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Letter

Reverse phenotyping in patients with skin capillary malformations and mosaic *GNAQ* or *GNA11* mutations defines a clinical spectrum with genotype-phenotype correlation.

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To the Editor

Post-zygotic mutations in *GNAQ*/*GNA11* genes encoding heterotrimeric G protein alpha subunits account for skin mosaic conditions with vascular or pigmentary anomalies (Shirley et al. 2013; Thomas et al. 2016; Couto et al. 2017; Siegel et al. 2018). We sought to delineate the phenotype of 32 patients with skin capillary malformations (CMs) harbouring an activating post-zygotic mutation in *GNA11* or *GNAQ* in affected skin. Nevus flammeus, ipsilateral segmental overgrowth, varicose veins and macrocephaly were associated with *GNAQ* mutations, whereas cutis marmorata, nevus anemicus, and ipsilateral hypotrophy were associated with *GNA11* mutations. Pigmentary anomalies were only associated with pigment skin type. Additional extracutaneous features included ocular and neurological anomalies of the Sturge-Weber syndrome, varicose veins with deep vein thrombosis, and hypertension with renal anomalies, encompassing a wide clinical spectrum.

All 32 patients (19 females, 13 males) aged 3 months to 67 years (median 14 years) were referred for genetic testing because of cutaneous CMs suggesting involvement of *GNA11* or *GNAQ*, and were included in the *Mosaic Undiagnosed Skin Traits And Related Disorders* (M.U.S.T.A.R.D. - NCT01950975) cohort, approved by our regional institutional review board and ethics committee. Five patients with phakomatosis pigmentovascularis (PPV) were previously reported (Thomas et al. 2016). Written informed consent from all participants or their parents was obtained. Ultradeep next generation sequencing of the whole coding sequence of *GNAQ/GNA11* (600 X to 6700 X) was performed on DNA from affected skin and venous blood on a MiSeq (Illumina®) according to standard protocol.

Patients were phenotypically classified based on clinical charts and photographs from

referring clinicians, with additional data from a standardised questionnaire addressing vascular, pigmentary, neurological and ocular involvement, or overgrowth. Cutaneous CMs were subclassified as suggested by Happle: nevus flammeus (port wine stain), nevus roseus (pale pink nevus), cutis marmorata/reticulate CM, and nevus anemicus (Figure 1) (Happle 2015). Patients with vascular and pigmentary manifestations were diagnosed with PPV and classified into one of the three types defined by Happle (Happle 2005). Patients with vascular anomalies and segmental asymmetry (overgrowth or hypotrophy) were assigned to one of the six types defined by Oduber et al (Oduber et al. 2011). Association between phenotypes and *GNAQ* or *GNA11* mutations was analysed using two-tailed Fisher's exact test.

Most patients (n=19) carried a post zygotic p.Arg183Gln *GNAQ* mutation in their CMs, and only one had a p.Arg183Gly *GNAQ* mutation (Table 1 S1). Twelve patients carried a *GNA11* mutation: p.Arg183Cys (n=10), p.Arg183His (n=1), p.Gln209His (n=1). A post zygotic *PIK3CA* mutation was excluded in 9 patients – two initially referred for suspicion of macrocephaly – capillary malformation – polymicrogyria syndrome (MCAP, OMIM#602501), the seven others with a Klippel-Trénaunay syndrome (KTS). Variant allele fraction in affected tissue ranged from 1 % to 17 % (median = 5%).

Cutaneous CMs (Figure 2A) involved the face in 21 patients, and multiple body segments in 23. Nevus flammeus was associated with *GNAQ* rather than *GNA11* mutations, yet not significantly (p=0.069). In contrast, nevus anemicus and cutis marmorata were associated with *GNA11* mutations (p= 0.030 and p=0.042, respectively). Nevus roseus did not show any association with either *GNAQ* or *GNA11* mutations. Nevus anemicus was always associated with other CMs (resulting in nevus vascularis mixtus), mainly cutis marmorata (10 patients).

