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Co-occurrence of *EIF1AX*, *SF3B1*, or *BAP1* variants in uveal melanomas: A case series and review

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ABSTRACT

Purpose: The purpose of this study is to present a case series of patients with co-occurrence of either BRCA1 associated protein-1 (*BAP1*), eukaryotic translation initiation factor 1A, X-chromosomal (*EIF1AX*), or splicing factor 3B subunit 1 (*SF3B1*) in the detection and treatment of a uveal melanoma (UM) prior to the development of metastatic disease.

Observations: This is a retrospective case series of ten patients with UM demonstrating co-occurrence of either *BAP1*, *EIF1AX*, or *SF3B1* variants treated at a single ocular oncology clinic by a senior ocular oncologist between 2020 and 2024. Charts were reviewed and data on medical history, demographics, tumor characteristics, genetic testing, follow up, as well as fundus photo and B-scan ocular ultrasound were collected. The average age of the patients was 58.5 years old. The mean length of follow up was 18.2 months. Four patients had guanosine nucleotide-binding protein alpha-11 (*GNA11*) variants and six had guanosine nucleotide-binding protein Q (*GNAQ*) variants. Four patients had germline *BAP1* variants. Four patients had a combination of *EIF1AX* and *BAP1* variants. Three patients had a combination of *EIF1AX* and *SF3B1* variants. Three patients had a combination of *SF3B1* and *BAP1* variants. Eight UM were gene expression profile (GEP) Class 1A and two UM were GEP Class 1B. Seven UM were preferentially expressed antigen in melanoma (PRAME) negative and three UM were PRAME positive. All patients had cytologic confirmation of the diagnosis of UM: seven had cytology results of spindle cells and three had results of mixed spindle and epithelioid cells. All patients were treated with Iodine-125 (I-125) plaque brachytherapy.

Conclusions and importance: We present a case series of patients with the co-occurrence of *EIF1AX*, *SF3B1*, or *BAP1*. With distinct genomic aberrations, transcriptional features, and clinical outcomes, *EIF1AX*, *SF3B1*, and *BAP1* are thought to be mutually exclusive. The present case series demonstrates rare exceptions to this general pattern and speculates on the early molecular steps of UM which may lead to these rare mutation combinations.

1. Introduction

Uveal melanoma (UM) is the most common primary cancer of the eye and its prognosis remains poor for disseminated disease.[1–3] Survival prognostication can be estimated by clinical features such as tumor size but is most accurately predicted by tumor genetics.[2] Further, our understanding of uveal melanoma initial pathogenesis and tumor evolution has expanded with genetic profiling and next generation sequencing (NGS).[4]

Advanced cytogenic, chromosomal and immunohistochemistry analysis have changed the landscape for predicting UM prognosis.[5–8] Gene expression profiling (GEP), preferentially expressed antigen in melanoma (PRAME), next generation sequencing (NGS) and other molecular techniques have emerged as molecular methods for predicting metastatic risk with great accuracy.[2,9] A combined GEP and PRAME classifier was found to have greater prognostic accuracy than GEP alone.[10] Furthermore, a large retrospective review of UM patients demonstrated concordance between GEP and NGS, as well as associations with clinical variables like tumor size as strong indicators of metastatic risk.[11] Two initiator variants, guanosine nucleotide-binding protein alpha-11 (*GNA11*) and guanosine nucleotide-binding protein Q (*GNAQ*), have been implicated in tumorigenesis in >95 % of cases but

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are not prognostically significant and are alone insufficient to induce metastasis.[4,12–15] Previously thought to be mutually exclusive, variants in eukaryotic translation initiation factor 1A (*EIF1AX*), splicing factor 3B subunit 1 (*SF3B1*), and BRCA1-associated protein 1 (*BAP1*), have been identified as important prognostic biomarkers and are associated with low, intermediate, and high metastatic risk in UM, respectively.[7,9,16,17]

In this report, we present a novel case series of patients with co-occurrence of either *EIF1AX*, *SF3B1*, or *BAP1* in the detection and treatment of a UM prior to disseminated disease, challenging the narrative of these variants being mutually exclusive and speculating on the early development of UM.

2. Methods

Among patients evaluated by a single ocular oncologist (ACS) from 2020 to 2024 when GEP, PRAME, and NGS were all routinely performed as part of clinical care, those patients with a combination of two of the following three mutations: EIF1AX, SF3B1, and BAP1 were included. Charts were reviewed and data related to demographic characteristics, affected eye, visual acuity, medical and ocular history, physical exam findings, treatment, tumor largest basal diameter (LBD), tumor height, cytology, GEP class, PRAME, and NGS results, and the presence/absence of metastatic disease were collected. Fundus photographs and B-scan ocular ultrasounds of the affected eyes were also collected.

