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SF3B1 and *BAP1* mutations in blue nevus-like melanoma

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Abstract

Blue nevi are melanocytic tumors originating in the cutaneous dermis. Malignant tumors may arise in association with or resembling blue nevi, so called ‘blue nevus-like melanoma’, which can metastasize and result in patient death. Identifying which tumors will behave in a clinically aggressive manner can be challenging. Identifying genetic alterations in such tumors may assist in their diagnosis and prognostication. Blue nevi are known to be genetically related to uveal melanomas (eg, both harboring *GNAQ* and *GNA11* mutations). In this study, we analyzed a large cohort (n=301) of various morphologic variants of blue nevi and related tumors including tumors diagnosed as atypical blue nevi (n=21), and blue nevus-like melanoma (n=12), screening for all gene mutations known to occur in uveal melanoma. Similar to published reports, we found the majority of blue nevi harbored activating mutations in *GNAQ* (53%) or *GNA11* (15%). In addition, rare *CYSLTR2* (1%) and *PLCB4* (1%) mutations were identified. *EIF1AX*, *SF3B1*, and *BAP1* mutations were also detected, with *BAP1* and *SF3B1* R625 mutations being present only in clearly malignant tumors (17% (n=2) and 25% (n =3) of blue nevus-like melanoma, respectively). In sequencing data from a larger cohort of cutaneous melanomas, this genetic profile was also identified in tumors not originally diagnosed as blue nevus-like melanoma. Our findings suggest that the genetic profile of coexistent *GNAQ* or *GNA11* mutations with *BAP1* or *SF3B1* mutations can aid the histopathological diagnosis of blue nevus-like melanoma and distinguish blue nevus-like melanoma from conventional epidermal-derived melanomas. Future studies will need to further elucidate the prognostic implications and appropriate clinical management for patients with tumors harboring these mutation profiles.

Blue nevi are common dermal melanocytic proliferations of the skin.[1] They derive their name from the blue color observed when viewed clinically, an appearance attributed to the Tyndall effect. The latter describes the phenomenon whereby longer wave lengths of light penetrate more deeply in the skin and are absorbed by deep dermal lying melanin pigment, whereas blue light, having a shorter wave length, is reflected closer to the skin surface.[2] The most frequent blue nevus subtype is the common or Jadasohn–Tièche blue nevus.[3] A range of other less frequent subtypes have also been described including cellular, epithelioid, sclerotic, and plaque-type blue nevi.[1,2,4]

The overwhelming majority of blue nevi are benign and are often only biopsied to rule out a primary melanoma or a metastasis. Rare malignant tumors showing some of the morphologic features seen in blue nevi are often termed ‘malignant blue nevi’ or ‘blue nevus-like melanoma’. The latter term is preferable because the term ‘malignant blue nevus’ includes the contradictory terms ‘malignant’ and ‘nevus’ (which by definition connotes a benign melanocytic proliferation). Blue nevus-like melanomas are indeed malignant tumors and should be treated accordingly.[5–8] If tumors resembling blue nevi show atypical clinico-pathologic features that are considered to fall short of malignancy, and there is consequently uncertainty with regard to their likely biological behavior, they are often designated ‘atypical cellular blue nevi’ (denoting a tumor of uncertain or indeterminate biologic potential). Unfortunately, no consensus histological criteria for atypical cellular blue nevi exist and diagnostic interobserver reproducibility is poor,[9] however, features proposed to render this diagnosis include one or more of the following features: asymmetry, hypercellular foci, focal cytological atypia, and mitoses (depending on the literature < 2 or < 3 per mm^2).[1,2,7,8]

Necrosis and atypical mitotic features are usually considered diagnostic of melanoma (blue nevus-like melanoma).

Distinguishing blue nevus-like melanoma from blue nevi histologically can be challenging. Many conventional histological criteria established to identify malignant behavior in more frequently occurring epidermal-derived melanomas cannot be applied to blue nevi. For example, blue nevi do not show maturation of cell nests toward deeper parts of the tumor, nor do malignant proliferations demonstrate ascending melanocytes in the epidermis (ie, pagetoid epidermal invasion), both useful diagnostic features frequently observed in epidermal-originating melanomas. Blue nevi also uniformly express HMB-45, an immunohistochemical marker which in epidermal-derived melanocytic tumors is usually only present in superficial melanocytes and if expressed in deeper dermal raises the possibility of melanoma. These features can make the distinction between benign and malignant dermal melanocytic proliferations difficult.

Blue nevi are genetically distinct from epidermal-derived common nevi and melanoma. Epidermal-derived nevi and melanoma frequently harbor mutations in *BRAF* and *NRAS*.[10–12] This is uncommon in blue nevi which frequently contain activating *GNAQ, GNA11* and less frequently *CYSLTR2* mutation.[13–16] All of these mutations were also identified in uveal melanoma.[13–15] Other genes mutated in uveal melanomas are *BAP1*, *EIF1AX*, and *SF3B1*.[17–19] Interestingly, mutations in these three genes are almost always mutually exclusive and have prognostic relevance. In uveal melanomas, *BAP1* mutations resulting in loss of BAP1 protein function are associated with a poor prognosis,[18] whereas *SF3B1* and *EIF1AX* mutations are generally associated with tumors having a favorable prognosis.[17,19]

The genetic similarity between blue nevi and uveal melanoma has been further demonstrated by a number of recent studies reporting *BAP1* mutations or protein loss occurring in blue nevus-like melanoma.[5,20–23] Similar to uveal melanocytic tumors, this fits the classic progression model, where benign tumors (nevi) frequently harbor an initial mutation (ie, a *GNAQ* or *GNA11* mutation) and acquisition of additional genetic alterations (ie, a *SF3B1*, *EIF1AX* or *BAP1* mutation) results in progression to a malignant neoplasm (melanoma).

The goal of the current study was to determine the extent to which the genetic alterations identified in uveal melanoma are also present in blue nevi, atypical cellular blue nevi, and blue nevus-like melanoma, and could potentially be applied to distinguish benign from malignant tumors. A targeted next-generation sequencing panel covering all known recurrent mutations in these genes in uveal melanoma was applied to a cohort of 301 blue nevi of various subtypes, including tumors diagnosed as atypical cellular blue nevi or blue nevus-like melanoma.

Materials and methods

Sample Selection

Samples of blue nevi, atypical cellular blue nevi and blue nevus-like melanoma were obtained by searching the databases of the Melanoma Institute Australia (n=26), the

Department of Dermatology University Hospital Essen (n=72), Dermatopathologie Friedrichshafen (n=106), Pathologie Salzburg (n =32) and Dermatopathologie bei Mainz (n=65), Germany. All cases were assessed histologically by at least two board-certified pathologists or dermatopathologists (RS, LJ, UH, RM, ME, AR, HK, HM and KGG). Of these samples, approximately one third (103) of the cases were described in a previous publication.[16] The study was performed in accordance with the guidelines of the ethics committee of the University of Duisburg-Essen and approved under the IRB-number 16–6951-BO.

DNA Isolation

DNA was isolated from formalin fixed paraffin-embedded tumor tissue cut in 10 m-thick sections. Sections were then deparaffinized and manually macrodissected according to standard procedures. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Targeted Sequencing

A custom amplicon-based sequencing panel covering ten genes (Table 1) was designed. Included were all seven genes known to occur in blue nevi and uveal melanoma (*GNAQ*, *GNA11*, *PLCB4*, *CYSLTR2, EIF1AX, SF3B1* and *BAP1*) as well as the most frequent activating gene mutations in cutaneous nevi and cutaneous melanomas (*BRAF*, *NRAS* and *KIT*). Coverage focused on the known relevant mutational hotspots for all genes with the exception of BAP1, where relevant inactivating mutations have been reported throughout the gene.[18]

Panel design and preparation took place applying the GeneRead Library Prep Kit from QIAGEN according to the manufacturer's instructions. NEB-Next Ultra DNA Library Prep Mastermix Set and NEB-Next Multiplex Oligos for Illumina from New England Biolabs were applied for adapter ligation and barcoding of individual samples. Sequencing was performed on an Illumina MiSeq next-generation sequencer, with up to 70 samples sequenced in parallel. Sequencing the sample cohort, an average coverage of 5753 reads was achieved with 495% of the target area having a minimum coverage of 30 reads.

