HHS Public Access

Author manuscript

Angiogenesis. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

Angiogenesis. 2017 August ; 20(3): 303–306. doi:10.1007/s10456-016-9538-1.

A Somatic *GNA11* Mutation is Associated with Extremity Capillary Malformation and Overgrowth

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Abstract

Background—Capillary malformation is a cutaneous vascular anomaly that is present at birth, darkens over time, and can cause overgrowth of tissues beneath the stain. The lesion is caused by a somatic activating mutation in *GNAQ*. In a previous study we were unable to identify a *GNAQ* mutation in patients with a capillary malformation involving an overgrown lower extremity. We hypothesized that mutations in *GNA11* or *GNA14*, genes closely related to *GNAQ,* also may cause capillary malformations.

Methods—Human capillary malformation tissue obtained from 8 patients that had tested negative for *GNAQ* mutations were studied. Lesions involved an extremity (n=7) or trunk (n=1). Droplet digital PCR (ddPCR) was used to detect *GNA11* or *GNA14* mutant cells (p.Arg183) in the specimens. Single molecule molecular inversion probe sequencing (smMIP-seq) was performed to search for other mutations in *GNA11.* Mutations were validated by sublconing and sequencing amplimers.

Results—We found a somatic *GNA11* missense mutation (c.547C>T; p.Arg183Cys) in 3 patients with a diffuse capillary malformation of an extremity. Mutant allelic frequencies ranged from 0.3%–5.0%. *GNA11* or *GNA14* mutations were not found in 5 affected tissues or in unaffected tissues (white blood cell DNA).

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The authors have no disclosures

Conculsions—*GNA11* mutations are associated with extremity capillary malformations causing overgrowth. Pharmacotherapy that affects *GNA11* signaling may prevent the progression of capillary malformations.

Keywords

capillary malformation; *GNAQ*; *GNA11*; vascular anomaly; extremity

INTRODUCTION

Capillary malformation is a congenital vascular lesion that affects the integument. Lesions darken, thicken, and cause disfigurement; adipose, muscle, and bone beneath the stain also can become overgrown. Somatic mutations in *GNAQ* (altering amino acid residue p.Arg183) result in constitutive activation of G-protein signaling and sporadic and syndromic (i.e., Sturge-Weber Syndrome) capillary malformations [1–3]. Mutations are enriched in the lesions' endothelial cell compartment [3]. In a previous study we were unable to identify *GNAQ* mutations in a cohort of capillary malformations involving the lower extremity [3]. We hypothesized that somatic *GNA11* mutations would also cause capillary malformations because *GNAQ* and *GNA11* share > 90% amino acid identity, and mutations affecting a different amino acid residue p.Gln209 in either *GNAQ* or *GNA11* cause congenital hemangiomas [4].

METHODS

The Committee on Clinical Investigation of Boston Children's Hospital approved this study. Two biopsy specimens were collected as part of a research protocol while the remaining samples were obtained during a clinically-indicated procedure. Capillary malformation was diagnosed based on history, physical examination, and histopathology. Tissues were flash-frozen and placed in −80°C until further processing. Capillary malformation specimens from 8 patients that previously tested negative for a *GNAQ* mutation by droplet digital PCR (ddPCR) were included in this study. Because the most common *GNAQ* mutation in capillary malformation affects p.Arg183, we designed a ddPCR assay to screen for mutations in the equivalent codon of *GNA11* or *GNA14*.

RESULTS

We found a somatic *GNA11* missense mutation (c.547C>T; p.Arg183Cys) in specimens from 3 patients (Table 1, Fig. 1). Individuals had a phenotype consistent with diffuse capillary malformation with overgrowth [5]. We validated the presence of the mutation in affected tissue using an orthologous method by subcloning and sequencing amplimers from an independent PCR reaction performed on separately extracted DNA. In 2 patients with *GNA11* mutations in affected tissue (participant 1, participant 5) we performed ddPCR on unaffected tissue (white blood cell DNA) and did not detect the mutant allele. We excluded false positive results, which could occur if our ddPCR assay had poor specificity, by not finding a *GNA11* or *GNA14* mutation in affected tissue from patients with capillary malformations caused by *GNAQ* mutations. To determine if other mutations in *GNA11* account for the ddPCR-negative samples, we performed single molecule molecular inversion

probe sequencing (smMIP-seq) [4] in 4 specimens that had sufficient DNA. No additional *GNA11* mutations were identified.

