Journal Pre-proof

GNA11 mutation as a cause of Sturge Weber Syndrome - expansion of the phenotypic spectrum of G-protein related mosaicism and the associated clinical diagnoses

Satyamaanasa Polubothu, Lara A-Olabi, Maria Carmen del Boente, Alisha Chacko, Georgios Eleftheriou, Mary Glover, David Jiménez-Gallo, Elizabeth A. Jones, Debra Lomas, Regina Fölster-Holst, Samira Syed, Monika Tasani, Anna Thomas, Martin Tisdall, Antonio Torrelo, Sarah Aylett, Veronica A. Kinsler

PII: S0022-202X(19)33478-5

DOI: https://doi.org/10.1016/j.jid.2019.10.019

Reference: JID 2220

To appear in: *The Journal of Investigative Dermatology*

Received Date: 29 June 2019

Revised Date: 1 October 2019

Accepted Date: 6 October 2019

Please cite this article as: Polubothu S, A-Olabi L, Carmen del Boente M, Chacko A, Eleftheriou G, Glover M, Jiménez-Gallo D, Jones EA, Lomas D, Fölster-Holst R, Syed S, Tasani M, Thomas A, Tisdall M, Torrelo A, Aylett S, Kinsler VA, *GNA11* mutation as a cause of Sturge Weber Syndrome - expansion of the phenotypic spectrum of G-protein related mosaicism and the associated clinical diagnoses, *The Journal of Investigative Dermatology* (2020), doi: https://doi.org/10.1016/j.jid.2019.10.019.

Title

GNA11 mutation as a cause of Sturge Weber Syndrome - expansion of the phenotypic spectrum of G-protein related mosaicism and the associated clinical diagnoses

Authors

Satyamaanasa Polubothu[1,2], Lara A-Olabi[1], Maria Carmen del Boente[3], Alisha Chacko[2], Georgios Eleftheriou[2], Mary Glover[2], David Jiménez-Gallo[4], Elizabeth A Jones[5,6], Debra Lomas[2], Regina Fölster-Holst[7], Samira Syed[2], Monika Tasani[2], Anna Thomas[1], Martin Tisdall[8,] Antonio Torrelo[9], Sarah Aylett[10], Veronica A Kinsler[1,2]

Affiliations

1. Genetics and Genomic Medicine, University College London GOS Institute of Child Health, London, UK
2. Paediatric Dermatology, Great Ormond St Hospital for Children NHS Foundation Trust, London, UK
3. Dermatology, Hospital del Niño Jesús, Pasaje Bertrés 224, 4000 San Miguel de Tucumán, Argentina
4. Dermatology, Puerta del Mar University Hospital, Cádiz, Spain
5. Manchester Centre for Genomic Medicine, Division of Evolution & Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK
6. Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Health Innovation Manchester, Manchester, UK

7. Campus Kiel, Dermatologie, Venerologie und Allergologie, Universitätsklinikum Schleswig-Holstein, Rosalind-Franklin-Str. 7, 24105, Kiel, Deutschland
8. Department of Neurosurgery, Great Ormond St Hospital for Children NHS Foundation Trust, London, UK
9. Dermatology, Hospital del Niño Jesús, Madrid, Spain
10. Paediatric Neurology, Great Ormond St Hospital for Children NHS Foundation Trust, London, UK

Corresponding author

Professor Veronica Kinsler, Genetics and Genomic Medicine, University College London Great Ormond Street Institute of Child Health, London WC1N 1EH; 020 7242 9789; v.kinsler@ucl.ac.uk; **Twitter Handle** @KinslerLab