Sequence Analysis

CLC Cancer Research Workbench from QIAGEN was applied for sequence analysis, applying a number of steps as previously reported[24] and demonstrated here briefly. The CLC workflow included adapter trimming and read pair merging before mapping to the human reference genome (hg19). Subsequently insertions and deletions as well as single nucleotide variant detection, local realignment, and primer trimming followed. Various databases (COSMIC, ClinVar, dbSNP, 1000 Genomes Project, HAPMAP, and PhastCons-Conservation_scores_hg19) were cross-referenced to obtain additional information regarding mutation type, single nucleotide polymorphisms, and conservation scores. Resulting csv files were further analyzed manually screening for mutations affecting the protein coding portion of the gene predicted to result in non-synonymous amino acid changes. Mutations were considered if the overall coverage of the mutation site was ≥30 reads, ≥5 reads reported the mutated variant and the frequency of mutated reads was ≥3%.

BAP1 Immunohistochemistry

BAP1 immunohistochemistry was performed applying a rabbit polyclonal antibody recognizing amino acids 430–729 of the BAP1 protein (clone C-4, Santa Cruz Biotechnology) as previously reported.[25] Nuclear staining was assessed for positivity.

Genetic Screening for Tumors with a Blue Nevus-Like Melanoma Mutation Profile

The database of sequenced melanoma samples (n=1121) in Essen was screened for tumors where an activating *GNAQ* or *GNA11* hotspot mutation had been identified. Tumors harboring these mutations, not reported to be of uveal or central nervous system origin, were assessed in more detail, screening available clinical data. If a cutaneous origin was reported, archived isolated tumor DNA was selected and sequenced applying the aforementioned 10 gene panel (Table 1). Particular attention was given to potential *BAP1*, *SF3B1* and *EIF1AX* mutations. When tissue block material was still available, BAP1 immunohistochemistry was also performed.

Associations of Gene Mutation Status with Clinical and Pathologic Parameters

We investigated associations of mutation status with available clinical and pathological parameters using Kruskal–Wallis tests, χ^2 tests and Fisher exact tests as appropriate. All statistical analyses were performed using IBM SPSS Statistics software (version 20.0; International Business Machines, Armonk, NY, USA). A P-value of ≤0.05 was considered statistically significant.

Results

Sample Cohort

The study cohort consisted of 301 blue nevus samples from 301 patients (174 females and 119 males, 8 cases unknown) with an average age of 48 years (range 3–99). The tumors included 176 (59%) common blue nevi, 69 (23%) cellular blue nevi, 21 (7%) atypical cellular blue nevi and 12 (4%) blue nevus-like melanoma. In addition, 18 (6%) combined nevi, harboring a blue nevus component, were assessed and 5 (2%) tumors were analyzed where genetic data suggested a blue nevus-like melanoma. All samples assessed were primary tumors, with the exception of samples suggested to be blue nevus-like melanoma by genetic analysis (5 tumor samples, including 4 metastases and 1 primary), defined as melanomas of cutaneous origin where sequencing identified activating *GNAQ* or *GNA11* mutations. An overview of available clinical data is listed in Table 2.

Distribution of Activating Oncogene Driver Mutations

Targeted amplicon sequencing of all genes, as described in the Material and methods section, was performed for the entire tumor cohort. Consistent with previously reported results, the most frequent activating mutations identified were in *GNAQ*, 53% (143 c.626A>T Q209L, 15 c.626A>C Q209P, 1 c.627A>T A209H and 1 c.548G>A R183Q) and GNA11, 15% (44 c.626A>T Q209L and 1 c.547C>T R183C) (Figure 1). Fifteen (5%) *BRAF* mutations (12 c.1799A>T V600E and 1 c.227G>C G469A) and 7 (2%) *NRAS* mutations (3 c.182A>G Q61R, 3 c.181C>A Q61K and 1 c.34G>C G12R) were detected,

most of these occurring in the combined nevi group (and was likely derived from the non-blue nevus component of the lesion). Three (1%) *PLCB4* mutations (2 c.1888G>A D630N and 1 c.1888_1889delGAinsTT D630F) were identified. Four tumors (1%) harbored activating *CYSLTR2* mutations (c.386T>A L129Q). Generally, activating mutations were found to be mutually exclusive, with the exception of a *GNA11* Q209L and *PLCB4* D630N occurring mutually in one common blue nevus sample. The mutations and distribution identified are further shown in Figure 1.

Distribution of Mutations in Combined Nevi

In combined nevi, which demonstrated a clear conventional epidermal or compound (epidermal and dermal) nevus component adjacent to or admixed with a blue nevus component, the mutations identified were primarily *BRAF* mutations (50%, 8 V600E and 1 G469A) and *NRAS* mutations (22%, 2 Q61R and 2 Q61K).

Distribution of Mutations in Atypical Cellular Blue Nevi and Blue Nevus-Like Melanoma

Our cohort included 21 tumors diagnosed as atypical cellular blue nevi and 12 tumors diagnosed as blue nevus-like melanoma (Table 3). In the atypical cellular blue nevi, 66% (n= 14) of tumors harbored activating *GNAQ* mutations. One (5%) *CLYSTR2* and one (5%) *PLCB4* mutation (Supplementary Figure 1) were identified. Two tumors (10%) were found to harbor *BRAF* V600E mutations. Two mutations in *EIF1AX* (exon 1 or 2) were noted, a P2L and Q33* mutation, respectively (Table 3 and Supplementary Figure 1).

The 12 tumors diagnosed as blue nevus-like melanoma harbored mutations in *GNAQ* in 25% (n=3), *GNA11* in 33% (n=4), and *BRAF* in 25% (n= 3) of cases (Figures 2, 3, 4 and Supplementary Figures 2). One tumor (8%) contained an *EIF1AX* P2L mutation. Three tumors (25%) harbored *SF3B1* R625 mutations (2 R625H and 1 R625C mutation, Figures 2, 3, Supplementary Figure 2). Two inactivating *BAP1* mutations were identified, with loss of protein confirmed by immunohistochemistry (Figure 4 and Supplementary Figure 3). All tumors demonstrating *SF3B1* or *BAP1* mutations additionally harbored activating *GNAQ* or *GNA11* mutations. In two tumors, the *SF3B1* (Figure 3) and *BAP1* (Figure 4) mutations were detected in the melanoma, but absent in the nevus portion of the tumor.

Tumors With a Blue Nevus-Like Melanoma Mutation Profile

By screening existing mutation data for melanomas harboring *GNAQ* or *GNA11* mutations not reported as tumors of uveal or CNS origin, we identified five tumors. Three of these were tumors demonstrated unusual or atypical clinical or histopathological features compared with conventional melanomas. In one case, the tumor was diagnosed as a MUP (melanoma of unknown primary) and presented as a subcutaneous tumor in the axilla. Genetic screening of the tumor revealed mutations in *GNA11*, *SF3B1*, and *BAP1* (case 34, Table 3 and Figure 5). In two other cases, the melanoma subtype was not documented in the original pathology and the original pathology slides were not available for review; both tumors were thick melanomas (>4 mm and >7.5 mm, case 35 and case 36 in Table 3, respectively). In the remaining two cases, the melanoma subtype was reported as superficial spreading melanoma, suggesting the primary tumor had a junctional melanocytic component, a feature not usually present in blue nevi (or blue nevus-like melanoma). One of

these two tumors (case 38, Table 3) also had a clear inactivating *BAP1* mutation, confirmed by loss of BAP1 expression with immunohistochemistry (Supplementary Figure 5) further fitting the genetic profile of a blue nevus-like melanoma.

Associations of Clinical and Pathological Parameters with Oncogene Mutation Status

Statistically significant associations of melanoma subtype with patient age and with mutation type were identified; full details are presented in Table 2. In all cases of blue nevus-like melanoma where follow-up data was available, this was screened and the relevant information is presented in Table 4. Associations with survival were not performed as the available data was too limited to allow a meaningful analysis.

