

## ORIGINAL ARTICLE

# Sturge–Weber Syndrome and Port-Wine Stains Caused by Somatic Mutation in *GNAQ*

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## ABSTRACT

**BACKGROUND**

The Sturge–Weber syndrome is a sporadic congenital neurocutaneous disorder characterized by a port-wine stain affecting the skin in the distribution of the ophthalmic branch of the trigeminal nerve, abnormal capillary venous vessels in the leptomeninges of the brain and choroid, glaucoma, seizures, stroke, and intellectual disability. It has been hypothesized that somatic mosaic mutations disrupting vascular development cause both the Sturge–Weber syndrome and port-wine stains, and the severity and extent of presentation are determined by the developmental time point at which the mutations occurred. To date, no such mutation has been identified.

**METHODS**

We performed whole-genome sequencing of DNA from paired samples of visibly affected and normal tissue from 3 persons with the Sturge–Weber syndrome. We tested for the presence of a somatic mosaic mutation in 97 samples from 50 persons with the Sturge–Weber syndrome, a port-wine stain, or neither (controls), using amplicon sequencing and SNaPshot assays, and investigated the effects of the mutation on downstream signaling, using phosphorylation-specific antibodies for relevant effectors and a luciferase reporter assay.

**RESULTS**

We identified a nonsynonymous single-nucleotide variant (c.548G→A, p.Arg183Gln) in *GNAQ* in samples of affected tissue from 88% of the participants (23 of 26) with the Sturge–Weber syndrome and from 92% of the participants (12 of 13) with apparently nonsyndromic port-wine stains, but not in any of the samples of affected tissue from 4 participants with an unrelated cerebrovascular malformation or in any of the samples from the 6 controls. The prevalence of the mutant allele in affected tissues ranged from 1.0 to 18.1%. Extracellular signal-regulated kinase activity was modestly increased during transgenic expression of mutant  $G\alpha_q$ .

**CONCLUSIONS**

The Sturge–Weber syndrome and port-wine stains are caused by a somatic activating mutation in *GNAQ*. This finding confirms a long-standing hypothesis. (Funded by the National Institutes of Health and Hunter’s Dream for a Cure Foundation.)

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**A** PORT-WINE STAIN IS A CUTANEOUS capillary malformation (Fig. 1A, 1B, and 1C) that occurs in approximately 3 of every 1000 newborns<sup>1,2</sup> and usually involves the head and neck.<sup>3</sup> The Sturge–Weber syndrome, also known as encephalofacial angiomatosis, is a neurocutaneous disorder that occurs as a sporadic congenital condition; it is characterized by a port-wine stain that affects the skin in the distribution of the ophthalmic branch of the trigeminal nerve (Fig. 1A and 1B) and is associated with venous-capillary abnormalities of the leptomeninges (Fig. 1D, 1E, and 1F) and the eye. It occurs in both male and female newborns, in approximately 1 in 20,000 to 50,000 live births.<sup>1</sup> A child born with a port-wine stain on the face has approximately a 6% chance of having the Sturge–Weber syndrome,<sup>2</sup> and this risk increases to 26% when the port-wine stain is located in the distribution of the ophthalmic branch of the trigeminal nerve.<sup>3</sup> Port-wine stains usually have underlying soft-tissue and bony-tissue overgrowth that may be mild or massive.<sup>4</sup> A long-standing but unproven hypothesis is that the Sturge–Weber syndrome and port-wine stains are caused by the same underlying somatic mutations,<sup>5,6</sup> with the precise clinical manifestations dependent on where and when in the developing fetus the somatic mutation occurs. We tested this hypothesis through whole-genome sequencing of affected and unaffected tissue to identify the causative somatic mutation.

## METHODS

### STUDY OVERSIGHT

All the sequencing was performed with approval from the institutional review board at the Johns Hopkins University or Duke University. Deidentified samples (see Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org) were obtained with written informed consent from persons with the Sturge–Weber syndrome or from the Brain and Tissue Bank for Developmental Disorders of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). The age, sex, race or ethnic group, and syndrome status (apparently non-syndromic port-wine stain or the Sturge–Weber syndrome) of the participants were confirmed and recorded from the source, along with information regarding the surgery, autopsy, or other procedure that was used for obtaining the tissue.