Tumors were sampled by either transscleral or transvitreal fine-needle aspiration. Tumor samples were cytologically evaluated intra-operatively and the tumor sample was tested for GEP, PRAME expression, and variant analysis with the Decision Dx-UM, Decision Dx-PRAME, and Decision UM-Seq panels (Castle Biosciences, Friendswood, TX). Variants were validated per the standard clinical protocol of Castle Biosciences, the clinical lab in which these samples were sequenced. All tumors possessing BAP1 variants underwent germline testing done at Invitae Corp. (San Francisco, CA). Patients treated with brachytherapy had an I-125 plaque placed at the site of the tumor after completion of the biopsy. Further details of our biopsy approach have been previously published.[18]

Approval for this study was obtained from the Sterling Institutional Review Board (IRB) governing Retina Consultants of Texas (Houston, TX). The review was performed in compliance with the provisions of the Health Insurance Portability and Accountability Act of 1996 and adhered to the Declaration of Helsinki, as amended in 2013. Informed consent was obtained from each patient. Guidelines by Kempen for reporting of case series were followed.[19]

3. Results

Among 289 patients with uveal melanoma treated with brachytherapy or enucleation, 10 patients were identified to have co-occurrence of EIF1AX, SF3B1, or BAP1 mutations. The mean age of patients in this cohort was 58.5 years. Tumor and patient characteristics are included in Tables 1 and 2. The mean length of follow up was 18.2 months. Four patients had *GNA11* variants and six had *GNAQ* variants. Four patients were found to have germline *BAP1* variants. Four patients had a combination of *EIF1AX* and *BAP1* variants. Three patients had a combination of *EIF1AX* and *SF3B1* variants. Three patients had a combination of *SF3B1* and *BAP1* variants. Mean tumor LBD and height were 11.2 mm (mm) (range: 6.5 mm–16.0 mm) and 3.5 mm (range: 1.4 mm–7.9 mm), respectively. Seven had cytology results of spindle cells and three had results of mixed spindle and epithelioid cells. All patients were treated with I-125 plaque brachytherapy. No metastatic disease has been identified in any of the patients to date.

Patient 1 presented with choroidal melanoma in the left eye

Table 1
Patient sex, tumor size, cytology, gene expression profile class (GEP), PRAME profile, next generation sequencing (NGS), germline BAP1 status, and follow up duration by patient.

Patient	Sex	LBD (mm)	Height (mm)	Cytology	GEP Class	PRAME	NGS Mutations (DNA variant, variant frequency, Tier)	Follow up duration (months)
1	M	12.0	2.9	Spindle	1A	–	GNA11 (c.37C > T, 38.0 %, II), BAP1 (c.1153C > T, 16.5 %, II), EIF1AX (c.547C > T, 19.2 %, II)	28.8
2	F	12.5	5.2	Spindle	1A	–	GNAQ (c.547C > G, 48.0 %, III), EIF1AX (c.4C > T, 97.6 %, II), SF3B1 (c.1873C > T, 49.2 %, II)	8.1
3	M	9.0	1.4	Spindle	1A	–	GNAQ (c.626A > C, 46.0 %, II), EIF1AX (c.17-2A > G, 92.8 %, II), SF3B1 (c.1997A > C, 34.2, II)	7.6
4	F	16.0	7.9	Mixed	1A	+	GNAQ (c.626A > T, 48.6 %, II), BAP1 (c.1216G > A, 49.7 %, III), SF3B1 (c.1873C > T, 51.3 %, II)	38.6
5	M	12.5	3.8	Mixed	1A	+	GNA11 (c.626A > T, 46.0 %, II), BAP1 (c.2001G > T, 5.8 %, III), EIF1AX (c.10A > G, 88.6 %, II)	19.2
6	F	6.5	1.7	Spindle	1A	–	GNA11 (c.626A > T, 34.8 %, II), BAP1 (c.2057-4G > T, 46.7 %, III), EIF1AX (c.16G > C, 50.8 %, II)	18.8
7	F	9.5	2.2	Mixed	1B	+	GNAQ (c.548G > A, 30.6 %, II), EIF1AX (c.4C > T, 52.7 %, II), SF3B1 (c.1874G > A, 27.8 %, II)	4.6
8	F	10.5	2.8	Spindle	1A	–	GNAQ (c.548G > A, 47.0 %, II), BAP1 (c.535C > T, 77.7 %, III), SF3B1 (c.1873C > T, 44.6 %, II)	0.9
9	F	11.0	1.4	Spindle	1B	–	GNAQ (c.626A > C, 48.9 %, II), BAP1 (c.1251-4A > G, 51.9 %, II), EIF1AX (c.11A > G, 75.0 %, II)	51.4
10	M	12.5	5.9	Spindle	1A	–	GNA11 (c.626A > T, 47.6 %, II), BAP1 (c.1838C > T, 51.7 %, III), SF3B1 (c.1997A > C, 48.9 %, II)	4.3

Abbreviations: Largest basal diameter (LBD); Gene expression profiling (GEP); Preferentially expressed antigen in melanoma (PRAME); Next generation sequencing (NGS); BRCA1 associated protein-1 (BAP1); Guanosine nucleotide-binding protein alpha-11 (GNA11); Eukaryotic translation initiation factor 1A, X-chromosomal (EIF1AX); Guanosine nucleotide-binding protein Q (GNAQ); Splicing factor 3B subunit 1 (SF3B1); Variant of potential clinical significance (Tier II); Variant of unknown clinical significance (Tier III).

Table 2
DNA variant, variant frequency, pathogenicity, associated conditions, and family history of tumors possessing BAP1 variants.