DISCUSSION

The most common mutation found in capillary malformation is *GNAQ* p.Arg183Gln [1,3,6]. Other *GNAQ* somatic missense mutations affecting residue 183, p.Arg183Leu and p.Arg183Gly, also have been observed in patients with capillary malformation [3]. Here, we report a second gene responsible for this lesion, *GNA11.* Consistent with our finding, functional overlap or redundancy between *GNAQ* and *GNA11* also have been observed in uveal melanoma [7], phakomatosis pigmentovascularis [8], melanomas associated with blue nevi [9], and congenital hemangiomas [4]. Although *GNA14* shares ~80% amino acid identity with *GNAQ* and *GNA11,* and can cause vascular tumors [10], we did not identify *GNA14* mutations in capillary malformations.

GNAQ and *GNA11* R183 mutations partially inactivate guanosine triphosphatase (GTPase) activity, resulting in constitutive activation of the mitogen-activated protein (MAP) kinase pathway; Q209 mutations completely inactivate the enzyme [11]. These mutations affect vascular endothelial growth factor-2 (VEGF-2) function [12–14]. Because VEGF2 is critical for both vasculogenesis and angiogenesis, mutations affecting this protein likely affect the pathophysiology of different types of vascular lesions (i.e., capillary malformation, congenital hemangioma). The more severe Q209 inhibition of GTPase may explain the presence of this mutation only in tumors and not malformations [4,11].

Currently, we do not know whether there are phenotypic differences between patients with *GNAQ* and *GNA11* p.Arg183 mutations. Among published reports, >95% of facial capillary malformations have a *GNAQ* p.Arg183 mutation [1,3,6]. In this study as well as a previous investigation [3], we have examined 10 extra-facial capillary malformations and have observed greater genetic heterogeneity: *GNA11* p.Arg183Cys in 3 legs and 1 arm, *GNAQ* p.Arg183Leu in 1 leg, and neither *GNA11* nor *GNAQ* in 4 legs and 1 trunk.

Our failure to find a *GNA11, GNA14,* or *GNAQ* mutation in several specimens could be because the mutant allele frequency was below our limit of detection. Intriguingly, participant 2 might be an example of this since her specimen had the *GNA11* p.Arg183Cys allele detected by ddPCR and smMIP-seq, but at levels below our true-positive threshold. Alternatively, mutations in another gene may be responsible for causing capillary malformations. Our identification of *GNA11* gain-of-function mutations in a subset of patients with capillary malformation indicate that drugs which suppress constitutive *GNAQ* or *GNA11* signaling may have therapeutic value.

