0.01	3.9x10 ⁻⁶	NA

[&] Due to the difference in the *de novo* mutation rates between cases and controls, we adjusted the *de novo* mutation rate in controls to match the per-person rate by dividing the original rate per control by 0.84 (=1.02/1.21).

* The p-values compare the number of *de novo* mutations between 52 case trios and 1,789 control trios using a two-tailed binomial exact test as shown previously (Sanders et al. 2012 Nature; Zaidi et al. 2013 Nature).

The odds ratio calculates the proportion of *de novo* mutations in a specific category to synonymous *de novo* mutations and then compare these ratios in case trios versus control trios. NA, not applicable.

Supplementary Table 5. Damaging <i>de novo</i> mutation in loss-of-function intolerant chromatin modifier genes in 55 cases, Related to Table 1																	
Salient genetic information											Salient clinical and segregation information						
Proband code	Gene	Coordinate (hg19)	Ethnicity	Inheritance	AA Change	gnomAD combined MAF**	pLI	Missense Z-score	OMIM phenotype	CADD	MetaS VM	Type of VOGM	Cutaneous manifestations or non CNS AVMs in kindred?	Vascular cutaneous manifestations on proband?	Vascular Birthmarks/AVMs on mother	Vascular Birthmarks/AVMs on father	Concordance with mutation type
KVGAM38	<i>KAT6A</i>	8:41832271:G:A	Mexican	<i>De novo</i>	p.Thr478Ile	<4.11E-06	1.00	2.14	Mental retardation (AD)	24.2	D	Choroidal	y	y	n	n	y
KVGAM26	<i>KMT2D</i>	12:49420060:C:T	European	<i>De novo</i>	p.Cys5230Tyr	<4.06E-06	1.00	3.10	Kabuki syndrome (AD)	22.4	D	Choroidal	y	y	n	n	y
KVGAM20*	<i>SIRT1</i>	10:69666624:TC:T	European	<i>De novo</i>	p.Arg341fs5	<3.63E-06	0.95	0.58	None	.	N/A	Choroidal	y	y	y	n	n
KVGAM29	<i>SMARCA2</i>	9:2086866:G:T	European	<i>De novo</i>	p.Arg855Leu	<4.06E-06	1.00	5.57	Nicolaidis-Baraitser syndrome (AD)	26.5	D	Mural	n	n	n	n	N/A

* This subject also carried a p.Val143Met mutation in CLDN14 transmitted from mother

** For variants not observed in public databases, their minor allele frequency is calculated as less than 1 out of total number of alleles sampled at the closest locus with allele number available

Gene	Case		Control		Fisher p value	Odds ratio
	# mutant allele	# ref allele	# mutant allele	# ref allele		
<i>EPHB4</i>	4	106	3	7,153	1.68E-06	89.97
<i>MYH7</i>	3	107	0	7,156	3.38E-06	Inf
<i>AOC2</i>	3	107	0	7,156	3.38E-06	Inf
<i>CLDN14</i>	3	107	0	7,156	3.38E-06	Inf
<i>OTOG</i>	2	108	0	7,156	2.27E-04	Inf
<i>NALCN</i>	2	108	0	7,156	2.27E-04	Inf
<i>LTBP2</i>	2	108	0	7,156	2.27E-04	Inf
<i>MLH3</i>	2	108	0	7,156	2.27E-04	Inf
<i>PKM</i>	2	108	0	7,156	2.27E-04	Inf
<i>TPSG1</i>	2	108	0	7,156	2.27E-04	Inf

Gene	Case		Control		Fisher p value	Odds ratio
	# mutant allele	# ref allele	# mutant allele	# ref allele		
<i>EPHB4</i>	4	106	92	121,318	1.98E-06	49.76
<i>AOC2</i>	3	107	39	121,373	8.07E-06	87.26
<i>CLDN14</i>	3	107	50	120,946	1.65E-05	67.82
<i>MPST</i>	2	108	17	116,829	1.48E-04	127.26
<i>DNAH12</i>	2	108	37	121,367	5.89E-04	60.74
<i>EMILIN3</i>	2	108	40	121,192	6.85E-04	56.11
<i>ABHD17A</i>	2	108	42	118,070	7.91E-04	52.06
<i>PRKDC</i>	1	109	0	121,412	9.05E-04	Inf
<i>SEPT1</i>	1	109	0	121,412	9.05E-04	Inf
<i>ADGRL4</i>	2	108	50	120,618	1.06E-03	44.67