Discussion

Our study presents the largest genetic screen of blue nevi and related tumors published to date. In addition to documenting the distribution of activating driver mutations in these tumors, it also further elucidates the unique genetic profile of malignant tumors, in particular highlighting the significance of the presence of *BAP1* and *SF3B1* R625 mutations in supporting a diagnosis of malignancy. This unique mutation profile may therefore have clinical implications, both from a diagnostic and treatment perspective.

Similar to previous studies,[5,13,14,16] the overwhelming majority of blue nevi in our cohort were found to harbor activating mutations in *GNAQ* and *GNA11*. *CYSLTR2* L129 and *PLCB4* D630 mutations were rare with a frequency of around 1% (4 and 3 cases, respectively). *BRAF* and *NRAS* mutations were mostly observed in combined nevi (Figure 1) and rarely in tumors diagnosed as blue nevi. One can debate if tumors harboring *BRAF* and *NRAS* mutations should be considered blue nevi or not. For benign neoplasms, we would argue this question is purely academic and of no real clinical relevance. For atypical or malignant melanocytic proliferations, we believe that the term ‘blue nevus’ should be used with caution if a *BRAF* or *NRAS* mutation is detected. If this term is included in the diagnosis, additionally stating the mutation status may ensure that all available treatment options are considered (ie, BRAF inhibitor therapy).

Similar to uveal melanoma, our analysis found that *EIF1AX*, *SF3B1*, and *BAP1* mutations also occur in blue nevus-like melanocytic proliferations. In our study, *SF3B1* R625 and inactivating *BAP1* mutations were only identified in tumors diagnosed as malignant (blue nevus-like melanoma). This is further supported by the available follow-up data. The *BAP1* mutant tumor where follow-up data was available metastasized (case 38, Tables 3 and 4). In 5 tumors harboring *SF3B1* mutations, follow-up data >1 year was available for 3 patients. In two cases, the patient died because of melanoma (case 23 and 24, Tables 3 and 4). Both cases had interesting clinical features. In case 24, the patient quickly died of liver metastasis, which in uveal melanoma typically occurs in patients whose tumor demonstrates BAP1 loss. Evidence for BAP1 alterations in case 24 was not observed in our study. Sequencing did not identify *BAP1* mutations and immunohistochemistry was suboptimal and inconclusive (probably reflecting lack of antigen preservation because of the age of the tissue block, Supplementary Figure 1). The other patient (case 23) was diagnosed with two other primary melanomas. We were unable to definitively ascertain which tumor metastasized and led to

the patient's death. The last case for whom follow-up was available (case 36, Tables 3 and 4) definitely metastasized and showed no loss of BAP1 (by IHC and sequencing). This data is intriguing as in uveal melanomas *SF3B1* mutations are associated with tumors which do not metastasize.[17,19] Although larger cohorts will be required, the preliminary data we present here argues that both *SF3B1* and *BAP1* mutant tumors can metastasize and affected patients should be regularly followed up after primary complete excision.

For *EIF1AX* mutations (in exon 1 and 2), which occur in uveal melanomas and are associated with a favorable prognosis,[17] their significance in blue nevus-like melanocytic proliferations is less clear. We identified *EIF1AX* mutations in two atypical cellular blue nevi and one blue nevus-like melanoma. On follow-up, the patients with atypical cases remained disease-free (Table 3), however the blue nevus-like melanoma recurred multiple times. Future studies will need to further elucidate the prognostic relevance of *EIF1AX*, *SF3B1*, and *BAP1* mutations.

The cases of genetically defined blue nevus-like melanoma we present pose a number of unresolved questions. In cases originally diagnosed as a metastasis or melanoma not otherwise characterized (cases 34–36 in Tables 3, 4 and Figure 5), the clinical and histopathological description is retrospectively consistent with unrecognized blue nevus-like melanoma. Case 34 demonstrates how blue nevus-like melanoma may occasionally be misdiagnosed as a metastasis because of its deep location and absence of an associated epidermal component. In the other two cases (cases 37+38 in Tables 3 and 4) superficial spreading melanomas (SSM) were originally diagnosed implying epidermal tumor involvement, a feature not usually observed in blue nevus-like melanoma (unfortunately the original slides were not available for review). Potentially these cases represent melanomas arising from pre-existing combined nevi. Although a question of debate whether these tumors might actually be bona-fide epidermal-derived melanomas, we believe the unique genetic profile of such cases should be clearly communicated in diagnostic pathology reports (eg, 'superficial spreading melanoma with the genetic profile of a blue nevus-like melanoma.'), as this could be relevant for patient management.

Our study has some limitations. Atypical cellular blue nevi and blue nevus-like melanoma are rare, which is why we could not avoid analyzing a heterogenous cohort from different institutions over different time periods. The original slides, clinical and follow-up data were not available in all cases, limiting the clinico-pathological associations that could be investigated. Our genetic analysis only covered the published relevant regions of 10 genes (Table 1) and paired normal DNA was not sequenced; as a result, rare mutations located outside the mutation hotspots or germline variants may not have been recognized. Our sequencing approach did not allow an assessment of copy number variations, which would have been valuable, as in particular chromosome 3 losses have been frequently reported in blue nevus-like melanomas.[5,20,26]

In our institutions, gene sequencing has become a routine part of the diagnostic evaluation of melanocytic tumors. If a tumor is histologically assessed as being clearly benign, no additional analysis is performed. However, in cases where the histopathologic diagnosis of an atypical cellular blue nevus or blue nevus-like melanoma is considered, IHC and genetic

analysis are performed. Our standard melanoma sequencing panel covers all frequent activating mutations (*BRAF*, *NRAS*, *KIT*, *NF1*, *GNAQ*, *GNA11*, etc). If a *GNAQ* or *GNA11* mutation is detected, the tumor is assumed to be a blue nevus-like melanocytic proliferation and sequencing of *EIF1AX*, *SF3B1*, and *BAP1* is performed. If a clear *SF3B1* R625 or inactivating *BAP1* mutation (with IHC loss) is observed, we interpret this as molecular evidence in support of a diagnosis of blue nevus-like melanoma. The implications of an *EIF1AX* mutation are still unclear. Complete tumor excision and regular patient follow-up could be considered.

Owing to the morphological and genetic similarities a uveal melanoma metastasis should be excluded before diagnosing a blue nevus-like melanoma. In patients with no known history of uveal melanoma, a fundoscopic examination by an ophthalmologist is recommendable.

We believe our findings clearly demonstrate that in addition to known inactivating *BAP1* mutations, *SF3B1* R625 mutations in conjunction with *GNAQ* and *GNA11* mutations are a genetic marker of malignancy in blue nevus-like melanocytic proliferations. This genetic profile can be applied as a diagnostic aid in cases where one is uncertain of a tumor's malignant potential based on histopathologic assessment. In addition, the genetic profile may be used to diagnose blue nevus-like melanoma in melanomas where such a diagnosis was not suspected from histopathologic evaluation. Nevertheless, future studies will need to further assess the prognostic relevance of *SF3B1* or *BAP1* mutations in blue nevus-like melanoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Zembowicz A, Phadke PA. Blue nevi and variants: an update. Arch Pathol Lab Med. 2011:327–336. [PubMed: 21366456]
2. Zembowicz A, Mihm MC. Dermal dendritic melanocytic proliferations: an update. Histopathology. 2004:433–451. [PubMed: 15500647]
3. Tièche M. Über benigne Melanome ("Chromatophorome") der Haut - "blaue Neavi". Virch Arch für path Anat. 1906:212–229.
4. Barnhill RL, Cerroni L, Cook M, et al. State of the art, nomenclature, and points of consensus and controversy concerning benign melanocytic lesions: outcome of an international workshop. Adv Anat Pathol. 2010:73–90. [PubMed: 20179431]