Patient	BAP1 DNA variant, variant frequency	Germline BAP1	Pathogenicity[a]	Associated Conditions	Reported Family History for each Patient
1	c.1153C > T, 16.5 %	No	Pathogenic[b]	BAP1-related tumor predisposition syndrome, Hereditary cancer-predisposing syndrome	Lung cancer (unspecified relative)
4	c.1216G > A, 49.7 %	Yes	Likely benign/Uncertain significance	BAP1-related tumor predisposition syndrome, Hereditary cancer-predisposing syndrome	None
5	c.2001G > T, 5.8 %	No	Uncertain significance	BAP1-related tumor predisposition syndrome	None
6	c.2057-4G > T, 46.7 %	Yes	Benign/Likely benign/ Uncertain significance	Uveal melanoma, BAP1-related tumor predisposition syndrome, Hereditary cancer-predisposing syndrome	None
8	c.535C > T, 77.7 %	No	Likely pathogenic/ Uncertain significance	Clear cell renal cell carcinoma, BAP1-related tumor predisposition syndrome, Hereditary cancer-predisposing syndrome	None
9	c.1251-4A > G, 51.9 %	Yes	Likely benign, Uncertain significance	BAP1-related tumor predisposition syndrome, Hereditary cancer-predisposing syndrome	None
10	c.1838C > T, 51.7 %	Yes	Benign/Likely benign	Uveal melanoma, BAP1-related tumor predisposition syndrome, Hereditary cancer-predisposing syndrome	Colon cancer with metastases to lungs (Father)

Abbreviations: BRCA1 associated protein-1 (BAP1).

[a] Pathogenicity based on report from clinical CLIA-certified laboratory in which germline sequencing was performed, based on the lab's assessment of computational tools and unified databases.

[b] BAP1-related tumor predisposition syndrome is associated with an increased risk for uveal melanoma, malignant mesothelioma, cutaneous melanoma, renal cell carcinoma, basal cell carcinoma, meningioma, and cholangiocarcinoma.

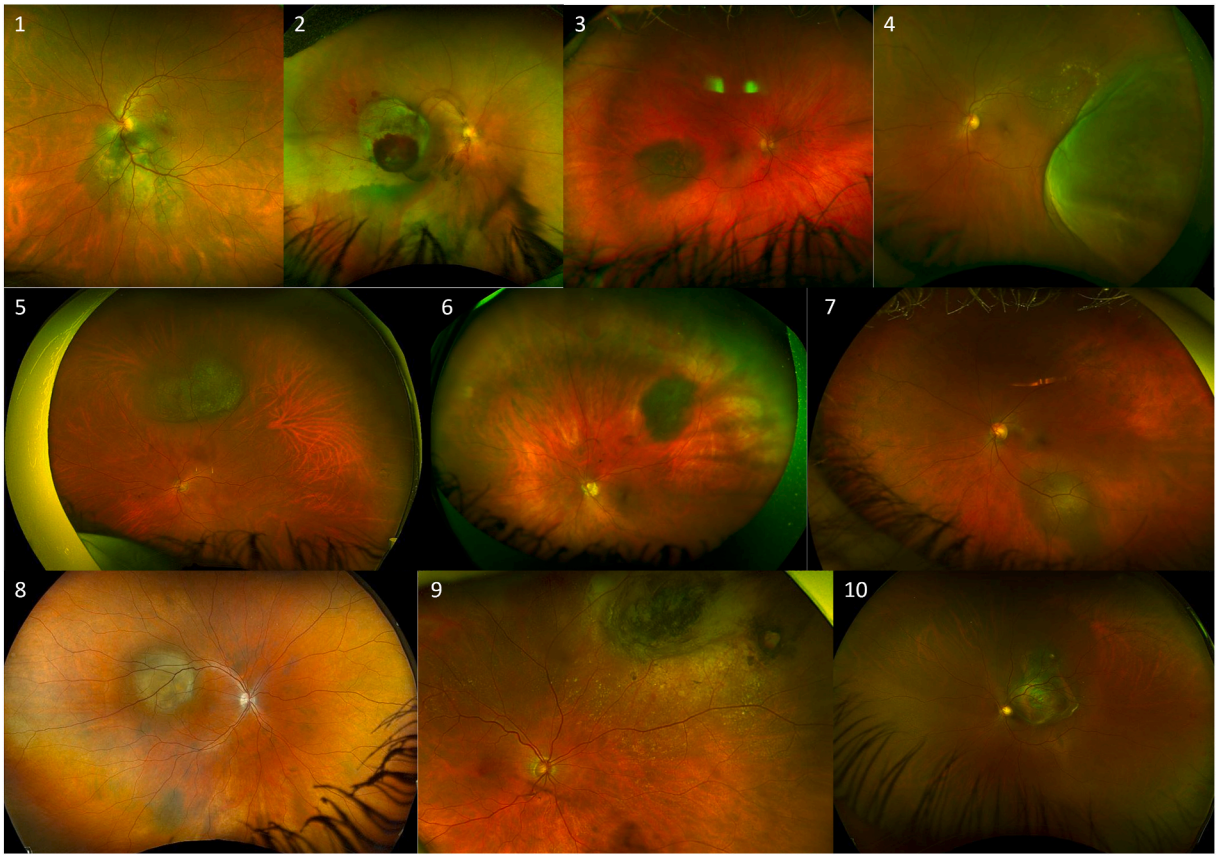

Fig. 1. Fundus photos demonstrating choroidal melanomas at presentation labeled with the corresponding patient case numbers.