### TISSUE SAMPLES

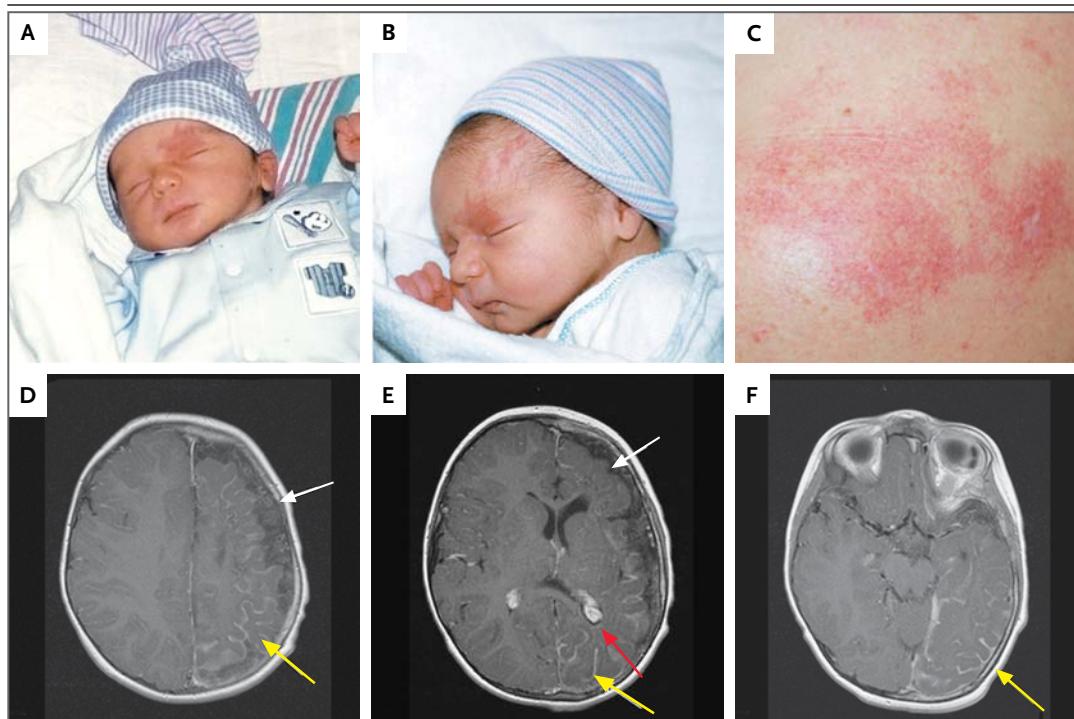
We obtained tissue samples from research participants and from the NICHD Brain and Tissue Bank for Developmental Disorders (Table S1 in the Supplementary Appendix). Included were samples of skin with port-wine stains and of visibly normal skin from participants with the Sturge–Weber syndrome (23 samples from 9 participants), samples of skin with port-wine stains and of visibly normal skin from participants without the Sturge–Weber syndrome (14 samples from 13 participants), samples of brain tissue from persons with the Sturge–Weber syndrome (50 samples from 18 persons), samples of brain tissue from presumably normal persons (controls; 6 samples from 6 persons), and samples of brain tissue from persons with the unrelated cerebral cavernous vascular malformation (4 samples from 4 persons). Amplicon sequencing and single-base extension interrogation (SNaPshot analysis) were used to investigate each tissue sample for the c.548G→A mutation. We assayed SNaPshot specificity by testing brain tissue from 5 normal controls (data not shown). When multiple samplings of biopsied tissue or multiple sequencing assays were performed, we considered the participant to be positive for the mutation if at least 1 tissue sample tested positive ( $\geq 1\%$  mutant allele) and to be negative if every tissue sample tested negative for the mutation ( $< 1\%$  mutant allele).

### WHOLE-GENOME SEQUENCING

Genomic DNA was purified from paired samples of affected tissue and unaffected tissue or blood from three participants (for a total of six samples). Whole-genome sequencing was performed on an Illumina HiSeq 2000 sequencer to a mean per-sample depth of coverage between 33X and 51X. Details regarding the preparation of the samples and bioinformatic methods are provided in the Supplementary Appendix.

### TARGETED AMPLICON SEQUENCING

GNAQ exon 4 and the adjacent intronic sequence were amplified by means of polymerase chain reaction in a two-stage reaction. Paired-end reads were generated with the use of an Illumina MiSeq sequencer and were evaluated at the c.548 position for base calls supporting the c.548G→A mutation. Samples were considered to have a mutation if the percentage of reads supporting a mutation exceeded 1% (10 times the expected base miscall



**Figure 1. Representative Photographs and Magnetic Resonance Imaging (MRI) Scans from Study Participants with the Sturge-Weber Syndrome or Isolated Port-Wine Stains.**

Photographs of a participant with the Sturge-Weber syndrome (Patient 36), obtained at birth, show a facial port-wine stain with a left-sided V1 distribution (Panels A and B). The child began having seizures at 7 months of age. An isolated port-wine stain birthmark on the left shoulder from a participant without the Sturge-Weber syndrome (Patient 10) shows a birthmark that is flat and red without evidence of hypertrophy or cobblestoning or any other associated vascular or lymphatic anomaly (Panel C). Axial contrast-enhanced MRI scans of the brain in a participant with the Sturge-Weber syndrome (Panels D, E, and F; Patient 36 at 17 months of age) show left-sided hemispheric leptomeningeal enhancement (yellow arrows), an enlarged and enhancing left-sided choroid plexus (red arrow), and left hemispheric brain atrophy (white arrows).

rate of 0.1%). Details regarding sequencing adapters, barcodes, and primer sequences are provided in the Supplementary Appendix.