Rank	Ingenuity Canonical Pathways	P-value	Corrected P-value	Ratio	Genes
1	Axonal Guidance Signaling	6.61E-08	1.33E-05	0.03	<i>SLIT3, ITGB1, SHH, EPHB4, NGEF, ITSNI, EPHA4, KEL, SLIT2, ADAMTS2, MET, EPHA6, SHANK2, RASA1, NRPI</i>
2	nNOS Signaling in Skeletal Muscle Cells	1.32E-07	2.64E-05	0.15	<i>CACNA1G, CACNG2, CACNA1B, RYR2, CACNA2D3, CACNA1A</i>
3	GABA Receptor Signaling	1.41E-06	2.81E-04	0.07	<i>CACNA1G, CACNG2, CACNA1B, GABRB1, KCNH2, CACNA2D3, CACNA1A</i>
4	Netrin Signaling	2.19E-06	4.34E-04	0.09	<i>CACNA1G, CACNG2, CACNA1B, RYR2, CACNA2D3, CACNA1A</i>
5	FcγRIIB Signaling in B Lymphocytes	6.92E-06	1.36E-03	0.08	<i>CACNA1G, CACNG2, CD79B, CACNA1B, CACNA2D3, CACNA1A</i>
6	Corticotropin Releasing Hormone Signaling	1.74E-05	3.41E-03	0.05	<i>CACNA1G, CACNG2, SHH, CACNA1B, KRT1, CACNA2D3, CACNA1A</i>
7	G Beta Gamma Signaling	5.62E-05	0.01	0.05	<i>CACNA1G, CACNG2, CACNA1B, CACNA2D3, CACNA1A, DNM2</i>
8	Synaptic Long Term Depression	7.41E-05	0.01	0.04	<i>CACNA1G, CACNG2, CACNA1B, RYR2, PPP2R2B, CACNA2D3, CACNA1A</i>
9	Ephrin Receptor Signaling	7.59E-05	0.01	0.04	<i>ITGB1, EPHA6, NGEF, EPHB4, ITSNI, EPHA4, RASA1</i>
10	Androgen Signaling	1.55E-04	0.03	0.04	<i>CACNA1G, CACNG2, CACNA1B, POLR2H, CACNA2D3, CACNA1A</i>
11	CCR5 Signaling in Macrophages	2.40E-04	0.05	0.05	<i>CACNA1G, CACNG2, CACNA1B, CACNA2D3, CACNA1A</i>

