Chromosome 3 Status Combined With *BAP1* and *EIF1AX* Mutation Profiles Are Associated With Metastasis in Uveal Melanoma

Kathryn G. Ewens,[1] Peter A. Kanetsky,[2] Jennifer Richards-Yutz,[1] Juliana Purrazzella,[1] Carol L. Shields,[3] Tapan Ganguly,[4] and Arupa Ganguly[1]

[1]Genetic Diagnostic Laboratory, Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States
[2]Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, Florida, United States
[3]The Ocular Oncology Service, Wills Eye Hospital, Thomas Jefferson University, Philadelphia, Pennsylvania, United States
[4]Penn Genomic Analysis Core, DNA Sequencing Facility, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Correspondence: Arupa Ganguly, Department of Genetics, Perelman School of Medicine, University of Pennsylvania, 415 Curie Boulevard, Philadelphia, PA 19104, USA; ganguly@mail.med.upenn.edu.

Submitted: April 8, 2014
Accepted: June 15, 2014

Citation: Ewens KG, Kanetsky PA, Richards-Yutz, et al. Chromosome 3 status combined with BAP1 and EIF1AX mutation profiles are associated with metastasis in uveal melanoma. *Invest Ophthalmol Vis Sci*. 2014;55:5160–5167. DOI:10.1167/iovs.14-14550

Purpose. Somatic mutations in *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* have been identified in uveal melanoma (UM). The aim of this study was to determine whether mutations in these genes in primary tumors were associated with metastases in individuals diagnosed with UM.

Methods. A total of 63 UM cases who developed a metastasis within 48 months of primary treatment and 53 UM controls who were metastasis-free over a similar time period were selected for the study. Primary UM cases were screened for mutations in *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1*. The association of these mutations with tumor characteristics, chromosome 3 copy number, and metastatic status was analyzed by logistic regression to estimate the odds of developing metastasis within 48 months.

Results. As expected, tumor diameter, thickness, cilio-choroidal location, and chromosome 3 monosomy were all significantly ($P < 0.02$) associated with the presence of metastasis. In univariate analysis, *GNA11* (odds ratio [OR] 2.5, 95% confidence interval [CI] 1.1–5.5) and *BAP1* (OR 6.3, 95% CI 2.7–14.4) mutations were positively associated and *EIF1AX* mutation (OR 0.13, 95% CI 0.034–0.47) was inversely associated with metastatic status at 48 months after UM treatment. After adjustment for covariates, a chromosome 3 monosomy/*BAP1*-mutation/*EIF1AX*–wild-type (WT) mutation profile was strongly associated (OR 37.5, 95% CI 4.3–414) with the presence of metastasis compared with a chromosome 3 disomy/*BAP1*-WT/*EIF1AX* mutation profile.

Conclusions. The results suggest that knowledge of mutations in *BAP1* and *EIF1AX* can enhance prognostication of UM beyond that determined by chromosome 3 and tumor characteristics. Tumors with chromosome 3 disomy/BAP1-*WT*/*EIF1AX*-*WT* have a 10-fold increased risk of metastasis at 48 months compared with disomy-3/*BAP1*-WT/*EIF1AX* mutant tumors.

Keywords: uveal melanoma, chromosome 3, somatic mutation, BAP1, EIF1AX, GNAQ, GNA11, SF3B1

Uveal melanoma (UM) is the most common adult-onset intraocular tumor and is associated with a high incidence of mortality due to metastasis, most often to the liver.[1–3] The primary risk factors for tumor metastasis in UM include large tumor size, location, extraocular extension, ciliary body involvement, cell type, and chromosomal abnormalities, including loss of chromosome 3, gain of 8q, and loss of 1p, 6q, and 8p.[4–17] Recent studies have focused the search for better prognostic indicators of UM on gene expression[18–22] and gene mutation analyses.[23–28]

