

GNAQ mutation R183Q as a potential cause of familial Sturge-Weber syndrome: A case report

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Abstract. Sturge-Weber syndrome (SWS) is a rare neurocutaneous disorder whose etiology remains unclear. To investigate the genetic contribution underlying this disease, the genetic variants of a 4-generation family with a history of SWS was analyzed in the present study. SWS was diagnosed in 3 of the family members (II-1, III-11 and IV-6). Sanger sequencing was performed to identify mutations in G protein subunit α_q (GNAQ) and RAS p21 protein activator 1 exons in the 3 patients with SWS and other unaffected family members. Notably, a non-synonymous single-nucleotide variant at codon 183 on exon 4 of the GNAQ gene was identified as the only pathogenic site. This variant generated a substitution of arginine (R) with glutamine and resulted in a change of function of the encoded protein. Evolutionary

conservation analysis revealed that the mutated residue 183 (R) of GNAQ is highly conserved across several vertebrate species. Furthermore, an immunofluorescence staining assay demonstrated that the substitution of arginine with glutamine resulted in a change in the sub-cellular localization of the GNAQ recombinant protein *in vitro*. These findings may aid in the development of novel diagnostic markers and/or therapeutic targets for the treatment of patients with familial SWS.

Introduction

Sturge-Weber syndrome (SWS), also known as encephalofacial angiomatosis, is a congenital neurocutaneous disorder characterized by facial port-wine stains, ipsilateral occipital leptomeningeal angioma and/or abnormal blood vessels in the eyes, which predisposes patients to glaucoma (1). SWS occurs in male and female newborns, in 1/20,000 to 1/50,000 live births (2). Patients with SWS frequently develop migraines, seizures, stroke-like episodes, hemiparesis, visual problems and mental retardation (3). Furthermore, the majority of treatments for the neurological complications associated with SWS are symptomatic instead of radical. The etiology of SWS remains unclear; however, evidence from whole-genome sequencing suggests that somatic mutations in the G protein subunit α_q (GNAQ) and RAS p21 protein activator 1 (RAS1) genes are causative factors of SWS and non-syndromic port-wine stains (4). Familial SWS is a rare genetic disorder that is dominantly inherited, which could affect family members with similar congenital deformities. To the best of our knowledge, the genetic factors predisposing to familial SWS have previously been unknown; however, systematic analysis of the genetic and molecular mechanisms underlying familial SWS is warranted.

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Case report

In the present study, a 4-generation family with a history of SWS was analyzed and demonstrated dominant inheritance of SWS (Fig. 1). SWS was diagnosed in 3 of the family members (II-1, III-11 and IV-6). The patients' clinicopathological characteristics are illustrated in Table I. The present study was reviewed and approved by the Ethics Committees of Peking University Shenzhen Hospital (Shenzhen, China) and informed consent was obtained from all patients. To systematically screen for candidate mutations associated with SWS, the full range of exons from the GNAQ and RASA1 genes of all family members were sequenced using Sanger sequencing Thermo Fisher Scientific, Inc. (Waltham, MA, USA). The primer sequences of exons from the GNAQ and RASA1 genes are presented in Tables II and III.

To identify the candidate mutation, all the genetic variants common to the three patients with SWS were compared with the control data from family member IV-5 (Fig. 2). A total of 8 case-specific genetic variants that coexisted in the 3 patients were identified. The case-specific variants were subsequently compared with genetic polymorphism datasets from the Single Nucleotide Polymorphism database [National Center for Biotechnology Information (NCBI); <http://www.ncbi.nlm.nih.gov/projects/SNP/>] and the 1000 Genome Project database (<http://www.1000genomes.org/>). Following these two comparative steps, a novel candidate mutation, potentially associated with familial SWS, was identified.

