# 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

*Maria C. Isales, MD, MPH,* Alexandra M. Haugh, BA,† Jeffrey Bubley, BA,† Anna E. Verzì, MD,† Bin Zhang, MS,† Emily Kudalkar, PhD,† Christina Y. Lee, BA,† Pedram Yazdan, MD,† Joan Guitart, MD,†‡ and Pedram Gerami, MD†‡*

**Abstract:** Blitz nevi/tumors are a distinct subset of melanocytic neoplasia which show mixed morphologic features of Spitz and blue nevus. Genomically, most blue nevi have GNAQ or GNA11 mutations while most Spitzoid neoplasms have either an HRAS mutation or translocations involving MET, ROS, BRAF, ALK1, NTRK1, and RET. The criteria used for the assessment of malignancy in blue and Spitzoid lesions are different, and these lesions have different prognostic markers. In this study, we assess the clinical, morphological, and genomic changes in 18 cases of Blitz nevi/tumors to better characterize this subset of neoplasms and determine their optimal genomic classification. Most lesions occurred on the extremities followed by the head and neck region typical of blue nevi. Histology showed most cases having a prominent plexiform growth pattern with cells aggregating around the adnexal structures and neurovascular bundles also typical of blue nevi. Using next generation sequencing, we detected the presence of somatic mutations in GNAQ or GNA11 in 4 of 7 cases (57%) of Blitz nevi with sufficient DNA available for sequencing. Normal skin samples in these 4 cases were sequenced to confirm that the GNAQ or GNA11 mutations were somatic mutations. All 4 cases were negative for immunohistochemical assessment for wild-type BRAF, RET, ALK, and NTRK1 and mutational analysis of HRAS was also negative in all cases. Hence, our study suggests that Blitz nevi/tumors are a distinct subset which genomically are best classified as a subset of blue nevi.

**Key Words:** Blitz nevus, blue nevus, epithelioid blue nevus, Spitz nevus, GNA11, GNAQ, sequencing

(*Am J Dermatopathol* 2017;0:1–7)

## INTRODUCTION

With the advent of molecular diagnostics and the development of novel prognostic markers for specific subtypes of melanocytic neoplasia, accurate classification of ambiguous melanocytic neoplasms is more relevant as well as more feasible than ever before. Several studies have established that HRAS mutations as well as translocations involving either ROS, RET, BRAF, ALK, NTRK1, or MET account for over half of the initiating genomic events in Spitzoid neoplasms while blue nevi typically have activating mutations in GNAQ or GNA11.[1,2] Loss of BRCA-1–Associated Protein 1 (BAP1) can occur in conventional nevi with BRAF or NRAS mutations and result in a melanocytic neoplasm with Spitzoid cytomorphology. In the vast majority of these cases, the prognosis is good and although aggressive behavior may rarely occur, it is overall uncommon.[3,4] Conversely, loss of BAP1 in malignant GNAQ- or GNA11-mutated neoplasms such as uveal melanoma or malignant blue melanocytic neoplasms is an adverse prognostic parameter associated with aggressive behavior.[5] These prognostic implications may have considerable impact on patient management, including the aggressiveness of the surgery, inclusion of sentinel lymph node biopsy, imaging studies, and even consideration of adjuvant therapy. With this in mind, it is more critical than ever before to accurately classify melanocytic neoplasms which may have vague or mixed morphologic features.

Blitz nevi or tumors, that is, those melanocytic tumors with mixed morphologic features of Spitz and blue nevi are a known diagnostic quandary in melanocytic neoplasms. The morphologic differential diagnosis of these cases could include Spitz nevi, epithelioid blue nevi (EBN), deep penetrating nevi (DPN), and pigmented epithelioid melanocytoma. Like blue nevi, Blitz nevi demonstrate both spindled and dendritic melanocytes with abundant pigment and melanophages. In addition, epithelioid melanocytes with abundant eosinophilic cytoplasm similar to those seen in Spitz nevi are present. Cohesive aggregates of heavily pigmented epithelioid melanocytes typical of pigmented epithelioid melanocytes or EBN are absent.[6] The cytoplasm of the epithelioid melanocytes in EBN is more granular in comparison to the glassy eosinophilic cytoplasm of Blitz nevi, and the melanocytes in Blitz nevi have a lower nuclear-to-cytoplasmic ratio with open chromatin typical of Spitzoid cells. DPN are a diverse group of lesions at the molecular level.[7] These lesions have been described as having a variety of cell types including epithelioid, spindle, and dendritic.

From the Departments of *Pathology, and †Dermatology, Feinberg School of Medicine, Northwestern University, Chicago, IL; and ‡Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Supported by the IDP Foundation.

P. Gerami has served as a consultant for Castle Biosciences, Inc, DermTech, Inc, and Myriad Genomics and has received honoraria for this. The remaining authors declare no conflicts of interest.

Reprints: Pedram Gerami, MD, Department of Dermatology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611 (e-mail: pgerami@nmff.org).