ORCiD IDs

Satyamaanasa Polubothu 0000-0001-7195-5670

Lara Al-Olabi 0000-0001-9947-6989

Alisha Chacko 0000-0002-3596-4462

George Eleftheriou 0000-0001-5674-9086

Maria Carmen del Boente 0000-0001-9390-6079

Mary Glover 0000-0002-6789-7241

David Jiménez-Gallo 0000-0003-1161-6698

Elizabeth Jones 0000-0002-0059-8829

Debra Lomas 0000-0003-4490-0038

Regina Fölster-Holst 0000-0001-9114-2351

Samira Syed 0000-0002-3872-5870

Monika Tasani 0000-0002-8139-3059

Anna C Thomas 0000-0001-6586-465X

Antonio Torrelo 0000-0002-5940-6916

Martin Tisdall 0000-0001-8880-8386

Sarah Aylett 0000-0001-9630-3222

Veronica A Kinsler 0000-0001-6256-327X

To the Editor,

GNA11 and *GNAQ* are highly homologous genes encoding different Gα subunits of the Gαq subfamily of heterotrimeric G-proteins. *GNAQ* mutation mosaicism has previously been found to cause Sturge-Weber syndrome (SWS) and isolated capillary malformations(Shirley et al., 2013). We recently described post-zygotic activating mutations in *GNA11* or *GNAQ* as causes of Phakomatosis pigmentovascularis (PPV)(Thomas et al., 2016), a group of conditions defined by the presence of both pigmentary and vascular birthmarks(Happle, 2005, Ota, 1947), and *GNAQ* mosaicism as a cause of Extensive or atypical dermal melanocytosis (EDM)(Thomas et al., 2016). Subsequently *GNA11* mosaicism was identified as a cause of capillary malformation and overgrowth in a limb(Couto et al., 2017). Systematic phenotypic description and phenotype-genotype studies have not yet been performed across this disease spectrum. Of note, somatic pathogenic variants in *GNAQ* and *GNA11* have been described in melanocytic tumours(Van Raamsdonk et al., 2009, Van Raamsdonk et al., 2010), and in congenital haemangiomas(Ayturk et al., 2016).

In this international cohort study of 45 patients, of whom 44 were children and 39 were recruited sequentially and prospectively from a single centre, we used deep phenotyping and DNA sequencing of skin biopsies to characterise the clinical spectrum, analyse phenotype-genotype correlations, and delineate predictors of adverse outcomes. Inclusion criteria were a clinical diagnosis of SWS, PPV or EDM. Cutaneous features recorded were the presence or absence of types of capillary malformation (port-wine stain; reticulate patterning; naevus anaemicus), types of pigmented lesion (dermal melanocytosis; café-au-lait macule; naevus spilus), and involvement of the forehead area (as previously

defined(Waelchli et al., 2014)) by vascular and/or pigmentary lesions. EDM was specifically defined, to distinguish it from common Mongolian blue spots, as 1) extending outside the lumbosacral area and 2) one of either not fading after the age of one year, or having very clearly delineated edges, or being very deeply pigmented.

DNA was extracted directly from affected skin from all 45 patients, with informed written consent under local Research Ethics Committee approval. All samples were sequenced for *GNAQ* and *GNA11* hotspot mutations affecting codons 183 and 209 in both genes, as previously described(Thomas et al., 2016) – briefly, initially by Sanger sequencing with restriction enzyme digest, and if negative by deep targeted next generation sequencing with a sensitivity of 1% mutant allele detection. Statistical analysis of two adverse clinical outcomes were modelled by multiple logistic regression on the basis of phenotype and genotype, namely any neurological or any ophthalmological abnormalities.

Clinical phenotyping data and outcome frequencies are shown in **Table 1**. Certain features are of note. Firstly, the majority of patients with PPV, independent of the subtype, developed multiple café-au-lait macules (CALMs) over time. This was also seen in EDM but not in SWS. Some of these CALMs would be considered “typical”, but most were atypical, with irregular or broken up edges, and/or large in size(Fois et al., 1993). Secondly, macrocephaly was seen in 9% where head circumference had been documented, all of whom had capillary malformations as part of the phenotype. Thirdly, asymmetry of growth was seen in 35%. Fourthly, two patients had renal hypertension. The first patient presented incidentally following a routine BP measurement but later developed severe headaches and proteinuria and was found to have an enlarged oedematous left

kidney with one renal cyst. The second was diagnosed during investigation for recurrent acute life-threatening events due to a central apnoea and was found to have stenosis of the superior right renal artery. Fifthly, one patient with PPV developed hypopigmented mycosis fungoides, and one patient with EDM developed a dermatofibromasarcoma protuberans. And lastly, two patients with PPV (type II or cesioflammea) died, both cases referred in for genotyping, so this may be a higher incidence than a true prospective cohort figure. One of these died in infancy, with recurrent apnoea, and classical changes of SWS on MRI/MRA, and the second died in adulthood, after sudden onset of multiple intracranial haemorrhages with no discoverable underlying MRI/MRA vascular abnormality, and no hypertension.

GNAQ or *GNA11* mutations were found in 49% of patients with these clinical diagnoses (**Table 1)**. Those with a diagnosis of SWS or PPV had a higher percentage of mutation positivity (71% and 63% respectively) than those with EDM (14%). Notably, one patient with SWS was *GNA11* mosaic, presenting with bilateral extensive (including forehead) reticulate vascular capillary malformation, glaucoma, hypotrophy of the right face and right leg, and a normal MRI/MRA (**Figure 1**). In addition, a *GNAQ*-mutant SWS patient could also have been classified as naevus vascularis mixtus(Hamm and Happle, 1986), bringing this diagnosis also into this genotypic spectrum.

