Case Report/Case Series

Novel Genetic Mutations in a Sporadic Port-Wine Stain

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IMPORTANCE Port-wine stains (PWSs) are common congenital cutaneous capillary malformations. A somatic *GNAQ* mutation was recently identified in patients with sporadic PWSs and Sturge-Weber syndrome. However, subsequent studies to confirm or extend this observation are lacking.

OBSERVATIONS We report a long-standing, unilateral facial PWS of a man in his early 70s confirmed by histopathological analysis. Staged surgical excision of the vascular malformation was performed, and genomic DNA was extracted from the vascular malformation specimen and normal skin. Targeted next-generation sequencing of the coding sequence of 275 known cancer genes including *GNAQ* was performed in both specimens. A single-nucleotide variant (c.548G>A, p.Arg183Gln) in *GNAQ* was identified in the PWS-affected tissue but not in the normal skin sample. In addition, this sequencing approach uncovered several additional novel somatic mutations in the genes *SMARCA4, EPHA3, MYB, PDGFR-β*, and *PIK3CA*.

CONCLUSIONS AND RELEVANCE Our findings confirm the presence of somatic mutations in *GNAQ* in the affected skin of a patient with congenital PWS, as well as alterations in several other novel genes of possible importance in the pathogenesis of PWS that may also offer substantial therapeutic targets.

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Port-wine stains (PWSs), or capillary malformation, are a common type of cutaneous vascular malformation with a prevalence of 0.3% to 0.5%.[1,2] Clinically, PWS often involves the head and neck as an isolated pink flat lesion that becomes darker and may thicken over time.[1] Port-wine stains may be part of a syndrome, including Sturge-Weber syndrome among others.[1] A long-standing hypothesis that PWSs are associated with an underlying somatic mutation was supported in a recent study. Twelve of 13 patients with nonsyndromic PWSs showed a mutation in a single-nucleotide variant (c.548G>A, p.Arg183Gln) in the *GNAQ* gene (OMIM 600998).[3]

In this report on a patient with a long-standing PWS lesion, we document the presence of a *GNAQ* c.548G>A mutation. Importantly, several novel mutations in the *SMARCA4, EPHA3, MYB, PDGFR*-β, and *PIK3CA* genes were also identified, extending the scope of mutational anomalies in this condition.

Report of a Case

A healthy man in his early 70s presented with a predominantly unilateral congenital facial vascular lesion. Physical examination revealed painless, dark red to violaceous macules, patches, coalescent plaques, and nodules on the right side of his face (**Figure 1**). There was marked hypertrophy of the bilateral lower lip, right cheek, right jaw, and right auricle (Figure 1). The lesion also extended to the left mentum and anterior neck. A clinical diagnosis of PWS was made, and Sturge-Weber syndrome was ruled out by appropriate studies. He had previously received laser and surgical therapy and most recently a staged surgical excision of the areas of soft-tissue hypertrophy over a 6-month period, during which an excisional biopsy of the previously untreated auricular nodule and a 1-cm diameter sample of normal skin area were obtained.

Histopathological Analysis

Compared with the normal skin sample, histopathological study of the auricular nodule showed extensive ectasia and dilatation of papillary dermal capillaries, postcapillary venules, and small veins in the superficial to mid-dermis (**Figure 2**A). The abnormal vascular structures exhibited different sizes and shapes throughout the lesional specimen (Figure 2A). The superficial abnormal vessels had very thin walls (Figure 2A), in contrast to the thick walls and hyalinized aspects of large vessels (Figure 2B). Those larger ectatic structures had thickened walls with multiple duplications of the basement membrane zones and entrapment of pericyte-like cells in the thickened areas (Figure 2B). The endothelial cells

Figure 1. Clinical Photograph of the Port-Wine Stain Lesion

Dark red to violaceous macules, patches, plaques, and nodules are prominent on the right side of the patient's face. Note the marked lower lip hypertrophy, as well as the deformed ear and partially blocked ear canal.