Cases with cohesive aggregates of heavily pigmented epithelioid melanocytes or epithelioid cells with heavily pigmented granular cytoplasm without spitzoid features are easily distinguished from Blitz nevi.

Previous studies have described the histomorphologic features of melanocytic neoplasms with the mixed features seen in Blitz nevi; however, the diagnostic terminology is varied and some have classified these lesions as a "variant of epithelioid blue nevus with some Spitzoid features," "Blitz nevus," and "combined blue nevus plus Spitz nevus."[8–10] In 1966, Kopf and Andrade first pictured and described one case of Blitz nevus in a series of Spitz nevi, and the most recent study by Ferrara et al (2010), classified a case of Blitz nevus/tumor as an atypical spitzoid neoplasm.[9,10] However, in the largest study to date, Groben et al[8] categorized 12 cases of Blitz nevi as a subtype of epithelioid combined nevi. The genomics of these cases has yet to be thoroughly studied. These cases show up frequently as consultation cases for second opinion and difficulties include criteria for malignancy as well as basic classification.

In this study, we identified 18 cases which showed mixed morphologic features of blue and Spitz nevi/tumors. We assessed the clinical and histologic features and outcomes for those cases for which it was available. Furthermore, in 7 cases in which tissue was available, we performed next generation sequencing using a 50 gene hot spot panel via the PGM ion torrent which included assessment for mutations in BRAF, NRAS, CKIT, GNAQ, GNA11, and HRAS among others. In addition, we performed immunohistochemical analysis for markers which could suggest the possibility of a translocation characteristic of Spitzoid neoplasms, such as wild-type BRAF, NTRK1, ALK, and RET.[1,2] Our findings suggest that Blitz nevi are a distinct subset of melanocytic neoplasm which genomically should be classified as a subset of blue nevus.

## MATERIALS AND METHODS

### Histology

After obtaining approval by the Institutional Review Board at Northwestern Memorial Hospital, we performed a retrospective search of our Dermatopathology Database for cases of Blitz nevus between January 1, 2006 and July 29, 2016. Search terms included "Blitz nevus," "epithelioid blue nevus," "combined blue nevus," "Spitz and blue nevus," and "atypical Spitz." Cases were included in the study if they met our histologic criteria for a Blitz nevus or Blitz tumor. The morphologic criteria used for the selection of the cases included (1) the tumor needed to include both epithelioid, spindle, and dendritic melanocytes (2) the epithelioid and/or spindle-shaped melanocytes needed to have some Spitzoid cytomorphologic features such as prominent eosinophilic or amphophilic cytoplasm often with large vesicular nuclei with open chromatin although most had a combination of eosinophilic and more vacuolated cytoplasm, and (3) all cases had numerous melanophages intervening between the aggregates of dermal melanocytes. Cases without an epithelioid component and consisting of only spindle-shaped and dendritic melanocytes were excluded as likely routine variants of blue nevi, whereas those consisting exclusively of epithelioid melanocytes were excluded as likely Spitzoid neoplasms. The tissue was formalin-fixed and paraffin-embedded and stained with hematoxylin and eosin. The slides were reviewed by 3 board-certified dermatopathologists (P.G., P.Y., and J.G.) and only cases with a unanimous impression of Blitz nevus or tumor were included in the study. Eighteen cases matching these criteria were identified. The following data points were collected for all cases: age at presentation, sex, site of lesion, ulceration status, mitotic rate, the presence or absence of pagetoid spread of melanocytes, the presence or absence of Kamino bodies, nuclear atypia, the presence or absence of a conventional blue nevus component, cell size, dermal or compound, and growth pattern. Among these 18 cases, 7 cases with sufficient tissue were sequenced.

### Immunohistochemistry

For cases with sufficient additional tissue, immunohistochemical (IHC) analysis was performed using unstained sections of formalin-fixed, paraffin-embedded material. All IHC was performed on the Leica BOND-MAX (Leica Biosystems, Buffalo Grove, IL) using the Bond Polymer Refine Detection Kit. The RET antibody (1:250, EPR2871; Abcam, Cambridge, MA) used a low pH retrieval. The BRAF (1:100, Clone pBR1; Spring Biosciences, Atlanta, GA), NTRK1 (Anti-TrkA, 1:100, Cline EP 1058Y; Abcam), and ALK (clone M7195; DAKO, Carpinteria, CA) immunostains used a high pH retrieval. The BRAF immunostain was a pan-BRAF antibody and used an additional rabbit anti-rat HRP (DAKO) link step.