(Fig. 1.1). Dimensions by B-scan ultrasound were 12.0 mm LBD and 2.9 mm height. Genetic analysis of the biopsy was Class 1A PRAME negative with variants in *GNA11*, *EIF1AX*, and *BAP1*.[20] Two years after I-125 plaque therapy, a slight elevation on the temporal margin of the treated tumor indicating local recurrence was treated with transpupillary thermotherapy.

Patient 2 presented with choroidal melanoma in the right eye (Fig. 1.2). Dimensions by B-scan ultrasound were 12.5 mm LBD and 5.2 mm height. Genetic analysis of the biopsy demonstrated a Class 1A PRAME negative profile with variants in *GNAQ*, *EIF1AX*, and *SF3B1*.[21]

Patient 3 presented with a lesion suspicious for choroidal melanoma in the right eye (Fig. 1.3). Dimensions by B-scan ultrasound were 9.0 mm LBD and 1.4 mm height. Eighteen months after initial diagnosis, the lesion height increased to 1.9mm representing aggressive behavior consistent with transformation into a choroidal melanoma. Genetic analysis of the biopsy was Class 1A PRAME negative with variants in *GNAQ*, *EIF1AX*, and *SF3B1*.[22,23]

Patient 4 presented with choroidal melanoma in the left eye

(Fig. 1.4). Dimensions by B-scan ultrasound were 16.0mm LBD and 7.9mm height. Genetic analysis of the biopsy was Class 1A PRAME positive with variants in *GNAQ*, *SF3B1*, and a *BAP1* variant which was also present in the patient's germline.[21,22,24]

Patient 5 presented with choroidal melanoma in the left eye (Fig. 1.5). Dimensions by B-scan ultrasound were 12.5 mm LBD and 3.8 mm height. Genetic analysis of the biopsy was Class 1A PRAME positive with variants in *GNA11*, *EIF1AX*, and *BAP1*.

Patient 6 presented with a choroidal melanocytic lesion suspicious for choroidal melanoma in the left eye (Fig. 1.6). Dimensions by B-scan ultrasound were 6.5 mm LBD and 1.7 mm height. Two years after initial diagnosis, the lesion LBD and height increased to 9.7 mm and 2.8 mm, respectively. Genetic analysis of the biopsy was Class 1A PRAME negative with variants in *GNA11*, *EIF1AX*, and *BAP1* which was also identified in the patient's germline.[22,25]

Patient 7 presented with choroidal melanoma in the left eye (Fig. 1.7). Dimensions by B-scan ultrasound were 9.5 mm LBD and 2.2 mm height. Genetic analysis of the biopsy was Class 1B PRAME positive with variants in *GNAQ*, *EIF1AX*, and a *SF3B1* mutation.[21]

Patient 8 presented with choroidal melanoma in the right eye (Fig. 1.8). Dimensions by B-scan ultrasound were 10.5 mm LBD and 2.8 mm height. Genetic analysis of the biopsy was Class 1A PRAME negative with variants in *GNAQ*, *SF3B1*, and *BAP1*.[21,26]

Patient 9 presented with choroidal melanoma in the right eye (Fig. 1.9). Dimensions by B-scan ultrasound were 11.0 mm LBD and 1.4 mm height. Genetic analysis of the biopsy was Class 1B PRAME negative with variants in *GNAQ*, *EIF1AX*, and *BAP1* which was also present in the patient's germline.[22]

Patient 10 presented with choroidal melanoma in the left eye (Fig. 1.10). Dimensions by B-scan ultrasound were 12.5 mm LBD and 5.9 mm height. Genetic analysis of the biopsy was Class 1A PRAME negative with variants in *GNA11*, *SF3B1*, and *BAP1* which was also present in the patient's germline.[23,27]

4. Discussion

Historically, the evolution of UM has been thought to occur through an initial Gq pathway mutation (*GNAQ*, *GNA11*, *CYSLTR2*, or *PLCB4*), and one of three other prognostic variants (*EIF1AX*, *SF3B1*, and *BAP1*), and several other chromosome copy number alterations.[13,28] Despite our expanded understanding of the genomic aberrations in UM, conflicting evidence on the progression of uveal melanoma development and metastasis exists.[4,28] Understanding prognostic risk using NGS will enable precision care of patients with UM and ultimately improve patient survival as delays between uveal melanoma diagnosis and treatment increase risk of metastatic death.[29] As such, the use of NGS has revealed biomarkers for early detection and potential targets for treatment. The following case series provides clinical insight on patients with UM who were found to have co-occurrence of either *EIF1AX*, *SF3B1*, or *BAP1* variants through NGS, previously thought to be mutually exclusive. The co-occurrence of these variants invites critical evaluation of the current understanding of the natural history of the disease.

