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Somatic *GNAQ* mutation in the *forme fruste* of Sturge-Weber syndrome

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Abstract

Objective

To determine whether the *GNAQ* R183Q mutation is present in the *forme fruste* cases of Sturge-Weber syndrome (SWS) to establish a definitive molecular diagnosis.

Methods

We used sensitive droplet digital PCR (ddPCR) to detect and quantify the *GNAQ* mutation in tissues from epilepsy surgery in 4 patients with leptomeningeal angiomatosis; none had ocular or cutaneous manifestations.

Results

Low levels of the *GNAQ* mutation were detected in the brain tissue of all 4 cases—ranging from 0.42% to 7.1% frequency—but not in blood-derived DNA. Molecular evaluation confirmed the diagnosis in 1 case in which the radiologic and pathologic data were equivocal.

Conclusions

We detected the mutation at low levels, consistent with mosaicism in the brain or skin (1.0%–18.1%) of classic cases. Our data confirm that the *forme fruste* is part of the spectrum of SWS, with the same molecular mechanism as the classic disease and that ddPCR is helpful where conventional diagnosis is uncertain.

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Glossary

ddPCR = droplet digital PCR; **LMA** = leptomeningeal angiomatosis; **SWS** = Sturge-Weber syndrome.

Sturge-Weber syndrome (SWS) is a rare, sporadic neurocutaneous disorder that occurs in 1 in 20,000 newborns, typically characterized by brain pathology—leptomeningeal angiomatosis (LMA), cortical atrophy and calcification, and layer 1 fusion—port-wine stain, and vascular glaucoma.[1] Clinical manifestations and severity are heterogeneous with drug-resistant epilepsy, hemiparesis and cognitive impairment the most common neurologic features, glaucoma the most frequent ocular presentation, and port-wine stain the predominant dermatological feature.[1] Sometimes, the characteristic meningeal lesions of SWS are seen without skin or ocular features[2,3]—this is referred to as *forme fruste* of SWS, or sometimes type III SWS, and diagnosis can be challenging.

A somatic mosaic mutation (c.548G>A; p.R183Q) of the *GNAQ* gene that disrupts the activity of the encoded guanosine triphosphatase is present in classic SWS and also in patients who only have a port-wine stain.[4] This mutation was found in studies from different populations to be present in the brain or skin of more than 80% of patients.[4,5] Enrichment of this mutation in endothelial cells of both SWS skin and brain specimens,[6,7] and SWS brain parenchyma not affected by LMA,[6] has also recently been reported.

Droplet digital PCR (ddPCR) is an ultra-sensitive technique recently reported for detection of the SWS mutation.[5,7] It uses microfluidics and surfactant chemistries to emulsify input DNA into thousands of uniformly sized droplets and then to amplify them with fluorescently labeled TaqMan probes before measuring fluorescence on a droplet reader, as we and others have previously described.[8,9] Based on fluorescence intensity, the number of mutation-positive and wild-type templates is quantified to calculate the frequency of a mutant allele. Here, we used this approach to screen 4 patients with *forme fruste* SWS including 1 in which the diagnosis was equivocal.

Methods

Patients

We ascertained 4 patients with *forme fruste* SWS through our epilepsy surgery programs at Austin Health, Royal Children's Hospital, Melbourne, and the Lady Cilento Children's Hospital, Queensland, Australia. Genomic DNA was extracted from the brain using the DNA Genotek PrepIt 2CD Kit (Ontario, Canada) or Qiagen AllPrep DNA/RNA Kit and peripheral blood using the Macherey-Nagel NucleoBond CB 100 Kit (Duren, Germany) or Qiagen QIAamp DNA Maxi Kit (Hilden, Germany).

Standard protocol approvals, registrations, and patient consents

The Human Research Ethics Committees of The Royal Children's Hospital, Melbourne, Australia (project no. 29077F), and Austin Health, Melbourne, Australia (project no. H2007/02961), approved this study. Informed consent was obtained from the patients, or their parents in the case of minors, for participation in the study.

