Published in final edited form as:

*J Invest Dermatol*. 2021 March ; 141(3): 685–688. doi:10.1016/j.jid.2020.03.978.

# Identification of a mosaic activating mutation in *GNA11* in atypical Sturge-Weber Syndrome

**Jeremy Thorpe**[1], **Laurence P. Frelin**[2], **Meghan McCann**[2], **Carlos A. Pardo**[3], **Bernard A. Cohen**[4], **Anne M. Comi**[2,3,5,&], **Jonathan Pevsner**[1,2,6,&,*]

[1]Program in Biochemistry, Cellular and Molecular Biology, Johns Hopkins School of Medicine, Baltimore, MD, USA

[2]Department of Neurology, Kennedy Krieger Institute, Baltimore, MD, USA

[3]Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD, USA

[4]Department of Dermatology, Johns Hopkins School of Medicine, MD, USA

[5]Department of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD, USA

[6]Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD, USA

To the Editor:

Sturge-Weber syndrome (Online Mendelian Inheritance in Man #185300) is a capillary malformation condition (Bichsel and Bischoff, 2019, Comi, 2015). Affected regions include the skin (typically with facial cutaneous vascular malformations called port-wine birthmarks), brain (often resulting in seizures, intellectual disability, and recurrent stroke-

*To whom correspondence should be addressed. Jonathan Pevsner, Ph.D., Dept. of Neurology, Kennedy Krieger Institute, 707 N. Broadway, Baltimore, MD 21205, Telephone: (443)923-2686, pevsner@kennedykrieger.org.

&Drs. Comi and Pevsner contributed equally to senior authorship.

Author contributions

Conceptualization: AMC, JP
Data Curation: JT, JP
Formal Analysis: JT, JP
Funding Acquisition: AMC, JP
Investigation: JT, LPF, MM, CAP, BAC, AMC, JP
Methodology: JT, LPF, MM, CAP, BAC, AMC, JP
Project Administration: AMC, JP
Resources: AMC, JP
Software: JT, JP
Supervision: AMC, JP
Validation: JT, LPF, MM, CAP, BAC, AMC, JP
Visualization: MM, CAP, BAC, AMC
Writing – JT, AMC, JP
Writing – AMC, JP



Data Availability Statement

Data from this study are available by contacting pevsner@kennedykrieger.org.

Conflict of Interest Statement

The authors declare no conflicts of interest.

like episodes), and eye (often causing glaucoma). We (Shirley et al., 2013) and others (Frigerio et al., 2015, Nakashima et al., 2014) reported that 90% of individuals with SWS or nonsyndromic PWB have a mosaic, activating mutation in *GNAQ*, encoding G protein subunit alpha q (Gαq). The same mutation at other sites on the skin can also result in cutaneous vascular and soft tissure overgrowth and underlying malformations (Gao et al., 2017, Ma et al., 2018, Tan et al., 2016). Identification of other noncanonical pathogenic mutations may further elucidate the pathophysiology of SWS and/or PWB as well as define genetic subtypes.

Based on targeted next-generation sequencing, we identified five individuals with SWS and/or PWB who were negative for Gαq R183Q mutations (Table 1). These studies were performed with Johns Hopkins Institutional Review Board approval. Written informed consent was obtained by the NIH NeuroBioBank that provided samples. We set the lower threshold for calling mutations at 10 times the sequencing error rate for Q30 base quality scores, i.e. a 1% mutant allele frequency. One case (SWS 3) had mutant allele frequencies of 0.58% and 0.22% in two independent affected brain samples. These values were below the 1% threshold for calling a mutation but significantly above background frequencies of other samples (range 0.02% to 0.12%, n=10, $p<0.00074$ two-tailed t-test).