### Molecular Analysis

Library preparation used 20–35 ng of DNA from 7 formalin-fixed, paraffin-embedded Blitz nevi and 4 normal skin samples. Genomic DNA was extracted using the Qiagen DNA FFPE tissue kit. Next Generation Sequencing was performed in our laboratory using Ion Ampliseq Cancer Hotspot Panel v2 kit along with the Ion Ampliseq Library Kit 2.0. The sequencing was performed on the Ion PGM platform (ThermoFisher Scientific). This PCR-based sequencing approach targets 207 amplicons which cover approximately 2800 COSMIC mutations from the following loci: ABL1, EGFR, GNAS, KRAS, PTPN11, AKT1, ERBB2, GNAQ, MET, RB1, ALK, ERBB4, HNF1A, MLH1, RET, APC, EZH2, HRAS, MPL, SMAD4, ATM, FBXW7, IDH1, NOTCH1, SMARCB1, BRAF, FGFR1, JAK2, NPM1, SMO, CDH1, FGFR2, JAK3, NRAS, SRC, CDKN2A, FGFR3, IDH2, PDGFRA, STK11, CSF1R, FLT3, KDR, PIK3CA, TP53, CTNNB1, GNA11, KIT, PTEN, and VHL. Ion Reporter software version 5.0 (ThermoFisher) was used for alignment to reference genome hg19 (National Center for Biotechnology Information Build 37). The default variant filter settings were used to exclude nonsynonymous variants and variants with a frequency >1% in the 6000 Genomes database. The variants were verified using the Integrative Genomics Viewer, version 2.3 (Broad Institute, Cambridge, MA), to exclude strand bias and homopolymers. The read distributions were compared between tumor and normal samples and a 2-way

Fisher exact test was used to determine the confidence level in each mutation.[11] The authenticity of individual calls was assessed by a certified clinical laboratory technologist to exclude potential artifacts. These somatic variants were interpreted using several tools including 3 databases, specifically the ExAC (Exome Aggregation Consortium) which provides exome sequencing data on 60,706 unrelated individuals; ClinVar, which is a public archive of variants found in patient samples; and PubMed. In addition, the Grantham score and SIFT algorithm were used to classify the proteins resulting from the genetic mutations as damaging, possibly damaging, or tolerated.[12]

## RESULTS

### Clinical Findings

Our study comprised 18 cases of Blitz nevi/tumors. There were 10 women and 8 men with an average age of 24 years (range 4–47) during diagnosis. The lesions ranged in size from 3 to 11 mm. The lesions had a widespread distribution with the most common site being the extremities in 8 cases (5 upper and 3 lower), head and neck in 4 cases, the back in 4 cases, the abdomen in 1 case, and the shoulder in 1 case. The lesions were described as blue, brown, black, or a combination of these colors (Fig. 1A). Before excision, the clinical considerations included Spitz nevus, blue nevus, nevus spilus, and dysplastic nevus. Follow-up was available for 10 of the 18 patients. The follow-up period ranged from 6 months to 7 years. No patients were reported to have had a recurrence. Clinical and histologic results are summarized in Table 1.

### Histologic Findings

Nine of the 18 lesions were compound, and 9 were purely dermal. Of note, all 4 lesions with a GNAQ/11 mutation were purely dermal. Epidermal hyperplasia was seen in 11 of 18 cases. None of the cases had Kamino bodies. Pagetoid spread was infrequent and seen in only 4 of 18 cases. In 5/18 cases, there was a wedge-like growth pattern (28%), 9/18 cases demonstrated a plexiform growth pattern (50%), and 4/18 cases (22%) demonstrated a combined wedge and plexiform growth. As part of the inclusion criteria, all cases had a combination of epithelioid, spindle, and dendritic melanocytes. The epithelioid and spindle-shaped melanocytes had a combination of cytoplasmic features with some cells being more eosinophilic and Spitzoid, whereas other cells had more amphophilic or vacuolated cytoplasm. All cases had aggregates of melanophages dispersed between the dermal melanocytes. Mitotic activity of approximately 1/mm$^2$ was seen in 12 cases. No mitoses were atypical. The nuclear atypia was moderate in most cases, but in 4 cases, there was high-grade nuclear atypia (Figs. 1B–E). None of the cases demonstrated ulceration. IHC analysis was performed on 7 cases, including the 4 cases displaying GNAQ or GNA11 mutations to assess for any possible expression of wild-type BRAF, NTRK1, RET, or ALK suggestive of a possible fusion gene. All 7 cases were negative by IHC.

### Molecular Findings

From the 18 cases of Blitz nevi or tumors, sufficient amounts of DNA with successful sequencing were achieved in 7 cases. Next generation sequencing was performed on these 7 Blitz nevi/tumors. Four of the 7 cases showed either a GNAQ or GNA11 mutation. The amino acid sequences in GNAQ and GNA11 are 90% homologous and have a similar function in melanocytes. Activating mutations in exon 5 (Q209) of GNAQ and GNA11 have been shown to disrupt GTPase activity and impair guanosine triphosphate (GTP) hydrolysis, leading to constitutive activity of G protein.[13–15]