#### PLASMIDS

Two specific mutations, c.548G→A (encoding p.Arg183Gln) and c.626A→T (encoding p.Gln209Leu), were introduced separately into *GNAQ* with the use of primers for site-directed mutagenesis (Table S4 in the Supplementary Appendix). Serum response element plasmid (pSRE)-Luc (Agilent Technologies) and pSV40-RL (Roche) were used as reporter plasmids for the luciferase assay.

#### CELL CULTURE AND WESTERN BLOTTING

Human embryonic kidney (HEK) 293T cell lysates were analyzed by means of Western blotting with the use of standard methods. Details of

culture conditions, antibodies, and methods of Western blotting are provided in the Supplementary Appendix.

#### LUCIFERASE ASSAY

*GNAQ*, *GNAQ* p.Arg183Gln or *GNAQ* encoding p.Gln209Leu, pSRE-Luc, and pSV40-RL were transfected into HEK293T cells, which were lysed after 20 to 24 hours of incubation. At the end of the incubation period, luciferase activity was measured.

#### PRIMER EXTENSION-BASED ASSAY

DNA was extracted from formalin-fixed, paraffin-embedded samples from participants with port-wine stains. Primers designed to amplify exon 4 of *GNAQ* (Table S5 in the Supplementary Appendix) were used to amplify genomic DNA from each of the samples. Amplicons were interrogated

ed for their sequence at position c.548 with the use of the SNaPshot Multiplex Kit (Life Technologies) and analyzed on an ABI Prism 3130 genetic analyzer (Life Technologies). Reference and mutant allele frequencies were calculated on the basis of the area of the resulting peaks.

## RESULTS

### IDENTIFICATION OF *GNAQ* SOMATIC VARIANT

To test the hypothesis that the Sturge–Weber syndrome is associated with a somatic mosaicism mutation, we sequenced the whole genomes of paired DNA samples from affected regions (biopsied tissue with port-wine stain or hemispherectomized brain tissue) and matched, presumably normal regions (blood or unaffected skin or brain tissue) from three persons with the Sturge–Weber syndrome. This sequencing resulted in the identification of 1294 somatic single-nucleotide variants found in at least one of three affected samples. We calculated the prevalence of the variant allele at each of these 1294 sites in all affected and normal samples and identified 658 single-nucleotide variants that were present in two or three affected samples and were not present in any normal samples (Fig. S1 in the Supplementary Appendix). We functionally annotated and ranked the 1294 somatic single-nucleotide variants (Table S6 in the Supplementary Appendix) using the Variant Annotation, Analysis and Search Tool (VAAST).<sup>7</sup> This resulted in the identification of one nonsynonymous somatic single-nucleotide variant that was present in all three affected samples and was not present in the samples that were presumed to be normal — a c.548G→A nucleotide transition in *GNAQ* on chromosome 9q21, encoding guanine nucleotide binding protein (G protein), q polypeptide ( $G\alpha_q$ ). The variant is predicted to result in the amino acid substitution p.Arg183Gln. The affected arginine residue, at position 183, is conserved in 24 human proteins paralogous to  $G\alpha_q$  (Fig. S2 in the Supplementary Appendix).

### DETECTION OF *GNAQ* SOMATIC VARIANT IN SAMPLES FROM PATIENTS WITH THE STURGE–WEBER SYNDROME

The results of our studies of skin samples were as follows: 100% of participants (9 of 9) with the Sturge–Weber syndrome were positive for the c.548G→A mutation in port-wine–stained skin, 86% of participants (6 of 7) with the syndrome

were negative for the mutation in visibly normal skin, and 92% of participants (12 of 13) with apparently nonsyndromic port-wine stains were positive for the mutation (Table 1). The mutation was also detected in brain samples from 83% of participants (15 of 18) with the Sturge–Weber syndrome, whereas 100% of brain samples (6 of 6 samples) from normal controls were negative. The results were negative in 100% of formalin-fixed, paraffin-embedded brain samples (4 of 4 samples) from persons with cerebral cavernous malformation (an unrelated cerebrovascular malformation) (Table 2), as well as in 99.3% of exomes from the 1000 Genomes database (664 of 669 exomes) (Table S7 in the Supplementary Appendix). In total, 88% of the participants (23 of 26) with the Sturge–Weber syndrome were positive for the c.548G→A mutation in either port-wine–stained skin or brain tissue.

Amplicon sequencing showed that mutant allele frequencies ranged from 1.0 to 18.1% and read depth ranged from 2446 to 93,008 (median, 12,947). Mutant allele frequencies in exomes in the 1000 Genomes database ranged from 1.0 to 1.5%, and read depth ranged from 100 to 453 (median, 271). *GNA11* mutations have also been found in patients with uveal melanoma.<sup>8</sup> We tested *GNAQ* Arg183Gln mutation–negative samples from participants with the Sturge–Weber syndrome and those with nonsyndromic port-wine stains for the presence of previously identified *GNA11* mutations (p.Arg183Cys, c.547C→T and c.546C→T; p.Arg183His, c.548G→A; p.Gln209Leu, c.626A→T and c.627G→A; and p.Gln209Pro, c.626A→C) using SNaPshot analysis. We did not detect any of these mutations (data not shown).