Droplet digital PCR

We used a commercially available ddPCR Mutation Detection Assay (ID: 10049047; Bio-Rad, Hercules, CA) to detect the *GNAQ* c.548G>A (p.R183Q) mutation and wild-type allele. Briefly, the ddPCR reaction mixture was assembled from a 2× ddPCR Supermix for Probes (No dUTP; Bio-Rad), 20× ddPCR Mutation Detection Assay, and 10 ng of DNA sample to a final volume of 23 μL. Twenty microliters of each reaction mixture was then loaded into the sample well of an 8-channel droplet generator cartridge (Bio-Rad), and droplets were generated with 70 μL of droplet generation oil (Bio-Rad) using the manual QX200 Droplet Generator. Following droplet generation, samples were manually transferred to a 96-well PCR plate, heat-sealed, and amplified on a C1000 Touch thermal cycler using the following cycling conditions: 95°C for 10 minutes for 1 cycle, followed by 40 cycles at 94°C for 30 seconds and 55°C for 60 seconds, 1 cycle at 98°C for 10 minutes and 12°C for infinite. Post-PCR products were read on the QX200 droplet reader (Bio-Rad) and analyzed using QuantaSoft software. We established the detection limit of the ddPCR assay by serially diluting mutant samples with wild-type DNA to obtain different mutant/(mutant + wild-type) ratios: 5%, 1%, 0.5%, 0.25%, and 0.1%. These mixed DNA samples were subjected to ddPCR as described above.

Results

Clinical report

Four patients presented during childhood with *forme fruste* or SWS type III with drug-resistant epilepsy (table 1) and LMA on MRI and histopathology (figures 1, A–C and 2, A–C, figure e-1, links.lww.com/NXG/A48, table 1), without port-wine stains. Fresh-frozen (cases 1, 2, and 4) or formalin-fixed paraffin-embedded (case 3) brain tissue was available following epilepsy surgery. The diagnoses of SWS type III for cases 1, 2, and 4 were definitive based on imaging and pathologic data (figure 1, A–C, figure e-1, links.lww.com/NXG/A48, table 1). In case 3, the diagnosis was less certain, as CT and MRI showed calcification in the left occipital region posteroinferiorly without convincing focal atrophy (figure 2, A and B, table 1). Pathologically, in the subarachnoid plane,

Table 1 Clinical characteristics of *forme fruste* cases of Sturge-Weber syndrome

Case	Leptomeningeal angiomatosis[a]	Seizure onset (mo)	Seizure types	Surgery	Age at surgery (y)	*GNAQ* R183Q, frequency (%) of the mosaic mutation
1	Definite	10	Focal impaired awareness seizures; left hemiclonic seizures	Right temporo-parieto-occipital disconnection	5	7.1
2	Definite	9	Right hemiclonic; focal impaired awareness seizures; myoclonic, atonic	Left temporo-parieto-occipital disconnection	2	5.8
3	Subtle	20	Focal impaired awareness seizures; tonic-clonic	Left occipital lesionectomy	21	2.1
4	Definite	12	Focal impaired awareness seizures; tonic-clonic	Left functional hemispherotomy	7	0.42

[a] Based on imaging and histopathologic analyses.

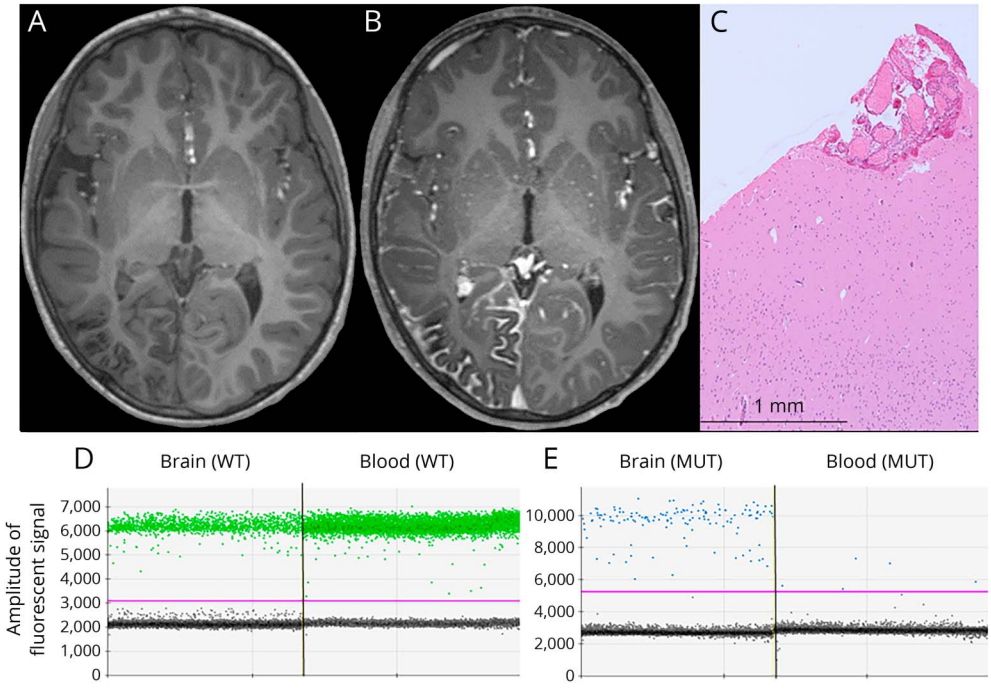
a small vascular malformation was seen with some arterial features, coupled with underlying parenchymal calcification and cortical dyslamination (figure 2C, table 1).