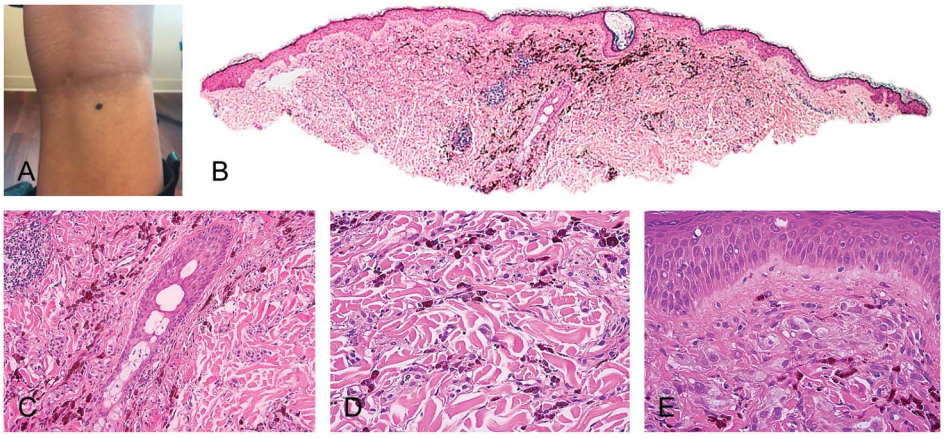


**FIGURE 1.** A, Blitz nevus with GNAQ mutation (case 16). Black-brown papule (6 × 5 mm) in the right inferior popliteal fossa. B, In this GNAQ-mutated melanocytic neoplasm, the overall architecture was both wedge-shaped and plexiform. There is an interstitial proliferation of melanocytic cells with prominent melanin pigmentation clustered around the adnexal structures, including the eccrine units and the neurovascular bundles. The predominantly intradermal cell population consists of epithelioid, spindled, and dendritic melanocytes surrounded by many melanophages (×2, H&E). C, This area of the nevus consists of a blue nevus-like organization of the cells, including a prominent interstitial pattern of cells with sclerotic collagen and cells with aggregation around the eccrine units (×20, H&E). D, There is a spectrum of cell types, including numerous small spindle to oval-shaped cells and dendritic cells interstitially dispersed between sclerotic collagen as well as many melanophages. Some cells have eosinophilic cytoplasm, whereas others have vacuolated, clear cytoplasm (×40, H&E). E, These epithelioid cells have large, open and vesicular nuclei with abundant cytoplasm. The cytoplasm of the cells had variable features including vacuolated cytoplasm, pale and amphophilic cytoplasm, and occasional cells having more of an eosinophilic cytoplasm typical of spitzoid cells (×40, H&E).

**TABLE 1.** Clinical and Histologic Features of Blitz Nevi

| Case | Sex | Age | Location | Clinical Information | Follow-up | Dermal/Compound |
|---|---|---|---|---|---|---|
| 1 | F | 25 | Abdomen, right | Brown-blue nevus, 3 mm | 7 yrs | Dermal |
| 2 | M | 31 | Arm, left | Nevus spilus with atypical blue/black papules | 6 yrs | Compound |
| 3 | F | 36 | Thigh, left upper | Brown papule, 5.5 mm | Not available | Dermal |
| 4 | M | 21 | Back, left upper | Deeply pigmented nevus. | Not available | Dermal |
| 5 | F | 7 | Arm, left | Present for 3 yrs and itchy | Not available | Compound |
| 6 | F | 29 | Shoulder, right anterior | Black/brown papule, 3 mm | 4 yrs | Compound |
| 7 | F | 47 | Calf, left | None provided. | Not available | Compound |
| 8 | F | 14 | Back | Irregular macule, 11 × 7 mm | 7 yrs | Dermal |
| 9 | M | 13 | Right ear pinna | None provided | Not available | Compound |
| 10 | F | 31 | Back, right upper | None provided | Not available | Compound |
| 11 | M | 12 | Neck, mid trapezial | Papule, blue nevus | 17 mo | Dermal |
| 12 | M | 12 | Preauricular, left | Dark gray pigmented lesion, 2 × 2 mm | Not available | Compound |
| 13 | F | 33 | Shin, left | None provided | Not available | Dermal |
| 14 | M | 34 | Back, right upper | Two-tone dark papule, 5 mm. | 10 mo | Dermal |
| 15 | F | 34 | Thigh, left upper | Brown-black, barely raised, 4 mm | 8 mo | Compound |
| 16 | F | 27 | Knee, right inferior popliteal fossa | Dark brown to black papule, 6 × 5 mm | 7 mo | Dermal |
| 17 | M | 4 | Cheek, left | Spitz nevus | 7 mo | Compound |
| 18 | M | 37 | Arm, right upper | R/o dysplasia | 4 mo | Dermal |