We next performed whole exome sequencing and somatic variant calling to identify candidate causal mutations (Table 1). Case SWS 3 had 20 reads covering the *GNAQ* p.R183Q locus with 18 reference C reads, one T (5%) predicted to produce the R183Q mutation, and 1 A (5%) predicted to be synonymous. Targeted amplicon sequencing of exon 4 of *GNAQ* validated a T minor allele frequency (MAF) of 0.27% (22/8,030 reads) in SWS 3, and revealed a 0.1% (9/8,028) MAF in a control (NA12878). We conclude that the mutant allele frequency was less than the conservative threshold of 1% or greater variant nucleotides, but this low-level mosaic variation was nonetheless pathogenic. We suggest that, in an appropriate clinical or research setting, a lower threshold of ≥0.25% for calling a mutation may be justified, particularly when supported by large read depth by targeted amplicon sequencing as reported here. Our results are consistent with those of Uchiyama et al. who suggested a minimum MAF threshold of 1% by next-generation sequencing, 0.25% using droplet digital PCR, and 0.1% using peptide nucleic acid combined with ddPCR (Uchiyama et al., 2016).

Case SWS 2, a 63-year old male, had negligible levels of *GNAQ* R183Q by targeted sequencing (0.02% and 0.06% in DNA from two separate brain samples) or by exome sequencing (22 C reads, 0 alternate alleles). However, we identified a 6.1% mutant allele frequency of *GNA11* p.R183Q mutation (93 C residues, 6 T residues) by whole exome sequencing, a finding validated by targeted next-generation sequencing in three separate affected tissue samples (MAFs in three brain regions were 0.7%, 1.9%, and 1.9%; MAF was 5.9% in affected skin and 0.06% in unaffected skin, and 0.12% in control individual NA12878) (Table 2). The human *GNA11* gene encodes G protein subunit alpha 11 (Gα11, NP_002058.2, 359 amino acids) that shares 90% amino acid identity with Gαq (NP_002063.2; also 359 amino acids). Germline mutations in *GNA11* can cause hypocalciuric hypercalcemia type II (HHC2; # 145981) and hypocalcemia dominant 2 (HYPOC2; OMIM # 615361). Couto et al. (Couto et al., 2017) reported a mosaic, activating

missense mutation in *GNA11* (c.547C >T; p.Arg183Cys) in three patients with a diffuse capillary malformation of an extremity, while Thomas et al. (Thomas et al., 2016) identified *GNA11* p.R183C or p.R183S in four patients with phakomatosis pigmentovascularis (PPV), including sites of vascular and pigmentary skin lesions. Associated pigmentary lesions in PPV include melanocytic nevi, café au lait macules, dermal melanocytosis and epidermal nevi and vascular lesions including capillary and venous malformations and nevus anemicus (Fernandez-Guarino et al., 2008). *GNA11* activating mutations have also been reported in uveal melanoma and blue nevi (Van Raamsdonk et al., 2010). Subject SWS 2 was diagnosed with “Meningeal Angiomatosis/clinical suspicion of Sturge-Weber syndrome” on post-mortem pathology; however there were atypical features both clinically and pathologically. He had extensive port-wine involvement of the face and body on his right side, but no history of seizures, hemiparesis or neurologic deficits prior to his acute hypertensive event, left thalamic and right sub-arachnoid bleeds, and infarct. Post-mortem macroscopic neuropathologic evaluation revealed thickened, vascular congested leptomeninges, with what appeared to be an under-developed vascularity. Microscopic evaluation demonstrated dilated venules, degenerated arterial walls with thickening and myxoid substance, calcification, and evidence of macrophage accumulation. Additional patients exhibiting pathologic *GNA11* mutations and features which overlap with SWS are needed to determine if they all display a milder neurologic phenotype, and later neurologic onset with hemorrhage, than the typical patient with SWS and a R183Q somatic mutation in *GNAQ*.