Recently, somatic mutations were identified in five genes in UM: two members of the q class of the G-protein α-subunits, *GNAQ*[24,25] and *GNA11*,[26] a tumor suppressor gene located on chromosome 3p21.1, BRCA1-associated protein1, *BAP1*,[23] the splicing factor *SF3B1*,[27–29] and the X-linked translation initiation factor *EIF1AX*.[28] Mutations unique to UM have been identified in up to 50% of tumors at position Q209 in exon 5 and R183 in exon 4 of *GNAQ*.[24,25] These same amino acids are mutated in *GNA11* in approximately 35% of tumors.[26] The mutations in *GNAQ* or *GNA11* occurred in approximately 80% of tumors in a mutually exclusive manner,[26,30,31] and are predicted to be early, initiating events in tumorigenesis.[24] Mutations at position R625 of *SF3B1* have been identified in both UM[27] and cutaneous melanoma,[32] as well as in breast and hematological cancers.[33–35] Thus far, mutations in exons 1 and 2 of *EIF1AX* have been described only in UM.[28]

Whole-exome sequencing of metastatic UM identified inactivating somatic mutations in *BAP1* in 84% of metastasizing tumors, suggesting that inactivation of *BAP1* is a key event occurring later in UM progression and coinciding with the

onset of metastatic behavior.[23] Somatic *BAP1* mutations also have been identified in a variety of other cancers, including cutaneous melanoma, mesothelioma, and colorectal, renal cell, and lung cancers.[36–46]

This study used a case-control design to investigate whether mutations in *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* in primary UM were associated with the development of metastasis within 48 months after primary treatment. The principal aim was to determine whether somatic mutations at these loci were independently associated with the presence of UM metastases after taking into consideration known risk factors, including tumor size, location, and chromosome 3 copy number. The ultimate goal of this work was to inform the development of enhanced prediction models that can distinguish patients at the time of initial cancer diagnosis who are at high risk for poor UM outcomes.

Methods

Patients

All 116 patients enrolled into this study were managed by the Ocular Oncology Service at Wills Eye Hospital, Philadelphia, PA, USA, between 1990 and 2013. In total, 63 UM cases who developed a metastasis within 48 months of primary treatment and 53 UM controls who had not developed a metastasis within this same period were selected for study. We choose 48 months as a cutoff point because our previous study showed that 93% of patients developed metastasis within 48 months.[15] Information on patient demographics and metastatic status and clinical and pathological data on tumors was obtained by a retrospective review of medical charts. Chromosome and mutation analysis of primary UM tumor samples were carried out by the Genetic Diagnostic Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania, United States.

All studies adhered to the tenets of the Declaration of Helsinki. The institutional review boards of University of Pennsylvania and Wills Eye Hospital approved this research. Written informed consent for use of tissues and data for research was obtained from all patients who participated in genetic testing.

DNA Extraction and Determination of Chromosome 3 Copy Number

Seventy-nine tumor samples were obtained by fine-needle aspiration biopsy (FNAB) and 37 by solid open biopsy of enucleated tumors. Genomic DNA was isolated as previously described.[15] Chromosome 3 copy number was analyzed in 54 tumors by microsatellite analysis[47] and in 62 tumors using Affymetrix Human 100K, single-nucleotide polymorphism (SNP)-5.0, or SNP-6.0 genotyping arrays (Affymetrix, Santa Clara, CA, USA).[15]

Mutation Screening of *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* Genes

Screening for somatic mutations in the *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* genes was performed using the following three different methods.

Taqman Genotyping of *GNAQ*, *GNA11*, and *SF3B1*. Custom assays were designed for five previously identified mutations in *GNAQ* (Gln209Leu and Gln209Pro),[24,25] *GNA11* (Gln209Leu),[26] and *SF3B1* (Arg625Cys and Arg625His).[27] The assays were performed using the StepOnePlus Real-Time PCR System following the manufacture's recommendations (Life Technologies, Carlsbad, CA, USA).