Supplementary Table 8. Mutation burden in cases compared to European ethnicity-matched controls in IPA-determined significant pathways, Related to Table 1 and Figure 3												
Control_Group	Pathway (# of intolerant genes)	Case_Carrier	Case_NonCarrier	CaseTotal	Control_Carrier*	Control_NonCarrier	ControlTotal	Enrichment	Confident_Interval	p-value		
Autism	nNOS Signaling in Skeletal Muscle Cells (n = 18)	4	41	45	196	2,690	2,886	1.34	[0.43, Inf]	0.37		
Autism	Axonal Guidance Signaling (n = 161)	14	31	45	279	2,607	2,886	4.22	[2.29, Inf]	6.94E-05		
Autism	Ephrin Receptor Signaling (n = 73)	8	37	45	127	2,759	2,886	4.69	[2.15, Inf]	8.36E-04		
Autism	Androgen Signaling (n = 55)	4	41	45	193	2,693	2,886	1.36	[0.44, Inf]	0.36		
Autism	CCR5 Signaling in Macrophages (n = 31)	3	42	45	138	2,748	2,886	1.42	[0.37, Inf]	0.37		
Autism	Corticotropin Releasing Hormone Signaling (n = 57)	5	40	45	244	2,642	2,886	1.35	[0.5, Inf]	0.33		
Autism	FcγRIIB Signaling in B Lymphocytes (n = 36)	3	42	45	149	2,737	2,886	1.31	[0.34, Inf]	0.42		
Autism	G Beta Gamma Signaling (n = 53)	4	41	45	189	2,697	2,886	1.39	[0.45, Inf]	0.34		
Autism	GABA Receptor Signaling (n = 45)	6	39	45	175	2,711	2,886	2.38	[0.97, Inf]	0.06		
Autism	Netrin Signaling (n = 32)	4	41	45	212	2,674	2,886	1.23	[0.4, Inf]	0.43		
Autism	Synaptic Long Term Depression (n = 65)	5	40	45	289	2,597	2,886	1.12	[0.42, Inf]	0.48		
ExAC	nNOS Signaling in Skeletal Muscle Cells (n = 18)	4	41	45	3,145	33,532	36,677	1.04	[0.34, Inf]	0.55		
ExAC	Axonal Guidance Signaling (n = 161)	14	31	45	3,878	32,799	36,677	3.82	[2.1, Inf]	1.52E-04		
ExAC	Ephrin Receptor Signaling (n = 73)	8	37	45	1,621	35,056	36,677	4.68	[2.18, Inf]	7.33E-04		
ExAC	Androgen Signaling (n = 55)	4	41	45	4,718	31,959	36,677	0.66	[0.22, Inf]	0.85		
ExAC	CCR5 Signaling in Macrophages (n = 31)	3	42	45	2,138	34,539	36,677	1.15	[0.3, Inf]	0.49		
ExAC	Corticotropin Releasing Hormone Signaling (n = 57)	5	40	45	3,466	33,211	36,677	1.20	[0.45, Inf]	0.42		
ExAC	FcγRIIB Signaling in B Lymphocytes (n = 36)	3	42	45	2,321	34,356	36,677	1.06	[0.28, Inf]	0.55		
ExAC	G Beta Gamma Signaling (n = 53)	4	41	45	2,726	33,951	36,677	1.22	[0.4, Inf]	0.43		
ExAC	GABA Receptor Signaling (n = 45)	6	39	45	2,995	33,682	36,677	1.73	[0.71, Inf]	0.16		
ExAC	Netrin Signaling (n = 32)	4	41	45	3,361	33,316	36,677	0.97	[0.32, Inf]	0.60		
ExAC	Synaptic Long Term Depression (n = 65)	5	40	45	4,494	32,183	36,677	0.89	[0.34, Inf]	0.66		

P values were calculated using the one-tailed Fisher's Exact Test. Values in red bold are P values exceeding the Bonferroni multiple-testing cutoff ($0.05/(2 \times 11) = 2.27E-03$)

* ExAC database does not provide individual level information, number of damaging mutations in each gene set carried by each individual was assumed to be 1.

Supplementary Table 9. Gene burden in cases compared to expectation for axonal guidance and Ephrin receptor signaling pathways, Related to Table 1 and Figure 3												
Axon guidance signaling					Ephrin receptor signaling							
LoF-intolerant genes with damaging mutations in VOGM cases	Observed	Expected	Enrichment	p-value	LoF-intolerant genes with damaging mutations in VOGM cases	Observed	Expected	Enrichment	p-value			
	18	7.14	2.52	3.02E-04		10	2.90	3.45	7.28E-04			
LoF-intolerant genes with synonymous mutations in VOGM cases	Observed	Expected	Enrichment	p-value	LoF-intolerant genes with synonymous mutations in VOGM cases	Observed	Expected	Enrichment	p-value			
	21	20.12	1.04	0.45		4	8.17	0.49	0.96			
LoF-intolerant genes with damaging mutations in autism controls	Observed	Expected	Enrichment	p-value	LoF-intolerant genes with damaging mutations in autism controls	Observed	Expected	Enrichment	p-value			
	372	348.77	1.07	0.11		166	141.56	1.17	0.02			
LoF-intolerant genes with damaging mutations in ExAC controls	Observed	Expected	Enrichment	p-value	LoF-intolerant genes with damaging mutations in ExAC controls	Observed	Expected	Enrichment	p-value			
	3878	3967.08	0.98	0.93		1621	1611.57	1.01	0.41			

P values were calculated using the one-tailed binomial test comparing observed number of variants in LoF-intolerant genes that belong to statistically significant canonical pathways of interest to the expected number of mutations in each set (see Methods). Values in bold red are P values exceeding the Bonferroni multiple-testing cutoff ($0.05/(2 \times 3) = 0.008$)

Supplementary table 10a. Gene burden in cases compared to expectation for axonal guidance pathway after removing genes in the ephrin signaling pathway, Related to Table 1 and Figure 3				
LoF-intolerant genes with damaging mutations in VOGM cases	Observed	Expected	Enrichment	p-value
	8	6.66	1.2	0.35

P values were calculated using the one-tailed binomial test comparing observed number of variants in LoF-intolerant genes that belong to statistically significant canonical pathways of interest to the expected number of mutations in each set (see Methods).