| Case | Epidermal Hyperplasia | Kamino Bodies | Growth Pattern | Pagetoid | Conventional Blue Component | Nuclear Atypia | Mitoses |
|---|---|---|---|---|---|---|---|
| 1 | No | No | Wedge | No | Yes | Focal high grade | 1/mm$^2$ |
| 2 | Yes | No | Wedge | No | Yes | Mild | None |
| 3 | No | No | Plexiform and wedge | No | No | Mild | None |
| 4 | Yes | No | Plexiform | Yes | Yes | Moderate | 1/mm$^2$ |
| 5 | Yes | No | Plexiform | No | Yes | Mild | 1/mm$^2$ |
| 6 | Yes | No | Wedge | No | Yes | Focal high grade | 1/mm$^2$ |
| 7 | No | No | Plexiform | No | Yes | Mild to moderate | 1/mm$^2$ |
| 8 | Yes | No | Plexiform | Yes | Yes | Focal high grade | 1/mm$^2$ |
| 9 | Yes | No | Wedge | No | Yes | Moderate | None |
| 10 | Yes | No | Plexiform | No | Yes | Moderate | 1/mm$^2$ |
| 11 | No | No | Plexiform | No | Yes | Moderate | 1/mm$^2$ |
| 12 | No | No | Plexiform | Yes | Yes | Focal high grade | 1/mm$^2$ |
| 13 | No | No | Plexiform and wedge | No | No | Moderate | 1/mm$^2$ |
| 14 | Yes | No | Plexiform and wedge | No | No | Mild | None |
| 15 | Yes | No | Plexiform | Yes | Yes | Moderate | None |
| 16 | No | No | Plexiform and wedge | No | No | Moderate | 1/mm$^2$ |
| 17 | Yes | No | Wedge | No | No | Moderate | 1/mm$^2$ |
| 18 | Yes | No | Plexiform | No | Yes | Moderate | None |

All 4 GNAQ and GNA11 mutations were seen in exon 5. We identified 2 novel mutations in exon 5 of GNAQ, c.664A>G, p.N222D, and c.671A>C, p.T224N. The other case with a GNAQ mutation c.627A>T, p.Q209H had been previously identified in uveal melanoma.[16] The GNA11 mutation was also a novel mutation located in exon 5 at c.622 G>T, p. G208C. Normal skin samples were obtained from the 4 cases in which a GNAQ or GNA11 mutation was identified. Next generation sequencing did not detect GNAQ or GNA11 mutations in the normal skin samples, confirming that these mutations were somatic rather than germline mutations. The molecular results are summarized in Table 2. The other 3 cases did not have an activating mutation in BRAF, NRAS, CKIT, HRAS, GNAQ, or GNA11. All 7 cases were also negative by IHC for ALK, wild-type BRAF, NTRK1, and RET.

## DISCUSSION

Classification of melanocytic neoplasia serves multiple purposes which includes better prediction of behavior, prognostication, and prediction of response to therapy. We have the opportunity now to do this at a more routine and reliable level than ever before by incorporating genomics into our classification system. In the case of Blitz nevi and/or Blitz tumors, there are mixed morphologic features of blue and

**TABLE 2.** Next Generation Sequencing: Somatic Mutations in 7 Cases of Blitz Nevi

| Case | GNAQ/GNA11 | PIK3CA | CTNNB1 | SMAD4 | VHL | SMARCB1 | NOTCH1 | MPL |
|---|---|---|---|---|---|---|---|---|
| 3 | GNA11 | — | — | Q256L | P81S | — | — | — |
| | G208C | — | — | 767A>T | 241C>T | — | — | — |
| | 622G>T | — | — | — | — | — | — | — |
| 7 | — | E707K | S37F | — | — | — | — | Y521H |
| | — | 2119G>A | 110C>T | — | — | — | — | 1561T>C |
| 9 | — | — | — | — | — | — | — | — |
| 13 | GNAQ | — | — | — | — | L54P | P2445T | — |
| | N222D | — | — | — | — | 161T>C | 7333C>A | — |
| | 664A>G | — | — | — | — | — | — | — |
| 14 | GNAQ | — | — | — | — | — | — | — |
| | Q209H | — | — | — | — | — | — | — |
| | 627A>T | — | — | — | — | — | — | — |
| 15 | — | — | — | — | — | — | — | — |
| 16 | GNAQ | — | — | V354L | — | — | — | — |
| | T224N | — | — | 1060G>C | — | — | — | — |
| | 671A>C | — | — | — | — | — | — | — |

Spitz nevi. This is problematic because Spitzoid neoplasms have many unique features. This includes different criteria for the identification of malignancy, such as pagetoid spread, expansile, or sheet-like growth, and mitotic activity, which are all features routinely seen in benign Spitz nevi.[1–4] In addition, loss of BAP1 is common in one variant of Spitz nevus/tumor and does not portend any adverse prognostic information. In fact, this BAP1-mutated variant of Spitz generally has indolent behavior.[3,4,17] Furthermore, even those Spitzoid melanocytic neoplasms judged to be malignant typically do considerably better than similarly staged conventional melanoma.[18,19] The initiating genomic event in over 50% of Spitz cases is either a translocation in RET, ROS, ALK, NTRK1, BRAF, or MEK or an activating mutation in HRAS.[1,2] Hence an ALK-translocated Spitzoid melanoma could be treated by an ALK inhibitor.