Sanger Sequencing of *BAP1* and *EIF1AX*. Sanger sequencing of exons 1 to 17 of *BAP1* was performed for 44 UM samples and for exons 1 and 2 of *EIF1AX* for all 116 samples following standard protocols.

Next-Generation Sequencing (NGS). Screening for mutations in the coding exons of *BAP1*, *GNAQ*, and *GNA11* of an additional 72 UM samples was done using multiplex-PCR followed by NGS on the Ion Torrent PGM (Personal Genome Machine) platform.[48] Multiplex PCR was performed using a primer mix containing primers for exons 1 to 17 of *BAP1* and exons 4 and 5 of *GNAQ* and *GNA11*. The sequencing libraries were barcoded and sequenced using the Ion PGM Sequencing 200 Kit (Invitrogen, Grand Island, NY, USA) following the manufacturer's instructions. For initial validation, two UM samples with known mutations were analyzed following the protocol detailed above. The sequencing data from these two tumors allowed assessment of background noise and filtering of false-positive variant calls due to homopolymer issues.

The sequence alignment and variant calling of the Ion Torrent PGM sequencing data were processed against the human hg19 reference sequence using Ion Torrent Suite v2.0 (Life Technologies). All variant calls were confirmed using NextGENe v2.3.0 software (SoftGenetics, State College, PA, USA). The variant output was annotated using SeattleSeq Annotation 137.[49] Variants were called if they passed the quality control filter, which required at least ×400 coverage for the reference sequence and ×80 (20%) coverage for the variant reads. Known SNPs were filtered out and variants excluded from further analysis if the *P* value was greater than 0.05, the variant frequency less than 20%, or it was intronic and more than 5 bp away from the exon–intron boundary. Sanger sequence validation of 12 variants identified on the Ion Torrent platform was performed; all variants were confirmed on Sanger sequencing when the *P* value was less than 0.05. These criteria preclude detection of low-level variation that may be present due to tumor heterogeneity.

Statistical Analyses

Logistic regression was used to assess the association between metastatic status and patient characteristics (sex and age), tumor characteristics (thickness, basal diameter, location), TNM (Tumor size and extent, lymph Node involvement, and presence of Metastasis) staging,[16] chromosome 3 status, and presence of somatic mutations in *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1*. Tumor location was coded as choroidal (1), ciliary body (2), iris (3), and choroidal with ciliary body involvement (4). TNM tumor staging was done according to the American Joint Committee on Cancer TNM system.[16] Logistic regression was used to determine associations between somatic mutations in *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* and patient and tumor characteristics. Here, the presence of a somatic mutation was considered the outcome (dependent) variable; to adjust for metastatic status, it was included in all models as an indicator variable (0 = absent; 1 = present).

Multivariate logistic regression modeling was used to determine the independent associations of mutations in *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* with metastatic status after adjusting for clinicopathological covariates shown to be statistically significantly associated with outcome in the univariate analyses. In the analysis of the joint effects of chromosome 3, *EIF1AX* and *BAP1* mutation status, disomy-3, and *BAP*-wild type (WT) were coded as 0 and monosomy-3 and the presence of *BAP1* mutations, both of which carry a risk of metastasis, were coded as 1. Because the presence of *EIF1AX* mutations was found to be protective, it was coded as 0 and the WT allele as 1. For the combined analysis, we defined the

Table 1. Association of Patient and Tumor Characteristics With Metastasis Assessed by Univariate Logistic Regression