A cutaneous vascular lesion in the previously delineated “forehead area” was confirmed to be a strong predictor of neurological and ophthalmological abnormalities as previously described(Waelchli et al., 2014) (OR 8.5, 95%CI 1.8-39.7, p=0.006, and OR 60, 95%CI 4.7-763.0, p=0.002 respectively). There were no neurological findings or glaucoma in SWS or PPV where there was no vascular involvement of the forehead area, however there was one

case of abnormal vasculature on MRI/MRA in a child with a clinical diagnosis of EDM, without cutaneous vascular lesions. Pigmentary lesions in the forehead area, without accompanying vascular lesions, were not used as an independent variable as the numbers were too low, however there are known associations of oculodermal melanocytosis with glaucoma and ocular melanoma(Shields et al., 2011, Teekhasaenee et al., 1990).

Genotype did not affect adverse outcome measures in childhood. Phenotypic modelling by genotype was not undertaken due to small numbers in each phenotypic variable, however, these data hint *GNA11* mosaicism may be associated with a telangiectatic capillary malformation, naevus vascularis mixtus and hypotrophy, and the cohort size is still too small to preclude these associations as a future possibility. Our recommendations for urgent ophthalmological assessment and for routine CNS MRI/MRA after birth for children with SWS and PPV continue for any child with a capillary malformation involving any part of the defined forehead area(Waelchli et al., 2014), and for anyone with relevant symptoms. This enables early detection and treatment of glaucoma, and of early detection of abnormal neurovasculature and consideration for prophylaxis with aspirin and/or anticonvulsants. Our in-house recommendations for EDM currently are for any child with this diagnosis as defined here to have a screening ophthalmological examination after birth or if any symptoms arise. For EDM we do not currently perform MRI/MRA in the absence of symptoms. Although the risk of cutaneous or ocular melanoma arising in patients with PPV and EDM appears to be relatively low, it is important that families and ophthalmologists are made aware of this as a possibility. Lastly, subsequent to this cohort study, we recommend that clinicians are alert to the possibilities of macrocephaly, hypertension, and asymmetric growth in children from birth, and throughout childhood.

Data availability

No datasets were generated or analysed during the current study.

Conflict of interest

The authors state no conflict of interest.

Acknowledgements

We gratefully acknowledge the participation of all patients and families in this study, and research coordination by Mrs Jane White. VAK, AT and the work presented in this study were funded by the Wellcome Trust (Grant WT104076MA). SP was funded by Caring Matters Now Charity and by the Newlife Foundation. The work was supported by the GOSHCC Livingstone Skin Research Centre, and by the UK National Institute for Health Research through the Biomedical Research Centre at Great Ormond St Hospital for Children NHS Foundation Trust, and the UCL GOS Institute of Child Health.

CREdiT statement

Conceptualisation: VK

Formal analysis: VK, SP

Writing: VK, SP

Investigation: SP, LA-O, AT

Figure preparation: SP

Resources: VK, SP, MCB, AC, GE, MG, DJ-G, EAJ, DL, RF-H, SS, MT, MT, AT and SA

Reviewing and editing: All authors

References

Ayturk UM, Couto JA, Hann S, Mulliken JB, Williams KL, Huang AY, et al. Somatic Activating Mutations in GNAQ and GNA11 Are Associated with Congenital Hemangioma. American journal of human genetics 2016;98(4):789-95.

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.

Fois A, Calistri L, Balestri P, Vivarelli R, Bartalini G, Mancini L, et al. Relationship between cafe-au-lait spots as the only symptom and peripheral neurofibromatosis (NF1): a follow-up study. European journal of pediatrics 1993;152(6):500-4.

Hamm H, Happle R. [Mixed vascular nevus. Report of 4 cases]. Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete 1986;37(7):388-92.

Happle R. Phacomatosis pigmentovascularis revisited and reclassified. Archives of dermatology 2005;141(3):385-8.

Ota M, Kawamura T, Ito N. . Phakomatosis pigmentovascularis. Japanese Journal of Dermatology 1947; 52:1-31.