Figure 2. Histopathological Analysis of Port-Wine Stain (PWS) Lesional Tissue

A, Extensive ectasia and dilatation of the papillary dermal capillaries in the superficial to mid-dermis were noted in the PWS lesional tissue. Arrowheads indicate ectatic vessels (hematoxylin-eosin, original magnification ×100). B, Various sizes and shapes of vessels, many filled with blood (yellow arrowhead), especially in the lower dermis, exhibit thickened hyalinized walls (black arrowhead) and entrapment of pericyte-like cells in the thickened walls (blue arrowheads) (hematoxylin-eosin, original magnification ×400).
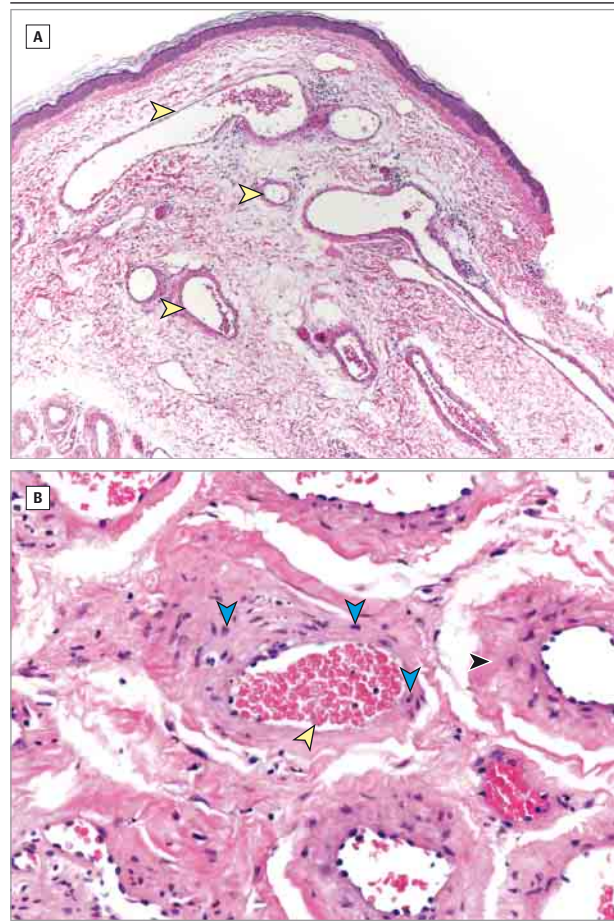
of some of the affected vessels were discontinuous along the basement membrane area (Figure 2B). When compared with the patient's normal skin, used as control, these histopathological changes were strikingly different and resembled those previously reported.[4]

Whole-Exon *GNAQ* Mutation Detection

DNA was isolated from formalin-fixed paired vascular malformation and normal tissue samples using standard methods. Samples were incubated in proteinase K overnight, followed by purification of the DNA (QIAamp DNA Mini Kit, Qiagen). DNA concentration was assessed using PicoGreen dsDNA detection (Life Technologies). Targeted next-generation sequencing was performed using a cancer genomic assay to detect mutations in 275 cancer genes previously implicated in tumorigenesis. The complete coding sequence of the target genes was captured using a solution-phase Agilent SureSelect hybrid capture kit (Agilent Technologies, Inc), and massively parallel sequencing was performed on an Illumina HiSeq 2500 sequencer (Illumina, Inc). Mutation calls were performed using Mutect and GATK software (Broad Institute).[5,6]

Samples were sequenced to a mean depth of 110 and 77 reads for lesional and normal skin, respectively. A c.548G>A nucleotide mutation was identified in the PWS sample at an allelic fraction of 0.05 (mean target coverage for *GNAQ* was 222 reads), whereas no such mutation was found in paired normal tissue (**Figure 3**). The percentage of *GNAQ* mutation was consistent with the percentage of lesional endothelial cells in the specimen. The endothelial cells made up approximately 5% of all cells in this lesional specimen. The variant affects guanine nucleotide-binding protein in a gain-of-function effect resulting from the substitution of amino acid p.Arg183Gln. In ad-

dition, several novel somatic mutations were also identified in our case, including *SMARCA4, EPHA3, MYB, PDGFR*-β, and *PIK3CA,* all present at less than 0.10 allelic fraction (**Table**). The complete gene list analyzed by sequencing is in the eTable in the Supplement.