Acknowledgments

Research reported in this manuscript was supported by the National Institutes of Health Award NICHD-081004 (AKG), National Institutes of Health Award NICHD-082606 (AKG), National Institutes of Health Award HL127030 (JB, AKG), National Institutes of Health Award AR-064231 (MLW), the Translational Research Program Mid-Career Award Boston Children's Hospital (AKG), and the Translational Neuroscience Center Pilot Study Award Boston Children's Hospital (JB, AKG). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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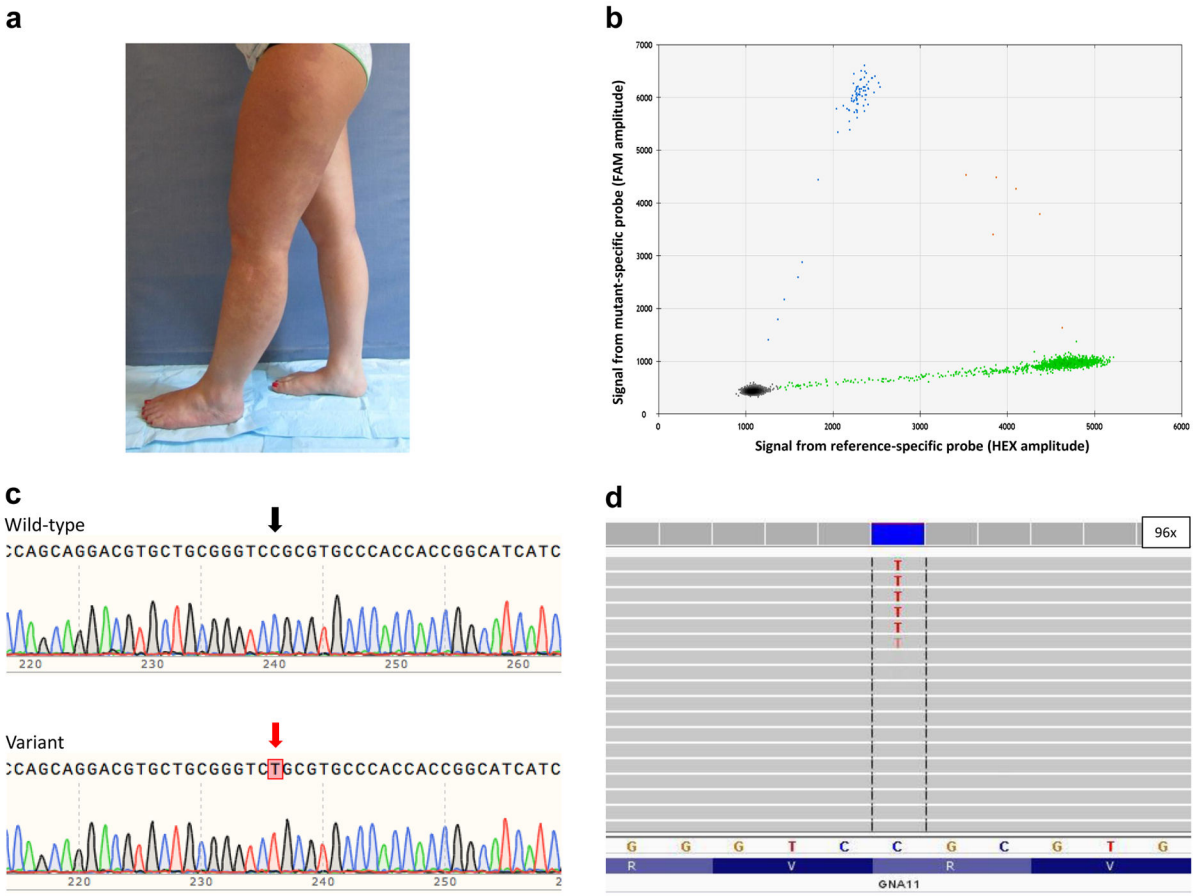

Figure 1.

GNA11 mutation in Participant 5. (**a**) Photograph depicting the patient's capillary malformation and hypertrophy of her left lower extremity. (**b**) Results from a ddPCR reaction displaying the presence of the *GNA11* c.547C>T (p.ArgR183Cys) mutation with a mutant allele frequency of ~5%. (**c**) Sanger sequencing of PCR-amplimer-subclones showing (*Top*) a clone with the wild-type sequence and (*Bottom*) another with the mutation at position 547 having a T (red arrow) instead of a C (black arrow). (**d**) Integrative Genomic Viewer screen-shot depicting MIP-seq coverage at the site of the mutation (c.547C>T) with ~5% mutant allele frequency. 96x indicates the depth of coverage at position 547.

Table 1

GNA11 Mutation Detection in Capillary Malformation

Patient	Age	Sex	Location	ddPCR	MIP-seq
1	9 y/o	F	Lower extremity	9/2871 (0.3%)	1/78 (1%)
2	9 y/o	F	Lower extremity	1/2214 (0%)[1]	1/270 (0.4%)
3	10 y/o	M	Lower extremity	0/4306 (0%)	0/146 (0%)
4	14 y/o	F	Lower extremity	0/3691 (0%)	0/61 (0%)
5	17 y/o	F	Lower extremity	75/1557 (5%)	5/94 (5%)
6	21 y/o	F	Posterior Trunk	1/7288 (0%)[1]	0/181 (0%)
7	25 y/o	F	Lower extremity	0/3815 (0%)	-
8	27 y/o	M	Upper extremity	90/3162 (3%)	-
			Lower extremity	21/4379 (0.5%)	-

Abbreviations: F, female; M, male; ddPCR, droplet digital polymerase chain reaction; MIP-seq, molecular inversion probes sequencing.

ddPCR and MIP-seq columns indicate number of droplets and read depth, respectively. The rate of variant/total alleles is also depicted in the aforementioned columns. Dash (−) indicates the assay was not performed.

[1]We considered a mutant allele frequency <1/000 to be background noise by ddPCR