Conversely, blue nevi have different criteria for malignancy.[19,20] In fact, loss of BAP1 has recently been identified as a marker of adverse prognosis for malignant blue melanocytic neoplasms.[21,22] The initiating genomic event in the group of blue melanocytic neoplasms and uveal melanomas is typically a mutation in GNAQ or GNA11 which has different therapeutic implications for a malignant blue melanocytic neoplasm or uveal melanoma. Most recently, GNAQ and GNA11 mutations have been identified in mucosal melanomas and have been associated with a poor prognosis.[23]

GNAQ and GNA11 are guanine-nucleotide binding alpha subunits of G proteins, which control G protein activity. The melanocytes in the dermis and uvea depend on endothelin-3 for migration, and the binding of endothelin-3 to the endothelin B receptor mediates signaling through GNAQ and GNA11. A mutation in either protein results in skin hyperpigmentation due to increased dermal melanocytes and melanin. The G protein is active when it is bound to GTP and inactive when the GTP is hydrolyzed to guanosine diphosphate. However, GTPase activity is blocked when GTP is in contact with substitutions of glutamine or arginine residues.[24,25] Two mutation hotpots have been previously identified in GNAQ and GNA11: Q209 and R183. Q209 mutations in exon 5 are significantly more prevalent in comparison to R183 mutations in exon 4. The overactive mitogen-activated protein kinase pathway results in increased cell proliferation and inhibition of apoptosis. It is believed that the inhibitory effect of Q209 mutations in exon 5 is more potent compared with the R183 mutations in exon 4.[26] Whereas both GNAQ p.Gln209Leu and GNAQ p. Arg183Gln mutations activate the extracellular signal-regulated kinase, only GNAQ p.Gln209Leu activates additional mitogen-activated protein kinase pathway members, p38 and Jun N-terminal kinase.[27,28]

In the original article from Van Raamsdonk et al,[25] mutually exclusive mutations in GNAQ or GNA11 were found in 62% of the blue nevi they assessed. However, somatic mutations in GNAQ have also been detected in 50% of melanoma-associated blue nevi, 46% of uveal melanomas, and 9.5% of mucosal melanomas.[23,28] Defining the genomics of Blitz nevi/tumors could be highly useful in their accurate classification. In our set of 18 cases of which 7 had tissue available for sequencing, we found 3 cases to have mutations in exon 5 of GNAQ while 1 case had a mutation in exon 5 of GNA11 (57%). This 57% value is relatively similar to the 62% of cases of blue nevi identified with GNAQ or GNA11 mutations by Van Raamsdonk.[25] These findings strongly suggest that Blitz nevi/tumors should be added to the spectrum of blue melanocytic neoplasms based on their genetics. Furthermore, Blitz lesions should have diagnostic criteria for malignancy more on par with those used to judge blue lesions, including size >1 cm, a multinodular configuration, necrosis, mitotic rate >2/mm$^2$, and infiltrative features.[20] It is likely but not yet tested that loss of BAP1 would be an adverse prognostic marker in Blitz neoplasms meeting morphologic criteria for malignancy. Conversely, loss of BAP1 in a Spitzoid neoplasm has no implications of adverse prognosis and in general is an indolent variant of Spitzoid neoplasms. Furthermore, because BAPomas rarely demonstrate significant pigmentation, a deep multinodular heavily

pigmented melanocytic neoplasm with BAP1 negativity would be suspect for a blue nevus-like melanoma.[3,4]

The Blitz neoplasms in our study demonstrate clinical and histologic features most characteristic of blue nevi. Twelve of these lesions were in locations highly typical of blue nevi, including 8 of 18 on the extremities and 4 of 18 on the head and neck. The remaining lesions were on the back (n = 4), the abdomen (n = 1), and the shoulder (n = 1). In addition, 9 cases presented with a blue, black, or gray color. Six cases had a 2-toned pattern of pigmentation suggestive of a combined morphologic pattern. All 4 lesions with GNAQ/11 mutations were purely dermal which also lends credence to their relationship with blue nevi. The architecture was predominantly plexiform in 13 cases with the cells aggregated around the adnexal structures and neurovascular bundles. This aggregation is another feature typical of blue nevi and related to their origin from a dermal pluripotent cell with potential to differentiate into Schwann cells or melanocytic cells.[27] All cases had many melanophages typical of heavy melanin production.

The mutations previously described in GNAQ and GNA11 are at amino acids Q209 and R183.[13,24–26,28,29] The mutations identified in our study involved exon 5 of GNAQ, c.664A>G, p.N222D; c.671A>C, p.T224N; and c.627A>T, p.Q209H. The GNA11 mutation had an exon 5 mutation at c.G208C, p.622G>T. The presence of these mutations near the 209 locus, which typically harbors blue nevus mutations and lies within the ras-like domain of GNAQ/GNA11, in combination with the absence of these mutations in the matched normal tissue as well as the positive predicted pathogenicity by Grantham, PolyPhen, and SIFT all suggest that these mutations are pathogenic and the initiating driver of these lesions.[14,15,24,25] It is unknown whether the phenotypic differences between blue nevi and Blitz nevi, such as the presence of epithelioid and plump spindle cells with some Spitzoid morphologic features, is related to the presence of the mutation in a site other than the typical Q209 or R183, but this is one possibility to explain the altered morphology.