Supplementary Table 10b. Mutation burden in cases compared to European ethnicity-matched controls for axonal guidance pathway after removing genes in the ephrin signaling pathway, Related to Table 1 and Figure 3										
Control_Sample	Pathway	Case_Carrier	Case_NonCarrier	CaseTotal	Control_Carrier*	Control_NonCarrier	ControlTotal	Enrichment	Confident_Interval	p-value
Autism	Axonal Guidance Signaling (n = 161)	6	39	45	197	2,689	2,886	2.10	[0.86, Inf]	0.09
ExAC	Axonal Guidance Signaling (n = 161)	6	39	45	2,726	33,951	36,677	1.92	[0.79, Inf]	0.11

P values were calculated using the one-tailed Fisher's Exact Test. Bonferroni multiple-testing cutoff for this test is $0.05/2 = 0.025$

* ExAC database does not provide individual level information, number of damaging mutations in each gene set carried by each individual was assumed to be 1.

Supplementary Table 11. Mutations in genes in the Ephrin receptor signaling pathway, Related to Table 1 and Figure 3

Proband code	Inheritance	Mutation Type	Gene	Coordinate (hg19)	Coding sequence variant	AA Modification	ExAC MAF*	gnomAD combined MAF*	pLI	Missense Z-score	OMIM phenotype	CADD	Meta SVM
VGAM105	Transmitted	LoF	<i>ITGB1</i>	chr10:33196073	N/A	Splice site	< 8.27E-06	< 4.15E-06	0.91	3.47	None	20.9	N/A
VGAM115	Transmitted	LoF	<i>EPHB4</i>	chr7:100417179	c.1295_1296del	p.Glu432fs1	< 9.06E-06	< 4.76E-06	0.99	2.84	Non-immune hydrops fetalis with/without atrial septal defect	.	D
KVGAM25	Transmitted	D-mis		chr7:100414876	c.1526C>G	p.Ala509Gly	3.30E-05	1.13E-05				25	D
KVGAM33	Transmitted	D-mis		chr7:100410537	c.1950G>T	p.Lys650Asn	< 8.24E-06	< 4.06E-06				29.9	D
KVGAM18	Transmitted	D-mis		chr7:100403202	c.2599T>C	p.Phe867Leu	< 9.03E-06	< 5.17E-06				31	D
KVGAM55	Transmitted	D-mis	<i>NGEF</i>	chr2:233745889	c.1909G>A	p.Asp637Asn	2.83E-05	1.22E-05	0.95	2.18	None	34	D
KVGAM7	Transmitted	D-mis	<i>ITSN1</i>	chr21:35260471	c.5033C>T	p.Thr1678Met	1.89E-05	8.13E-06	1.00	3.44	None	34	D
VGAM114	Transmitted	D-mis	<i>EPHA4</i>	chr2:222299011	c.G2347G>A	p.Gly783Ser	< 8.28E-06	< 4.07E-06	1.00	3.15	None	34	D
KVGAM45	Transmitted	D-mis	<i>EPHA6</i>	chr3:96706498	c.775C>T	p.Arg259Cys	< 8.38E-06	< 3.23E-05	0.95	0.93	None	34	D
KVGAM42	Transmitted	LoF	<i>RASA1</i>	chr5:86672323	c.2125C>T	p.Arg709*	< 8.26E-06	< 3.23E-05	1.00	2.96	CM-AVM1; Parkes Weber Syndrome; Somatic basal cell carcinoma	40	N/A

* For variants not observed in public databases, their minor allele frequency is calculated as less than 1 out of total number of alleles sampled at the closest locus with allele number available

Supplementary Table 12. Transmitted VOGM mutations in Mendelian AVM genes, Related to Figure 3