Gain of function variants in *CYSLTR2*, *GNAQ*, *GNA11*, or *PLCB4* initiate tumorigenesis in UM and play a critical role in the G-protein couple receptor transmembrane signaling pathway.[14,15,30–32] Eliminating intrinsic GTPase activity allows subsequent constitutive activation of the mitogen-activated protein kinase (MAPK) pathway resulting in an uninhibited cascade of growth signals that induce cell proliferation.[17,33] These ubiquitous variants in UM are thought to occur early in disease development but do not play a role in metastatic potential.[34]

The effects of the three variants implicated in malignant progression on cell proliferation are more varied and their presumed mutual exclusivity are less well understood. *BAP1* is a known tumor suppressor gene that encodes a nuclear deubiquitinase involved in cell growth and cancer pathogenesis.[17] Inactivating variants in *BAP1* impair homologous recombination resulting in unrepaired damage to DNA. *BAP1* variants have been associated with the highest risk of metastasis among the three variants and are closely correlated with GEP Class 2 tumors.[35] However, none of the *BAP1*-variant tumors in this series exhibited Class 2 GEP, suggesting some of the variants may be nonpathogenic. The clinical laboratory that evaluated these variants reported that one was pathogenic, one was benign, and five were variants of uncertain significance.

Four patients in this series were found to have *BAP1* variants in their germline as well as their UM. All tumors possessing *BAP1* variants underwent germline testing; however, even if a germline variant was not detected, the presence of a germline variant cannot be definitively excluded.[36] Germline variants in *BAP1* may predispose patients to *BAP1*-Tumor predisposition syndrome (*BAP1*-TPDS), a syndrome in which patients are at increased risk of developing multiple cancers including UM, cutaneous melanoma, malignant mesothelioma, and renal cell carcinoma among others.[37] Recently published guidelines recommend patients with *BAP1*-TPDS be offered annual multidisciplinary screening given their increased risk for a wide range of cancers.[38]

SF3B1 encodes a core component of the RNA spliceosome which processes precursor mRNA into mature transcripts. Aberrant splicing from variants in *SF3B1* are thought to impact the *ATM* and *p53* DNA repair pathways.[17] *SF3B1* variants have been associated with a moderate risk of metastasis and are closely correlated with GEP Class 1B and PRAME expression.[35,39–41] *In vitro* and *in vivo* laboratory research has proposed the co-occurrence of *SF3B1* and *BAP1* variants results in significant disruption of the cell's capacity to buffer DNA damage from endogenous sources and consequently leads to DNA damage and senescence.[42] Other research has speculated that *SF3B1* and *BAP1* variants can independently lead to oncogenesis and/or malignant progression, reducing the need of their co-occurrence in the natural course of the disease.[43] In this cohort, three tumors were found to have *SF3B1* c.1873C > T missense variants at approximately 50 % allelic frequency. Variants at different sites in the *SF3B1* coding sequence have been associated with PRAME expression when allelic frequency is high.[44] This variant has been found to be potentially pathogenic and has variable association with PRAME expression. Limited literature exists on the presence of *SF3B1* and *EIF1AX* variants with germline origin. A possible germline variant of *SF3B1* (*p.K666N*) was found among bone marrow samples of 40 patients with myeloid neoplasm in a non-comparator study.[45] Nevertheless, variants in the *EIF1AX* and *SF3B1* gene highlighted in the present study could not be proven to be germline despite showing a high allelic frequency.

EIF1AX encodes a protein that is involved in the initiation of protein synthesis through ribosome stabilization and recognition of the start codon.[17] The mechanism in which mutated *EIF1AX* induces oncogenic behavior is not fully understood but the mutated gene has been implicated in a variety of other tumor types such as gliomas, lung adenocarcinomas, endometrial carcinomas, and thyroid lesions.[46] *EIF1AX* variants have been associated with a low risk of metastasis and are closely correlated with GEP Class 1A.[35,47] Similar to theories regarding the independent oncogenicity of *BAP1* and *SF3B1* variants, the paucity of *EIF1AX* and *SF3B1* mutation co-occurrences suggests their effects on oncogenic progression can be substituted for one another.[48]

While mostly thought to be mutually exclusive, there are rare reports of mutation co-occurrences in the literature.[7,16,42,44] Three cases of *EIF1AX* variants co-occurring with *SF3B1* variants have been reported including one case treated with local resection and subsequent death from metastatic disease at 34 months and another treated with enucleation with subsequent death from metastatic disease at 58 months.[7,16] Four cases of *BAP1* with *SF3B1* mutation co-occurrence have been reported with one treated with local resection followed by death due to metastatic disease 12 months after primary tumor management and two other patients treated with enucleation who died of other causes 94 and 99 months later.[7,16,44] Our case series includes three co-occurrences of *EIF1AX* with *SF3B1*, three co-occurrence of *BAP1* with *SF3B1*, and four

co-occurrences of *BAP1* with *EIF1AX*, continuing to challenge the existing narrative of mutual exclusivity of these variants.

Given the advent of routine NGS in the clinical care of UM in the past two years, the current understanding of the natural history of UM continues to evolve. Recently, it has been suggested that *EIF1AX*, *SF3B1*, and *BAP1* affect different cellular processes and a combination of these variants in cells may lead to cellular senescence.[7,16,42,44] In contrast, the rare cases of co-existing variants in *BAP1*, *EIF1AX*, or *SF3B1* described in this series challenge this narrative. It is possible that other genetic or epigenetic alterations might explain these rare tumor cases and allow proliferation of tumor cells or enhance the expression of one variant over the other in early tumor cell development, ultimately affecting the downstream progression and metastatic potential of a UM.