5. Costa S, Byrne M, Pissaloux D, et al. Melanomas associated with blue nevi or mimicking cellular blue nevi: 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. 2016:368–377. [PubMed: 26645730]
6. Loghavi S, Curry JL, Torres-Cabala CA, et al. Melanoma arising in association with blue nevus: a clinical and pathologic study of 24 cases and comprehensive review of the literature. Mod Pathol. 2014:1468–1478. [PubMed: 24743221]
7. Martin RC, Murali R, Scolyer RA, et al. So-called 'malignant blue nevus': a clinicopathologic study of 23 patients. Cancer. 2009:2949–2955. [PubMed: 19472395]
8. Murali R, McCarthy SW, Scolyer RA. Blue nevi and related lesions: a review highlighting atypical and newly described variants, distinguishing features and diagnostic pitfalls. Adv Anat Pathol. 2009:365–382. [PubMed: 19851128]
9. Barnhill RL, Argenyi Z, Berwick M, et al. Atypical cellular blue nevi (cellular blue nevi with atypical features): lack of consensus for diagnosis and distinction from cellular blue nevi and malignant melanoma ('malignant blue nevus'). Am J Surg Pathol. 2008:36–44. [PubMed: 18162768]
10. Cancer Genome Atlas Network. Genomic classification of cutaneous melanoma. Cell. 2015:1681–1696. [PubMed: 26091043]
11. Shain AH, Yeh I, Kovalyshyn I, et al. The genetic evolution of melanoma from precursor lesions. N Engl J Med. 2015:1926–1936. [PubMed: 26559571]
12. Bastian BC. The molecular pathology of melanoma: an integrated taxonomy of melanocytic neoplasia. Annu Rev Pathol. 2014:239–271. [PubMed: 24460190]
13. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature. 2009:599–602.
14. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in GNA11 in uveal melanoma. N Engl J Med. 2010:2191–2199. [PubMed: 21083380]
15. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. Nat Genet. 2003:19–20. [PubMed: 12447372]
16. Moller I, Murali R, Muller H, et al. Activating cysteinyl leukotriene receptor 2 (CYSLTR2) mutations in blue nevi. Mod Pathol. 2016:350–356. [PubMed: 27934878]
17. Martin M, Masshofer L, Temming P, et al. Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. Nat Genet. 2013:933–936. [PubMed: 23793026]
18. Harbour JW, Onken MD, Roberson ED, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. Science. 2010:1410–1413. [PubMed: 21051595]
19. Harbour JW, Roberson ED, Anbunathan H, et al. Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. Nat Genet. 2013:133–135. [PubMed: 23313955]
20. Yeh I, Mully TW, Wiesner T, et al. Ambiguous melanocytic tumors with loss of 3p21. Am J Surg Pathol. 2014:1088–1095. [PubMed: 24705312]
21. Dai J, Tetzlaff MT, Schuchter LM, Elder DE, Elenitsas R. Histopathologic and mutational analysis of a case of blue nevus-like melanoma. J Cutan Pathol. 2016:776–780. [PubMed: 27152652]
22. Perez-Alea M, Vivancos A, Caratu G, et al. Genetic profile of GNAQ-mutated blue melanocytic neoplasms reveals mutations in genes linked to genomic instability and the PI3K pathway. Oncotarget. 2016:28086–28095. [PubMed: 27057633]
23. Vivancos A, Caratu G, Matito J, et al. Genetic evolution of nevus of Ota reveals clonal heterogeneity acquiring BAP1 and TP53 mutations. Pigment Cell Melanoma Res. 2016:247–253. [PubMed: 26701415]
24. van de Nes J, Gessi M, Sucker A, et al. Targeted next generation sequencing reveals unique mutation profile of primary melanocytic tumors of the central nervous system. J Neurooncol. 2016:435–444. [PubMed: 26744134]
25. van de Nes JA, Nelles J, Kreis S, et al. Comparing the prognostic value of BAP1 mutation pattern, chromosome 3 status, and BAP1 immunohistochemistry in uveal melanoma. Am J Surg Pathol. 2016:796–805. [PubMed: 27015033]

26. Held L, Eigentler TK, Metzler G, et al. Proliferative activity, chromosomal aberrations, and tumor-specific mutations in the differential diagnosis between blue nevi and melanoma. Am J Pathol. 2013:640–645. [PubMed: 23261261]

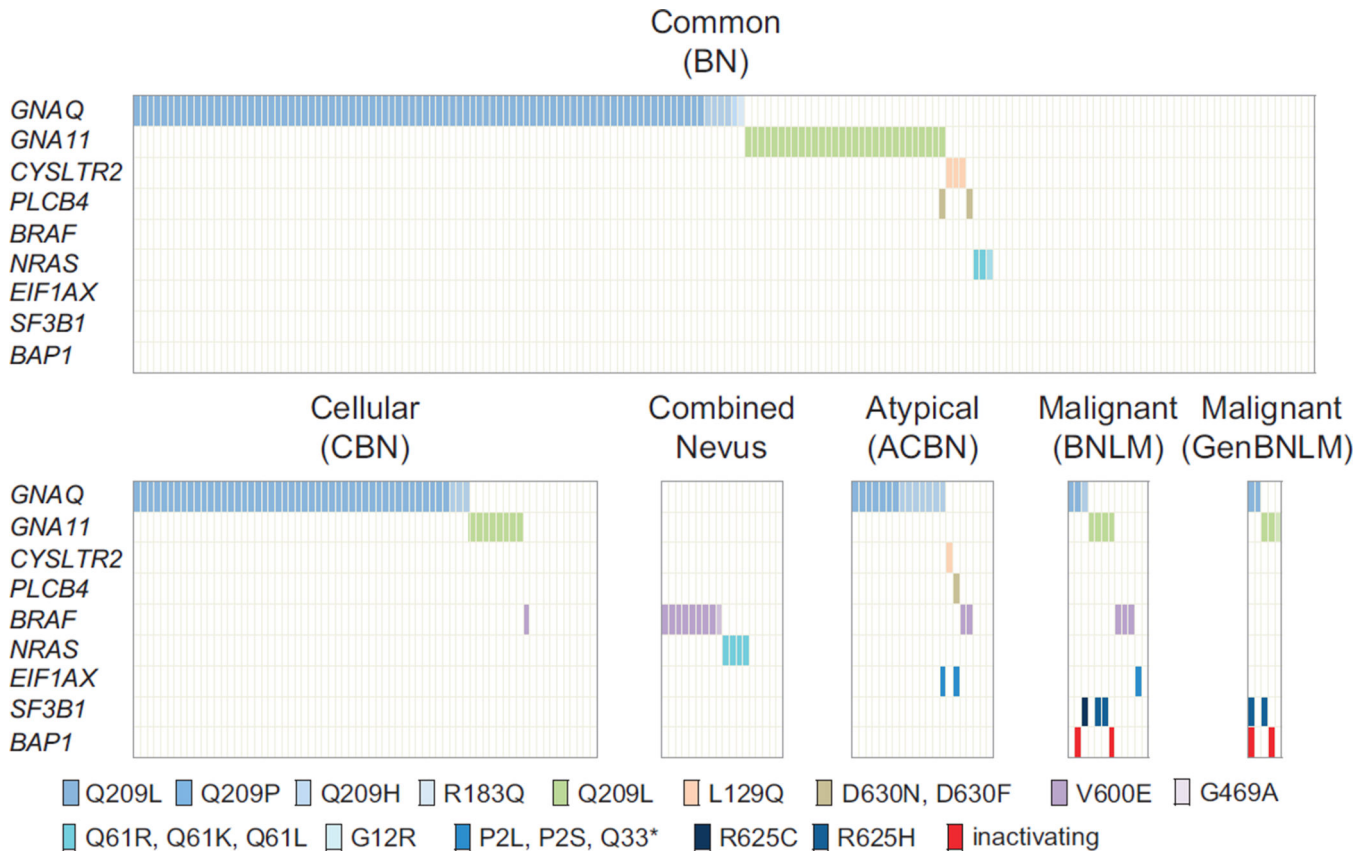

Figure 1.

Mutations identified in a cohort of blue nevi and blue nevus-like melanomas. Shown are the activating mutations identified by targeted next-generation sequencing in a cohort of 301 blue nevi. The protein alterations occurring through the different mutations are signified by different colors, annotated at the bottom of the figure. ACBN, atypical cellular blue nevus; BNLM, blue nevus-like melanoma; BN, blue nevus; CBN, cellular blue nevus; combined nevus, nevus having a blue and conventional nevus compartment; GenBNLM, genetic BNLM.