### EFFECT OF MUTATION ON MAPK SIGNALING PATHWAY

The somatic substitutions in *GNAQ* encoding p.Gln209Leu and p.Arg183Gln are found in patients with uveal melanoma. The more common p.Gln209Leu has been shown to overactivate the mitogen-activated protein kinase (MAPK) pathway.<sup>9</sup> We examined whether p.Arg183Gln would likewise overactivate the MAPK pathway. As shown in Figure 2A, cells transfected with *GNAQ* p.Gln209Leu or *GNAQ* p.Arg183Gln, as compared with cells transfected with nonmutant *GNAQ*, showed significant activation of extracellular signal-regulated kinase (ERK) ( $P < 0.05$ ). However, the activation induced by p.Arg183Gln was

modest as compared with the activation induced by p.Gln209Leu. We also examined the effect of these substitutions on additional downstream signaling pathways.  $G\alpha_q$  p.Gln209Leu strongly activated p38 and Jun N-terminal kinase (JNK), other MAPK pathway members, whereas p.Arg183Gln did not (Fig. 2B and 2C). Neither substitution had an effect on the AKT signaling pathway (Fig. 1D). These data show that p.Arg183Gln has a gain-of-function effect that activates downstream signaling pathways. However, the effect of p.Arg183Gln in MAPK signal transduction appeared to be both weaker and less promiscuous with respect to the activation of downstream effectors than the effect of the substitution p.Gln209Leu that is found more commonly in uveal melanoma tissue.

#### EFFECT OF GNAQ MUTATION ON SRE PROMOTER ACTIVITY

A different substitution in GNAQ encoding a variant at the same amino acid residue, p.Arg183Cys, was previously shown to overstimulate the serum response element (SRE) in a promoter reporter assay.<sup>10</sup> We investigated whether the p.Arg183Gln substitution had the same stimulatory effect on SRE promoter activity. We transfected HEK 293T cells with pSRE-Luc, pSV40-RL (reporter constructs), and GNAQ, GNAQ p.Arg183Gln, or GNAQ p.Gln209Leu plasmids and measured luciferase activity after 24 hours. Both p.Gln209Leu and p.Arg183Gln showed significantly increased reporter activity as compared with nonmutant GNAQ ( $P < 0.05$ ), confirming that the p.Arg183Gln mutation is a gain-of-function or activating mutation (Fig. 2F). In this assay, the p.Gln209Leu substitution again showed a stronger effect than did p.Arg183Gln.

#### DISCUSSION

Rudolf Happle first suggested that sporadic asymmetric or scattered birth defects involving the skin are caused by somatic mosaic mutations that would be lethal if they occurred in very early embryonic development.<sup>11</sup> Somatic mosaic activating mutations have been identified in several disorders, including the McCune–Albright syndrome<sup>12</sup> and the Proteus syndrome.<sup>13</sup> In the current study, we found that a specific somatic mosaic activating mutation in GNAQ is associated with both the Sturge–Weber syndrome, a neuro-

**Table 1. Somatic Mutation of GNAQ in Skin Samples.\***

Patient No.	Mutation Present†	PWS	SWS	Mutant Allele Frequency‡	Total No. of Samples Assayed
				percent	
1	Yes	Yes	Yes	3.60	1
1	No	No	Yes	0.11	1
2	Yes	Yes	Yes	3.17	1
2	No	No	Yes	0.13	1
3	Yes	Yes	Yes	6.06–6.46	2
3	No	No	Yes	0.62–0.93	2
4	Yes	Yes	Yes	3.50–4.51	2
4	No	No	Yes	0.13–0.90	2
5	Yes	Yes	Yes	3.38	1
5	No	No	Yes	0.11	1
6	Yes	Yes	Yes	3.99	1
7	Yes	Yes	Yes	2.05–2.16	2
7	Yes	No	Yes	0.09–2.00	2
8	Yes	Yes	Yes	4.08	1
8	No	No	Yes	0.06	1
9	Yes	Yes	No	5.58	1
10	Yes	Yes	No	2.76	1
10	Yes	No	No	1.14	1
11	Yes	Yes	No	6.70	1
12	No	Yes	No	0.00	1
13	Yes	Yes	No	5.90	1
14	Yes	Yes	No	6.20	1
15	Yes	Yes	No	14.20	1
16	Yes	Yes	No	1.70	1
17	Yes	Yes	No	4.50	1
18	Yes	Yes	No	5.30	1
19	Yes	Yes	No	4.70	1
20	Yes	Yes	No	4.30	1
21	Yes	Yes	No	18.10	1
22	Yes	Yes	Yes	5.00	1

\* Data are shown for c.548G→A, which results in the p.Arg183Gln amino acid substitution. This position corresponds to position 80,412,493 on chromosome 9 (Genome Reference Consortium Human Build 37). PWS denotes port-wine stains, and SWS Sturge–Weber syndrome.

† The p.Arg183Gln substitution was considered to be present if the mutant allele frequency was more than 1%.