Pigmentary anomalies associated with CMs, (Figure 2B) consisted of extensive dermal melanocytosis, scleral melanocytosis, or café-au-lait macules, and led to a diagnosis of PPV in 11 patients. They were not preferentially associated with either *GNAQ* or *GNA11* mutations but instead with pigment skin types III-V (11/20 patients), and were absent in skin types I-II (p=0.006). Similar findings had previously been reported (Polubothu and Kinsler 2017). All PPV cesioflammea patients carried *GNAQ* mutations, whereas all PPV cesiomarmorata patients carried *GNA11* mutations. No patients had PPV spilorosea. Three PPV patients could not be classified.

In patients with facial CMs (Figure 2C), macrocephaly or hemifacial overgrowth were associated with a *GNAQ* mutation. Thirteen patients had Sturge-Weber syndrome, with seizures, brain MRI anomalies (leptomeningeal angiomatosis, cortical atrophy with calcifications), or glaucoma, without preferential *GNAQ* or *GNA11* genotype. In patients with extra-cephalic CMs (Figure 2D), proportionate ipsilateral segmental overgrowth was found in 50%, mainly with nevus flammeus-type CMs and *GNAQ* mutations. In contrast, all patients with ipsilateral segmental hypotrophy (inverse Klippel-Trénaunay syndrome)(Danarti et al. 2007) carried a *GNA11* mutation. Varicose veins were present in 7 patients with *GNAQ* mutations. Three of them experienced deep vein thrombosis. Most patients with overgrowth could be clinically classified as type 1 (CM type) or 2 (CM-venous malformation type), but types 3 (Klippel-Trénaunay type) and 6 (reticular CM type) were also found. Two patients could not be classified.

Six patients had arterial hypertension, associated with renal artery dysplasia in two, and renal hypoplasia in two. No preferential association with either *GNAQ* or *GNA11* mutations was found.

Our results suggest that clinical features in CM patients may differentiate between *GNAQ* and *GNA11* mutations, although more patients need to be studied to corroborate our findings. A limitation to our study is the absence of information on evolution. CMs may be classified differently at birth than later in life, particularly cutis marmorata/reticulate CM, which may evolve into a homogeneous CM with time. Likewise, with age, nevus roseus may darken and EDM may fade away. Also, extensive skin involvement in most patients and high frequency of extracutaneous manifestations reflect ascertainment bias, as only severe forms were referred. Yet, posterior phenotyping has allowed us to better define the highly variable mosaic *GNAQ/GNA11*-related clinical spectrum. Association of overgrowth with a slow flow vascular anomaly can be diagnosed as KTS(Vahidnezhad et al. 2016). However, *GNAQ/GNA11*-related overgrowth, previously reported (Couto et al. 2017), was proportionate and milder than in *PIK3CA*-related KTS (Keppler-Noreuil et al. 2014; Keppler-Noreuil et al. 2015). The term ‘‘diffuse capillary malformation with overgrowth’’ (DCMO) has been suggested for extensive reticular/homogeneous CMs with proportionate overgrowth (Lee et al. 2013), and may apply to many patients in our series. No correlation was found between *GNAQ/GNA11* mutations and the classification of vascular anomalies associated with deregulated growth by Oduber et al., since our patients belonged to four of the six subtypes, or could not be classified at all (Oduber et al. 2011). Varicose veins and thrombosis may be associated with overgrowth. To our knowledge, hypertension and renal anomalies had not previously been reported in association with *GNAQ/GNA11* mutations. They are reminiscent of the vascular manifestations of neurofibromatosis type I, due to mutations in a small G protein, and should be considered in the clinical assessment and follow-up of *GNAQ/GNA11* patients.

Data availability statement

No datasets were generated"

Conflict of interest statement

The authors have no conflict of interest to declare.

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CRediT statement

PV defined goals of the project, supervised the work and acquired financial support. JA, LB, EB, OB, NB, ACB, CC, BD, MF, JM, AG, SH-R, AM, AM, JM, CM, FM-P, M-LM, FP, JP, AP, SW, MW, PV provided clinical details and samples. MJ collected data and wrote the original draft. MJ, VC, PV synthesized study data. VC, AS, PK, CP validated genetic results. VC and PV reviewed and edited the manuscript.