Discussion

Port-wine stains are congenital cutaneous vascular malformations that affect 20 million individuals worldwide.[2] Clinically, PWSs are geographic or plaquelike pink, purple, or red macules and patches.[1] The lesions grow with the child.[1] They usually darken and may develop nodules and associated soft-tissue hypertrophy in adult life.[1] Port-wine stains may also be associated with other syndromes, especially Sturge-Weber syndrome, in which patients also have seizures, glaucoma,

Figure 3. A Single-Nucleotide Mutation Identified by Whole-Exon *GNAQ* Mutation Detection
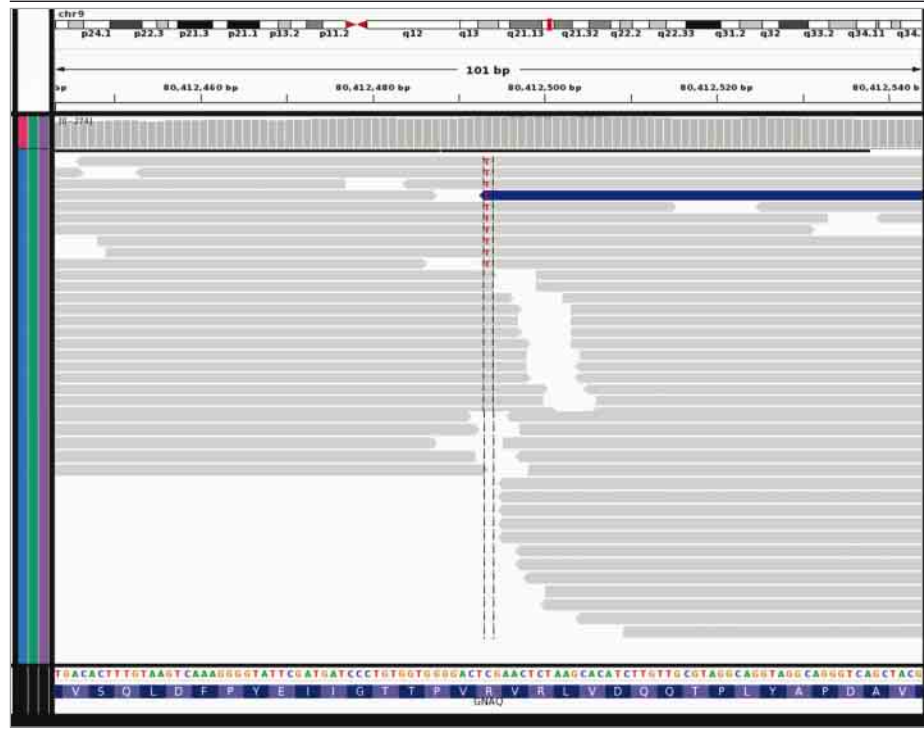
A single c.548G>A nucleotide mutation was identified in a tissue sample of a port-wine stain lesion, whereas no such mutation was found in paired normal tissue by whole-exon *GNAQ* mutation detection.

Table. *GNAQ* and Other Novel Somatic Mutations Identified in Our Patient

Gene	Mutation	Position	Coverage	AF	Canonical	AA Change
GNAQ	G/A	9:80412493	222	0.05	p.R183Q	Present
SMARCA4	G/C	19:11105624	125	0.07	p.E514Q	Present
EPHA3	C/G	3:89445047	180	0.07	p.S456C	Present
PIK3CA	GG/AA	3:178952090	174	0.05	p.G1049N	Present
PDGFR-β	C/T	5:149505035	64	0.06	p.E594K	Present
MYB	G/A	6:135516982	170	0.10	p.G349R	Present