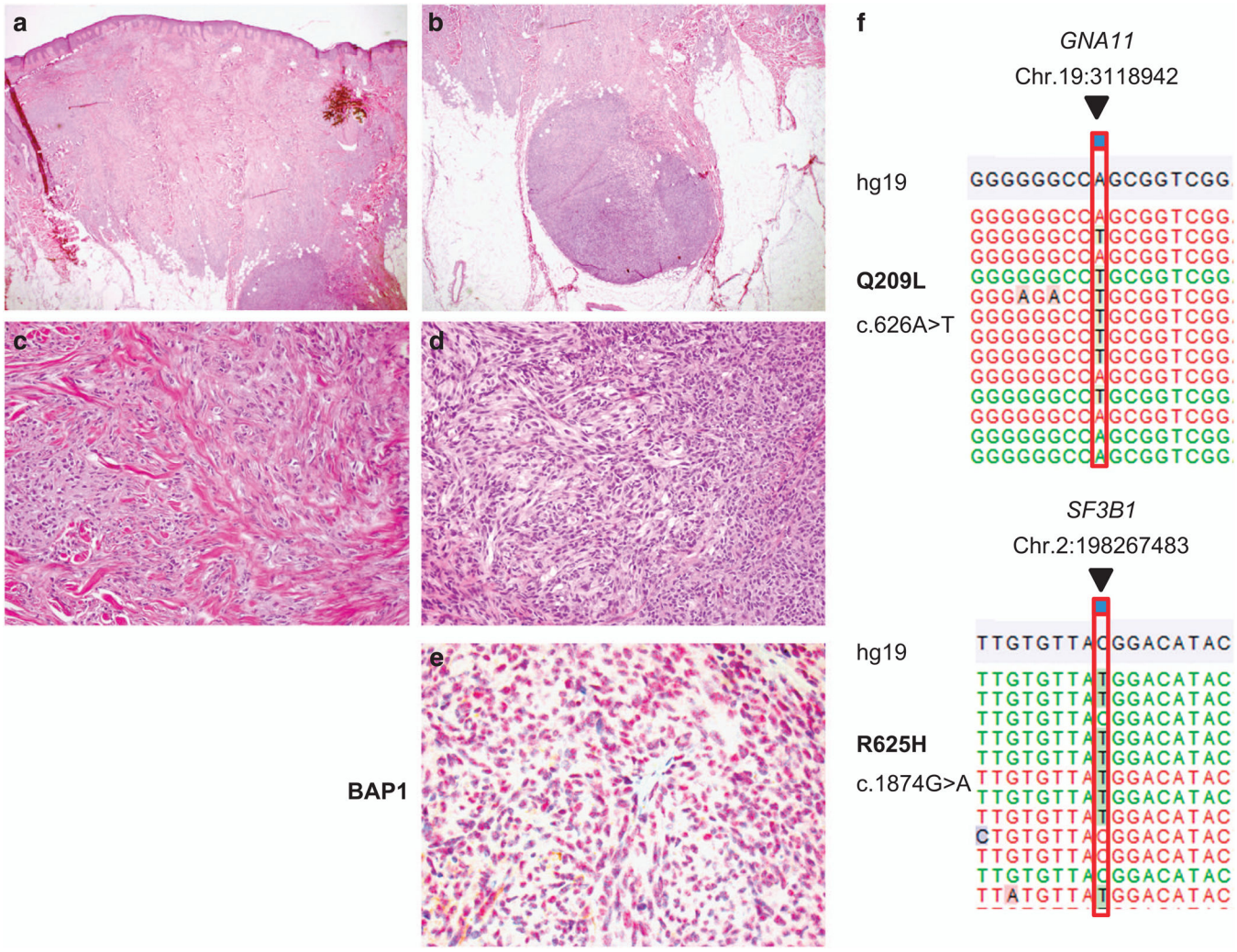

Figure 2.

Blue nevus-like melanoma with a *GNA11* and *SF3B1* mutation. Demonstrating a case of a tumor histopathologically diagnosed as a blue nevus-like melanoma. In addition to a larger dermal component (**a, c** × 20), a nodule of cells is demonstrated in the subcutis (**b, d** ×200). The BAP1 immunohistochemistry (**e** ×400) showed retained BAP1 protein expression. (**f**) The *GNA11* Q209L (c.626A>T) and *SF3B1* R625H (c.1874G>A) mutations identified in the tumor. Annotation according to human genome assembly 19 (hg19). (The case demonstrated refers to number 23 in Table 3).

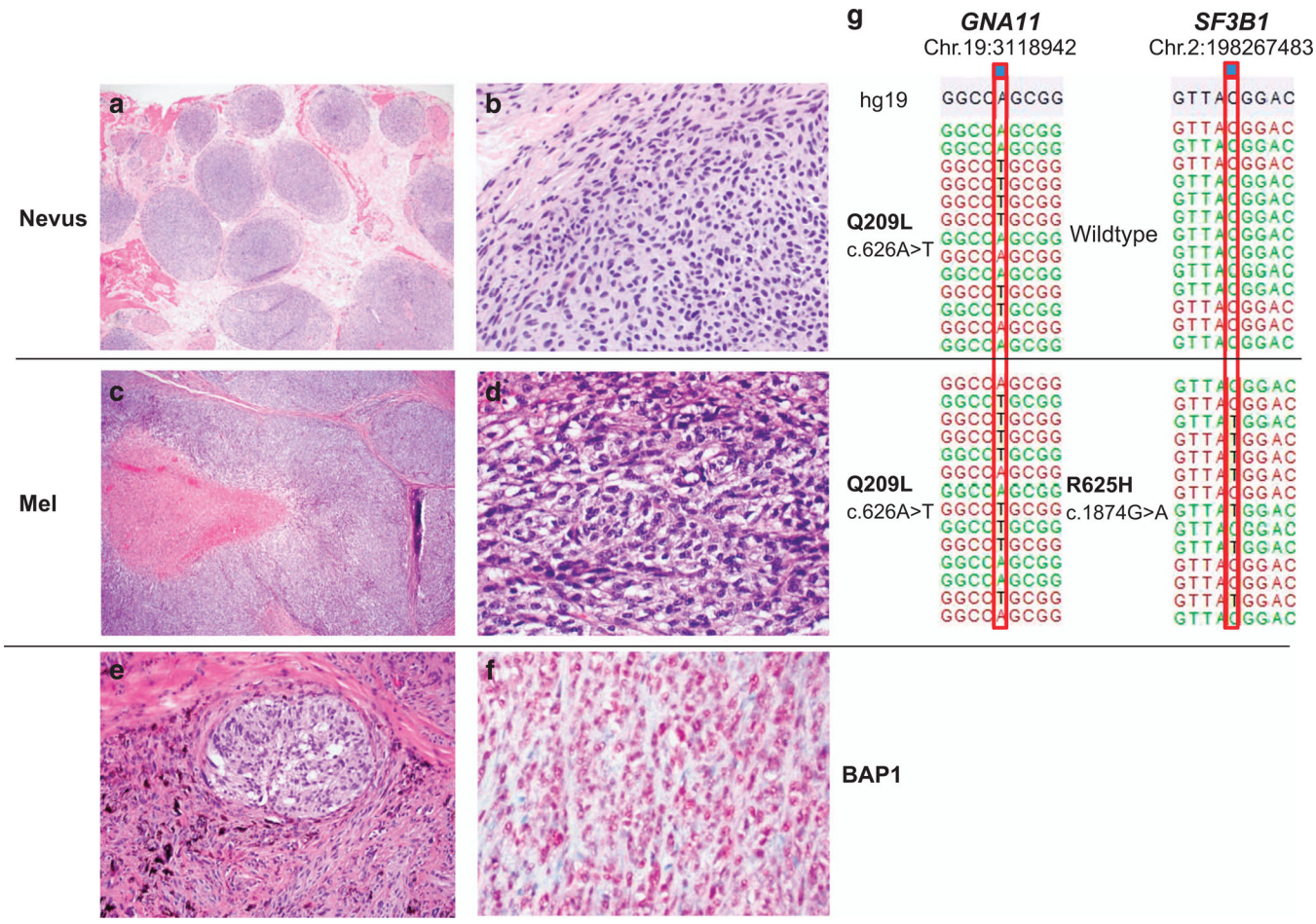

Figure 3.