Family	Type of VOGM	Ethnicity	Gene	Mutation	Domain affected	ExAC MAF*	gnomAD MAF*	pLI	MetaSVM	CADD
KVGAM6	Mural	European	<i>ACVRI</i> ^{&}	p.(G39S)	Activin receptor	< 8.24E-06	3.24E-05	0.96	D	24.8
KVGAM44	Mural	European	<i>ACVRI</i> ^{&}	p.(N373S)	Kinase domain	< 8.26E-06	< 4.07E-06	0.96	T	24.1
VGAM100	Choroidal	Mexican	<i>ACVRL1</i>	p.(R484Q)	Kinase domain	< 8.28E-06	< 3.24E-05	0.01	D	33

[&] *ACVRI* is a paralog of *ACVRL1*

* For variants not observed in public databases, their minor allele frequency is calculated as less than 1 out of total number of alleles sampled at the closest locus with allele number available

Supplementary Table 13. Distribution of highly pathogenic mutations in VOGM patients with different subphenotypes, Related to Table 1, Figure 3, and STAR Methods

Proband Code	Gender	Ethnicity	Sub-Phenotype at Diagnosis (weeks)	Fetal Age	Postnatal Age at Diagnosis	Gene	Amino Acid Change	Type	Transmission	Proband with Capillary Malformation	Relatives with Capillary Malformation	Other phenotypes
KVGAM1	M	European	Choroidal		7 mo.					n	n	Neurodevelopmental delay
KVGAM10	M	European	Choroidal	34		<i>KEL</i>	p.Gln321*	stopgain	<i>De novo</i>	n	y	Pulmonary hypertension, neurodevelopmental delay
KVGAM15	M	European	Choroidal		3 d.					n	y	Patent ductus arteriosus, neurodevelopmental delay
KVGAM18	M	European	Choroidal	40		<i>EPHB4</i>	p.Phe867Leu	Dmis	Transmitted	n	y	Cryptorchidism, strabismus, cerebral palsy
KVGAM2	M	Undetermined	Choroidal	33						n	n	n
KVGAM20	F	European	Choroidal		6 mo.	<i>CLDN14</i> <i>SIRT1</i>	p.Val143Met p.Arg341fs5	Dmis Frameshift deletion	Transmitted <i>De novo</i>	y	y	n
KVGAM22	F	European	Choroidal		5 mo.					n	n	n
KVGAM24	M	European	Choroidal		8 yr.					n	n	Recurrent epistaxis
KVGAM25	M	European	Choroidal		19 d.	<i>EPHB4</i>	p.Ala509Gly	Dmis	Transmitted	n	n	Pectus excavatum
KVGAM26	M	European	Choroidal		1 d.	<i>KMT2D</i>	p.Cys5230Tyr	Dmis	<i>De novo</i>	n	n	Hypothyroidism, low set ears, long and everted palpebral fissures, neurodevelopmental delay, seizures
KVGAM30	M	European	Choroidal		5 d.					n	y	Neurodevelopmental delay, seizures
KVGAM31	F	European	Choroidal		11 mo.					n	n	Neurodevelopmental delay
KVGAM32	M	European	Choroidal		7 d.					n	y	n
KVGAM33	F	Mexican	Choroidal		1 d.	<i>EPHB4</i>	p.Lys650Asn	Dmis	Transmitted	n	y	Neurodevelopmental delay
KVGAM34	M	European	Choroidal	35						n	n	Neurodevelopmental delay, attention deficit hyperactivity disorder
KVGAM35	M	European	Choroidal	37						n	n	n
KVGAM37	M	European	Choroidal	36						n	y	Neurodevelopmental delay
KVGAM38	M	Mexican	Choroidal		6 d.	<i>KAT6A</i>	p.Thr478Ile	Dmis	<i>De novo</i>	y	n	Neurodevelopmental delay, seizures
KVGAM4	M	Undetermined	Choroidal		2 d.					n	n	n
KVGAM40	M	European	Choroidal		3 mo.					n	y	Cerebral palsy, hip dysplasia, visual impairment, gastroesophageal reflux disease, neurodevelopmental delay, seizures
KVGAM52	F	European	Choroidal	32						n	n	n
KVGAM54	F	European	Choroidal	38						n	y	n
KVGAM55	F	European	Mural	20		<i>NGEF</i>	p.Asp637Asn	Dmis	Transmitted	n	n	n