References

Couto JA, Ayturk UM, Konczyk DJ, Goss JA, Huang AY, Hann S, et al. A somatic *GNA11* mutation is associated with extremity capillary malformation and overgrowth. Angiogenesis. 2017;20(3):303–6.

Danarti R, König A, Bittar M, Happle R. Inverse Klippel-Trenaunay Syndrome: Review of Cases Showing Deficient Growth. Dermatology. 2007;214(2):130–2.

Happle R. Phacomatosis pigmentovascularis revisited and reclassified. Arch Dermatol. 2005;141(3):385–8.

Happle R. Capillary malformations: a classification using specific names for specific skin disorders. J Eur Acad Dermatol Venereol JEADV. 2015;29(12):2295–305.

Keppler-Noreuil KM, Rios JJ, Parker VER, Semple RK, Lindhurst MJ, Sapp JC, et al. PIK3CA-Related Overgrowth Spectrum (PROS): Diagnostic and Testing Eligibility Criteria, Differential Diagnosis, and Evaluation. Am J Med Genet A. 2015;0(2):287–95.

Keppler-Noreuil KM, Sapp JC, Lindhurst MJ, Parker VER, Blumhorst C, Darling T, et al. Clinical delineation and natural history of the *PIK3CA*-related overgrowth spectrum. Am J Med Genet A. 2014;164(7):1713–33.

Lee MS, Liang MG, Mulliken JB. Diffuse capillary malformation with overgrowth: A clinical subtype of vascular anomalies with hypertrophy. J Am Acad Dermatol. 2013;69(4):589–94.

Oduber CEU, van der Horst CMAM, Sillevis Smitt JH, Smeulders MJC, Mendiratta V, Harper JI, et al. A proposal for classification of entities combining vascular malformations and deregulated growth. Eur J Med Genet. 2011;54(3):262–71.

Polubothu S, Kinsler VA. The ethnic profile of patients with birthmarks reveals interaction of germline and postzygotic genetics. Br J Dermatol. 2017;176(5):1385–7.

Shirley MD, Tang H, Gallione CJ, Baugher JD, Frelin LP, Cohen B, et al. Sturge–Weber Syndrome and Port-Wine Stains Caused by Somatic Mutation in *GNAQ*. N Engl J Med. 2013 May 23;368(21):1971–9.

Siegel DH, Cottrell CE, Streicher JL, Schilter KF, Basel DG, Baselga E, et al. Analyzing the Genetic Spectrum of Vascular Anomalies with Overgrowth via Cancer Genomics. J Invest Dermatol. 2018;138(4):957–67.

Thomas AC, Zeng Z, Rivière J-B, O'Shaughnessy R, Al-Olabi L, St.-Onge J, et al. Mosaic Activating Mutations in *GNA11* and *GNAQ* Are Associated with Phakomatosis Pigmentovascularis and Extensive Dermal Melanocytosis. J Invest Dermatol. 2016;136(4):770–8.