Cellular blue nevus progressing to melanoma by *SF3B1* mutation. A tumor arising in the scrotum of a 39-year-old male. Upon initial excision, a melanoma was diagnosed (**c** × 20 and **d** × 400), which demonstrated both highly pleomorphic cells and areas of necrosis (**c**). Near the surgical margins, a benign dermal melanocytic proliferation was present with features of a cellular blue nevus (**a** × 40 and **b** × 400). In **e** (×200), mainly benign blue nevus cells are present with a focal nodule of malignant cells infiltrating in the center of the field of view. The BAP1 immunohistochemistry showed retained BAP1 protein expression both in the benign and malignant portions of the lesion (**f** × 400). Genetic results are shown in **g**. The *GNA11* Q209L (c.626A>T) mutation was detected in both the nevus and melanoma portions of the tumor however, the *SF3B1* R625H (c.1874G>A) mutation was only identified in the melanoma. Annotation according to human genome assembly 19 (hg19). (The case demonstrated refers to number 27 in Table 3). Mel, melanoma.

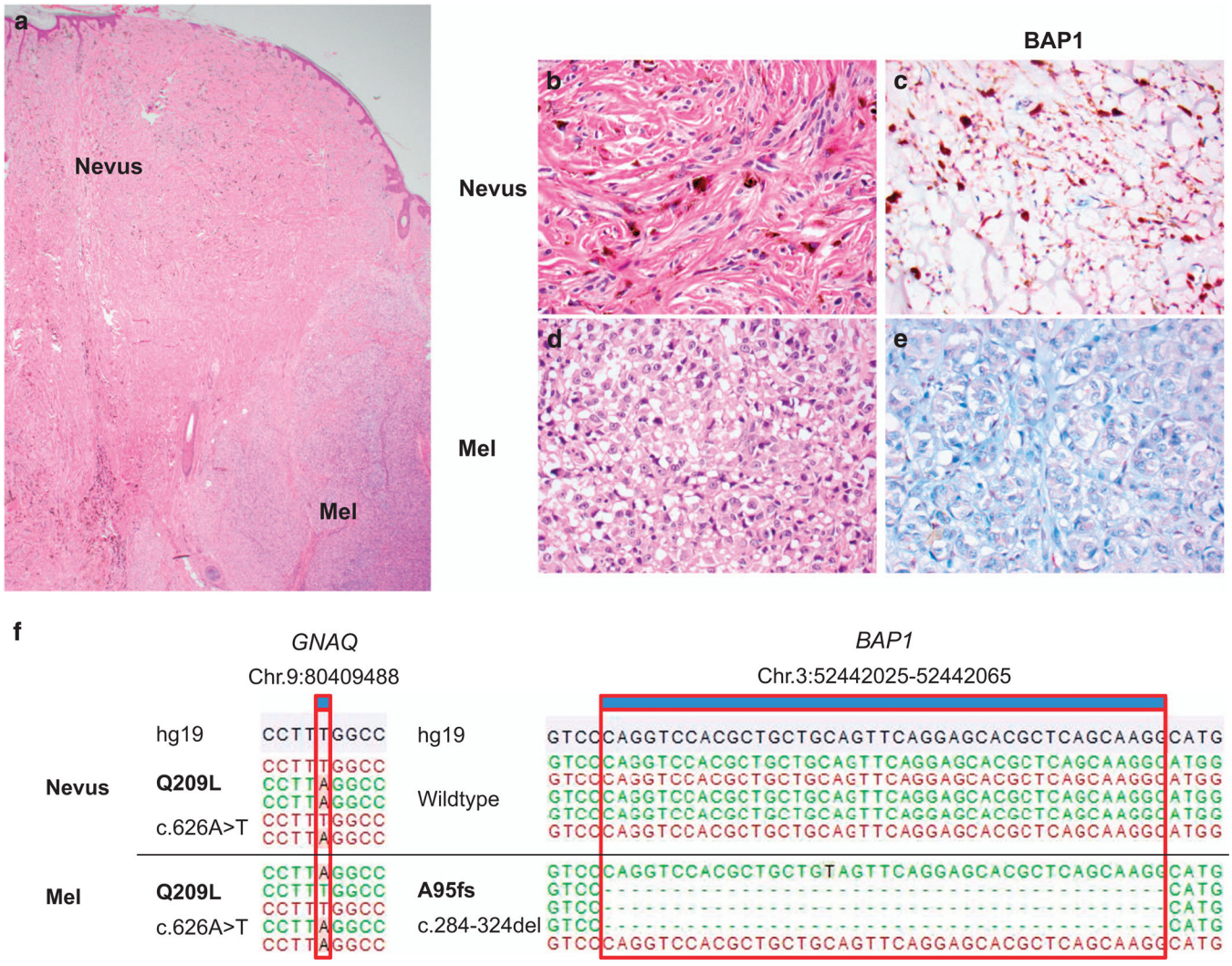

Figure 4.

Blue nevus progressing to melanoma by inactivating *BAP1* mutation. A case demonstrating a residual blue nevus proliferation (**a** × 20) in the top left and progression to melanoma in the bottom right. High magnification of the nevus and melanoma portions of the lesion with BAP1 immunohistochemistry is demonstrated in **b–e** ×400. The top panel demonstrated morphologically benign pigmented nevus cells with the corresponding BAP1 expression in immunohistochemistry. The bottom panel demonstrates the melanoma fraction with epithelioid cells and lack of BAP1 expression (right). (**c**) The genetic data, demonstrating the same *GNAQ* Q209L (c.626A>T) mutation in both the nevus and melanoma portions of the tumor. On the right, the inactivating *BAP1* mutation leading to a frame-shift mutation (A95fs, c.284-324del) was only found in the melanoma, not the nevus portion of the tumor. The notation is according to human genome assembly 19 (hg19). (The case presented is number 30 in Table 3). Mel, melanoma.