## Acknowledgements

We thank individuals with Sturge-Weber syndrome and their families for participating in this study. We are grateful to the NIH NeuroBioBank for providing us the samples. We thank Macrogen Clinical Laboratories for kindly donating resources (to J.P.) to perform whole exome sequencing. We thank N. Varg for helpful discussions. A.M.C. was supported by funding from the Celebrate Hope Foundation and the Faneca 66 Foundation. J.P. was supported by NIH grants U01 MH106884 and U54 HD079123, and by the Sturge-Weber Foundation. This work was supported by grants from the National Institutes of Health (Lawton, Comi, and Marchuk) [grant number U54NS065705]. The Brain Vascular Malformation Consortium [grant number U54NS065705] is a part of the NIH Rare Diseases Clinical Research Network (RDCRN), supported through the collaboration between the NIH Office of Rare Diseases Research (ORDR) at the National Center for Advancing Translational Science (NCATS) and the National Institute of Neurological Disorders and Stroke (NINDS).

## References

Bichsel C, Bischoff J. A somatic missense mutation in GNAQ causes capillary malformation. Curr Opin Hematol2019;26(3):179–84. [PubMed: 30870248]

Comi AM. Sturge-Weber syndrome. Handb Clin Neurol2015;132:157–68. [PubMed: 26564078]

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. Angiogenesis2017;20(3):303–6. [PubMed: 28120216]

Exome Aggregation C. http://exac.broadinstitute.org/gene/ENSG00000173612; 2019[accessed.

Fernandez-Guarino M, Boixeda P, de Las Heras E, Aboin S, Garcia-Millan C, Olasolo PJ. Phakomatosis pigmentovascularis: Clinical findings in 15 patients and review of the literature. J Am Acad Dermatol2008;58(1):88–93. [PubMed: 18045734]

Frigerio A, Wright K, Wooderchak-Donahue W, Tan OT, Margraf R, Stevenson DA, et al.Genetic Variants Associated with Port-Wine Stains. PLoS One2015;10(7):e0133158. [PubMed: 26192947]

Gao L, Yin R, Wang H, Guo W, Song W, Nelson JS, et al.Ultrastructural characterization of hyperactive endothelial cells, pericytes and fibroblasts in hypertrophic and nodular port-wine stain lesions. The British journal of dermatology2017;177(4):e105–e8. [PubMed: 28182251]

Ma G, Yu Z, Liu F, Wang L, Yu W, Zhu J, et al.Somatic GNAQ mutation in different structures of port-wine macrocheilia. The British journal of dermatology2018;179(5):1109–14. [PubMed: 29878304]

Nakashima M, Miyajima M, Sugano H, Iimura Y, Kato M, Tsurusaki Y, et al.The somatic GNAQ mutation c.548G>A (p.R183Q) is consistently found in Sturge-Weber syndrome. J Hum Genet2014;59(12):691–3. [PubMed: 25374402]

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 Med2013;368(21):1971–9. [PubMed: 23656586]

Tan W, Nadora DM, Gao L, Wang G, Mihm MC Jr., Nelson JS. The somatic GNAQ mutation (R183Q) is primarily located within the blood vessels of port wine stains. J Am Acad Dermatol2016;74(2):380–3. [PubMed: 26775782]

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. J Invest Dermatol2016;136(4):770–8. [PubMed: 26778290]

Uchiyama Y, Nakashima M, Watanabe S, Miyajima M, Taguri M, Miyatake S, et al.Ultra-sensitive droplet digital PCR for detecting a low-prevalence somatic GNAQ mutation in Sturge-Weber syndrome. Sci Rep2016;6:22985. [PubMed: 26957145]

Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, et al.Mutations in GNA11 in uveal melanoma. N Engl J Med2010;363(23):2191–9. [PubMed: 21083380]