Abbreviations: A, adenine; AA, amino acid; AF, allelic fraction; C, cytosine; G, guanine; T, thymine.

abnormal cerebral vasculature, and mental retardation.[1] Histopathologically, the lesions have been characterized by ectatic papillary dermal capillaries and postcapillary venules in the superficial dermis.[7] There is a substantial decrease in nerve density and increase in vessel-to-nerve ratio when compared with normal skin, thus suggesting a possible deficit in neural modulation.[7] Many treatments have been tried for PWS with varying degrees of efficacy. Currently, the pulsed-dye laser is the most effective treatment for PWS, particularly for early stages of superficial lesions in young patients.[2]

As far as the etiology of PWS is concerned, there are data to suggest that vascular endothelial growth factor (VEGF) and VEGF receptor could contribute to the pathogenesis of capillary malformations by inducing vessel proliferation and/or vasodilatation.[8] An inactivating mutation of *RASA1* on 5q encoding a guanosine triphosphatase-activating protein with negative regulation of Ras activity has been detected in some families with multiple capillary malformations.[9] With regard to the hypothesis suggesting a somatic mutation, a breakthrough was recently made in a study using amplicon sequenc-

ing and SNaPshot assays to test affected and unaffected tissue from 50 patients with Sturge-Weber syndrome, nonsyndromic PWSs, and normal tissue as controls for somatic mutations.[3] Eighty-eight percent of patients with Sturge-Weber syndrome and 92% of patients with nonsyndromic PWSs showed a single-nucleotide variant (c.548G>A, p.Arg183Gln) *GNAQ* mutation.[3] The data indicated that there is a unifying mechanism for nonsyndromic PWSs and provided a possible molecular basis associated with these malformations.

The *GNAQ* gene codes for G alpha q, a G-protein subunit that is involved in intracellular downstream signaling of transmembrane proteins.[3] GNAQ is involved in signaling within the mitogen-activated protein kinase (MAPK) and phospholipase C pathways, and cellular signals implicated in cell growth are regulated by these pathways. GNAQ is implicated in transmitting signals from receptors at the cell membrane to the MAPK pathway, which is also implicated in cellular growth. An activating mutation would therefore increase the signaling down this pathway, which may lead to the capillary malformations seen in Sturge-Weber syndrome and PWS.[3]

In addition to the *GNAQ* mutation, we also identified novel somatic mutations including the *SMARCA4, EPHA3, MYB, PDGFR*-β, and *PIK3CA* genes in our patient. Although these mutations have been implicated in the pathogenesis of different tumor types, little is known about their role in the development of vascular malformations. First, *SMARCA4*, also known as Brahma-related gene 1 (*BRG1*), is a mammalian SWItch/ sucrose nonfermentable (SWI/SNF)–like adenosine triphosphate–dependent chromatin-remodeling complex.[10] Mutated *BRG1* has been found to play a role in embryonic vascular development, specifically in promoting venous specification, the loss of which has been shown to result in poor remodeling of vessels; thin, blunt-ended vessels; or vessels that fail to interconnect.[10] The importance of this finding may relate directly to the structural abnormalities affecting the capillaries and veins that are present in the PWS.

In addition, the Eph receptors are a large family of receptor tyrosine kinases that bind ephrin ligands and are divided into EphA or EphB depending on their ephrin-binding preference.[11] The Eph/ephrin signaling system is involved in the regulation of angiogenesis through various mechanisms. EphA3 plays a role in cell adhesion, repulsion, and motility.[11] Although the effect of the *EphA3* mutation is not known, it is possible that its presence upregulates the function of EphA3, resulting in a repulsion of endothelial cells, contributing to the ectatic vascular structure seen in PWSs.

The *MYB* gene family plays a role in transcriptional transactivation.[12] The most studied protein in the MYB family, c-Myb, has been shown to function in cell cycle progression, hematopoiesis, and apoptosis.[12] Also, studies have shown that a decrease in c-Myb was associated with a decrease in vascular smooth muscle proliferation.[12] These studies intimate an important relationship between mutated c-Myb and the dilated thin vessels with abnormal muscle coat found in PWSs.