Mutation detection in the brain-derived genomic DNA by ddPCR

We established the detection limit for the *GNAQ* mutation detection ddPCR by assaying serially mixed mutant and wild-type samples in triplicate. The mutant allele at a frequency ≥0.25% was consistently detected, while detection of the mutant allele at 0.1% was only achieved in 2 of the 3 wells (figure e-2, links.lww.com/NXG/A48, table e-1). Thus, the detection limit in our hands was 0.25% mutant allele frequency, comparable with a previously reported limit (0.1%) for a similar assay.[5]

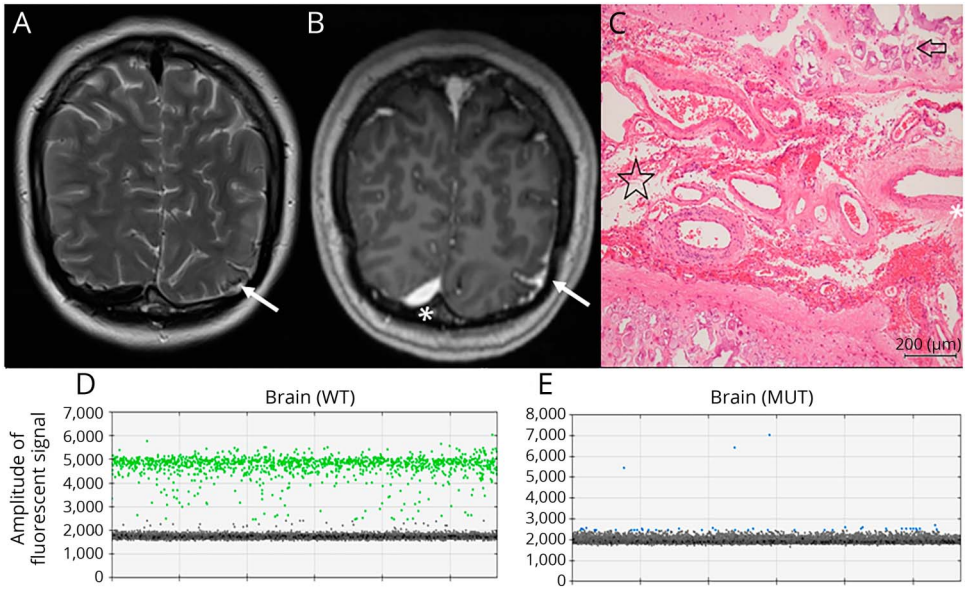
Genomic DNA isolated from the resected brain tissue and that from the peripheral blood were analyzed using ddPCR. The *GNAQ* p.R183Q mutant allele was detected only in genomic DNA extracted from the brain tissue (7.1% frequency in case 1, 5.8% in case 2, 2.1% in case 3, and 0.42% in case 4) but not in genomic blood-derived DNA from 3 patients (figures 1, D, E and 2, D, E, figures e-1, e-3 to e-7, links.lww.com/NXG/A48). Blood-derived DNA was not available from case 3. It should be noted that although very low, the 0.42% mutant allele frequency of case 4 was above our established

Figure 1 Imaging, histopathology, and molecular evaluation of case 1 with definite leptomeningeal angiomatosis



(A) Precontrast T1-weighted axial MRI scan showing right temporal and occipital atrophy and right occipital cortical calcification. (B) Postcontrast T1-weighted axial MRI scan showing leptomeningeal enhancement. (C) Hematoxylin and eosin–stained image of the neocortex showing a small area of densely clustered leptomeningeal vessels. (D) Identification of the wild-type *GNAQ* allele in green (present in the brain and blood) by digital PCR. (E) Identification of the mutant *GNAQ* R183Q allele (in blue) in the brain-derived but not blood-derived DNA—rare blue dots in blood are signal from droplets containing multiple DNA templates (supplemental data, links.lww.com/NXG/A48). Droplets without DNA templates are gray. Y-axis, amplitude of fluorescent signal. WT = wild-type *GNAQ* probe; MUT = mutant *GNAQ* R183Q probe.