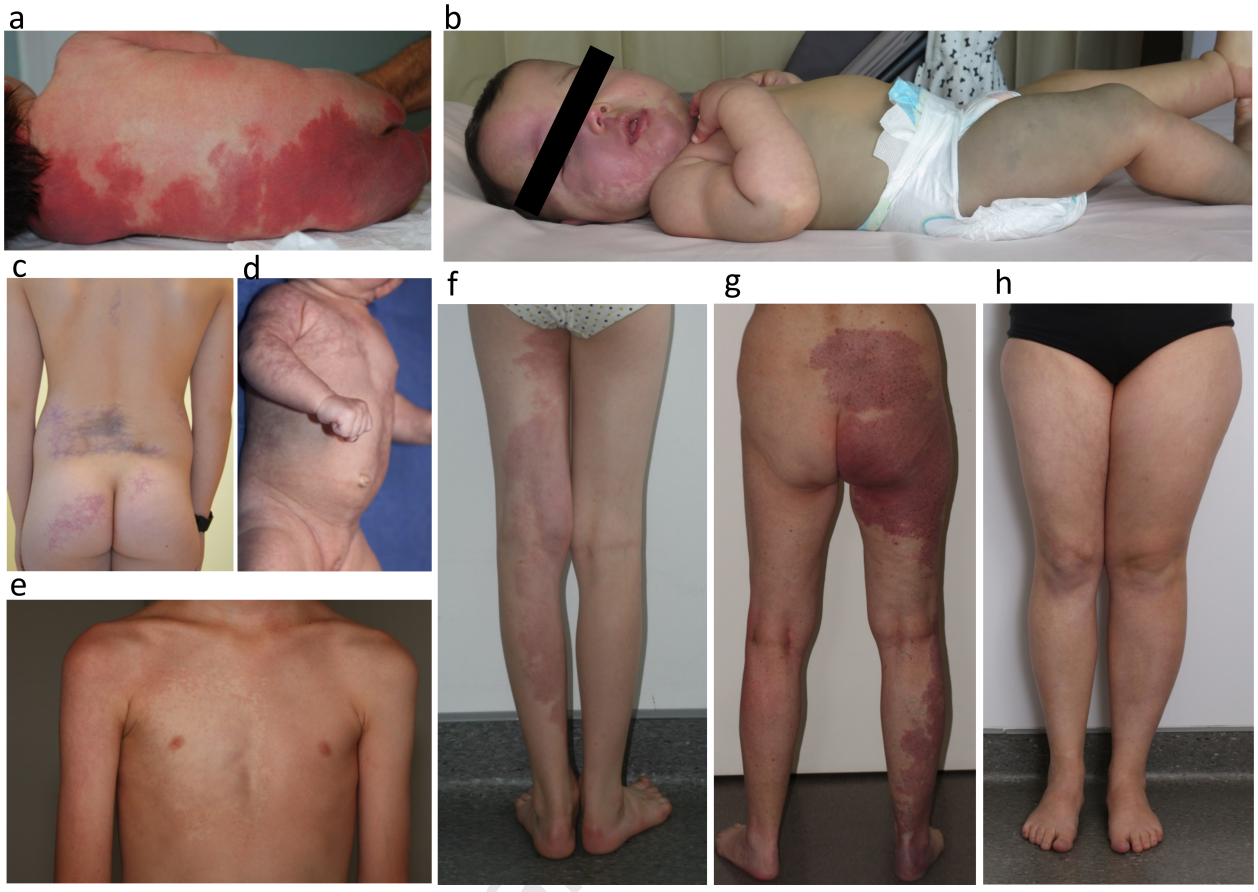
Vahidnezhad H, Youssefian L, Uitto J. Klippel-Trenaunay syndrome belongs to the PIK3CA-related overgrowth spectrum (PROS). Exp Dermatol. 2016;25(1):17–9.