The variant allele frequencies, a metric characterized as the measurement of the specific variant allele proportion within a genomic locus, of each UM variant may also support the significance of the rare co-occurrence of these variants in the following case series. In our series, *BAP1*, *EIF1AX*, and *SF3B1* occurred at relatively high allelic frequencies suggesting that these variants are likely significant and contributing directly to the progression of the tumor, rather than an insignificant passenger variant.[49] Further, the patients in our cohort who possess high-risk (*BAP1*) variants but have low-risk (Class 1) GEP classifications may be the result of the presence of UM subclones with a spectrum of variants early in the development of the tumor. The more aggressive and proliferating cells, such as those that possess *BAP1* variants, may result in clonal dominance as the tumor matures and eventually progresses to a high-risk (GEP Class 2) classification. Alternatively, high allelic frequencies may also reflect nonpathogenic germline variants, so further study is necessary to fully understand the significance of these variants.

Given the possibility of early naive tumor cells expressing these variants with potential for deleterious downstream progression, opportunities may exist for targeted therapy early in the development of UM, prior to the progression of metastatic disease. In this case series, four patients who possessed a germline *BAP1* variant and variants in either *EIF1AX* and *SF3B1* were successfully identified and treated with plaque radiotherapy. Previous reports of these rare co-occurrences have been described in both Class 1 GEP and Class 2 GEP tumors, further supporting a differential selection process in early tumor development and final metastatic risk profile of UM being selected after a series of genetic alterations and interactions between identified variants and other non-identified genetic and epigenetic variants.

Our findings that high-risk variants can be associated with low-risk GEP classification suggests that these variants may co-occur early in the development of UM.[35,42] The tumors sampled in our report were small to medium size and two were identified as nevi before more rapid growth and a clinical decision to treat them. The early identification and treatment of these tumors with a low-risk GEP classification was imperative in preventing morbidity and mortality associated with delayed treatment of UM.[29] The presence of multiple driver variants and low-risk GEP classification may be part of the early natural history of UM and represents an opportunistic time to treat these tumors. To date, no metastatic disease has been identified in any of the patients in our cohort despite all having at least one moderate to high-risk mutation. Longer follow-up will be necessary to confirm this outcome but the Class 1 designation in all these tumors suggests such a favorable prognosis is quite likely, especially in the seven patients whose tumors were PRAME negative, including the three patients with a germline *BAP1* mutation.

This study reports on a rare series of patients found to have UM early in its development with variant co-occurrences, successfully treated with plaque brachytherapy. The study is limited by a retrospective approach, small sample size, and relatively short follow up period. Genetic profiling of UM has only become routine clinical practice in the past two years and our understanding of the genetic markers associated with UM will continue to improve with future studies. Future studies investigating the natural history of UM with genetic data extracted earlier in the disease course will provide valuable insights into accurate prognostication of UM using NGS.

CRediT authorship contribution statement

Henry C. Skrehot: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Amer F. Alsoudi:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Amy C. Schefler:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Consent to participate

Informed consent was obtained from each patient.

Statements and declarations

The authors have no financial support or conflicts of interest to disclose.

Compliance with ethical standards

Approval for this study was obtained from the Sterling Institutional Review Board (IRB) governing Retina Consultants of Texas (Houston, TX). The review was performed in compliance with the provisions of the Health Insurance Portability and Accountability Act of 1996 and adhered to the Declaration of Helsinki, as amended in 2013. Informed consent was obtained from each patient.

Ethics approval

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence, treatment, and survival. *Ophthalmology*. 2011;118:1881–1885. https://doi.org/10.1016/j.ophtha.2011.01.040.
2. Onken MD, Worley LA, Char DH, et al. Collaborative Ocular Oncology Group report number 1: prospective validation of a multi-gene prognostic assay in uveal melanoma. *Ophthalmology*. 2012;119:1596–1603. https://doi.org/10.1016/j.ophtha.2012.02.017.
3. Krantz BA, Dave N, Komatsubara KM, et al. Uveal melanoma: epidemiology, etiology, and treatment of primary disease. *Clin Ophthalmol Auckl NZ*. 2017;11:279–289. https://doi.org/10.2147/OPTH.S89591.
4. Field MG, Durante MA, Anbunathan H, et al. Punctuated evolution of canonical genomic aberrations in uveal melanoma. *Nat Commun*. 2018;9:116. https://doi.org/10.1038/s41467-017-02428-w.
5. Damato B, Dopierala JA, Coupland SE. Genotypic profiling of 452 choroidal melanomas with multiplex ligation-dependent probe amplification. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2010;16:6083–6092. https://doi.org/10.1158/1078-0432.CCR-10-2076.
6. Berus T, Halon A, Markiewicz A, et al. Clinical, histopathological and cytogenetic prognosticators in uveal melanoma - a comprehensive review. *Anticancer Res*. 2017;37:6541–6549. https://doi.org/10.21873/anticanres.12110.