Tumor Variable	All Tumors, $n = 116$ (Frequency)	Case/Control Status: Controls: No Metastasis, $n = 53$ (Frequency)	Case/Control Status: Cases: Metastasis, $n = 63$, (Frequency)	Logistic Regression: OR	Logistic Regression: 95% CI Lower–Upper	Logistic Regression: *P* Value*
Sex						0.182
Female	47 (0.40)	25 (0.47)	22 (0.35)	Reference		
Male	69 (0.60)	28 (0.53)	41 (0.65)	1.7	0.79–3.5	
Age, y, median, range†	61, 22–88	57, 22–83	62, 22–88	1.8	0.50–6.5	0.374
Basal diameter, mm, median, range	13.0, 5.0–22	10, 5.0–19	16, 8–22	1.5	1.3–1.7	**<0.001**
Thickness, mm, median, range	6.6, 1–16.5	1.0, 1–13.5	8.7, 2–16.5	1.5	1.3–1.8	**<0.001**
Location of tumor						**0.018**
Choroid	79 (0.68)	43 (0.81)	36 (0.57)	Reference		
Ciliary body	4 (0.034)	2 (0.038)	2 (0.032)	1.2	0.16–8.9	
Cilio-choroid	31 (0.27)	6 (0.11)	25 (0.40)	5.0	1.8–13.5	
Iris	2 (0.017)	2 (0.038)	0 (0)	Not estimable		
TNM tumor stage‡						**<0.001**
Stage I	21 (0.18)	19 (0.37)	2 (0.03)	Reference		
Stage IIA	26 (0.23)	20 (0.39)	6 (0.10)	2.8	0.51–15.9	
Stage IIB	32 (0.28)	7 (0.14)	25 (0.40)	33.9	6.3–182	
Stage IIIA	25 (0.22)	4 (0.078)	21 (0.33)	49.9	8.2–304	
Stage IIIB	6 (0.053)	1 (0.020)	5 (0.08)	47.5	3.5–636	
Stage IV	4 (0.035)	0	4 (0.06)	Not estimable		
Chromosome 3						
Disomy	41 (0.35)	30 (0.57)	11 (0.18)	Reference		**<0.001**
Monosomy	75 (0.65)	23 (0.43)	52 (0.82)	6.2	2.6–14.4	

P values in bold indicate statistically significant association.

* Overall *P* value from Wald test.

† Odds ratio for age is based on natural log-transformed values.

‡ Tumor staging according to American Joint Committee on Cancer TNM system.[16] In this analysis, iris melanoma was not included in the TNM staging and, because information on lymph node involvement was not available for any sample, it could not be used in determining tumor stage.

reference multilocus genotype as disomy-3/*BAP1*-WT/*EIF1AX*-mutant. Statistical analyses were carried out using SPSS 20 (IBM, New York, NY, USA), and *P* values less than or equal to 0.05 were considered statistically significant.

Results

Demographic and UM Characteristics

A description of patient and UM characteristics and their association with metastatic status is presented in Table 1. Comparing cases with controls, tumor diameter ($P < 0.001$), thickness ($P < 0.001$), location ($P = 0.018$), and TNM stage ($P < 0.001$) were significantly associated with metastatic status, but sex and age were not (Table 1). Monosomy-3 was present in 75 UM samples (65%) and was significantly associated with metastasis ($P < 0.001$, Table 1).

Table 2 shows associations between patient and tumor characteristics and *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* mutation status after adjusting for metastatic status. *GNAQ* mutations were significantly associated with basal diameter (odds ratio [OR] 1.1, 95% confidence interval [CI] 1.0–1.3), whereas both *GNAQ* and *GNA11* mutations were associated with cilio-choroidal location (OR 0.39, 95%CI 0.15–0.99 and OR 3.5, 95% CI 1.4–8.9, respectively). *BAP1* mutations were associated with tumor thickness (OR 1.2, 95% CI 1.0–1.4). *SF3B1*, *EIA1AX*, and *BAP1* mutations were all significantly associated with chromosome 3 status (OR 0.20, 95% CI 0.045–0.87; OR 0.26, 95% CI 0.078–0.87; and OR 23.6, 95% CI 6.3–88.2, respectively).

