*GNAQ* Mutations in Diffuse and Solitary Choroidal Hemangiomas

Francis JH1,2,Milman T3, Grossniklaus H4, Albert DM5, Folberg R6, Levitin GM7, Coupland SE8, Catalanotti F1, Kandoth C1, Busam KJ1,2, Abramson DH1,2.

1Memorial Sloan-Kettering Cancer Center, New York, NY

2Weill Cornell Medical Center, New York, NY

3Departments of Ophthalmology and Pathology, Wills Eye Hospital and Thomas Jefferson University Hospital, Sidney Kimmel Medical College of Thomas Jefferson University, Philadelphia, PA

4Department of Ophthalmology, Emory University School of Medicine, Atlanta, GA 30322

5McPherson Eye Research Institute, University of Wisconsin-Madison, Madison, WI

6Oakland University William Beaumont School of Medicine, Rochester, MI

7New York Eye and Ear Infirmary, New York, NY

8Department of Cellular and Molecular Medicine, University of Liverpool, Liverpool, UK

Short title: Mutations in choroidal hemangiomas

The Fund for Ophthalmic Knowledge supported this study, Research to Prevent Blindness and Cancer Center Support Grant (P30 CA008748), Cycle for Survival, and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology. The sponsor or funding organization had no role in the design or conduct of this research.

Corresponding author:

 Jasmine H. Francis MD

 Ophthalmic Oncology Service

 Memorial Sloan-Kettering Cancer Center

 1275 York Ave, New York, NY 10065

 francij1@mskcc.org

 Fax: 646 227 7275

**Abstract**

Purpose: *GNAQ* mutations have been identified in capillary malformations (both Sturge-Weber associated and non-syndromic) and melanocytic intraocular neoplasms. This study investigates the presence of *GNAQ* mutations in diffuse- (the vascular malformation associated with Sturge-Weber) and solitary choroidal hemangiomas.

Participants: Tissue from 11 patients with the following diagnoses: cutaneous capillary malformation (n = 3), diffuse choroidal hemangioma (n = 1), solitary choroidal hemangioma (n = 6), choroidal nevus (n =1)

Methods: Ten specimens were interrogated with Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a hybridization capture-based next-generation sequencing assay for targeted deep sequencing of all exons and selected introns of 341 key cancer genes in formalin-fixed, paraffin-embedded tumors. Digital polymerase chain reaction was used to detect *GNAQ* Q209 mutation in one specimen.

Main Outcomes: Detection of *GNAQ* codon-specific mutation

Results: Activating somatic *GNAQ* mutations (c.547C>T; p.Arg183Cys) were found in 100% (3 of 3) cutaneous capillary malformations and the diffuse choroidal hemangioma. Somatic *GNAQ* mutations (c.626A>T;p.Gln209Leu) were found in 100% (6 of 6) solitary choroidal hemangiomas and (c.626A>C;p.Gln209Pro) in the choroidal nevus.

Conclusion: *GNAQ* mutations occur in both diffuse and solitary hemangiomas, however, at distinct codons. An R183 codon is mutant in diffuse choroidal hemangioma, consistent with other Sturge-Weber vascular malformations. By contrast, solitary choroidal hemangiomas have mutations in the Q209 codon, similar to other intraocular melanocytic neoplasms.

**Introduction**

Mutations in *GNAQ* or *GNA11* result in dysregulation of the mitogen-activated protein kinase (MAPK), which influences gene transcription and results in cellular proliferation.1 A group of vascular and pigmented neoplasms is recognized to have *GNAQ/11* mutations, and this includes both benign and malignant variants (Table 1).2-20 21 22 Many of these lesions have been identified in the eye or the periocular tissues. That is, the most frequently mutated lesions include 82-95% of choroidal nevi and melanomas, which have mutually exclusive mutations in *GNAQ* or *GNA11*, and most commonly at the Q209 codon.11 With similar frequency, capillary malformations (nevus flammeus, either non-syndromic or related to Sturge-Weber) have *GNAQ* mutations, but at codon R183.2

The eyes of patients with Sturge-Weber syndrome develop an intraocular vascular malformation, termed diffuse choroidal hemangioma. There is no published information regarding the specific genetic aberrations in these intraocular lesions. Also, there is no published genetic information regarding the non-syndromic counterpart, solitary choroidal hemangiomas. These lesions are discovered later in life, can be asymptomatic and unlike hemangiomas in Sturge-Weber syndrome are not associated with ipsilateral glaucoma. In this study, we investigated whether diffuse and solitary choroidal hemangiomas harbor *GNAQ/11* mutations with particular attention to the codon involved.

