Unilateral Vestibular Schwannoma and Meningiomas in a Patient with PIK3CA-Related Segmental Overgrowth: Co-occurrence of Mosaicism for Two Rare Disorders

John R. Mills*1, Ann M. Moyer*1, Benjamin R. Kipp1, Andrzej B. Poplawski2, Ludwine M. Messiaen2, Dusica Babovic-Vuksanovic3

1Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN USA
2Medical Genomics Laboratory, Department of Genetics, University of Alabama, Birmingham, AB, USA
3Department of Clinical Genomics, Mayo Clinic, Rochester, MN USA

*These authors contributed equally

Corresponding Author:
Dusica Babovic-Vuksanovic, M.D.
Department of Clinical Genomics
Mayo Clinic
200 First Street SW
Rochester, MN 55905
dbabovic@mayo.edu
Phone:507-284-7511

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ABSTRACT

A 28-year-old female with PIK3CA-related segmental overgrowth presented with headaches.

She also had a unilateral vestibular schwannoma (VS), as well as three small (<2 cm) meningiomas, which according to the Manchester consensus diagnostic criteria for
neurofibromatosis 2 (NF2) is sufficient for a clinical diagnosis. Analysis of blood revealed a mosaic \textit{PIK3CA} c.2740G>A (p.Gly914Arg) mutation, confirming the diagnosis of \textit{PIK3CA}-related overgrowth, but no mutations in \textit{NF2} were detected. Although VS has not previously been reported in \textit{PIK3CA}-related segmental overgrowth, meningiomas have, raising the question of whether this patient’s VS and meningiomas represent coincidental NF2 or phenotypic extension of her overgrowth syndrome. Genetic analysis of the VS revealed a heterozygous \textit{NF2} mutation c.784C>T (p.Arg262Ter) and loss of a portion of 22q, including \textit{NF2}, \textit{SMARCB1}, and \textit{LZTR1} genes. These results suggest that the patient has two different mosaic disorders, NF2 and \textit{PIK3CA}-related overgrowth. The \textit{PIK3CA} mutation was also present in the VS. Confirmation of the clinical diagnosis of mosaic NF2 in this patient has implications for monitoring and highlights the possibility of co-occurrence of mosaicism for multiple rare disorders in a single patient.
INTRODUCTION

PIK3CA-Related Overgrowth Spectrum (PROS) includes disorders of the brain as well as segmental body overgrowth and typically results from somatic mutations in PIK3CA (1-3). Megalencephaly-capillary malformation syndrome (MCAP syndrome; OMIM #602501) is one of the PROS syndromes and is characterized by the major findings of megalencephaly or hemimegalencephaly associated with neurologic findings and cutaneous capillary malformations with focal or generalized somatic overgrowth. In addition to these major findings, patients may also demonstrate digital anomalies, cortical malformations and variable connective tissue dysplasia (4, 5). Both benign and malignant tumors have occurred in patients with MCAP syndrome which include reports of meningiomas (5-7). VS is not associated with PROS disorders (8). Most patients with MCAP syndrome have somatic mosaicism for a PIK3CA missense mutation (1-3).

Another syndrome that is associated with somatic mosaicism and has a propensity towards the development of tumors is NF2. Patients with neurofibromatosis 2 (NF2) classically develop bilateral VS. In addition, patients may also develop schwannomas involving other nerves, meningiomas, ependymomas, astrocytomas, posterior subcapsular lens opacities, or mononeuropathy. NF2 demonstrates autosomal dominant inheritance. Approximately 50% of individuals with NF2 have an affected parent while the remaining 50% have de novo mutations. In individuals without a family history of NF2, 25-30% are mosaic for an NF2 mutation (9). NF2, which is typically diagnosed based on clinical criteria, has an overall prevalence of approximately 1:60,000 (10). To establish the diagnosis of NF2, the Manchester (modified NIH) diagnostic criteria are often applied which includes multiple meningiomas AND unilateral VS or any two of schwannoma, glioma, neurofibroma, or cataract (11). In tumor tissue, two NF2...
mutations should be identified: one that represents the “first-hit” and should be identified in all
cells of an individual, and one that represents the “second-hit” and is unique to each tumor
within an individual. In cases of suspected NF2, schwannomatosis should be considered in the
differential diagnosis due to phenotypic overlap. In NF2, bilateral VS are almost universal
whereas in schwannomatosis, schwannomas are typically non-intradermal and nonvestibular.
However, there are rare cases of unilateral VS reported in patients with schwannomatosis (12).
Furthermore, both conditions may involve the development of meningiomas. In patients with
unilateral VS lacking other features typical of NF2, or in the absence of a proven germline or
mosaic NF2 mutation, genetic testing for *LZTR1* and *SMARCB1* mutations may confirm a
diagnosis of schwannomatosis.