According to the NCBI (<http://www.ncbi.nlm.nih.gov/>), the human GNAQ gene is located on chromosome 9q21, and contains 7 exons and 8 introns, encoding guanine nucleotide-binding protein (G protein), q polypeptide (Gαq). As illustrated in Fig. 2, one non-synonymous single-nucleotide variation (nsSNV) was present exclusively in the 3 patients with SWS. The variation was predicted to be located on the fourth exon of GNAQ and resulted in the substitution of amino acid p.Arg183Gln (p.R183Q). Evolutionary conservation analysis was performed using MegAlign software version 5 (DNASTAR®, Inc., Madison, WI, USA). Multiple protein alignments revealed that the Arg183 residue on the GNAQ gene is highly conserved across several vertebrate species (Fig. 3), which indicates that the p.R183Q mutation is likely to disrupt the normal functions of Gαq. In addition, the functional significance of the SWS-associated mutation was predicted using several complementary nsSNV scoring algorithms, including SIFT (5), PolyPhen2 (6), PhastCons (7) and GERP scores (8). As hypothesized, the p.R183Q mutation results in a change to the function of the encoded protein Gαq.

To detect the sub-cellular localization of Gαq, the mutant (R183Q) and wild-type (WT) genotypes of the human GNAQ gene were overexpressed in 293T cells. The cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The coding sequence of human GNAQ (WT) was amplified using reverse transcription-polymerase chain reaction (RT-PCR) analysis with the following primers: Forward, 5'-CCGGAATTCTATGACTCTGGAGTCCATCATGGCGT-3' and reverse, 5'-CGCGGATCCACGCCATGATGGACTCCA GAGTCAT-3'. Reverse transcription was performed using a PrimeScript™ Reverse Transcription Reagent kit (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol. The thermocycling conditions were

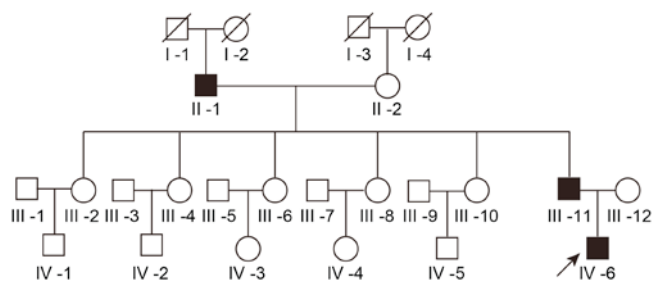


Figure 1. A 4-generation family tree of familial Sturge-Weber syndrome. Black boxes represent the affected individuals. The arrow beside the black box indicates the proband.

as follows: 37°C for 60 min; 95°C for 5 min; and then held at 4°C. PCR was performed using the Takara EmeraldAmp PCR Master mix (Takara Biotechnology Co., Ltd.). The PCR thermocycling conditions were as follows: 98°C for 2 min, 98°C for 20 sec, 60°C for 30 sec and 72°C for 2 min for 36 cycles, with a final step at 72°C for 5 min. Subsequently, the PCR products were double-digested at 37°C for 60 min using the restriction endonucleases *EcoRI* and *BamHI* (Takara Bio, Inc., Otsu, Japan), and the digested products were sub-cloned into the plasmid enhanced green fluorescent protein (EGFP)-C1 vector (Thermo Fisher Scientific, Inc.) to produce a pEGFP-C1-GNAQ fusion protein-expressing vector. The coding sequence of EGFP-GNAQ was confirmed through DNA sequencing by Thermo Fisher Scientific, Inc. The GNAQ mutant was synthesized by Thermo Fisher Scientific, Inc. The nuclei of 293T cells were labeled using Hoechst 33258 and normal 293T cells transfected with the empty vector (pEGFP-C1) were used as controls. Immunofluorescence analysis was performed as described previously (9). The data showed that the GNAQ (WT) recombinant protein was predominantly detected in the cytoplasm, whereas the GNAQ (R183Q) was mainly detected in the nuclei of the 293T cells (Fig. 4). This result suggests that the change of subcellular localization contributes to the generation of a loss-of-function Gαq protein. The results of the present study demonstrated that the mutation p.R183Q may be a risk factor that contributes to the pathogenesis of familial SWS.