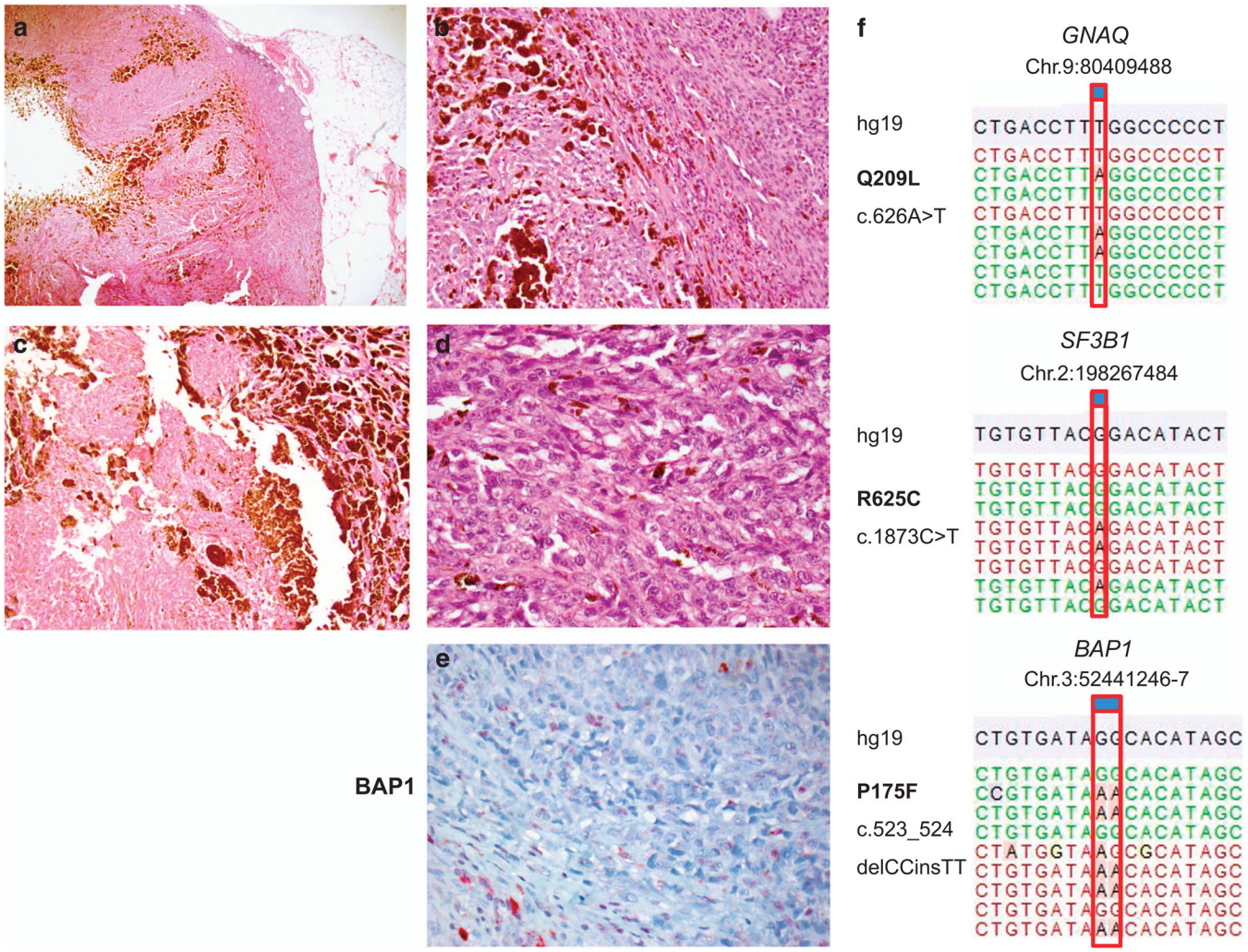

Figure 5.

Metastasized melanoma harboring a *GNAQ, SF3B1*, and *BAP1* mutation. Example of a genetically diagnosed blue nevus-like melanoma. The tumor was originally diagnosed as a melanoma of unknown primary (MUP). The morphology demonstrated in **a** ×40, **b** ×100, **c** ×200, and **d** ×400 shows heavy pigmentation, reminiscent of a blue nevus-like melanocytic tumor, with cytological features of malignancy. BAP1 expression was lost in the tumor by immunohistochemistry (**e** ×400). Interestingly, this tumor showed presence of mutations in *GNAQ* Q209L (c.626A>T), *SF3B1* R625C (c.1873C>T), and *BAP1* P175F (c. 523_524delCCinsTT). Annotation according to human genome assembly 19 (hg19). (The case presented is number 34 in Table 3).

Table 1

Genes covered in the applied sequencing panel

No.	Gene	Chr.	Location GRCh37	Target exons	Selection of mutations covered	Mutation type	Primer pairs
1	*BRAF*	7	140453065	11, 15	G463, G465, V600	Activating	4
2	*NRAS*	1	115256411	1, 2	G12, G13, Q61	Activating	5
3	*KIT*	4	55593572	11, 13, 17	L576, K642, N822	Activating	8
4	*GNAQ*	9	80409369	4, 5	R183, Q209	Activating	6
5	*GNA11*	19	3114932	4, 5	R183, Q209	Activating	3
6	*CYSLTR2*	13	49281314	1	L129	Activating	1
7	*PLCB4*	20	9389740	20	D630	Activating	1
8	*SF3B1*	2	198267458	14	R625	Change of function	1
9	*EIF1AX*	X	20156647	1, 2	Mutations in exons 1 and 2	Change of function	3
10	*BAP1*	3	52436293	all	Mutations in all exons	Inactivating	41

Table 2

Blue nevi cohort, clinical and genetic overview

		Histological blue nevus subtype													
		Common BN		Cellular BN		Combined nevus		Atypical cellular BN		BNLM		Genetically BNLM		Total	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Total		176	56	69	23	18	6	21	7	12	4	5	2	301	
Age															
Median	$P < 0.001$	53		35		42		29		55		39		48	
Range		7–99		8–82		13–67		3–79		23–88		20–66		3–99	
Sex															
Female	$P = 0.11$	101	57	45	65	12	67	9	43	5	42	2	40	174	58
Male		73	42	20	29	6	33	10	48	7	58	3	60	119	40
Missing data		2	1	4	6	0	0	2	10	0	0	0	0	8	3
Site															
Head/neck	$P = 0.29$	47	27	15	22	6	33	5	24	5	42	3	60	81	27
Trunk		42	24	27	39	6	33	7	33	3	25	2	40	87	29
Upper extremity		31	18	8	12	0	0	2	10	0	0	0	0	41	14
Lower extremity		26	15	10	15	4	22	3	14	0	0	0	0	43	14
Missing data		30	17	9	13	2	11	4	19	4	33	0	0	49	16
Activating mutations															
GNAQ	$P < 0.001$	91	52	50	73	0	0	14	67	3	25	2	40	160	53
GNA11		30	17	8	12	0	0	0	0	4	33	3	60	45	15
CYSLTR2		3	2	0	0	0	0	1	5	0	0	0	0	4	1
PLCB4		2	1	0	0	0	0	1	5	0	0	0	0	3	1
BRAF		0	0	1	1	9	50	2	10	3	25	0	0	15	5
NRAS		3	2	0	0	4	22	0	0	0	0	0	0	7	2
Wild-type		48	27	10	15	5	28	3	14	2	17	0	0	68	23

Abbreviations: BN, blue nevus; BNLM, blue nevus-like melanoma.

Table 3

Genetic alterations in atypical blue nevi and blue nevus-like melanoma

Nr	Type ACBN	More detailed diagnosis given	Age:	Sex:	Location	BRAF 600	NRAS 12,13,61	GNA11 183,209	GNAQ 183,209	CYSLTR2 129	PLCB4 630	EIF1AX Ex.1+2	SF3B1 625	BAP1 inact.	BAP1 IHC	Status	Int.
1	ACBN	atypical pigmented cellular blue nevus	47	F	upper chest	-	-	-	-	-	-	-	-	-	+	N.K.	-
2	ACBN	animal type blue nevus	N.K.	N.K.	N.K.	-	-	-	Q209L	-	-	-	-	-	+	N.K.	-
3	ACBN	atypical cellular blue nevus	79	M	right neck	-	-	-	Q209P	-	-	-	-	-	+	N.K.	-
4	ACBN	atypical cellular blue nevus	42	M	left occipital	-	-	-	Q209P	-	-	-	-	-	+	F	1
5	ACBN	atypical cellular blue nevus	23	F	right hand	-	-	-	Q209P	-	-	-	-	-	N.A.	N.K.	-
6	ACBN	atypical cellular blue nevus	3	F	scalp	-	-	-	Q209P	-	-	-	-	-	+	N.K.	-
7	ACBN	atypical cellular blue nevus	44	M	right thigh	-	-	-	-	-	D630F	P2L	-	-	+	F	13y
8	ACBN	atypical cellular blue nevus	66	M	N.K.	-	-	-	Q209L	-	-	-	-	-	+	N.K.	-
9	ACBN	atypical cellular blue nevus	29	M	N.K.	-	-	-	Q209L	-	-	-	-	-	+	N.K.	-
10	ACBN	atypical neuronevus Masson	26	M	gluteal left	-	-	-	Q209L	-	-	-	-	-	+	N.K.	-
11	ACBN	atypical neuronevus Masson	35	W	gluteal left	-	-	-	Q209L	-	-	-	-	-	+	N.K.	-
12	ACBN	atypical epithelold blue nevus	N.K.	N.K.	N.K.	-	-	-	-	-	-	-	-	-	+	N.K.	-
13	ACBN	pigmented epithelioid melanocytoma	17	M	right jaw	-	-	-	-	L129Q	-	-	-	-	+	F	1y
14	ACBN	pigmented epithelioid melanocytoma	17	F	right back	-	-	-	Q209P	-	-	-	-	-	+	F	6y
15	ACBN	pigmented epithelioid melanocytoma	26	F	right cheek	-	-	-	Q209P	-	-	Q33*	-	-	+	F	2y
16	ACBN	atypical neuronevus Masson	28	M	gluteal left	-	-	-	Q209L	-	-	-	-	-	+	N.K.	-
17	ACBN	atypical neuronevus Masson	66	F	gluteal	-	-	-	Q209P	-	-	-	-	-	+	N.K.	-
18	ACBN	atypical spindle cell dermal melanocyte tumor	63	M	back of left foot	-	-	-	Q209L	-	-	-	-	-	+	N.K.	-
19	ACBN	atypical epithelioid dermal melanocyte tumor	29	M.	upper back	-	-	-	-	-	-	-	-	-	+	N.K.	-
20	ACBN	atypical cellular blue nevus	46	F	upper arm right	V600E	-	-	-	-	-	-	-	-	N.A.		
21	ACBN	atypical cellular blue nevus	28	F	lower right calf	V600E	-	-	-	-	-	-	-	-	+	F	4y
	BNLM	**Diagnosis given**	**Age**	**Sex**	**Location**	**BRAF**	**NRAS**	**GNA11**	**GNAQ**	**CYSLTR2**	**PLCB4**	**EIF1AX**	**SF3B1**	**BAP1**	**BAP1**	**Status**	**Int.**
22	BNLM	blue nevus-like melanoma	88	M	posterior left scalp	-	-	-	-	-	-	P2S	-	-	+	†	*
23	BNLM	blue nevus-like melanoma	61	M	scalp	-	-	Q209L	-	-	-	-	R625H	-	+	†	*
24	BNLM	blue nevus-like melanoma	70	M	left back	-	-	-	Q209P	-	-	-	R625C	-	N.K.#	†	*