**CASE REPORT**

A 28-year-old female with a history of *PIK3CA*-related segmental overgrowth presented
to Mayo Clinic with daily headaches, dizziness, and near syncopal episodes. At birth, she was
noted to be large (10 pounds, 1 ounce), had asymmetric macrocephaly, mottled skin, postaxial
polydactyly and bilateral two-three toe syndactyly. By 6 months of age, she had developed right
hemihypertrophy characterized by generalized body and head asymmetry that was uniform and
proportional. Multiple vascular lesions developed on her right temple in this time period, as well
as port-wine stains, and a cutis marmorata-like appearance to her skin was evident. While
several of her vascular lesions have spontaneously regressed or faded over time, others have
developed throughout her lifetime. In addition, she began to have seizures that resolved around
age 6 years and experienced global developmental delays. She was noted to have a recessed
midface with very prominent forehead and lower face that was asymmetric which required
craniofacial surgery at 2.5 years of age due to concern of optic nerve compression. A port-wine stain distributed over multiple portions of her body bilaterally was faint but still evident. A complete ophthalmologic exam indicated no signs of posterior subcapsular lenticular opacity, although epiretinal membranes were noted. No cutaneous features of NF were noted. No additional neurofibromas were noted on MRI of her cervical, thoracic, or lumbar regions. Her family history, including both biological parents, was unremarkable.

During evaluation for recurrent headaches, computed tomography (CT) revealed a 5.4 cm left-sided mass in the left cerebellopontine angle, large ventricles, three small distinct masses located in the right lateral frontal convexity dura, left occipital convexity dura adjacent to the superior sagittal sinus, and along the left transverse sinus (8mm, 18mm, and 15mm in diameter, respectively) consistent with meningiomas, and a Chiari malformation (Figure 1A). She underwent surgical debulking in hopes of correcting her headaches, dizziness and near syncope episodes. On pathologic examination, the mass was confirmed to be a VS (Figure 1B).

Although this patient’s overall clinical presentation was consistent with MCAP, VS is a feature not previously described in the PROS syndromes (4, 5). Furthermore, while meningiomas have been described in PIK3CA-related syndromes, they are also a feature of NF2 (13, 14). According to the Manchester consensus diagnostic criteria for NF2 and the more recent Baser criteria, with the presence of both VS and at least 2 meningiomas at <30 years of age, this patient meets the criteria for a clinical diagnosis of NF2 (11, 15, 16). Therefore, the question of whether this patient’s newly diagnosed VS represented a previously unreported manifestation of MCAP or coincidental mosaic NF2 was raised.

METHODS
Genomic DNA was isolated from peripheral blood cells. Targeted next-generation sequencing (NGS) of 7 key cancer genes (including PIK3CA) was performed at Baylor Medical Genetics Laboratories. Separately, NF2 genetic analysis using long-range RT-PCR with direct Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) was performed at the Medical Genomics Laboratory (MGL) at the University of Alabama at Birmingham.

Tumor DNA was extracted from formalin-fixed paraffin-embedded (FFPE) VS tissue after tumor macrodissection and was tested for mutations in NF2, SMARCB1, and LZTR1 in the MGL at the University of Alabama, using PCR and bidirectional sequencing of all exons and copy number analysis using MLPA. Tumor DNA was tested for PIK3CA mutations by a 50-gene NGS panel including PIK3CA, followed by Sanger sequencing confirmation at Mayo Clinic.

RESULTS & DISCUSSION

A pathogenic PIK3CA variant, c.2740G>A, p.Gly914Arg, was detected in the blood with a variant allele fraction (VAF) of 9% based on NGS reads, consistent with somatic mosaicism, and confirming the diagnosis of MCAP. However, initial testing of blood using Sanger sequencing did not reveal any mutations in NF2.