**Methods**

This retrospective study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC), New York Eye and Ear Infirmary and University of Washington School of Medicine and Public Health and adhered to the Declaration of Helsinki. At the participating institutions, hemangioma specimens were retrieved from the archives of the Department of Pathology. The specimens were obtained through a search of the respective database, patient and tissue records. The cohort included 11 specimens: 1 diffuse choroidal hemangioma, three cutaneous capillary malformations (2 related to SW and one non-SW), six solitary choroidal hemangiomas and one choroidal nevus. An ophthalmic pathologist at each institution made the initial pathological diagnosis. The correct diagnosis was confirmed and the amount of lesional tissue assessed for suitability for inclusion in the study by MSKCC pathology (KB). 10 of 11 samples were investigated with the Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) Assay. Due to a small quantity of tissue, one specimen (number 8) was studied using the digital droplet polymerase chain reaction (ddPCR) assay for the *GNAQ* residue Q209 mutation.

*Isolation and purification of DNA*

Microdissection was performed on the ten formalin-fixed and paraffin-embedded (FFPE) samples on 10µm-thick unstained sections, using hematoxylin and eosin-stained (H&E) sections as a guide. The DNeasy Tissue Kit (Qiagen) was used for DNA extraction according to manufacturer’s recommendations. The Nano-Drop 8000 (Thermo Scientific) and Qubit (Life Technologies) were employed to quantify the extracted DNA. The minimum concentration of formalin fixed paraffin embedded DNA was 250ng.

*Exon-capture Sequencing*

Genetic alterations in *GNAQ/11* were profiled using the IMPACT assay (Integrated Mutation Profiling of Actionable Cancer Targets). As previously described in detail,23,24 this assay employs solution phase hybridization-based exon capture and massively parallel DNA sequencing to interrogate all protein-coding exons and select introns of 410 oncogenes, tumor suppressor genes, and members of pathways considered actionable by targeted therapies. The concentration of genomic DNA and DNA sequence library are both tested at the beginning of the assay before and after exon capture. If the yield is too low at any of these steps, the sample is designated as "failed": this designation did not apply to any specimens studied.

*Digital drop polymerase chain reaction assay (ddPCR)*

Assays specific for the detection of GNAQ-p.Q209R-626A>G were designed and ordered through BioRad (Hercules, CA): (Unique Assay ID: dHsaMDS971099482).

Cycling conditions were tested to allow for optimal annealing/extension temperature in addition to optimal separation of positive from empty droplets. All reactions were executed on a QX200 ddPCR system (BioRad, Hercules CA). Each sample was assessed for technical duplicates. PCR reactions contained primers and probes, DNA and digital PCR Supermix for probes. Reactions were partitioned into a median of ~16,000 droplets per well employing the QX200 droplet generator. Emulsified PCRs were run on a 96-well thermal cycler utilizing cycling conditions identified during the optimization step (95°C 10’;40 cycles of 94°C 30’’ 55°C 1’, 98°C 10’, 4°C hold). Plates were read and analyzed with the QuantaSoft software (BioRad, Hercules, CA) to evaluate the number of droplets positive for mutant DNA, wild-type DNA, both, or neither.

**Results**

All ten specimens contained activating somatic *GNAQ* mutations in either c.547C>T; p.Arg183Cys (p.R183Q), c.626A>C;p.Gln209Pro (p.Q209P) or c.626A>T;p.Gln209Leu (p.Q209R). Table 2 provides a summary of the results.

**Discussion**

Sturge-Weber syndrome (encephalofacial angiomatosis) is a phakomatosis and manifests clinically as a vascular malformation involving the skin, neural tissue, and eye. It occurs sporadically from an activating mutation in *GNAQ* at codon R183.2 Published reports have confirmed the presence of this mutation in many characteristic features of Sturge-Weber including cutaneous capillary malformations (Nevus Flammeus)2,3,6 in addition to gingival lesions,5,6 leptomeningeal angiomatosis2,7,8 and even the adjacent cortex/white matter.7 However, there are no published results that identify the presence of the hallmark genetic aberration in the intraocular tumor associated with this syndrome. In this study, we confirm that the intraocular manifestation of Sturge-Weber, a diffuse choroidal hemangioma, likewise has a mutation in *GNAQ* at the R183Q codon.