GNAQ and *GNA11*

A total of 52 (46%) of 113 tumors carried *GNAQ* mutations, with the two common *GNAQ* mutations (Gln209Leu/Pro) accounting for 46 of these mutations. Three additional *GNAQ* mutations were found in six tumors: Gln209Arg, Arg183Gln, and Arg183Tyr (Supplementary Table S1). The presence of *GNAQ* mutations was not associated with metastatic status (OR 0.99, 95% CI 0.47–2.1; Table 3).

The *GNA11* mutations were present in 41 (35%) of 116 UMs and all but one of these mutations were Gln209Leu (Supplementary Table S1). There was a significant association of *GNA11* mutation status with metastatic status (OR 2.5, 95% CI 1.1–5.5), but after adjustment for tumor characteristics, there was insufficient evidence to demonstrate statistical significance (OR 2.3, 95% CI 0.75–7.3; Table 3). The *GNAQ* and *GNA11* mutations were present in a mutually exclusive pattern in a total of 93 (82%) of 113 tumors.

SF3B1 and *EIF1AX*

Genotypes for two previously identified mutations in *SF3B1*, Arg625Cys, and Arg625His[27] were determined in 110 tumors. Eight tumors carried the Arg625Cys and three carried the Arg625His mutation (Supplementary Table S1). All three Arg625His mutations were present in tumors with monosomy-3. Mutations at Arg625 were not significantly associated with metastatic status (OR 1.0, 95% CI 0.30–3.6; Table 3).

Sixteen different mutations in exons 1 and 2 of *EIF1AX* were identified in 18 of 111 tumors (Supplementary Table S2). Fourteen missense changes, one splice site mutation, and one

Table 2. Association of Patient Demographics and Uveal Melanoma Characteristics With *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* Mutations Assessed by Logistic Regression and Adjusted for Metastatic Status

	GNAQ, *n* = 113			*GNA11*, *n* = 116		
Tumor Variable	**WT, *n* = 61 (0.54)**	**Mutant, *n* = 52 (0.46)**	**OR (95% CI)***	**WT, *n* = 75 (0.65)**	**Mutant, *n* = 41 (0.35)**	**OR (95% CI)***
Patient sex						
Female	26 (0.43)	21 (0.40)	Reference	31 (0.41)	16 (0.39)	Reference
Male	35 (0.57)	31 (0.60)	1.1 (0.52–2.3)	44 (0.59)	25 (0.61)	0.99 (0.44–2.2)
Patient age, mo, median, range†	60, 22–84	61, 22–88	0.65 (0.17–2.4)	60, 22–88	61, 22–84	1.5 (0.37–6.1)
Basal diameter, mm, median, range	12, 5–22	14, 6–22	**1.1 (1.0–1.3)**	12, 5–22	13, 5–22	0.93 (0.82–1.0)
Thickness, mm, median, range	7.0, 1.0–15.0	6.4, 2.0–16.5	1.0 (0.90–1.1)	5.7, 1.0–16.5	8.0, 1.5–15.0	1.1 (0.95–1.2)
Location of tumor			*P* = 0.20‡			***P* = 0.014‡**
Choroid	36 (0.59)	40 (0.77)	Reference	60 (0.80)	19 (0.46)	Reference
Ciliary body	3 (0.05)	1 (0.019)	0.29 (0.029–3.0)	1 (0.01)	3 (0.07)	9.7 (0.93–101)
Cilio-choroid	21 (0.34)	10 (0.20)	**0.39 (0.15–0.99)**	13 (0.17)	18 (0.44)	**3.5 (1.4–8.9)**
Iris	1 (0.02)	1 (0.019)	1.0 (0.60–17.2)	1 (0.01)	1 (0.03)	4.4 (0.25–77.1)
TNM tumor stage			*P* = 0.12‡			*P* = 0.14‡
Stage I	14 (0.23)	7 (0.14)	Reference	17 (0.23)	4 (0.10)	Reference
Stage IIA	12 (0.20)	13 (0.26)	2.2 (0.66–7.5)	19 (0.26)	7 (0.18)	1.4 (0.35–5.9)
Stage IIB	12 (0.20)	19 (0.37)	3.6 (0.92–14.0)	23 (0.31)	9 (0.22)	1.1 (0.24–5.2)
Stage IIIA	17 (0.28)	7 (0.14)	0.94 (0.22–4.1)	9 (0.12)	16 (0.40)	5.0 (1.0–23.9)
Stage IIIB	2 (0.03)	4 (0.078)	4.6 (0.58–36.4)	4 (0.05)	2 (0.05)	1.4 (0.16–12.3)
Stage IV	3 (0.05)	1 (0.020)	0.78 (0.058–10.7)	2 (0.03)	2 (005)	2.6 (0.22–23.3)
Chromosome 3			*P* = 0.96			*P* = 0.053
Disomy	22 (0.36)	19 (0.36)	Reference	33 (0.44)	8 (0.20)	Reference
Monosomy	39 (0.64)	33 (0.64)	0.98 (0.44–2.3)	42 (0.56)	33 (0.80)	2.6 (0.99–6.7)