7. Robertson AG, Shih J, Yau C, et al. Integrative analysis identifies four molecular and clinical subsets in uveal melanoma. *Cancer Cell*. 2017;32:204–220.e15. https://doi.org/10.1016/j.ccell.2017.07.003.
8. Abdel-Rahman MH, Cebulla CM, Verma V, et al. Monosomy 3 status of uveal melanoma metastases is associated with rapidly progressive tumors and short survival. *Exp Eye Res*. 2012;100:26–31. https://doi.org/10.1016/j.exer.2012.04.010.
9. Schefler AC, Kim RS. Recent advancements in the management of retinoblastoma and uveal melanoma. *F1000Research*. 2018;7. https://doi.org/10.12688/f1000research.11941.1. F1000 Faculty Rev-476.
10. Harbour JW, Correa ZM, Schefler AC, et al. 15-Gene expression profile and PRAME as integrated prognostic test for uveal melanoma: first report of collaborative ocular oncology group study No. 2 (COOG2.1). *J Clin Oncol Off J Am Soc Clin Oncol*. 2024;42:3319–3329. https://doi.org/10.1200/JCO.24.00447.
11. Alsoudi AF, Skrehot HC, Chévez-Barrios P, et al. Comprehensive molecular profiling of uveal melanoma evaluated with gene expression profiling, preferentially expressed antigen in melanoma expression, and next-generation sequencing. *Retina Phila Pa*. 2024;44:1580–1589. https://doi.org/10.1097/IAE.0000000000004153.
12. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in GNA11 in uveal melanoma. *N Engl J Med*. 2010;363:2191–2199. https://doi.org/10.1056/NEJMoa1000584.
13. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature*. 2009;457:599–602. https://doi.org/10.1038/nature07586.
14. Vader MJC, Madigan MC, Versluis M, et al. GNAQ and GNA11 mutations and downstream YAP activation in choroidal nevi. *Br J Cancer*. 2017;117:884–887. https://doi.org/10.1038/bjc.2017.259.
15. Nell RJ, Menger NV, Versluis M, et al. Involvement of mutant and wild-type CYSLTR2 in the development and progression of uveal nevi and melanoma. *BMC Cancer*. 2021;21:164. https://doi.org/10.1186/s12885-021-07865-x.
16. Thornton S, Coupland SE, Olohan L, et al. Targeted next-generation sequencing of 117 routine clinical samples provides further insights into the molecular landscape of uveal melanoma. *Cancers*. 2020;12:1039. https://doi.org/10.3390/cancers12041039.
17. Barbagallo C, Stella M, Broggi G, et al. Genetics and RNA regulation of uveal melanoma. *Cancers*. 2023;15:775. https://doi.org/10.3390/cancers15030775.
18. Kim RS, Chevez-Barrios P, Divatia M, et al. Yield, techniques, and complications of transvitreal and transscleral biopsies in small uveal melanoma. *JAMA Ophthalmol*. 2018;136:482–488. https://doi.org/10.1001/jamaophthalmol.2018.0561.
19. Kempen JH. Appropriate use and reporting of uncontrolled case series in the medical literature. *Am J Ophthalmol*. 2011;151:7–10.e1. https://doi.org/10.1016/j.ajo.2010.08.047.
20. Njauw C-NJ, Kim I, Piris A, et al. Germline BAP1 inactivation is preferentially associated with metastatic ocular melanoma and cutaneous-ocular melanoma families. *PLoS One*. 2012;7, e35295. https://doi.org/10.1371/journal.pone.0035295.
21. Chang MT, Asthana S, Gao SP, et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat Biotechnol*. 2016;34:155–163. https://doi.org/10.1038/nbt.3391.
22. Landis CA, Masters SB, Spada A, et al. GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. *Nature*. 1989;340:692–696. https://doi.org/10.1038/340692a0.
23. Cancer Genome Atlas Research Network, Ley TJ, Miller C. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368:2059–2074. https://doi.org/10.1056/NEJMoa1301689.
24. Gupta MP, Lane AM, DeAngelis MM, et al. Clinical characteristics of uveal melanoma in patients with germline BAP1 mutations. *JAMA Ophthalmol*. 2015;133:881–887. https://doi.org/10.1001/jamaophthalmol.2015.1119.
25. Pilarski R, Cebulla CM, Massengill JB, et al. Expanding the clinical phenotype of hereditary BAP1 cancer predisposition syndrome, reporting three new cases. *Genes Chromosomes Cancer*. 2014;53:177–182. https://doi.org/10.1002/gcc.22129.
26. Peña-Llopis S, Vega-Rubín-de-Celis S, Liao A, et al. BAP1 loss defines a new class of renal cell carcinoma. *Nat Genet*. 2012;44:751–759. https://doi.org/10.1038/ng.2323.
27. Bodian DL, McCutcheon JN, Kothiyal P, et al. Germline variation in cancer-susceptibility genes in a healthy, ancestrally diverse cohort: implications for individual genome sequencing. *PLoS One*. 2014;9, e94554. https://doi.org/10.1371/journal.pone.0094554.
28. Shain AH, Bagger MM, Yu R, et al. The genetic evolution of metastatic uveal melanoma. *Nat Genet*. 2019;51:1123–1130. https://doi.org/10.1038/s41588-019-0440-9.