 Solitary choroidal hemangioma is a circumscribed vascular tumor histopathologically similar to the diffuse counterpart, although its clinical presentation and features are distinct. One of the central questions in this present study was whether solitary and diffuse choroidal hemangiomas share the same mutational profile. We discovered that they both exhibit mutations in *GNAQ*, but at different codons: diffuse hemangiomas at R183Q, which is consistent with Sturge-Weber, and solitary hemangiomas at Q209. The latter is similar to other solitary uveal neoplasms: choroidal nevi and uveal melanoma.

The significance of these specific codons has been debated in the literature in relation to other *GNAQ* mutated lesions. Mutations in either R183 or Q209 both have a gain-of-function effect with hyperactivation of downstream MAPKinase pathways.1 However, they have a distinct impact on G-alpha protein structure and different modulation of signal intensity.2,5,9,25 For example, mutations in codon 183 partially inactivates guanosine triphosphatase (GTPase),3 which activates extracellular signal-related kinase (ERK),2 but not p38 nor Jun N-terminal Kinase (JNK). It has been proposed that these “weaker and less promiscuous activated effects” are also a result of partial regulation by a member of the RGS family (regulator of G-protein signaling).2 In contrast, mutations in codon 209 result in *complete* inactivation of GTPase, and subsequent activation of both p38 and JNK, thereby resulting in a more profound activation of downstream MAPKinase signaling.2,3 In table 1, note that Q209 inhibition appears to occur more often in lesions that are solitary and deeply or viscerally located.

The question becomes how we can relate this knowledge to diffuse and solitary hemangiomas. It has been proposed that the more severe Q209 inhibition of GTPase, compared to R183, may explain the presence of this mutation in “tumors” (which we interpret as mass-producing neoplasms with a potential for significant growth) and *not* stationary malformations.3,11,13Akin to Happle’s theory, which states that less severe mutations are more sustainable in the germline,26 could it be possible that less severe codon-specific mutations are supportable more often in malformations than tumors? Both Sturge-Weber and Phakomatosis Pigmentovascularis have the less severe R183 mutations identified in their characteristic malformations.9 In line with this, we identified the R183 mutation in diffuse capillary hemangioma, which is consistent with this lesion being a malformation related to Sturge-Weber. In contrast, the codon Q209 mutation in solitary choroidal hemangiomas is more compatible with a non-stationary neoplastic process in line with the clinical behavior of these lesions, rather than with a developmental aberration that yields a stationary malformation. As such, it would point toward solitary choroidal hemangiomas as acquired lesions (and therefore absent at birth –a contentious subject), much along the same lines as other *GNAQ* Q209 mutant lesions such as choroidal nevi, uveal melanomas. To add complexity to this topic, it may not simply be distinct genomics that differentiate the final phenotype, but we have to also consider the contribution of epigenetics and eventually environmental factors.

Both Sturge-Weber associated cutaneous capillary malformations and non-syndromic capillary malformations share mutations at the same R183 codon of *GNAQ*. Cuoto et al. also advanced the hypothesis that an early versus late origin of the mutation defines whether the pathology is syndromic or non-syndromic4. If this proves to be accurate, then it would follow that syndromic and non-syndromic capillary malformations may have the same mutation but at different time points in embryologic development. If solitary choroidal hemangiomas were merely the non-syndromic counterpart to diffuse choroidal hemangiomas, one might expect them to share codon-specific *GNAQ* mutations and to be detected at birth. However, solitary hemangiomas are typically detected in adulthood with a characteristic progressive clinical course and have distinct codon mutations at Q209, suggesting they are different from being merely the non-syndromic version of diffuse choroidal hemangiomas.

The 183-codon-specific mutation in capillary hemangiomas is recognized to be rich in endothelial cells. Cuoto et al. hypothesized that the pathogenesis of capillary hemangiomas occurs from the aberrant communication between mutant endothelial cells and wild-type perivascular cells4. Presumably, even though solitary choroidal hemangiomas and choroidal nevi/melanomas share mutations in the same gene (*GNAQ*), at the same codon (Q209), the mutation-harboring cell type differs in these lesions: occurring at the endothelial cell in solitary hemangiomas and the melanocyte in nevi/melanomas. We invite future studies to prove this hypothesis to be correct and to confirm our present findings with a larger scale study. With the recent discovery of a natural compound (FR900359), which targets and deactivates the aberrant G alpha q signaling,27 the identification of *GNAQ* mutations in both diffuse and solitary choroidal hemangiomas may have promising clinical applications.