Table 2. Continued

	SF3B1, *n* = 110			*EIF1AX*, *n* = 111		
Tumor Variable	**WT, *n* = 99 (0.90)**	**Mutant, *n* =11 (0.10)**	**OR (95% CI)***	**WT, *n* = 93 (0.84)**	**Mutant, *n* = 18 (0.16)**	**OR (95% CI)***
Patient sex						
Female	38 (0.38)	6 (0.54)	Reference	39 (0.42)	7 (0.39)	Reference
Male	61 (0.62)	5 (0.46)	0.51 (0.14–1.8)	54 (0.58)	11 (0.61)	1.5 (0.51–4.7)
Patient age, mo, median, range†	61, 22–88	52, 24–78	0.22 (0.037–1.3)	61,22–88	53.5, 22–84	0.90 (0.15–5.6)
Basal diameter, mm, median, range	12, 5–22	15, 6–22	1.2 (0.98–1.4)	14, 5–22	11, 6–18	1.0 (0.87–1.2)
Thickness, mm, median, range	6.6, 1.0–16.5	6.5, 2–11.7	1.0 (0.83–1.2)	7.3, 1.0–16.5	5.6, 2.0–12.5	1.0 (0.86–1.3)
Location of tumor			*P* = 1.0‡			*P* = 0.55‡
Choroid	66 (0.67)	8 (0.73)	Reference	58 (0.62)	17 (0.94)	Reference
Ciliary body	4 (0.040)	0	Not estimable	4 (0.043)	0	Not estimable
Cilio-choroid	27(0.27)	3 (0.27)	0.91 (0.21–4.0)	29 (0.31)	1 (0.06)	0.20 (0.024–1.7)
Iris	2 (0.020)	0	Not estimable	2 (0.022)	0	Not estimable
TNM tumor stage			*P* = 0.95‡			*P* = 0.34‡
Stage I	19 (0.20)	1 (0.09)	Reference	17 (0.19)	3 (0.17)	Reference
Stage IIA	22 (0.23)	3 (0.27)	2.7 (0.26–28.3)	15 (0.16)	10 (0.56)	**4.8 (1.1–21.7)**
Stage IIB	27 (0.28)	3 (0.27)	2.6 (0.20–32.6)	26 (0.29)	4 (0.22)	2.5 (0.41–15.5)
Stage IIIA	21 (0.22)	3 (0.27)	3.4 (0.25–45.4)	23 (0.25)	1 (0.056)	0.77 (0.064–9.4)
Stage IIIB	5 (0.052)	1 (0.09)	4.7 (0.20–110)	5 (0.066)	0	Not estimable
Stage IV	3 (0.031)	0	Not estimable	4 (0.044)	0	Not estimable
Chromosome 3			***P* = 0.032‡**			***P* = 0.029‡**
Disomy	33 (0.33)	7 (0.64)	Reference	27 (0.29)	13 (0.72)	Reference
Monosomy	66 (0.67)	4 (0.36)	**0.20 (0.045–0.87)**	66 (0.71)	5 (0.28)	**0.26 (0.078–0.87)**