‡ The mutant allele frequency was calculated as the percentage of mutant alleles divided by total alleles (with ranges shown in the case of multiple samples).

cutaneous disorder, and apparently nonsyndromic port-wine stains. GNAQ encodes  $G\alpha_q$ , a member of the q class of G-protein alpha subunits that mediates signals between G-protein–coupled recep-

**Table 2. Somatic Mutation of GNAQ in Brain-Tissue Samples.\***

Patient No.	Mutation Present	SWS	Mutant Allele Frequency <i>percent</i>	Total No. of Samples Assayed
7	Yes	Yes	5.57–5.63	2
23	Yes	Yes	5.56–5.78	2
24	Yes	Yes	2.67–3.51	2
25	No	Yes	0.02–0.10	2
26	Yes	Yes	0.13–3.06	4
27	Yes	Yes	2.19–5.12	2
28	Yes	Yes	6.95–8.13	4
29	Yes	Yes	6.04–11.15	5
30	Yes	Yes	4.14	1
31	Yes	Yes	4.78	1
32	Yes	Yes	0.22–1.48	4
33	Yes	Yes	4.04–5.74	2
34	No	Yes	0.05–0.12	2
35	Yes	Yes	0.05–1.51	7
36	Yes	Yes	0.35–6.03	5
37	Yes	Yes	5.74–6.49	2
38	No	Yes	0.03–0.05	2
39	Yes	Yes	1.83	1
40	No	No	0.11	1
41	No	No	0.05	1
42	No	No	0.08	1
43	No	No	0.09	1
44	No	No	0.04	1
45	No	No	0.04	1
46	No	No, CCM	0.00	1
47	No	No, CCM	0.00	1
48	No	No, CCM	0.00	1
49	No	No, CCM	0.00	1

\* Data are shown for c.548G→A, which results in the p.Arg183Gln amino acid substitution. Samples were obtained from persons with SWS, normal controls, and patients with a cerebral cavernous malformation (CCM).

tors and downstream effectors. We have identified somatic mosaic GNAQ encoding p.Arg183Gln amino acid substitutions in skin and brain tissue from patients with the Sturge–Weber syndrome and in skin tissue with nonsyndromic port-wine stains and have shown that this mutation, much like the GNAQ variant encoding p.Gln209Leu, activates downstream MAPK signaling. Gα<sub>q</sub> Arg183

is conserved in the guanosine triphosphate (GTP) binding pocket of all human Gα subunits, where it plays a critical role in the hydrolysis of GTP, the key step required for inactivation of the protein. Substitution of cysteine at this position results in a reduction in the intrinsic GTPase activity, leading to increased signaling activity.<sup>10,14–18</sup>

Activating mutations in genes encoding Gα subunits have previously been shown to be associated with relevant phenotypes, including the McCune–Albright syndrome, which is characterized by skeletal abnormalities and abnormal skin pigmentation.<sup>12</sup> Activating somatic GNAQ mutations have been identified in blue nevi and the more extensive nevi of Ota.<sup>9</sup> When these melanocytic nevi are colocalized with port-wine stains, the disorder is termed phakomatosis pigmentovascularis, which is occasionally found in association with the Sturge–Weber syndrome.<sup>19</sup> Mutations in GNAQ were also identified in a chemical mutagenesis screen for a dark-skin phenotype in laboratory mice.<sup>20</sup> Two of the dark-skin mutant alleles were identified at positions corresponding to human Gα<sub>q</sub> p.Val179Met and p.Phe335Leu. These germline-derived amino acid substitutions cause an increase in the number of neural-crest cells that differentiate into melanoblasts. The abnormal early melanocytic development resulting from these mutations in the neural-crest cells is mediated through endothelin, a G-protein–coupled receptor.<sup>20</sup> Since endothelin also has important roles in vasculogenesis,<sup>21</sup> dysregulation of this G-protein–coupled receptor as a result of the Gα<sub>q</sub> p.Arg183Gln mutation in persons with the Sturge–Weber syndrome and those with nonsyndromic port-wine stains may also bring about vascular malformation.