Discussion

Gαq is a member of the q class of G-protein α subunits that mediates signals between G protein-coupled receptors (GPCRs) and downstream effectors. GPCRs are involved in RAS-associated signaling pathways. Gαq Arg183 is conserved in the GTP-binding pocket of all human Gα subunits, which serves an essential role in the hydrolysis of GTP. Results from previous studies have demonstrated that substituting Arg183 with a cysteine residue reduces the intrinsic GTPase activity and increases the signaling activity (10-12). The p.R183Q mutation identified in the present study is located in the GTP-binding domain of GNAQ. Therefore, it was hypothesized that the substitution of arginine with glutamine affects the functions of Gαq by changing its subcellular localization.

Previous studies have established that activating mutations in genes that encode Gα subunits are associated with various diseases, including McCune-Albright syndrome,

Table I. Clinicopathological characteristics of the 3 patients with SWS from the 4-generation family.

Family member	Gender	Age, years	Presence of SWS	Location	Size, cm
I-1	M	74	No		
I-2	F	68	No		
I-3	M	73	No		
I-4	F	70	No		
II-1	M	52	Yes	Right arm	2
II-2	F	48	No		
III-1	M	51	No		
III-2	F	55	No		
III-3	M	39	No		
III-4	F	42	No		
III-5	M	36	No		
III-6	F	37	No		
III-7	M	36	No		
III-8	F	38	No		
III-9	M	37	No		
III-10	F	35	No		
III-11	M	37	Yes	Left arm	2
III-12	F	38	No		
IV-1	M	24	No		
IV-2	M	12	No		
IV-3	F	10	No		
IV-4	F	8	No		
IV-5	M	8	No		
IV-6	M	7	Yes	Right face	4

SWS, Sturge-Weber syndrome; M, male; F, female.

Table II. Primer sequences for reverse transcription-polymerase chain reaction amplification of G protein subunit αq exons.

Exon	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Size, bp
1	AGACTATCCGCTCCCACCGC	GCCTCTGGTCCGAGTGATCTGC	694
2	CATAGGTAGTGTTTCCAATTCTAGA	ATGATACTTACAACTCTCTCCTTA	660
3	AAGGAAGGTGTTACCTGAATTGTA	TTAGATTTATGAGGTTGGCATAAG	420
4	ATGTAATCCATAGATGGATAACCTT	TTGTTTTGAAGCCTACACATGATT	430
5	GTATTAATTGAATTGACTTGGATGA	TCAGAACCTCTGCATAGTGTTA	514
6	ATGACAGTGTCCAGATTCACAAC	ACACTGGGGTTTGGAGACAAAAC	530
7	TTCATGAGCAATGACACAGTATTT	GACGGCAATAAATTAGTATTATGC	398

which exhibits characteristic skeletal abnormalities and abnormal skin pigmentation (13). Activating GNAQ mutations have been found in patients with blue nevi and nevi of Ota (14). In addition, GNAQ mutations were previously identified in a chemical mutagenesis screen for a dark-skin phenotype in laboratory mice (15). An activating GNAQ mutation may have oncogenic functions (16). The results of several studies revealed that mutations in the Q209 and R183 residues of the GNAQ gene, including the Q209L substitution located in the Ras-like domain, are likely to exhibit involvement in the process of tumorigenesis via the upregulation of

the mitogen-activated protein kinase pathway (16). R183 is located in the GTP-binding pocket of the $G\alpha$ subunit and has an important role in GTP hydrolysis; however, a markedly lower potential to activate signal transmission was found for the R183 mutant compared with the Q209 mutant (17,18).

In the current study, the R183Q mutation of the GNAQ gene was identified as a potential cause for familial SWS through the sequencing of all of the exons of the GNAQ and RASA1 genes in 3 patients with familial SWS. The functional study confirmed that the genetic mutation R183Q affected the functions of the encoded protein, $G\alpha q$. To the best of our

Table III. Primer sequences for reverse transcription-polymerase chain reaction amplification of RAS p21 protein activator 1 exons.