KVGAM7	F	European	Choroidal		1 yr.	<i>ITSN1</i>	p.Thr1678Met	Dmis	Transmitted	n	y	Pulmonary valve stenosis, neurodevelopmental delay
KVGAM8	M	European	Choroidal	39						n	y	Neurodevelopmental delay, seizures
KVGAM9	F	European	Choroidal	38						n	n	Patent ductus arteriosus, patent foramen ovale, seizures
VGAM100	F	Mexican	Choroidal	34		<i>ACVRL1</i> <i>CLDN14</i>	p.R484Q p.Ala113Pro	Dmis Dmis	Transmitted Transmitted	n	n	Strabismus
VGAM101	M	European	Choroidal	36		<i>EFNB2</i>	p.Arg277His	Dmis	Transmitted	y	y	Hip dysplasia, neurodevelopmental delay, seizures
VGAM104	M	European	Choroidal		3 d.					y	n	Cerebral palsy, neurodevelopmental delay
VGAM107	F	European	Choroidal		4 d.					n	n	Medulloblastoma, seizures
VGAM108	M	European	Choroidal		2 mo.					n	y	Asthma
VGAM109	M	Mexican	Choroidal		3 d.					y	y	Cerebral palsy, neurodevelopmental delay, seizures
VGAM111	M	European	Choroidal	31						n	n	Cerebral palsy, gastroesophageal reflux disease, renal agenesis, precocious puberty, mitral insufficiency, neurodevelopmental delay, seizures
VGAM112	M	European	Choroidal	36						n	n	n
VGAM113	M	European	Choroidal		2 d.					n	n	Chiari Type I malformation
VGAM114	F	European	Choroidal		11 mo.	<i>EPHA4</i>	p.Gly783Ser	Dmis	Transmitted	y	y	n
VGAM115	M	European	Choroidal		3 mo.	<i>EPHB4</i>	p.Glu432fs1	Frameshift deletion	Transmitted	n	y	Neurodevelopmental delay, seizures
VGAM47	M	European	Choroidal	29						n	n	Hypospadias
KVGAM11	M	European	Mural		6 mo.					n	y	n
KVGAM29	M	European	Mural	38		<i>SMARCA2</i>	p.Arg855Leu	Dmis	<i>De novo</i>	n	n	Seizures
KVGAM3	F	European	Mural	31						n	n	n
KVGAM36	F	European	Mural		3 mo.					n	y	n
KVGAM41	M	European	Mural		14 mo.					n	y	Major depressive disorder with psychotic features, attention deficit hyperactivity disorder, hydrocephalus, migraines
KVGAM42	F	Mexican	Mural		6 mo.	<i>RASA1</i>	p.Arg709*	stopgain	Transmitted	y	y	Cerebral palsy, seizures
KVGAM43	M	African	Mural		14 mo.					n	y	n
KVGAM44	M	European	Mural		9 mo.	<i>ACVRL1</i>	p.Asu373Ser	T-mis (high CADD = 24.1)	Transmitted	n	y	Atrial septal defect, partial anomalous pulmonary venous return
KVGAM45	F	European	Mural		6 mo.	<i>EPHA6</i> <i>KEL</i>	p.Arg259Cys p.Gly202Ser	Dmis T-mis (high CADD = 22.6)	Transmitted <i>De novo</i>	n	n	Neurodevelopmental delay, seizures
KVGAM49	M	Undetermined	Mural		16 mo.					n	n	n
KVGAM5	M	Mexican	Mural	28						n	n	Cystic fibrosis
KVGAM51	F	European	Mural	31		<i>CLDN14</i>	p.Val143Met	Dmis	Transmitted	n	n	n
KVGAM6	F	European	Mural	35		<i>ACVRL1</i>	p.Gly39Ser	Dmis	Transmitted	n	n	n
VGAM102	M	European	Mural		3 d.					n	n	Attention deficit hyperactivity disorder, recurrent epistaxis
VGAM105	M	European	Mural		2 mo.	<i>ITGB1</i>	Splice site	splice site	Transmitted	n	n	Chiari Type I malformation
VGAM110	F	European	Mural	39						y	n	n
VGAM46	M	European	Mural		1 yr.					n	n	n

Gender M = Male, F = Female; Dmis = Missense variants with 'D' annotation per MetaSVM; T-mis = Missense variants with 'T' or '.' annotation per MetaSVM; y = Yes; n = No