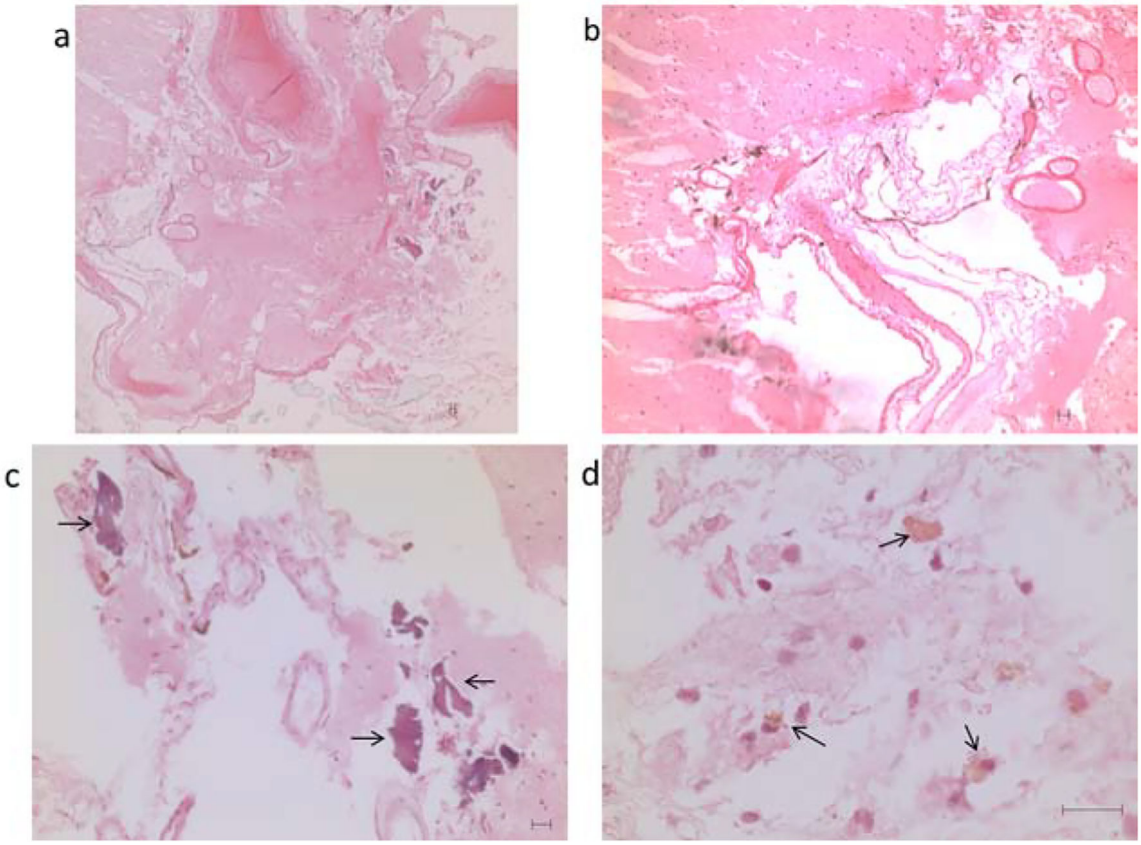


**Figure 1.**

Hematoxylin and eosin stained sections from post-mortem cortex of Case SWS 2. A. Low magnification image of the superficial cerebral cortex and leptomeninges. Abnormally dilated venous vessels and degenerating arteriolar vessels seen, along with evidence of calcification. B. High magnification of thickened leptomeninges. C. Detailed image of meningeal calcification (arrows). D. High magnification images of leptomemingeal macrophages accumulated in the subarachnoid compartment (arrows). All images bar magnification 20 μM.

**Table 1.**

Demographs and results of targeted GNAQ p.R183 sequencing and whole exome sequencing.