Figure 2 Imaging, histopathology, and molecular evaluation of case 3 with subtler MRI findings



(A) Precontrast T2-weighted coronal MRI scan showing subtle signal change and calcification in the left occipital region (arrow) posteroinferiorly involving the occipital cortex or leptomeninges. Calcification was confirmed on CT (not shown). (B) Postcontrast T1-weighted coronal MRI scan showing leptomeningeal enhancement in the same region. Enhancement in the right occipital region (asterisk) is due to the normal transverse sinus. (C) Hematoxylin and eosin stained image showing subarachnoid angiomatosis (starred) between adjacent cerebral gyrae with cortical calcification (arrow). (D) Identification of the wild-type *GNAQ* allele (in green) in the brain by digital PCR. (E) Identification of the mutant *GNAQ* R183Q allele (in blue) in the brain. Droplets without genomic DNA templates are gray. Y-axis, amplitude of fluorescent signal. WT = wild-type *GNAQ* probe; MUT = mutant *GNAQ* R183Q probe.

detection limit (figures e-2, e-3, and e-7, links.lww.com/NXG/A48, table e-1).

For case 1, fluorescent droplets were observed in the blood-derived genomic DNA below the expected amplitude, but these did not overlap with the true positive signal in the brain-derived genomic DNA when fluorescence intensity was viewed on 2D plots (figure e-3, links.lww.com/NXG/A48). Instead, this is fluorescent signal from droplets containing multiple genomic templates, a phenomenon not infrequently observed when running ddPCR assays.

Discussion

The important discovery of a recurrent, somatic *GNAQ* mutation provided the first insights into the molecular biology of SWS. Initial reports focused on classic SWS,[4–6,10,11] and here, we extend these findings to *forme fruste* cases, a far more subtle, sometimes unrecognized, form of SWS. Our findings confirm that *forme fruste* cases are caused by the somatic *GNAQ* p.R183Q mutation present at low to very low levels in brain tissue due to mosaicism, consistent with a few reported cases.[4,11] It is intriguing that the mutation was only present in the brain tissue of these *forme fruste* cases, and not in blood (of 3 cases), suggesting that the mutation may have arisen later during development than for classic cases, although we did not have other tissues available from our cases to confirm this. As MRI and even pathologic diagnosis can be equivocal for subtle LMA lesions, as for case 3 (figure 2, table 1), molecular evaluation may have specific diagnostic value. The relatively low level of the *GNAQ* mutation in the brain tissue of case 3 is consistent with the milder imaging and pathologic manifestations; however, case 4 had an even lower mutant load in terms of percentage mosaicism in the tissue tested, suggesting that there are other, as yet unidientified, influences on genotype-phenotype correlation.

In formalin-fixed paraffin-embedded samples, low-level somatic mosaic mutations are challenging to detect because the DNA is of low quality and often has impurities. Despite these challenges, we were able to identify the somatic mutation in case 3 from a 3-year-old pathologic specimen. This and other sensitive mutation detection technologies are showing increasing utility in elucidating the role of somatic mosaicism in brain-specific neurologic disorders, as shown recently for tuberous sclerosis,[12] in addition to SWS.

Author contributions

M.S.H., I.E.S., and S.F.B. initiated and directed the project. M.S.H., J.A.D., H.D., Z.Y., L.M., E.O., and G.G. performed molecular genetics experiments. A.S.H., S.M., W.M., B.N., M.W., K.P., R.J.L., I.E.S., and S.F.B. conducted clinical phenotyping. R.K. performed histopathologic analyses. M.S.H., N.C.J., P.J.L., A.D., and S.F.B. provided equipment and reagents. M.S.H., A.S.H., S.M., I.E.S., and S.F.B. wrote the paper. All authors discussed the results and commented on the manuscript.

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