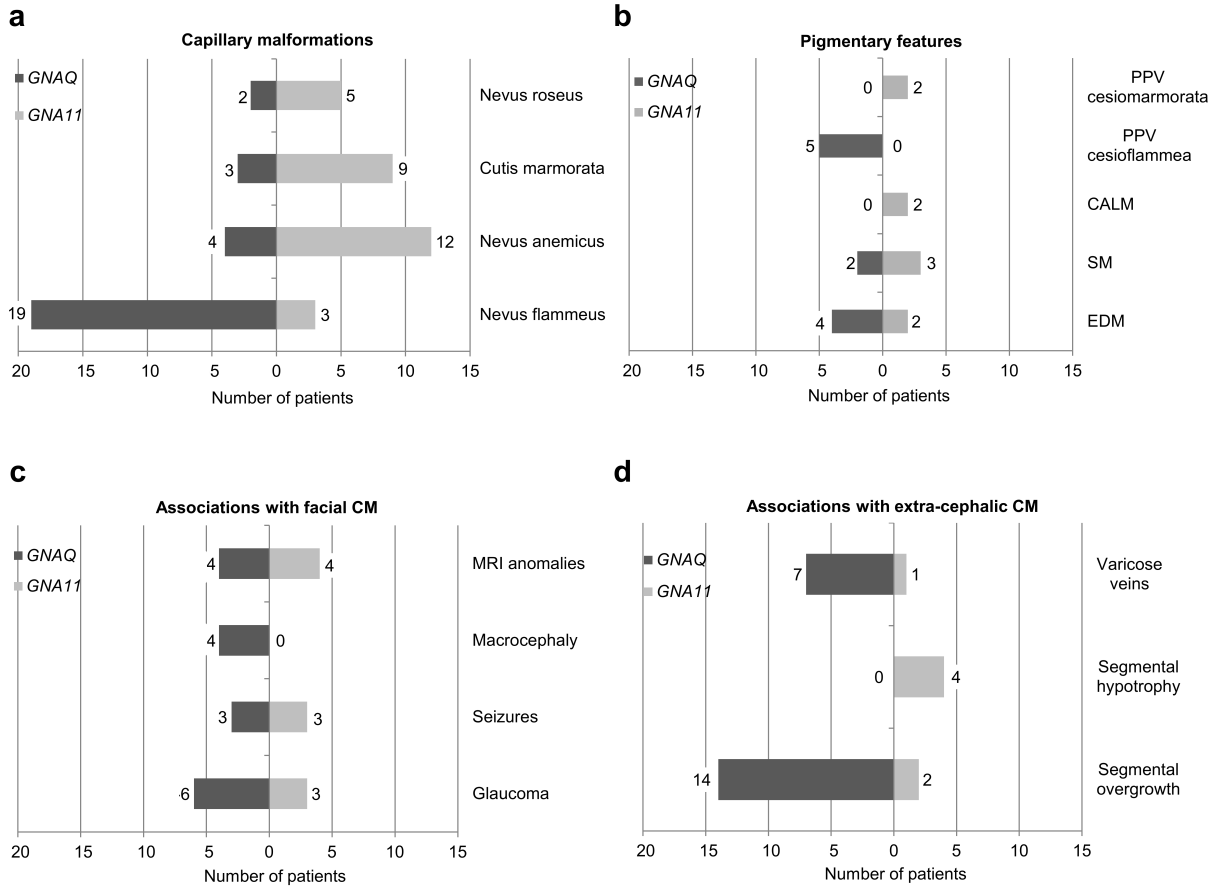
Legends to figures.

Figure 1. Cutaneous vascular or pigmentary phenotypes associated with *GNAQ* and *GNA11* mutations. (a) Patient 3886. Naevus vascularis mixtus (naevus flammeus and naevus anemicus; *GNAQ* p.Arg183Gly). (b) Patient 4396. PPV cesioflammea and naevus anemicus, macrocephaly, and SWS (*GNAQ* p.Arg183Gln). (c) Patient 1085. Naevus vascularis mixtus (naevus anemicus and cutis marmorata; *GNA11* p.Gln209His) (d) Patient 4037. PPV cesiomarmorata and naevus anemicus (*GNA11* p.Arg183Cys). (e) Patient 2846. Naevus vascularis mixtus (naevus roseus and naevus anemicus; *GNA11* p.Arg183Cys). (f) Patient 2824. Naevus flammeus and ipsilateral limb overgrowth. (*GNAQ* p.Arg183Gln-). (g) Patient 4849. Naevus flammeus, overgrowth (length and girth) and varicose veins (*GNAQ* p.Arg183Gln). (h) Patient 2579 Naevus vascularis mixtus (naevus anemicus and cutis marmorata) with ipsilateral lower limb hypotrophy (*GNA11* p.Arg183His). Patients or their parents consented to the publication of their image.

Figure 2. Distribution of clinical findings with respect to GNAQ and GNA11 mutations.