Shields CL, Kligman BE, Suriano M, Viloria V, Iturralde JC, Shields MV, et al. Phacomatosis pigmentovascularis of cesioflammea type in 7 patients: combination of ocular pigmentation (melanocytosis or melanosis) and nevus flammeus with risk for melanoma. Archives of ophthalmology (Chicago, Ill : 1960) 2011;129(6):746-50.

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. The New England journal of medicine 2013;368(21):1971-9.

Teekhasaenee C, Ritch R, Rutnin U, Leelawongs N. Ocular findings in oculodermal melanocytosis. Archives of ophthalmology (Chicago, Ill : 1960) 1990;108(8):1114-20.

Thomas AC, Zeng Z, Riviere JB, 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. The Journal of investigative dermatology 2016;136(4):770-8.

Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature 2009;457(7229):599-602.

Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, et al. Mutations in GNA11 in uveal melanoma. The New England journal of medicine 2010;363(23):2191-9.

Waelchli R, Aylett SE, Robinson K, Chong WK, Martinez AE, Kinsler VA. New vascular classification of port-wine stains: improving prediction of Sturge-Weber risk. The British journal of dermatology 2014;171(4):861-7.

Table 1

Demographic and phenotypic features and outcomes of the cohort of 45 patients, by genotype. Where the totals do not add to 45 this is due to missing data. This cohort includes eight patients from our previous publication(Thomas et al., 2016). In 21/22 cases these affected codons 183 of *GNAQ* (p.(R183Q)) or *GNA11* (p.(R183C) in all except one p.(R183S)(Thomas et al., 2016)) and in one case of EDM the mutation affected codon 209 (p.(Q209P)(Thomas et al., 2016)). We have subdivided capillary malformations into port wine stain/naevus flammeus, and pale pink telengiectatic naevi, to relect recent publications suggesting clinical subdivision may be helpful.

Feature, diagnosis or outcome	*GNAQ mutant*	*GNA11 mutant*	*Double wild type*	*Total n (%)*
Male	9	5	4	18/45 (40%)
White Caucasian	6	2	5	13/42 (31%)
Diagnosis of SWS	4	1	2	7/45 (16%)
Diagnosis of PPV	8	7	9	24/45 (69%)
Diagnosis of EDM	2	0	12	14/45 (31%)
Presence of any capillary malformation	11	8	10	29/45 (64%)
Presence of port wine stain (naevus flammeus)	9	3	4	16/45 (36%)
Presence of a pale pink capillary malformation (naevus roseus)	1	0	3	4/45 (9%)
Presence of reticulate or telengiectatic vascular patterning	5	5	1	11/45 (24%)
Presence of naevus anaemicus	1	2	0	3/45 (7%)
Presence of pigmentary abnormalities of any type	10	7	18	35/43 (81%)
Presence of dermal melanocytosis	9	4	14	27/42 (64%)
Presence of café-au-lait macular pigmentation	5	3	7	15/36 (42%)

Forehead involvement vascular	6	4	4	14/39 (36%)
Forehead involvement pigmentary	3	2	3	8/41 (20%)
Forehead involvement either vascular or pigmentary	7	5	7	19/40 (48%)
Epidermal naevus	0	0	0	0/45 (0%)
Hypertrophy	6	2	3	11/37 (28%)
Hypotrophy	0	1	1	2/37 (5%)
Macrocephaly	2	1	0	3/34 (9%)
Ophthalmological abnormalities	7	4	2	13/28 (46% of those tested)
Glaucoma	6	3	2	11/30 (37% of those tested)
Heterochromia irides	0	1	0	1/36 (3%)
Any neurological abnormality	8	3	4	15/41 (37%)
Neurodevelopmental abnormalities	7	2	2	11/41 (27%)
Seizures	6	0	2	8/42 (19%)
MRI/MRA head abnormalities	6	1	4	11/20 (55% of those scanned)
All other internal organ anomalies (including renal)	0	3	0	3 (however most not screened)
Renal or renovascular anomalies	0	2	0	2 (however most not screened)
Hypertension	0	2	0	2 (however most not measured)

Figure Legends

Figure 1 - Clinical features of *GNA11*- variant Sturge Weber syndrome

Clinical features of the patient with *GNA11*-variant Sturge Weber syndrome at birth and with a congenital telangiectatic capillary malformation on the right side of the face extending onto the forehead, subtle facial asymmetry, and iris heterochromia (a-c). There is extension of the capillary malformation onto right arm and leg with associated right leg undergrowth (d-f). Pictures a, b and d were taken at 4 months of age, and pictures c, e and f at the six years of age.

The parent/guardian of this patient consented to the publication of the images.