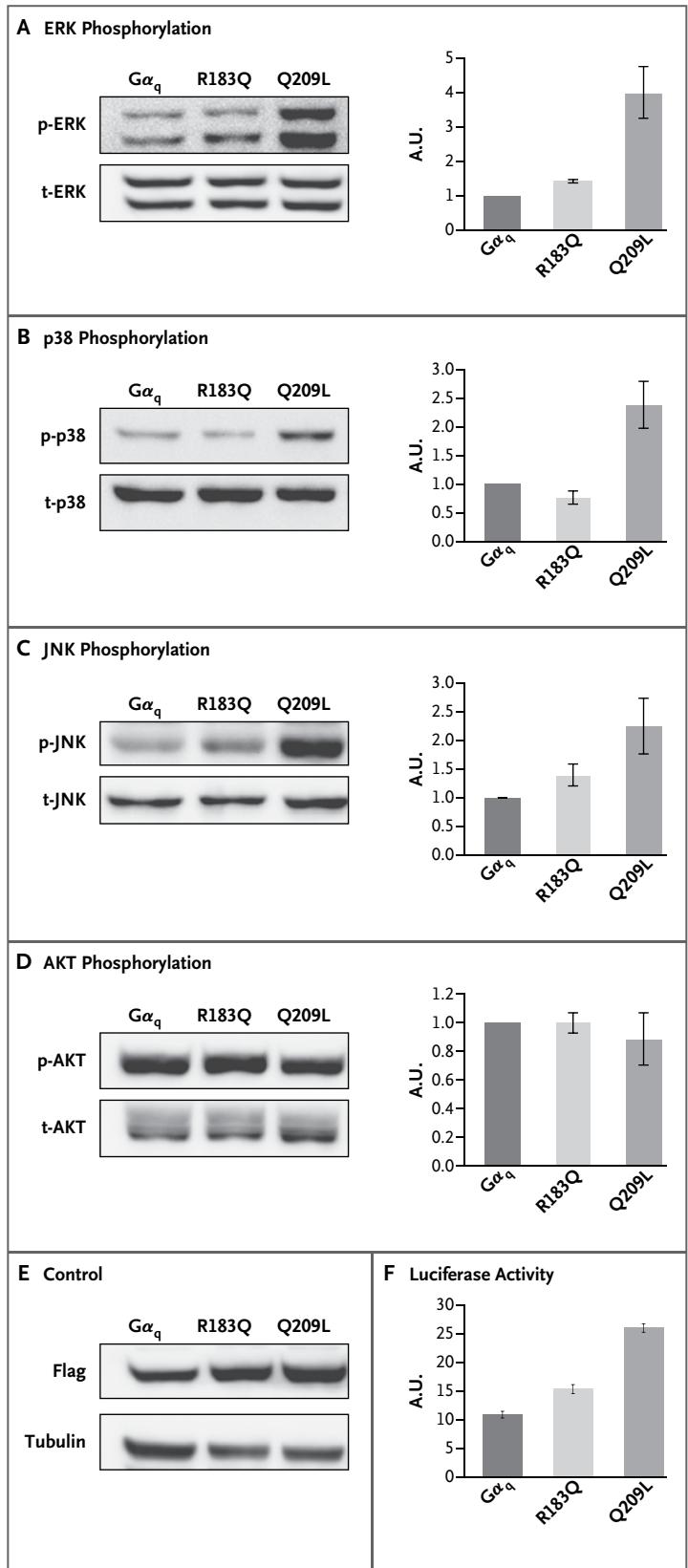
A somatic activating mutation may have oncogenic potential. In fact, somatic mutations of GNAQ in melanocytes are associated with uveal melanoma. The most common mutation, causing Gα<sub>q</sub> p.Gln209Leu, is an activating mutation that leads to increased downstream signaling through the MAPK pathway. The activation of this pathway increases cell proliferation and inhibits apoptosis.<sup>9</sup> A few uveal melanomas have been reported to harbor a somatic mutation in GNAQ encoding p.Arg183Gln, although the functional consequence of this substitution has not been reported.<sup>8</sup> The pathogenesis of uveal melanoma is likely to be very different from the pathogenesis of nonsyndromic port-wine stains and the

**Figure 2. Downstream Effectors of  $G\alpha_q$ .**

Plasmid encoding nonmutant  $G\alpha_q$ , p.Arg183Gln, and p.Gln209Leu were transfected into human embryonic kidney (HEK) 293T cells. Strongly increased phosphorylation of extracellular signal-regulated kinase (ERK) is seen with  $G\alpha_q$  p.Gln209Leu, and weaker but marked activation with  $G\alpha_q$  p.Arg183Gln ( $P < 0.05$  for both comparisons) (Panel A). Increased phosphorylation of p38 is seen with  $G\alpha_q$  p.Gln209Leu ( $P < 0.05$ ) but not with  $G\alpha_q$  p.Arg183Gln (Panel B). Increased phosphorylation of Jun N-terminal kinase (JNK) is seen with  $G\alpha_q$  p.Gln209Leu ( $P < 0.05$ ), and weaker activation with  $G\alpha_q$  p.Arg183Gln ( $P = 0.052$ ) (Panel C). No change in phosphorylation of AKT is seen with either the  $G\alpha_q$  p.Arg183Gln or the p.Gln209Leu construct (Panel D). A control for transfection efficiency, transfected into HEK 293T cells, shows similar amounts of the three transfected, Flag-tagged proteins (Panel E). A serum-response-element (SRE) luciferase assay (Panel F) shows the relative luciferase activity expressed under the control of the SRE promoter, coexpressed with *GNAQ* encoding p.Arg183Gln and p.Gln209Leu, as compared with nonmutant  $G\alpha_q$  ( $P < 0.05$  for both comparisons). AU denotes arbitrary units, the prefix p antibody recognizing phosphorylated antigen, and the prefix t antibody recognizing total antigen.