**References**

1. Akinleye A, Furqan M, Mukhi N, et al. MEK and the inhibitors: from bench to bedside. J Hematol Oncol 2013;6:27.

2. Shirley MD, Tang H, Gallione CJ, et al. Sturge-Weber syndrome and port-wine stains caused by somatic mutation in GNAQ. N Engl J Med 2013;368:1971–1979.

3. Couto JA, Huang L, Vivero MP, et al. Endothelial Cells from Capillary Malformations Are Enriched for Somatic GNAQ Mutations. Plast Reconstr Surg 2016;137:77e–82e.

4. Couto JA, Ayturk UM, Konczyk DJ, et al. A somatic GNA11 mutation is associated with extremity capillary malformation and overgrowth. Angiogenesis 2017;20:303–306.

5. Martins L, Giovani PA, Rebouças PD, et al. Computational analysis for GNAQ mutations: New insights on the molecular etiology of Sturge-Weber syndrome. J Mol Graph Model 2017;76:429–440.

6. Ma G, Yu Z, Liu F, et al. Somatic GNAQ mutation in different structures of Port-wine Macrocheilia. Br J Dermatol 2018.

7. Sundaram SK, Michelhaugh SK, Klinger NV, et al. GNAQ Mutation in the Venous Vascular Malformation and Underlying Brain Tissue in Sturge-Weber Syndrome. Neuropediatrics 2017;48:385–389.

8. Nakashima M, Miyajima M, Sugano H, et al. The somatic GNAQ mutation c.548G>A (p.R183Q) is consistently found in Sturge-Weber syndrome. J Hum Genet 2014;59:691–693.

9. Thomas AC, Zeng Z, Rivière J-B, et al. Mosaic Activating Mutations in GNA11 and GNAQ Are Associated with Phakomatosis Pigmentovascularis and Extensive Dermal Melanocytosis. J Invest Dermatol 2016;136:770–778.

10. 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.

11. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in GNA11 in uveal melanoma. N Engl J Med 2010;363:2191–2199.

12. Lim YH, Bacchiocchi A, Qiu J, et al. GNA14 Somatic Mutation Causes Congenital and Sporadic Vascular Tumors by MAPK Activation. Am J Hum Genet 2016;99:443–450.

13. Ayturk UM, Couto JA, Hann S, et al. Somatic Activating Mutations in GNAQ and GNA11 Are Associated with Congenital Hemangioma. Am J Hum Genet 2016;98:789–795.

14. Bean GR, Joseph NM, Gill RM, et al. Recurrent GNAQ mutations in anastomosing hemangiomas. Mod Pathol 2017;30:722–727.

15. Joseph NM, Brunt EM, Marginean C, et al. Frequent GNAQ and GNA14 Mutations in Hepatic Small Vessel Neoplasm. Am J Surg Pathol 2018:1.

16. Isales MC, Haugh AM, Bubley J, et al. Genomic Assessment of Blitz Nevi Suggests Classification as a Subset of Blue Nevus Rather Than Spitz Nevus: Clinical, Histopathologic, and Molecular Analysis of 18 Cases. Am J Dermatopathol 2018;40:118–124.

17. Vader MJC, Madigan MC, Versluis M, et al. GNAQ and GNA11 mutations and downstream YAP activation in choroidal nevi. Br J Cancer 2017;117:884–887.

18. Francis JH, Wiesner T, Milman T, et al. Investigation of Somatic GNAQ, GNA11, BAP1 and SF3B1 Mutations in Ophthalmic Melanocytomas. Ocul Oncol Pathol 2016;2:171–177.

19. Mudhar HS, Doherty R, Salawu A, et al. Immunohistochemical and molecular pathology of ocular uveal melanocytoma: evidence for somatic GNAQ mutations. Br J Ophthalmol 2013;97:924–928.

20. Küsters-Vandevelde HVN, van Engen-van Grunsven IACH, Coupland SE, et al. Mutations in g protein encoding genes and chromosomal alterations in primary leptomeningeal melanocytic neoplasms. Pathol Oncol Res 2015;21:439–447.

21. Emley A, Nguyen LP, Yang S, Mahalingam M. Somatic mutations in GNAQ in amelanotic/hypomelanotic blue nevi. Hum Pathol 2011;42:136–140.