Table 2. Continued

Tumor Variable	*BAP1*, $n = 111$		
	WT, $n = 55$ (0.50)	Mutant, $n = 56$ (0.50)	OR (95% CI)*
Patient sex			
Female	24 (0.44)	20 (0.36)	Reference
Male	31 (0.56)	36 (0.64)	1.1 (0.49–2.6)
Patient age, mo, median, range†	58, 24–84	61, 22-88	0.71 (0.17–3.0)
Basal diameter, mm, median, range	11, 5–19	14, 5–22	1.1 (0.94–1.2)
Thickness, mm, median, range	5.1, 1.0–12.7	8.2, 1.5–16.5	**1.2 (1.0–1.4)**
Location of tumor			$P = 0.64$‡
Choroid	45 (0.82)	30 (0.54)	Reference
Ciliary body	0	3 (0.05)	Not estimable
Cilio-choroid	10 (0.18)	21 (0.38)	1.9 (0.72–5.1)
Iris	0	2 (0.04)	Not estimable
TNM tumor stage			$P = 0.14$‡
Stage I	14 (0.26)	5 (0.09)	Reference
Stage IIA	20 (0.36)	4 (0.07)	0.42 (0.90–2.0)
Stage IIB	12 (0.22)	19 (0.35)	1.9 (0.45–8.1)
Stage IIIA	6 (0.11)	19 (0.35)	3.6 (0.76–17.2)
Stage IIIB	2 (0.04)	4 (0.07)	2.2 (2.6–19.4)
Stage IV	1 (0.02)	3 (0.06)	2.6 (0.18–37.2)
Chromosome 3			**$P < 0.001$**‡
Disomy	36 (0.66)	3 (0.05)	Reference
Monosomy	19 (0.34)	53 (0.95)	**23.6 (6.3–88.2)**

Values in bold indicate statistically significant association.

* OR and upper and lower 95%CI for association of gene mutation status (wild-type or mutant) with each variable after adjusting for metastatic status.

† OR for age is based on natural log-transformed values.

‡ Overall *P* value from Wald test.

variant of unknown significance in the 5′ untranslated region were identified. Seven, including the splice site mutation, were in novel sites not previously described by Martin et al.[28]

Among the 93 tumors without *EIF1AX* mutations, 57 metastasized and 36 did not (Table 3). A comparison of the frequency of tumors carrying an *EIF1AX* mutation that did, or did not, metastasize (3 [12%] of 18 vs 15 [84%] of 18) suggested that the presence of an *EIF1AX* mutation is protective. The effect of an *EIF1AX* mutation is estimated to be a decreased odds of metastasis (OR 0.13, 95% CI 0.034–0.47; Table 3), which remained significant in the model adjusted for tumor characteristics (OR 0.13, 95% CI 0.024–0.68).

BAP1

A total of 52 unique mutations were identified in 56 (50%) of 111 tumors. These mutations included 13 missense changes, 8 nonsense, 12 5′- or 3′-splice site mutations located within 5 bp of the exon–intron boundary, 15 frameshift deletions leading to premature termination, 2 in-frame deletions, 1 complex in-frame in/del, and 1 tumor with deletion of exons 12 and 17 (Supplementary Table S3). To the best of our knowledge, the same mutations (or a different substitution at the same site) have been previously reported for only 6 of these 52 mutations. *BAP1* mutations were associated with significantly increased odds of metastasis (OR 6.3, 95% CI 2.7–14.4; Table 3), which remained significant after adjustment for tumor characteristics (OR 3.6, 95% CI 1.2–10.2).