Sturge–Weber syndrome. Melanomas frequently have several somatic mutations.<sup>22</sup> We found no evidence of accumulating mutations on whole-genome sequencing of our three paired samples (affected and unaffected tissue) from participants with the Sturge–Weber syndrome. In addition, the Sturge–Weber syndrome, nonsyndromic port-wine stains, and melanocytic nevi are thought to originate during fetal development; therefore, the effects of the same *GNAQ* somatic mutation may be quite different, depending on the cell type and the point in development at which they arise. There are reported cases of uveal melanoma associated with phakomatosis pigmentovascularis,<sup>23</sup> and the coincidence of the blue nevus and port-wine stain phenotypes in a patient with the Sturge–Weber syndrome may indicate an increased risk of uveal melanoma, although such coincidences are rare.

We have shown that the  $G\alpha_q$  p.Arg183Gln substitution can activate ERK and does not activate p38 or JNK in the same way that p.Gln209Leu does. We propose that the moderate activation of ERK, the differential effect on p38 and JNK pathways, or both may contribute to the port-wine stains and syndromic characteristics of the Sturge–Weber syndrome. This may occur through



either upstream regulation of  $G\alpha_q$  or downstream modulation of the G-protein-coupled receptor signaling cascade. To provide insight into possible mechanisms underlying the partial activation of  $G\alpha$  downstream signaling, we considered an interesting corollary in other  $G\alpha$  proteins. The regulator of G-protein signaling (RGS) proteins serve as GTPase-activating proteins for  $G\alpha$  proteins, inhibiting downstream activation. Of these, RGS4 regulates  $G\alpha_q$  and  $G\alpha_i$ , whereas RGS2 is selective for  $G\alpha_q$ .<sup>24</sup> On examination of the ability of RGS4 to regulate  $G\alpha_{i1}$  with activating mutations in positions p.Arg178Cys and p.Gln204Leu, homologous to  $G\alpha_q$  p.Arg183Gln and p.Gln209Leu, it was found that all regulatory ability was lost for p.Gln204Leu, whereas GTPase activity was partially maintained for p.Arg178Cys.<sup>18</sup> Thus, the weaker and less promiscuously activating effects of  $G\alpha_q$  p.Arg183Gln, as compared with  $G\alpha_q$  p.Gln209Leu, may be a result of partial regulation by a member of the RGS family.  $G\alpha_q$  is also able to initiate sustained RhoA and Rac1 activation independently of PLC- $\beta$ , through direct interaction with Trio, a guanine nucleotide exchange factor. It has been shown that  $G\alpha_q$ -mediated oncogenic proliferation, mediated through p38 and JNK, is significantly reduced after Trio knockdown without affecting PLC or ERK activation levels.<sup>25</sup> This provides a possible mechanism, related to altered affinity of protein-protein interactions with both regulators (RGS family) and cascade activators (Trio), to explain the non-oncogenic proliferation seen in the Sturge-Weber syndrome and nonsyndromic port-wine stains. We hypothesize that only the weaker effect of somatic  $G\alpha_q$  p.Arg183Gln would be compatible with the abnormal but nonlethal development of the cerebrovascular system seen in the Sturge-Weber syndrome. We also hypothesize that during vulnerable periods in embryonic development, moderately increased baseline signaling downstream of  $G\alpha_q$ , or dysregulated signaling through G-protein-coupled receptors such as that for endothelin,<sup>21</sup> may result in the malformed, progressively dilated, and abnormally innervated blood vessels underlying port-wine stains. There is some evidence in the literature to support this hypothesis. Shirazi et al. reported the localization of phosphorylated ribosomal protein S6 (RPS6), which is downstream of MAPK signaling, to endothelial cells lining the luminal wall of abnormal blood vessels in port-

wine stain tissue from patients with the Sturge-Weber syndrome.<sup>26</sup>

The nonsyndromic port-wine stains may represent a late origin of the somatic  $GNAQ$  mutation in vascular endothelial cells, whereas the Sturge-Weber syndrome mutation may occur earlier in development, in progenitor cells that are precursors to a larger variety of cell types and tissues, leading to the syndromic phenotype. We found that 0.7% of samples of blood from the 1000 Genomes database (5 of 669 samples) that were tested for the presence of the  $GNAQ$  mutation encoding p.Arg183Gln were positive. The reported prevalence of port-wine stains<sup>1,2</sup> is 0.3 to 0.5%. We therefore hypothesize that the 0.7% prevalence in this database represents the occurrence of port-wine stains in this population.

Our data indicate that there is a single underlying mechanism for the Sturge-Weber syndrome and nonsyndromic port-wine stains and add a molecular basis for a decades-old hypothesis regarding the cause of these malformations. The scientific and translational novelty of this discovery lies in the association of both apparently nonsyndromic port-wine stains and the Sturge-Weber syndrome with a mutation in a specific gene, a specific genetic mechanism, and a set of potential pathways, which provides a foundation for further scientific and clinical research.

The views expressed in this article are those of the authors and do not necessarily represent the official views of the National Institutes of Health.

