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SHORT COMMUNICATION

The somatic *GNAQ* mutation c.548G > A (p.R183Q) is consistently found in Sturge–Weber syndrome

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Sturge–Weber syndrome (SWS) is a neurocutaneous disorder characterized by capillary malformation (port-wine stains), and choroidal and leptomeningeal vascular malformations. Previously, the recurrent somatic mutation c.548G > A (p.R183Q) in the G-α q gene (*GNAQ*) was identified as causative in SWS and non-syndromic port-wine stain patients using whole-genome sequencing. In this study, we investigated somatic mutations in *GNAQ* by next-generation sequencing. We first performed targeted amplicon sequencing of 15 blood–brain-paired samples in sporadic SWS and identified the recurrent somatic c.548G > A mutation in 80% of patients (12 of 15). The percentage of mutant alleles in brain tissues of these 12 patients ranged from 3.6 to 8.9%. We found no other somatic mutations in any of the seven *GNAQ* exons in the remaining three patients without c.548G > A. These findings suggest that the recurrent somatic *GNAQ* mutation c.548G > A is the major determinant genetic factor for SWS and imply that other mutated candidate gene(s) may exist in SWS.
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Sturge–Weber syndrome (SWS; MIM no. 185300) is a rare neurocutaneous disorder characterized by facial cutaneous vascular malformations (port-wine stains), and ocular and cerebral vascular malformations result in neurological impairment including seizures and intellectual disability.[1,2] The prevalence is estimated at ~ 1/20 000–50 000.[2] Because the occurrence of SWS is sporadic with no heritability, it is thought to be caused by somatic mutations.[3] Previously, a somatic mutation in the gene encoding the q class of guanine nucleotide-binding protein (G-protein)-α subunit (guanine nucleotide-binding protein, Q polypeptide; *GNAQ*) was identified in both SWS patients and those with non-syndromic port-wine stains.[4] Here, we investigated the presence of somatic *GNAQ* mutations in 15 SWS patients using targeted next-generation sequencing.

PATIENTS AND METHODS

Study population

A total of 15 SWS patients were recruited for this study, and complete paired sets of peripheral blood leukocytes and surgically resected brain tissues were obtained from all patients. Patients were diagnosed as having SWS if they had two or more of the following symptoms: (1) craniofacial vascular malformation, (2) early-onset seizure, (3) contralateral hemiplegia or ateliosis, (4) intellectual disability and (5) ocular findings including choroidal vascular malformations, glaucoma, buphthalmia and hemianopia. All of the individuals underwent preoperative neuroimaging with magnetic resonance imaging. Detailed clinical features are shown in Table 1. All the brain specimens were histopathologically examined and confirmed as truly leptomengial angioma. Experimental protocols were approved by the Institutional Review Board of Yokohama City University School of Medicine and Juntendo University Graduate School of Medicine. Written informed consent was obtained from patients or parents of pediatric patients.

Deep sequencing of *GNAQ* c.548G > A

Genomic DNA of RNAlater (ThermoFisher Scientific, Waltham, MA, USA)-treated brain tissue blocks and peripheral blood leukocytes was extracted using Puregene Core Kit A (Qiagen, Valencia, CA, USA) and PAXgene Blood DNA kit (Qiagen), respectively, according to the manufacturer's instruction. With genomic DNA obtained from leukocytes and tissues, the 173-bp target region including c.548G > A in *GNAQ* exon 4 was PCR-amplified using the following primers: forward 5′-ATTGTGTCTTCCCTCCTCTA-3′ and reverse 5′-GGTTT CATGGACTCAGTTAC-3′. A single-indexed sequencing library was prepared using the SureSelect XT Library Prep Kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on an Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) with 101-bp paired-end reads. Image analysis and base calling were performed by sequence control software real-time analysis and CASAVA software v1.8.2 (Illumina). Quality-filtered reads were mapped to the human reference genome sequence (UCSC hg19, NCBI build 37) and aligned using Novoalign (Novocraft Technologies, Jaya, Malaysia). The aligned read files in the BAM format were sorted and indexed using SAMtools.[5] Data analysis including allele counting was performed by Integrative Genomics Viewer software.[6,7] We defined the 1% cutoff line for the presence of mutant alleles out of total reads based on the Shirley's criteria.[4]

GNAQ mutation screening

In the remaining subjects without c.548G > A, *GNAQ* mutation screening was performed using additional brain tissues. Primers were designed for the entire

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Table 1 Clinical features of SWS individuals and deep-sequencing results of c.548G>A in *GNAQ*

				LAs		Seizures				c.548G>A mutation		
Patients	*Sex*	*Age*	*PWS*	*Region*	*Size*	*Onset age*	*Type*	*ID*	*GL*	*Read depth (brain/blood)*	*Mutant allele frequency (%)[a] (brain/blood)*	*Assessed brain samples*
1	M	6	Face	Left	Hemisphere	3 Mon	CP	+	+	289 312/2 676 243	5.69/0.03	1
2	M	7	Face	Left	T, P, O	11 Mon	CP	+	+	1 170 532/591 208	4.03/0.05	1
3	Fe	6	Face	Right	Hemisphere	8 Mon	Pa	+	–	566 890/1 128 199	0.03–0.06/0.03	2
4	M	4	Face	Right	Hemisphere	4 Mon	Pa	+	–	996 691/1 556 151	0.16–0.28/0.03	2
5	M	5	Face	Right	T, P, O	8 Mon	Pa	–	+	490 977/471 499	5.04/0.04	1
6	M	10	Face	Right	T, O	12 Mon	CP	+	+	930 173/409 196	6.14/0.04	1
7	M	4	Face	Left	Hemisphere	4 Mon	Pa	+	+	639 834/635 419	4.11/0.04	1
8	M	3	Face	Right	Hemisphere	7 Mon	Pa	–	+	253 920/230 876	6.17/0.44	1
9	M	3	Face	Left	F, P	5 Mon	CP	–	–	1 277 805/1 128 803	7.59/0.03	1
10	M	3	Face	Left	Hemisphere	3 Mon	Pa	+	+	622 186/1 128 803	8.06/0.01	1
11	Fe	3	Head	Left	Hemisphere	2 Mon	Pa	–	–	387 543/432 693	0.03–0.04/0.03	2
12	F	2	Face	Right	Hemisphere	1 Mon	Pa	–	–	65 437/20 297	3.77/0.04	1
13	M	7	Face	Right	F	4 Years	CP	+	–	611 093/441 137	3.91/0.03	1
14	Fe	2	—	Right	Hemisphere	3 Mon	Pa	–	–	578 857/1 244 217	8.94/0.04	1
15	M	3	Face	Left	O	11 Mon	Pa	–	–	739 125/887 628	3.66/0.03	1