(A) Types of capillary malformations. (B) Pigmentary features and PPV types. (C) Associations with facial capillary malformations. (D) Associations with extra-cephalic capillary malformations. PPV: phacomatosis pigmentovascularis ; CALMs: café-au-lait macules ; SM: scleral melanocytosis; EDM: extensive dermal melanocytosis ; CM : capillary malformation ; MRI: magnetic resonance imaging.




a
Capillary malformations
GNAQ
GNA11
2
5
Nevus roseus
3
9
Cutis marmorata
4
12
Nevus anemicus
19
3
Nevus flammeus
20 15 10 5 0 5 10 15
Number of patients
b
Pigmentary features
GNAQ
GNA11
0
2
PPV cesiomarmorata
5
0
PPV cesioflammea
0
2
CALM
2
3
SM
4
2
EDM
20 15 10 5 0 5 10 15
Number of patients
c
Associations with facial CM
GNAQ
GNA11
4
4
MRI anomalies
4
0
Macrocephaly
3
3
Seizures
6
3
Glaucoma
20 15 10 5 0 5 10 15
Number of patients
d
Associations with extra-cephalic CM
GNAQ
GNA11
7
1
Varicose veins
0
4
Segmental hypotrophy
14
2
Segmental overgrowth
20 15 10 5 0 5 10 15
Number of patients

Patients	*Gene*	Variation (amino acids)	Allelic fraction (%)	Pigment Skin type	Cutaneous capillary malformation	Overgrowth	Hypotrophy	Neurological anomalies	Glaucoma	Venous anomalies	Pigmentary anomalies	Other features
PED1064	*GNAQ*	p.(Arg183Gln)	6.0	IV	Naevus flammeus: extensive, F, L ; anaemic naevus: F,L	L, Macrocephaly	-	-	+	-	EDM SM	Arterial hypertension, renal artery dysplasia
PED1153	*GNAQ*	p.(Arg183Gln)	3.0	II	Naevus flammeus: L; cutis marmorata: F, extensive	Macrocephaly	-	Seizures, ID, LA, brain atrophy	+	-	-	-
PED1500	*GNAQ*	p.(Arg183Gln)	5.0	IV	Naevus flammeus: extensive, F , L	Lips	-	ID	-	-	EDM	Dental anomalies
PED1991	*GNAQ*	p.(Arg183Gln)	6.5	IV	Naevus flammeus: extensive, extrafacial	L, mild	-	-	-	VV, partial agenesia	-	-
PED2333	*GNAQ*	p.(Arg183Gln)	3.0	III	Naevus flammeus: extensive, F, L	L, mild	-	Seizures, LA, brain atrophy	+	VV, thrombosis	-	Arterial hypertension, Renal atrophy
PED2355	*GNAQ*	p.(Arg183Gln)	6.0	III	Naevus flammeus: extensive F, L	-	-	-	-	-	SM	-
PED2360	*GNAQ*	p.(Arg183Gln)	8.0	III	Naevus flammeus: LL	L, mild	-	-	-	VV	-	-
PED2379	*GNAQ*	p.(Arg183Gln)	4.8	II	Naevus flammeus: LL	L, mild	-	-	-	VV, thrombosis	-	-
PED2586	*GNAQ*	p.(Arg183Gln)	10.0	III	Naevus flammeus: L	L, mild	-	-	-	VV, partial agenesia, thrombosis	-	-
PED2613	*GNAQ*	p.(Arg183Gln)	4.0	III	Naevus flammeus: F, UL; cutis marmorata: LL	L + F, mild	-	-	-	-	-	Scoliosis
PED2824	*GNAQ*	p.(Arg183Gln)	4.0	II	Naevus flammeus: LL	L, mild	-	-	-	-	-	Scoliosis
PED3248	*GNAQ*	p.(Arg183Gln)	7.0	V	Naevus flammeus: UL	UL, mild	-	-	-	-	EDM	-
PED3396	*GNAQ*	p.(Arg183Gln)	5.0	II	Naevus flammeus: F, UL	Macrocephaly	-	LA	-	-	-	-
PED3886	*GNAQ*	p.(Arg183Gln)	7.1	III	Naevus flammeus: extensive F, T, L; anaemic naevus: T	L, mild	-	-	-	-	-	-
PED4046	*GNAQ*	p.(Arg183Gln)	17.0	II	Naevus flammeus: F	F	-	-	-	-	-	-
PED4110	*GNAQ*	p.(Arg183Gln)	4.0	II	Cutis marmorat: extensive F + L; anaemic naevus: F	L, mild	-	-	+	-	-	-
PED4396	*GNAQ*	p.(Arg183Gln)	4.0	IV	Naevus flammeus: F; naevus roseus: L; anaemic naevus: F	Macrocephaly	-	-	+	-	EDM	-
PED4720	*GNAQ*	p.(Arg183Gly)	5.0	IV	Naevus flammeus: F	-	Microcephaly	Seizures, ID, LA	+	-	-	-
PED4849	*GNAQ*	p.(Arg183Gln)	3.8	III	Naevus flammeus: L	L, mild	-	-	-	VV, partial agenesia	-	Arterial hypertension
PED4965	*GNAQ*	p.(Arg183Gln)	6.0	II	Naevus flammeus:	-	-	-	-	VV	-	-