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## REFERENCES

1. Comi AM. Update on Sturge-Weber syndrome: diagnosis, treatment, quantitative measures, and controversies. *Lymphat Res Biol* 2007;5:257-64.
2. Piram M, Lorette G, Sirinelli D, Herbreteau D, Giraudeau B, Maruani A. Sturge-Weber syndrome in patients with facial port-wine stain. *Pediatr Dermatol* 2012;29:32-7.
3. Ch'ng S, Tan ST. Facial port-wine stains — clinical stratification and risks of neuro-ocular involvement. *J Plast Reconstr Aesthet Surg* 2008;61:889-93.
4. Greene AK, Taber SF, Ball KL, Padwa BL, Mulliken JB. Sturge-Weber syndrome: soft-tissue and skeletal overgrowth. *J Craniofac Surg* 2009;20:Suppl 1:617-21. [Erratum, *J Craniofac Surg* 2009;20:1629-30.]
5. Gasparini G, Perugini M, Vetrano S, Cassoni A, Fini G. Angiodysplasia with osteohypertrophy affecting the oromaxillofacial area: clinical findings. *J Craniofac Surg* 2001;12:485-9.
6. Vissers W, Van Steensel M, Steijlen P, Renier W, Van De Kerkhof P, Van Der Vleuten C. Klippel-Trenaunay syndrome and Sturge-Weber syndrome: variations on a theme? *Eur J Dermatol* 2003;13:238-41.
7. Yandell M, Huff C, Hu H, et al. A probabilistic disease-gene finder for personal genomes. *Genome Res* 2011;21:1529-42.
8. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in *GNA11* in uveal melanoma. *N Engl J Med* 2010;363:2191-9.
9. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of *GNAQ* in uveal melanoma and blue naevi. *Nature* 2009;457:599-602.
10. Takasaki J. A novel  $G\alpha(q11)$ -selective inhibitor. *J Biol Chem* 2004;279:47438-45.
11. Happle R. Lethal genes surviving by mosaicism: a possible explanation for sporadic birth defects involving the skin. *J Am Acad Dermatol* 1987;16:899-906.
12. Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel AM. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N Engl J Med* 1991;325:1688-95.
13. Lindhurst MJ, Sapp JC, Teer JK, et al. A mosaic activating mutation in *AKT1* associated with the Proteus syndrome. *N Engl J Med* 2011;365:611-9.
14. Conklin BR, Chabre O, Wong YH, Federman AD, Bourne HR. Recombinant Gq alpha: mutational activation and coupling to receptors and phospholipase C. *J Biol Chem* 1992;267:31-4.
15. Orth JHC, Preuss I, Fester I, Schlosser A, Wilson BA, Aktories K. Pasteurella multocida toxin activation of heterotrimeric G proteins by deamidation. *Proc Natl Acad Sci U S A* 2009;106:7179-84.
16. Coleman DE, Berghuis AM, Lee E, Linder ME, Gilman AG, Sprang SR. Structures of active conformations of Gi alpha 1 and the mechanism of GTP hydrolysis. *Science* 1994;265:1405-12.
17. Kimple AJ, Bosch DE, Giguère PM, Siderovski DP. Regulators of G-protein signaling and their  $G\alpha$  substrates: promises and challenges in their use as drug discovery targets. *Pharmacol Rev* 2011;63:728-49.
18. Berman DM, Wilkie TM, Gilman AG. GAIP and RGS4 are GTPase-activating proteins for the Gi subfamily of G protein alpha subunits. *Cell* 1996;86:445-52.
19. Al Robaee A, Banka N, Alfadley A. Phacomatosis pigmentovascularis type IIB associated with Sturge-Weber syndrome. *Pediatr Dermatol* 2004;21:642-5.
20. Van Raamsdonk CD, Fitch KR, Fuchs H, de Angelis MH, Barsh GS. Effects of G-protein mutations on skin color. *Nat Genet* 2004;36:961-8.
21. Kajio F, Nakazawa M. Vascular effects of endothelin-1 in stage 21 chick embryos. *Heart Vessels* 1997;12:300-5.
22. Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. *Cell* 2012;150:251-63.
23. Shields CL, Kligman BE, Suriano M, et al. Phacomatosis pigmentovascularis of cesioflammea type in 7 patients: combination of ocular pigmentation (melanocytosis or melanosis) and nevus flammeus with risk for melanoma. *Arch Ophthalmol* 2011;129:746-50.
24. Hepler JR, Berman DM, Gilman AG, Kozasa T. RGS4 and GAIP are GTPase-activating proteins for Gq alpha and block activation of phospholipase C beta by gamma-thio-GTP-Gq alpha. *Proc Natl Acad Sci U S A* 1997;94:428-32.
25. Vaqué JP, Dorsam RT, Feng X, et al. A genome-wide RNAi screen reveals a trio-regulated Rho GTPase circuitry transducing mitogenic signals initiated by G protein-coupled receptors. *Mol Cell* 2013;49:94-108.
26. Shirazi F, Cohen C, Fried L, Arbiser JL. Mammalian target of rapamycin (mTOR) is activated in cutaneous vascular malformations in vivo. *Lymphat Res Biol* 2007;5:233-6.

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