In the tumor DNA extracted from FFPE tissue from the patient’s VS, a heterozygous c.784C>T (p.Arg262Ter) pathogenic mutation in NF2 was identified, along with heterozygous loss of 22q (chr22:19,765,795-36,390,067; GRCh38/hg38), which includes the NF2, SMARCB1, and LZTR1 genes. No additional mutations were identified in either SMARCB1 or LZTR1. The absence of NF2 mutations in blood and presence of two mutations in the VS suggests that the
patient is mosaic for NF2. As Sanger sequencing has poor sensitivity for detecting VAFs <15%, more sensitive targeted approaches were used on the original blood-derived DNA, including co-amplification at lower denaturing temperature (COLD)-PCR, High-Resolution Melting Curve analysis and NGS. The \textit{NF2}:c.784C>T mutation was not detected by any of these methods, including in none out of 1119 reads generated by NGS. In addition to the \textit{NF2} variants, the same c.2740G>A (p.Gly914Arg) \textit{PIK3CA} mutation previously identified at mosaic levels in blood was identified in the VS by NGS. In the VS, this variant was identified in 10972 of 27593 reads (40%).

These results suggest that this patient has two rare mosaic disorders, MCAP and NF2. While the \textit{PIK3CA} mutation was present at a high enough level to be identified in the patient’s blood, the \textit{NF2} mutation was not. This may reflect a difference in the distribution of cells with the variant. Interestingly, mutations in both \textit{NF2} and \textit{PIK3CA} were identified in the patient’s VS. While the presence of two \textit{NF2} mutations in the VS but not in blood strongly suggests that the patient has mosaic NF2 which alone is known to cause VS, it is unclear how the \textit{PIK3CA} mutation may have contributed to the VS. The VS could represent a sporadic case which is not uncommon in individuals presenting with a unilateral VS in the absence of other clinical signs of NF2, however, this is unlikely as sporadic cases of unilateral VS typically present around 50 years of age whereas the patient reported in this study was only 28 years old at the time of diagnosis (17). Meningioma tissue was not available for testing.

The presence of both the \textit{NF2} and \textit{PIK3CA} mutation in the lesional tissue indicates that during development, a common precursor cell likely received two different mutational hits. A potential scenario is that the \textit{PIK3CA} mutation arose early leading to an expansion of cells and subsequently a \textit{NF2} mutation was acquired in a single \textit{PIK3CA} mutation-containing clonal cell
within the neural crest. This is supported by the presence of the PIK3CA mutation in blood and at near heterozygous levels within the VS (40% of reads). The p.Gly914Arg PIK3CA mutation is a well-studied and has only been documented in the mosaic state arising postzygotically during development.

Merlin, the protein encoded by NF2, is a tumor suppressor which acts as a negative regulator of the PI3K/Akt/mTOR pathway. Loss of NF2 and activating mutations in PIK3CA both increase downstream mTOR signaling. The VS also contained a second hit to the NF2 gene. This may indicate that the p.Gly914Arg PIK3CA mutation is not sufficient to replace the need for the second NF2 hit. Interestingly, this is not a “hot-spot” mutation, having been reported in once in the COSMIC database, and is postulated to be only modestly activating (3). In contrast, this is a recurrent mutation in patients with a MCAP diagnosis and has only been reported in the mosaic form.

With the currently available clinical material, it is not possible to determine which of the two NF2 mutations—the loss of a portion of 22q or the missense mutation c.278C>T—was the “first-hit” in the patient’s VS. The presence of two hits in NF2 and the absence of “second-hit” mutations in both LZTR1 and SMARCB1 make a diagnosis of schwannomatosis very unlikely.

This case highlights the utility of genetic testing in clinical scenarios where a patient presents with features that may be overlapping among multiple disorders. In this case, the patient had a clear diagnosis of MCAP, along with meningiomas that may be part of her known disease. The additional finding of a VS however, which is not known to be part of MCAP, instigated further genetic testing to confirm the diagnosis and to clarify that this individual did have multiple distinct disorders—MCAP and NF2—rather than an additional manifestation of her known disease.
REFERENCES


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**LEGENDS**

**Figure 1**: Patient MRI demonstrating VS (A) and a corresponding hematoxylin and eosin stained section (B).

**Figure 1**