In addition to the 4 GNAQ/GNA11 mutations identified by next generation sequencing, several nevi were found to harbor additional mutations. Of the 8 additional genetic mutations reported in Table 2, 3 mutations were predicted to have an impact on protein structure/function based on SIFT score and previously reported mutations. A CTNNB1 S37F mutation identified in a case without a GNAQ/GNA11 mutation has been identified in other benign tumors such as pilomatricoma and craniopharyngioma.[30] Two GNAQ/GNA11-mutated neoplasms harbored point mutations in the tumor suppressor genes VHL and SMARCB1. VHL P81S is a mutational hotspot that has been shown to be significantly influenced by tumor microenvironment and can be responsible for altered tumor metabolism and apoptotic resistance.[31] However, the largest study to date by Amendola et al[32] has suggested that this mutation is likely benign, as it is found in a large proportion of the general population as a germline mutation. The SMARCB1 L54P mutation is novel yet other nearby missense mutations have been implicated in hereditary schwannomatosis when they occur in the germline.[33] These tumor suppressor gene mutations are unlikely to signify malignant transformation as studies have proposed a multihit model for pathogenesis in both VHL and SMARCB1 tumorigenesis and SMARCB1 germline mutations lead to the development of benign tumors. These mutations also have unclear pathogenesis in melanocytic neoplasms. All other identified mutations are novel and by SIFT and Grantham were considered unlikely to be pathogenic.

In summary, fine-tuning the classification of melanocytic neoplasms has potential prognostic and therapeutic value. We provide an example in this series of cases with ambiguous or mixed morphologic features which can be more precisely categorized when assessed by molecular diagnostics. Based on our studies including the clinical presentation and the genetic findings, the melanocytic neoplasms classified as Blitz nevi or Blitz tumors, that is, those cases with mixed Spitz and blue nevus should primarily fall in the category of blue nevus. It would be interesting to assess the value of BAP1 as a prognostic marker in a group of malignant neoplasms with these features. Based on the genetics, one would expect the same prognostic value in these lesions as seen in malignant blue melanocytic neoplasms and uveal melanoma. The presence of pathogenic mutations in exon 5 within 4 of 7 sequenced cases while absent in matched control tissue substantiates the importance of these mutations. We can only speculate as to the source of the Spitzoid cytomorphology. One possibility is the presence of the nonconventional mutations in GNAQ and GNA11. These novel mutations were present in the key exon of importance, but were not the mutations most commonly reported in blue nevi. Interestingly, large series of conventional blue nevi assessed for mutations did not identify these specific alterations.[24,25,28,29] Hence, we theorize that these alternative mutations are the reason for the distinct and different morphological features seen in our cases including less intense pigment production and more cytomorphologic variability when compared with conventional blue nevi. Given our small study size, larger studies would be necessary for more definitive genotypic–phenotypic correlation. Last, we have found that many of these fusion-based Spitzoid neoplasms have a considerably better prognosis even when considered as malignant Spitzoid melanoma than similarly staged conventional melanomas[18,19] highlighting the importance of recognizing and excluding these GNAQ/GNA11-mutated Blitz neoplasms.

## REFERENCES


1. Bastian BC, LeBoit PE, Pinkel D. Mutations and copy number increase of HRAS in Spitz nevi with distinctive histopathologic features. *Am J Pathol.* 2000;157:967–972.
2. Wiesner T, He J, Yelensky R, et al. Kinase fusions are frequent in spitz tumors and spitzoid melanomas. *Nat Commun.* 2014;5:3116.
3. Murali R, Wiesner T, Scolyer RA. Tumors associated with BAP1 mutations. *Pathology*. 2013;45:116–126.
4. Wiesner T, Kutzner H, Cerroni L, et al. Genomic aberrations in spitzoid melanocytic tumors and their implications for diagnosis, prognosis, and therapy. *Pathology*. 2016;48:113–131.
5. Harbour JW, Onken MD, Roberson EDO, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science*. 2010;330:1410–1413.
6. Yazdan P, Haghighat Z, Guitart J, et al. Epithelioid and fusiform blue nevus of chronically sun-damaged skin, an entity distinct from the