| Case | Age | Sex | Race[1] | Sample | region (source)[2] | Status[3] | GNAQ AF[4] | WES | WES Candidates[5] |
|---|---|---|---|---|---|---|---|---|---|
| SWS 1 | 15y 89 d | F | C | SWS1-B | brain (surg) | Aff | 0.10 | 1 | none |
| SWS 2 | 63y 235d | M | C | SWS2-B | brain (PM) | Aff | 0.06 | 2 | none |
| | | | | SWS2-BPC | brain (PM) | Aff | ND | | |
| | | | | SWS2-SA | skin (PM) | Aff | 0.02 | 3 | GNA11 p.R183C |
| | | | | SWS2-SA2 | skin (PM) | Aff | ND | | |
| | | | | SWS2-SUA | skin (PM) | Unaff | ND | 4 | none |
| SWS 3 | 2y 6mo | M | U | SWS3-BA | brain (surg) | Aff | 0.58 | | |
| | | | | SWS3-B1 | brain (surg) | Aff | 0.22 | 5 | GNAQ p.R183Q |
| | | | | SWS3-B2 | brain (surg) | Aff | ND | | |
| SWS 4 | 19y 352 d | M | C | SWS4-B1 | brain (PM) | Aff | 0.05 | 6 | none |
| | | | | SWS4-B2 | brain (PM) | Aff | 0.12 | | |
| SWS 5 | 5y 12d | F | C | SWS5-BU | brain (PM) | Unaff | 0.05 | 7 | none |
| | | | | SWS5-BA | brain (PM) | Aff | 0.07 | 8 | GPRC6A[6] |

1. Ethnicity was Caucasian (C) or unknown (U).

2. Brain samples were from right hemisphere (SWS2) or bilateral (SWS1, SWS4, SWS5). Surg, surgical specimen; PM, postmortem.

3. Samples were from regions presumed to be affected (Aff) or unaffected (Unaff).

4. Targeted amplicon sequencing of *GNAQ* exon 4 was performed using forward primer 5'-GGGTATTCGATGATCCCTGTGGTGGG-3' and reverse primer 5'-CCTTTCCGTAGACAGCTTTGGTGTGATG-3".

5. Whole exome sequencing was performed on eight samples using the Illumina HiSeq 2500 platform with Agilent SureSelect_V5 enrichment kit targeting 50,390,601 base pairs. Read length was 150 base pairs with total reads ranging from 35.9 to to 40.4 million. The mean depth of coverage of targeted regions was 69X.

6. We identified a candidate gene mutation in case SWS 5, which had no evidence for mosaic mutations in *GNAQ* or *GNA11*. Utilizing DNA from affected brain tissue, we identified a mosaic mutation in G protein-coupled receptor class C group 6 member A (*GPRC6A*). DNA from affected brain tissue had a mosaic predicted nonsense mutation (63 C residues, 4 A residues; 6.0% mutant frequency). The GPRC6A G-protein coupled receptor senses high concentrations of extracellular calcium (and possibly amino acids and/or osteocalcin) and couples to Gαq (Pi et al., 2005). However, germline loss of function variants are extremely common (e.g. 6% population allele frequency for SNP rs550458778, and 39 loss of function variant alleles listed in the ExAC database (Exome Aggregation, 2019)). We therefore conclude that, while the mutation we observed appears somatic rather than germline, it is not pathogenic.

**Table 2.**

Targeted sequencing data for *GNA11*.

| | | Nucleotide call | | | | |
|---|---|---|---|---|---|---|
| **Sample** | **Region** | **C** | **A** | **T** | **G** | **AF**[1] |
| SWS 2 | brain | 7990 | 4 | 6 | 1 | 0.07 |
| SWS 2 | brain | 7813 | 9 | 151 | 3 | 1.9 |
| SWS 2 | brain | 7831 | 7 | 157 | 1 | 1.9 |
| SWS 2 | skin (affected) | 7471 | 6 | 469 | 4 | 5.9 |
| SWS 2 | skin (unaffected) | 8011 | 7 | 5 | 0 | 0.06 |
| NA12878 | blood | 7985 | 4 | 10 | 1 | 0.12 |

[1]AF, mutant allele frequency (percent of T/total). Targeted amplicon sequencing of *GNA11* exon 4 was performed using forward primer 5'-GTGCTGTGTCCCTGTCCTG −3' and reverse primer 5'- GGCAAATGAGCCTCTCAGTG −3".