Nr	Type ACBN	More detailed diagnosis given	Age:	Sex:	Location	BRAF 600	NRAS 12,13,61	GNA11 183,209	GNAQ 183,209	CYSLTR2 129	PLCB4 630	EIF1AX Ex.1+2	SF3B1 625	BAP1 inact.	BAP1 IHC	Status	Int.
25	BNLM	blue nevus-like melanoma	63	F	head right side	-	-	Q209L	-	-	-	-	-	-	N.A.	F	*
26	BNLM	blue nevus-like melanoma	35	M	gluteal region left	-	-	-	Q209L	-	-	-	-	-	+	N.K.	-
27	BNLM	blue nevus-like melanoma	39	M	scrotum left	-	-	Q209L	-	-	-	-	R625H	-	+	A	*
28	BNLM	blue nevus-like melanoma	61	F	N.K.	-	-	-	-	-	-	-	-	-	+	N.K.	-
29	BNLM	blue nevus-like melanoma	28	F	N.K.	-	-	Q209L	-	-	-	-	-	P190R	neg	N.K.	-
30	BNLM	blue nevus-like melanoma	49	F	occipital	-	-	-	Q209L	-	-	-	-	A95fs	neg	N.K.	-
31	BNLM	blue nevus-like melanoma	51	M	buttock	V600E	-	-	-	-	-	-	-	-	N.A.	N.K.	-
32	BNLM	blue nevus-like melanoma	84	F	N.K.	V600E	-	-	-	-	-	-	-	-	+	N.K.	-
33	BNLM	blue nevus-like melanoma	23	M	scalp	V600E	-	-	-	-	-	-	-	-	+	N.K.	-
	Gen-BNLM	**Clinical Information**	**Age**	**Sex**	**Location**	**BRAF**	**NRAS**	**GNA11**	**GNAQ**	**CY5LTR2**	**PLCB4**	**EIF1AX**	**SF3B1**	**BAP1**	**BAP1**	**Status**	**Int.**
34	GenBNLM	MUP - "metastasis" left axilla	39	F	axillary left	-	-	-	Q209L	-	-	-	R625C	P175F	neg	N.K.	-
35	GenBNLM	melanoma NOCTT >4 mm	20	W	gluteal right	-	-	-	Q209L	-	-	-	-	-	N.A.	F	3y
36	GenBNLM	melanoma NOC TT 7.5 mm	64	M	temporal right	-	-	Q209L	-	-	-	-	R625C	-	+	A	*
37	GenBNLM	superficial spreading melanoma	66	M	pectoral	-	-	R183C	-	-	-	-	-	-	+	F	1y
38	GenBNLM	superficial spreading melanoma	31	M	temporal right	-	-	Q209L	-	-	-	-	-	Q253*	neg	A	*

Abbreviations: A, alive; ACBN, atypical cellular blue nevus; blue, mutations altering function; BNLM, blue nevus-like melanoma (also known as ‘malignant blue nevus’); F, free of disease; GenBNLM, genetic blue nevus-like melanoma; Green, activating mutations; IHC, immunohistochemistry; Int., interval (available follow-up period); Max., maximum; Met., metastasis; MUP, melanoma of unknown primary; N.A., not analyzed; N.K., not known; NOC, not otherwise characterized; red, loss of function mutations; TT, tumor thickness.

[a] Signifies cases where more detailed follow-up information.

[b] BAP1 immunohistochemistry was inconclusive (demonstrated in Supplementary Figure 2).

Table 4

Available clinical information of malignant tumors

No.	Type	More detailed diagnosis given	Gene mutations	Clinical info
22	BNLM	Blue nevus-like melanoma	*EIF1AX*	Multiple local/in-transit recurrence 16, 18, 23, 26, and 28 months after diagnosis. Patient died 30 months after diagnosis (Alzheimer's disease, heart failure, and metastasized melanoma).
23	BNLM	Blue nevus-like melanoma	*GNA11 SF3B1*	The patient had two other primary melanomas, one diagnosed 12 months prior to the BNLM, another nodular melanoma diagnosed ~48 months (4 years) later which had a positive SLNB. Patient died of metastatic disease ~ 72 months (6 years) after diagnosis.
24	BNLM	Blue nevus-like melanoma	*GNAQ SF3B1*	Developed liver metastases and died of disease within 12 months.
25	BNLM	Blue nevus-like melanoma	*GNA11*	Diagnosed with a non-ulcerated 7 mm tumor, shortly afterwards full tumor excision with wide excision margin and SLNB neg., no further disease to date (11 months follow-up).
26	BNLM	Blue nevus-like melanoma	*GNAQ*	Diagnosed with a tumor thickness >6mm (pT4b), SLNB pos. (1/1 LN), LAD neg. (0/10), no further disease to date (13 months follow-up).
27	BNLM	Blue nevus-like melanoma	*GNA11 SF3B1*	Presented with an unusual tumor of the scrotum. Diagnostically a metastasis was considered, however upon histological and genetic data the diagnosis of a malignant blue nevus was made. Currently free of disease (4 months follow-up).
35	GenBNLM	Melanoma NOC TD > 4 mm	*GNAQ*	SLNB (2/2 pos.), LAD neg. (0/9), irregular follow-up, last seen 34 months after diagnosis with no sign of disease in staging.
36	GenBNLM	Melanoma NOC TD 7.5 mm	*GNA11, SF3B1*	Cervical LN metastasis ~144 months (12 years) after diagnosis. Sequence analysis led to the genetic diagnosis of a blue nevus-like melanoma. Subsequently multiple additional LN and soft tissue metastasis in the neck area have been excised (~146–164 months after diagnosis).
37	GenBNLM	Superficial spreading melanoma TT 1.45 mm CL II	*GNAQ*	Diagnosed with a tumor thickness of 1.45 mm and Clark level II, excised with 1 cm safety margin, SLNB pos. (1/3 LN).
38	GenBNLM	Superficial spreading melanoma	*GNA11, BAP1*	No tumor thickness given on initial diagnosis, local recurrence 25 months later, SLNB was performed and pos., LAD pos. (1/5), 42 months after diagnosis recurrence mastoid right, radical neck dissection. Fifty months after diagnosis liver metastasis, partial liver resection, Chemotherapy with gemcitabine and treosulfan, later ipilimumab, mixed response. Fifty-six months after diagnosis cutaneous nuchal and abdominal metastasis. Sixty-nine months after diagnosis scrotal metastasis. Seventy-seven months after diagnosis re-induction ipilimumab. Eighty-one months after diagnosis progressive disease. One-hundred three months after diagnosis genetic analysis identifying a genetic profile typical of a blue nevus-like melanoma.

Abbreviations: BNLM, blue nevus-like melanoma; GenBNLM, genetic blue nevus-like melanoma; LAD, lymphadenectomy; neg, negative; NOC, not otherwise characterized; pos, positive; SLNB, sentinel lymph node biopsy; TT, tumor thickness.