22. Francis JH, Grossniklaus HE, Habib LA, et al. BRAF, NRAS, and GNAQ Mutations in Conjunctival Melanocytic Nevi. Invest Ophthalmol Vis Sci 2018;59:117–121.

23. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J Mol Diagn 2015;17:251–264.

24. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017.

25. Huang Z, Li Y, Zhao Z, et al. GNAQ mutation R183Q as a potential cause of familial Sturge-Weber syndrome: A case report. Oncol Lett 2017;13:2665–2669.

26. 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.

27. Onken MD, Makepeace CM, Kaltenbronn KM, et al. Targeting nucleotide exchange to inhibit constitutively active G protein α subunits in cancer cells. Sci Signal 2018;11:eaao6852.

Table 1: Published literature on GNAQ mutations

|  |  |
| --- | --- |
| Diagnosis  | Mutated Codon |
| GNAQp.R183 | GNA11p.R183 | GNAQp.Q209 | GNA11p.Q209 |
| SW capillary malformation | 12/13 (88%)2,3, 18/20 (90%)6 |   |   |   |
| non-SW capillary malformation | 23/26 (92%)2,3 |  |  |  |
| SW gingval lesion | 1 case5, 19/20 (95%)6 |   |   |   |
| SW CNS (leptomeningeal angiomatosis) | 15/18 (83%)2, 9/9 (100%)7, 12/15 (80%)8 |  |  |  |
| SW CNS (cortex/white matter) | 7/9 (78%)7 |   |   |   |
| Extensive dermal melanocytosis | 1/3 (33%)9 |  | 1/3 (33%)9 |  |
| Phakomatosis pigmentovascularis | 2/8 (25%)9 | 4/8 (50%)9 |   |   |
| Lobular capillary hemangioma |  | 2/3 (66%)12 |  |  |
| Capillary malformation extemity |   | 3/7 (43%)4 |   |   |
| Congenital hemangioma |  |  | 8/16 (50%)13 | 4/16 (25%)13 |
| Anastamosing hemangioma |   |   | 9/13 (69%)14 |   |
| Hepatic small vessel neoplasm |  |  | 2/3 (66%), 2/8 (25%)15 |  |
| Common blue nevus |   | 1/41 (2.4%)10,11 | 4/10 (40%)21, 39/60 (65%)10,11 | 4/60 (6.7%)10,11 |
| Blitz nevi |  |  | 1/7 (14%)16 |  |
| Uveal melanoma | 4/145 (2.8%)10,11 | 3/145 (2.1%)10,11 | 73/163 (44.8%)10,11 | 52/163 (31.9%)10,11 |
| Melanosis oculi | 1/11 (9.1%)10,11 |  | 2/20 (10%)10,11 | 1/20 (5%)10,11 |
| Choroidal nevi |   |   | 7/16 (44%)17 | 8/16 (50%)17 |
| Melanocytoma (iris, optic n) |  |  | 2/6 (33%)18 |  |
| Melanocytoma (ciliochoroid) |   |   | 2/2 (100%)19 |   |
| Melanocytoma (CNS) |  |  | 8/16 (50%)20 |  |
| Conjunctival blue nevus |   |   | 2/2 (100%)22 |   |
| SW = Sturge-Weber, CNS = central nervous system |
|

Table 2: Pathological diagnosis and GNAQ mutations

|  |  |  |
| --- | --- | --- |
| **Specimen** | **Pathological Diagnosis** | **GNAQ mutation** |
| 1 | SW Diffuse Choroidal Hemangioma | p.R183Q |
| 2 | SW Cutaneous Capillary Malformation | p.R183Q |
| 3 | Non SW Cutaneous Capillary Malformation |  p.R183Q |
| 4 | SW Cutaneous Capillary Malformation | p.R183Q |
| 5 | Solitary Choroidal Hemangioma  | p.Q209R |
| 6 | Solitary Choroidal Hemangioma  | p.Q209R |
| 7 | Solitary Choroidal Hemangioma  | p.Q209R |
| 8 | Solitary Choroidal Hemangioma\*  | p.Q209R |
| 9 | Solitary Choroidal Hemangioma  | p.Q209R |
| 10 | Solitary Choroidal Hemangioma  | p.Q209R |
| 11 | Choroidal Nevus | p.Q209P |
| \* = by ddPCR assay, SW = Sturge-Weber |   |