epithelioid blue nevus of the Carney complex. *Am J Surg Pathol.* 2013; 37:81–88.
7. Bender RP, McGinniss MJ, Esmay P, et al. Identification of HRAS mutations and absence of GNAQ and GNA11 mutations in deep penetrating nevi. *Mod Pathol.* 2013;26:1320–1328.
8. Groben PA, Harvell JD, White WL. Epithelioid blue nevus: neoplasm sui generis or variation on a theme? *Am J Dermatopathol.* 2000;22:473–488.
9. Kopf AW, Andrade R. Benign Juvenile Melanoma. In: Kopf AW, Andrade R, eds. *Year Book of Dermatology*. Chicago, IL: Year Book Medical Publishers Inc; 1966:7–52.
10. Ferrara G, Zalaudek I, Savarese I, et al. Pediatric atypical spitzoid neoplasms: a review with emphasis on "red" ("spitz") tumors and "blue" ("Blitz") tumors. *Dermatol Case Rev.* 2010;220:306–310.
11. Dees ND, Zhang Q, Kandoth C, et al. MuSiC: identifying mutational significance in cancer genomes. *Genome Res.* 2012;22:1589–1598.
12. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc.* 2009;4:1073–1081.
13. Yilmaz I, Gamsizkan M, Sari SO, et al. Molecular alterations in malignant blue nevi and related blue lesions. *Virchows Arch.* 2015;467:723–732.
14. O'Hayre M, Vazquez-Prado J, Kufareva I, et al. The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. *Nature*. 2013;13:412–424.
15. Berman DM, Wilkie TM, Gilman AG. GAIP and RGS4 are GTPase activating proteins for the Gi subfamily of G protein alpha subunits. *Cell*. 1996;86:445–452.
16. De Lange MJ, Razzaq L, Versluis M, et al. Distribution of GNAQ and GNA11 mutation signatures in uveal melanoma points to a light dependent mutation mechanism. *PLoS One*. 2015;10:e0138002.
17. Wiesner T, Obenauf AC, Murali R, et al. Germline mutations in BAP1 predispose to melanocytic tumors. *Nat Genet.* 2011;43:1018–1021.
18. Gerami P, Cooper C, Bajaj S, et al. Outcomes of atypical spitz tumors with chromosomal copy number aberrations and conventional melanomas in children. *Am J Surg Pathol.* 2013;37:1387–1394.
19. Gerami P, Scolyer RA, Xu X, et al. Risk assessment for atypical spitzoid melanocytic neoplasms using FISH to identify chromosomal copy number aberrations. *Am J Surg Pathol.* 2013;37:676–684.
20. Hung T, Argenyi Z, Erickson L, et al. Cellular blue nevomelanocytic lesions: analysis of clinical, histological and outcome data in 37 cases. *Am J Dermatopathol.* 2016;38:499–503.
21. Costa S, Byrne M, Pissaloux D, et al. Melanomas associated with blue nevi or mimicking cellular blue nevus: clinical, pathologic, and molecular study of 11 cases displaying a high frequency of GNA11 mutations, BAP1 expression loss, and a predilection for the scalp. *Am J Surg Pathol.* 2016;40:368–377.
22. Chan MP, Andrea AA, Harms PW. Genomic copy number analysis of a spectrum of blue nevi identifies recurrent aberrations of entire chromosomal arms in melanoma ex blue nevus. *Mod Pathol.* 2016;29:227–239.
23. Sheng X, Kong Y, Li Y, et al. GNAQ and GNA11 mutations occur in 9. 5% of mucosal melanomas and are associated with poor prognosis. *Eur J Cancer.* 2016;65:156–163.
24. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue nevi. *Nature*. 2009; 457:599–602.
25. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in GNA11 in uveal melanoma. *N Engl J Med.* 2010;363:2191–2199.
26. Urtatiz O, van Raamsdonk CD. GNAQ and GNA11 in the endothelin signaling pathway and melanoma. *Front Genet.* 2016;7:1–10.
27. Mull AN, Zolekar A, Wang YC. Understanding melanocytes stem cells for disease modeling and regenerative medicine applications. *Int J Mol Sci.* 2015;16:30458–30469.
28. Shirley MD, Tang H, Galion CJ, et al. Sturge-Weber Syndrome and port-wine stains caused by somatic mutation in GNAQ. *N Engl J Med.* 2013; 368:1971–1979.
29. Perez-Alea M, Vivancos A, Caratu G. Genetic profile of GNAQ-mutated blue melanocytic neoplasms reveals mutations in genes linked to genomic instability and PI3K pathway. *Oncotarget*. 2016;7:28086–28095.
30. Kazakov DV, Sima R, Vanecek T, et al. Mutations in exon 3 of the CTNNB1 gene (beta-catenin gene) in cutaneous adnexal tumors. *Am J Dermatopathol.* 2009;31:248–255.
31. Desimone MC, Rathmell WK, Threadgill DW. Pleiotropic effects of the trichloroethylene-associated P81S VHL mutation on metabolism, apoptosis, and ATM-mediated DNA damage response. *J Natl Cancer Inst.* 2013;105:1355–1364.
32. Amendola LM, Dorschener MO, Robertson PD, et al. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Res.* 2015;25:305–315.
33. Smith MJ, Walker JA, Shen Y, et al. Expression of SMARCB1 (INI1) mutations in familial schwannomatosis. *Hum Mol Genet.* 2012;21:5239–5245.