					mild limb							
PED1077	*GNA11*	p.(Arg183Cys)	4.9	II	Cutis marmorata + anaemic naevus: F, L	-	L	ID, LA	-	-	-	Iris heterochromia
PED1085	*GNA11*	p.(Gln209His)	4.0	III	Cutis marmorata + anaemic naevus: T	-	-	-	-	-	-	Arterial hypertension, Renal artery dyslasia
PED1494	*GNA11*	p.(Arg183Cys)	5.0	IV	Naevus roseus + anaemic naevus: F; Cutis marmorata: T, L	-	F	Seizures	+	-	SM, self-resoluting EDM	Epidermal naevus
PED2082	*GNA11*	p.(Arg183Cys)	5.5	III	Naevus flammeus: F; naevus roseus + anaemic naevus: F, T, L	-	-	LA	+	-	Naevus spilus	-
PED2363	*GNA11*	p.(Arg183Cys)	3.0	II	Naevus flammeus: F, L; cutis marmorata + anaemic naevus: T, L	-	mild LL	-	-	-	-	-
PED2579	*GNA11*	p.(Arg183His)	3.0	II	Cutis marmorata + anaemic naevus: T, LL	-	mild LL	-	-	-	-	-
PED2846	*GNA11*	p.(Arg183Cys)	4.0	II	Naevus roseus: F; Cutis marmorata + anaemic naevus: T, L	-	-	-	-	-	-	-
PED3078	*GNA11*	p.(Arg183Cys)	6.0	II	Naevus flammeus: F, T, L	L, mild	-	Seizures, LA brain atrophy	-	VV	-	Scoliosis, lipoma, Arterial hypertension, Renal atrophy
PED3577	*GNA11*	p.(Arg183Cys)	7.1	III	Naevus roseus + anaemic naevus: F; cutis marmorata: T, L	-	-	Seizures, ID, LA	+	-	SM	-
PED3767	*GNA11*	p.(Arg183Cys)	3.7	III	Cutis marmorata + anaemic naevus: LL	-	-	-	-	-	CALS	-
PED4037	*GNA11*	p.(Arg183Cys)	3.5	III	Naevus roseus: F; cutis marmorata + anaemic naevus: F, T, L	-	-	-	-	-	EDM	-
PED4729	*GNA11*	p.(Arg183Cys)	14.0	NA	NA	LL, mild	-	-	-	-	Bilateral SM	-

F: Face; L: Limbs, EDM: Extensive dermal melanocytosis; SM: Scleral melanocytosis; ID: Intellectual disability; LA: Leptomeningeal angiomatosis; VV: Varicose vein; UL: Upper Limb; LL: Lower limb; NA: Not available.

Supplementary Table 1 : Genotype and phenotype from the 32 affected individuals.