Abbreviations: CP, complex partial seizure; F, frontal lobe; Fe, female; GL, glaucoma; ID, intellectual disability; LAs, leptomeningeal angiomas; M, male; mon, months; O, occipital lobe; P, parietal lobe; Pa, partial seizure; PWS, port-wine stain; SWS, Sturge–Weber syndrome; T, temporal lobe.
[a]Percent of mutant allele frequency was calculated by mutant allele reads/total reads.

Table 2 Primer sequences for PCR amplification of *GNAQ*

Exon	*Forward (5′–3′)*	*Reverse (5′–3′)*	*Size (bp)*
1	GACACCCCGGTGAATGAG	GGACACGAAAAGGAACAAGC	849
2	AAAAGATGCTGTTGCCATTG	CCAAATATGCCTTTCATTGA	755
3	GATGGGAGAGCTGAATACGC	AGTTTGCAATTTGGGGGAGG	715
4	TTGCCTCTGGGGAGTATGAG	CGATTTTACTCAACCA-CAAGCA	971
5	TACCATTTTGCTTGGCACAG	GACACACCCATCACACAAAC	814
6	TGACAGTGTTCCAGATTCA-CAA	GGAATGCAATGTTTTGTGTCA	650
7	GCCTTGGCTTTCAAGTCATC	GAATTAGCGGGGGAAGAAAA	814

GNAQ (NM_002072.4) gene covering the coding region, intron–exon boundaries, as well as 5′- and 3′-untranslated regions (Table 2). A dual-indexed sequencing library was prepared with the Nextera DNA Sample Preparation Kit (Agilent Technologies) and sequenced on an Illumina MiSeq (Illumina) with 150-bp paired-end reads. Read alignment, sorting and indexing were performed as described above. Somatic single-nucleotide variant calling was performed by MuTect algorithms with the default setting.[8] Variants that passed the MuTect filters were annotated using ANNOVAR software.[9] Novel somatic single-nucleotide variants were selected based on the following four criteria: (1) mutant alleles observed with $\geqslant 2$ reads in the brain and <2 reads in blood leukocytes, (2) variants unregistered in dbSNP 137 except for clinically associated single-nucleotide polymorphisms (flagged),[9] (3) variants unregistered in 6500 ESP, 1000 Genomes databases[10,11] or 575 in-house control exomes and (4) non-synonymous variants.

RESULTS AND DISCUSSION

The recurrent somatic *GNAQ* c.548G>A mutation was identified in 12 of 15 SWS samples (80%) in the present study. The total read depth ranged from 20 284× to 2 674 940× (mean depth, 708 528×). Mutant allele frequencies ranged from 3.66 to 8.94% (mean, 5.59%; Table 1). In the remaining three subjects without c.548G>A (patients 3, 4 and 11; Table 1), we performed *GNAQ* mutation screening. The mean depth of coverage of the coding sequences ranged from 18 080 to 27 967 (average 23 050.6), and 500 or more reads covered 100% of coding sequences. However, no other somatic mutations were found in *GNAQ*.

Heterotrimeric G-proteins are composed of three subunits, α, β and γ, and act as signal transducers when coupled with seven transmembrane receptors in a diverse range of signaling pathways.[12,13] *GNAQ* encodes the q class of G-α subunit that activates phospholipase Cβ, thereby leading to the generation of inositol triphosphate and diacylglycerol.[14,15] Somatic mutations in *GNAQ* were previously reported as oncogenic gain-of-function mutations causing melanocytic neoplasms including melanomas and nevi.[16–18]

Van Raamsdonk *et al*.[17] showed that somatic mutations in the Q209 and R183 residues of GNAQ, especially the Q209L substitution located in the Ras-like domain, were likely to be involved in tumorigenesis through upregulation of the MAP kinase pathway. R183 is located in the GTP-binding pocket of the G-α subunit and has an important role in GTP hydrolysis; however, the potential of the R183 mutant to activate signal transmission was markedly lower than that of the Q209 mutant.[19,20] Recently, Shirley *et al*.[4] identified the somatic *GNAQ* mutation c.548G>A (pR183Q) in 88% of SWS patients (23 of 26) and 92% of patients with non-syndromic port-wine stains (12 of 13). This substitution was suggested to induce moderate activation of the extracellular signal-regulated kinase (ERK) pathway, which may lead to pathologic but not significantly neoplastic growth of capillaries in the skin and brain. In this study, we found the same recurrent somatic c.548G>A (pR183Q) mutation in 80% of patients (12 of 15), which supports the previous findings. However, we could not identify any other causative somatic mutation in *GNAQ*. Therefore it remains unclear whether mutations in other genes can be causative of SWS, although the recurrent somatic *GNAQ* mutation c.548G>A (p.R183Q) appears to be the major determinant in this disorder.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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