29. Stålhammar G. Delays between uveal melanoma diagnosis and treatment increase the risk of metastatic death. *Ophthalmology*. 2023;S0161–6420(23). https://doi.org/10.1016/j.ophtha.2023.11.021, 00856–4.
30. Urtatiz O, Van Raamsdonk CD. Gnaq and Gna11 in the endothelin signaling pathway and melanoma. *Front Genet*. 2016;7:59. https://doi.org/10.3389/fgene.2016.00059.
31. Johansson P, Aoude LG, Wadt K, et al. Deep sequencing of uveal melanoma identifies a recurrent mutation in PLCB4. *Oncotarget*. 2016;7:4624–4631. https://doi.org/10.18632/oncotarget.6614.
32. Moore AR, Ceraudo E, Sher JJ, et al. Recurrent activating mutations of G-protein-coupled receptor CYSLTR2 in uveal melanoma. *Nat Genet*. 2016;48:675–680. https://doi.org/10.1038/ng.3549.
33. Isaacson AL, Sompallae RR, Guseva NV, et al. Genomic profiling of metastatic uveal melanoma shows frequent coexisting BAP1 or SF3B1 and GNAQ/GNA11 mutations and correlation with prognosis. *Am J Clin Pathol*. 2022;158:177–186. https://doi.org/10.1093/ajcp/aqac019.
34. Helgadottir H, Höiom V. The genetics of uveal melanoma: current insights. *Appl Clin Genet*. 2016;9:147–155. https://doi.org/10.2147/TACG.S69210.
35. Harbour JW, Onken MD, Roberson EDO, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science*. 2010;330:1410–1413. https://doi.org/10.1126/science.1194472.
36. Stout LA, Hunter C, Schroeder C, et al. Clinically significant germline pathogenic variants are missed by tumor genomic sequencing. *NPJ Genomic Med*. 2023;8:30. https://doi.org/10.1038/s41525-023-00374-9.
37. Masoomian B, Shields JA, Shields CL. Overview of BAP1 cancer predisposition syndrome and the relationship to uveal melanoma. *J Curr Ophthalmol*. 2018;30:102–109. https://doi.org/10.1016/j.joco.2018.02.005.
38. Lalloo F, Kulkarni A, Chau C, et al. Clinical practice guidelines for the diagnosis and surveillance of BAP1 tumour predisposition syndrome. *Eur J Hum Genet EJHG*. 2023;31:1261–1269. https://doi.org/10.1038/s41431-023-01448-z.
39. Field MG, Decatur CL, Kurtenbach S, et al. PRAME as an independent biomarker for metastasis in uveal melanoma. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2016;22:1234–1242. https://doi.org/10.1158/1078-0432.CCR-15-2071.
40. Yavuzyigitoglu S, Koopmans AE, Verdijk RM, et al. Uveal melanomas with SF3B1 mutations: a distinct subclass associated with late-onset metastases. *Ophthalmology*. 2016;123:1118–1128. https://doi.org/10.1016/j.ophtha.2016.01.023.
41. Drabarek W, van Riet J, Nguyen JQN, et al. Identification of early-onset metastasis in SF3B1 mutated uveal melanoma. *Cancers*. 2022;14:846. https://doi.org/10.3390/cancers14030846.
42. Yu L, Zhou D, Zhang G, et al. Co-occurrence of BAP1 and SF3B1 mutations in uveal melanoma induces cellular senescence. *Mol Oncol*. 2022;16:607–629. https://doi.org/10.1002/1878-0261.13128.
43. Küsters-Vandevelde HVN, Creytens D, van Engen-van Grunsven ACH, et al. SF3B1 and EIF1AX mutations occur in primary leptomeningeal melanocytic neoplasms; yet another similarity to uveal melanomas. *Acta Neuropathol Commun*. 2016;4:5. https://doi.org/10.1186/s40478-016-0272-0.
44. Harbour JW, Roberson EDO, Anbunathan H, et al. Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nat Genet*. 2013;45:133–135. https://doi.org/10.1038/ng.2523.
45. Mestre J, Chaparro L, Manzanares A, et al. Beyond myeloid neoplasms germline guidelines: validation of the thresholds criteria in the search of germline predisposition variants. *EJH*. 2024;5:1021–1027. https://doi.org/10.1002/jha2.1012.
46. Gargano SM, Badjatia N, Nikolaus Y, et al. Characterization and clinical significance of EIF1AX mutations and Co-mutations in cytologically indeterminate thyroid nodules: a 5-year retrospective analysis. *Acta Medica Acad*. 2021;50:4–12. https://doi.org/10.5644/ama2006-124.322.
47. Ewens KG, Kanetsky PA, Richards-Yutz J, et al. Chromosome 3 status combined with BAP1 and EIF1AX mutation profiles are associated with metastasis in uveal melanoma. *Investig Ophthalmol Vis Sci*. 2014;55:5160–5167. https://doi.org/10.1167/iovs.14-14550.
48. Martin M, Maßhöfer L, Temming P, et al. Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. *Nat Genet*. 2013;45:933–936. https://doi.org/10.1038/ng.2674.
49. Boscolo Bielo L, Trapani D, Repetto M, et al. Variant allele frequency: a decision-making tool in precision oncology? *Trends Cancer*. 2023;9:1058–1068. https://doi.org/10.1016/j.trecan.2023.08.011.