Platelet-derived growth factor (PDGF) has an important function in angiogenesis, as well as cell proliferation and growth. Whereas the role of PDGF has not been studied in PWSs, Roach et al[13] show that PDGF-BB, one of the PDGF ligands, inhibits adipogenesis in hemangioma-derived stem cells through PDGFR-β. The implication of a mutation in *PDGFR*-β in PWSs is unclear. It is possible that the mutated receptor binds PDGF-BB irreversibly, sending continuous downstream signaling to prevent adipogenesis, thereby preventing the PWS vascular involution.

Finally, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (*PIK3CA*), is a gene that encodes the p110α catalytic subunit of class IA phosphatidylinositol-3-kinase (PI3K).[14] Various mutations have been reported in *PIK3CA* and are associated with activation of the PI3K-AKT pathway, which normally regulates cell growth, angiogenesis, and survival, among other functions.[15] *PIK3CA* mutations have been associated with segmental overgrowth diseases such as CLOVES (congenital lipomatous asymmetric overgrowth of the trunk with lymphatic, capillary, venous, and combined-type vascular malformations, epidermal nevi, and skeletal anomalies) syndrome[14] and megalencephaly–capillary malformation syndrome.[15] Both of these syndromes are associated with cutaneous capillary and venous malformations, persistent nevus flammeus, or widespread cutis marmorata, as well as with multiple systemic manifestations.[14,15] Further study is warranted to elucidate the role of a mutation in *PIK3CA* and its apparent limitation to a cutaneous vascular anomaly (PWS), without additional systemic manifestations.

In our patient's PWS, many vessels, both capillaries and small veins, have abnormal shapes and often very thin walls, whereas others have thick walls with abnormal basement membrane zone abnormalities. We present the finding of several novel mutations in a patient with PWS in addition to confirming the presence of the *GNAQ* mutation. A mutation in these genes may have a variety of functions and interactions that may affect vascular cell growth and differentiation. A c-Myb mutation possibly affects vascular smooth muscle cell thickness and abnormal vascular smooth muscle organization. An EphA3 mutation may lead to intraendothelial cell interactions, possibly repulsion. PDGFR-β mutations may play a role in the maintenance of endothelial cell integrity in PWS. Also, Brg1 mutations may play a role in venous morphological abnormalities. The role of PIK3CA is unclear, but it may function through the AKT pathway, whereas GNAQ functions through the MAPK pathway.

It is important to highlight some study limitations. First, the patient has undergone multiple therapies and a limited amount of untreated skin was available for study; hence, only a single sample was studied. In spite of this apparent limitation, the findings confirmed the *GNAQ* mutation and also revealed mutations in 5 novel genes possibly important in the evolution of the PWS. Second, the lesional tissue was screened using a next-generation sequencing platform that includes 275 genes implicated in tumorigenesis. Because the PWS, especially in nodular growth, may show appendageal tumors, neural hamartomas, and prominent increased vessels and stroma, we consider the neoplasm platform to be most appropriate for our study. In addition, the platform includes somatic genes that are potentially actionable and informative in clinical application. Finally, the similar mutational frequency of all of the observed somatic mutations within this sample suggests that they coexist in 1 cellular population and may cooperate in the pathogenesis of PWSs. In addition, the genetic mutation frequencies correlate with the percentage of endothelial cells in the lesions histologically. Thus, the low allelic fraction of these gene mutations suggests that they may be vascular cell lineage restricted. Future studies will be needed to confirm our findings by laser-guided microdissection or by immunohistochemical staining using mutation-specific antibody or fluorescence-based in situ hybridization assays.

Conclusions

Considering the cumulative function of these genes, and their possible effect if mutated, should serve to widen our understanding of PWSs as a polygenic abnormality and provides a foundation for further scientific and clinical research, including a search for downstream targets as candidates for new therapeutic agents.

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