Exon	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Size, bp
1	GAGTAGAGCGGGCTTCAACATGA	GGAGCCAGCTTCCCAAATTCCTGA	600
2	GGCATTAAAACCTCTAGTAGTAT	TAAATATATTTGAGGATCAGCACAG	480
3	TAGCCCCAAGTAATTTACTGTGAT	ATTCAGAATCTTGCAGCAAAGTC	630
4	GGCACTGGGTATTTATAGTCCAAG	GTGAGAGACAGTGGAAATAAAACC	475
5	ACTCTAATTCCTTACATTTTTCAAT	TTATAATATGCTAGCCTGATTATGT	420
6	TTAAGAGAGGTTTGGAAAGATATT	GAGTAAGAGAGAATTGGGATTAAT	690
7	AGACCTATAACAGTCTGTTATTATC	TCACCTAGTGTACTTCTGAG	590
8	AAGCTGGTAAGATACACCAGAGA	GACTGTCTAAAGAATTCTTGGG	539
9	CTTGATGGGTATGGAAAATCAGAA	AGGTTGTTCTGATTACCTAAAATC	520
10	CTTTGGAAAGCAAAGATCATTTA	ATGCTTATATATGGGCTCTAAACAA	410
11	AGGCATTTTTGTACAGATTTGTTAT	AAGTGCATCTGCTACTTTTAGAAG	449
12	ACTGATAACATTTTAAATAGTGCTG	AGGTCAATTTAAAATGGAGTTTTTC	527
13	TGAGAATAGAAATGGCAGTCTAG	TTTCATAAAGAAGTAACGTTTTTCT	360
14	ATGTCTGAATGTTTTAAGTATGTC	GCTATTAGAACACCTGGATATGGTA	460
15	GAGGTTAAAGATGGTAGGAAAAG	TAACAGTGTGTACAGTTGAACCAT	500
16	CTCTCTACCTATCAGAGTTTAGTG	GTGCTACTAAGCTATGAATTACTAT	480
17	AATGATGCCAGAGATTTTAAACCT	TTGTGGCTTCTAGTACTTATCTGC	600
18	CATTGTAATTAGTACTTTCAACGC	AATTTCTGGTGCAACTTGATGG	518
19	GGCTAATTGGGAATAAATGGTAAC	ACCATGACTTTTTTCAGAGTATAATA	450
20	ATAAAGTTTTTCCTTACCCCTTGCT	GCCTCATCTTGTAAGGGTTTAA	519
21	GGTTTAGAGTGAATTTTATGGGTT	GGTTTGAGACACAAATAGGGGTA	462
22	ACATTCTGAGTTCGAATGGAAG	CTTTCATCGCTAAAAATTTCACTGC	335
23	TGTGTTGCTGCTGCTACACCTAAT	AAGGCAGACAAAATTTGCAGGC	400
24	ATGTGAATACAAAGGTTACAAAGT	CAGACTCAAGAGCAAACCTGAT	744
25	GAATTGTGGTAATATTTTATGCATC	CAGATAGCTTACAAGGTTGCTG	395

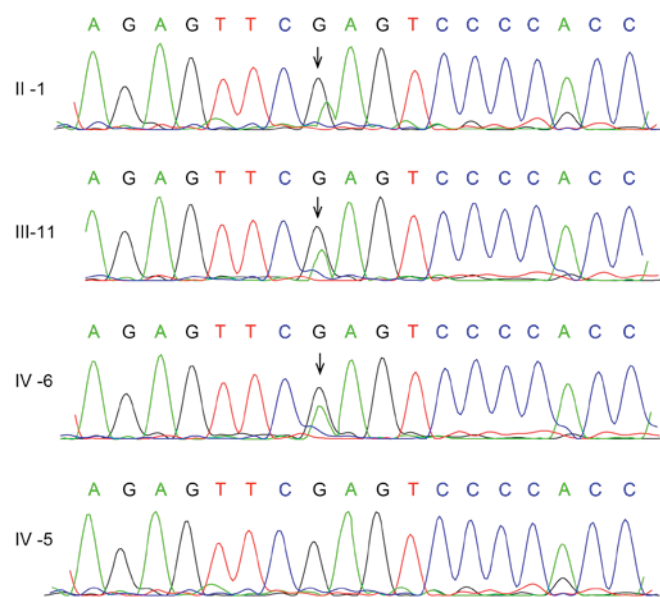


Figure 2. A missense mutation in the G protein subunit α_q gene identified in patients with familial SWS. Trace chromatograms were developed using Sanger sequencing and revealed a validated missense mutation (p.R183Q). The three upper panels illustrate the sequencing chromatograms of the patients with familial, SWS II-1, III-11 and IV-6, while the lower panel illustrates the sequencing chromatogram of the control group, IV-5. SWS, Sturge-Weber syndrome.



Figure 3. Evolutionary conservation analysis of amino acids affected by the missense mutation. The identification numbers of guanine nucleotide-binding protein (G protein), α_q polypeptide were as follows: Human (NP_0,02063.2), Chimpanzee (XP_0,033,12167.1), Rhesus (XP_0,011,00833.1), Bovine (NP_0,011,03472.1), Rat (NP_112,298.1) and Mouse (NP_03,2165.3). The mutant alleles are boxed, and the asterisk (*) shows the conserved residue.

knowledge, this is the first genetic mutation to be associated with the pathogenesis of familial SWS. The results of the present study, in addition to data from previous studies, suggest that an imbalance in the hydrolysis of GTP is the common cause of certain congenital neurocutaneous disorders, such as SWS. These findings may aid in the development of novel diagnostic markers and/or therapeutic targets for the treatment of patients with familial SWS.

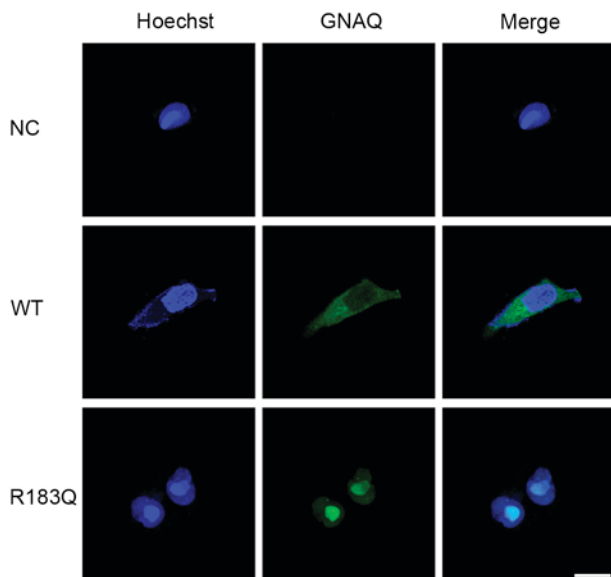


Figure 4. Immunolocalization analysis of GNAQ (WT) and GNAQ (R183Q) in transfected 293T cells. Green represents the anti-GNAQ staining, nuclei were stained with Hoechst 33,258. Cells were transfected with plasmid DNA (pEGFP-C1 vector) encoding the GNAQ (WT) or GNAQ (R183Q). Normal 293T cells transfected with the empty vector (pEGFP-C1) served as controls. Cells transfected with GNAQ (WT) illustrated GNAQ recombinant protein expression predominantly in the cytoplasm, whereas expression of the GNAQ recombinant protein from the GNAQ (R183Q) transfected cells was mainly detected as patches distributed across the entire nucleus. Scale bar, 10 μ m. GNAQ, G protein subunit α ; WT, wild-type; R183Q, p.Arg183Gln; EGFP, enhanced green fluorescent protein; NC, normal control.

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