Three tumors with mutations in *BAP1* were disomic for chromosome 3. The mutations in UM11 (intron 6, c.438-2A>G abolished a 3′-splice acceptor site) and UM42 (exon 12, c.1147C>T, Arg383Cys, rs201844078) were present in heterozygous states, implying that a knockout of the WT allele had not occurred. These patients were free of metastasis at 48 months (and remained metastasis-free for 67 and 78 months, respectively). However UM13 developed metastases to the spleen within a year of initial treatment. The NGS reads of UM13 were reviewed and indicated 86% of the reads represented the mutant allele of the Arg60X mutation, implicating a copy neutral loss of heterozygosity event in the tumor. Nineteen of the 72 tumors with monosomy 3 did not carry a *BAP1* sequence mutation. Of these, 10 developed metastasis and 9 did not.

Chromosome-3 Interactions With *EIF1AX* and *BAP1*

We examined the joint effects of chromosome 3 status with *EIF1AX* or *BAP1*. We found that the combination of monosomy-3 with the *EIF1AX*-WT (risk) allele was significantly associated with metastasis (OR 29.7, 95% CI 3.6–244, Table 4) and remained significant after adjustment for tumor variables (OR 31.4, 95% CI 3.0–332). Tumors with monosomy-3 and either *BAP1*-WT or mutant alleles had significantly increased odds of metastasis (OR 3.3, 95% CI 1.0–10.8 and OR 11.5, 95% CI 4.2–31.3, respectively).

Finally, we investigated the presence of metastasis at 48 months when the effects of chromosome 3, *BAP1*, and *EIF1AX* status were considered jointly (Table 5). Tumors with monosomy-3/*BAP1*-WT/*EIF1AX*-WT, or monosomy-3/*BAP1*-mutant/*EIF1AX*-WT had significantly increased odds of developing a metastasis (OR 13.5, 95% CI 1.4–128 and OR 45.6, 95% CI 5.3–394, respectively) compared with tumors with the reference multilocus genotype of disomy-3/*BAP1*-WT/*EIF1AX*-mutant. Even after adjustment for tumor thickness, diameter, and location, these three loci combinations remained significant with high risk of metastasis (OR 19.1, 95% CI 1.5–251 and OR 37.5, 95% CI 3.4–414, respectively). The lowest risk of metastasis is associated with the reference variable, disomy-3/*BAP1*-WT/*EIF1AX*-mutant tumors.

Discussion

Uveal melanoma is a rare ocular tumor associated with significant morbidity. Hence, many affected individuals wish to be tested for the most sensitive prognostic marker(s). There are several traditional markers, including larger tumor size, tumor location, and chromosome 3 monosomy, that are well-established poor prognosticators for UM metastasis.[4–15] The aim of this article was to evaluate the association of *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX*, and *BAP1* mutation profiles with metastasis while taking the prior known tumor characteristics into account with the goal of providing new genomic information that can enhance patient prognostic characterization.

With this background, we used a case-control study design that included 63 UMs from patients with documented metastasis within 48 months of diagnosis and 53 UMs from patients whose tumors had not metastasized within the same time period. The distribution of tumor stages in our collection representing TNM stages I to IIIC was 18%, 23%, 28%, 22%, 5.3%, and 0%, respectively, which is similar to that found by Kujala et al.[17] In our sample set, the proportion of patients with metastases at diagnosis (TNM stage IV) was 3.5% (Table 1), which is within the generally accepted range of 1% to 4%.[16] Furthermore, the thickness and basal diameter of the tumors in our cohort and the percentage of tumors with ciliary body

Table 3. Logistic Regression Analysis of Association of *GNAQ*, *GNA11*, *SF3B1*, and *EIF1AX* and *BAP1* Mutations With Metastatic Status.

			Case/Control Status		Logistic Regression	
Gene	Mutation Status*	No. (Frequency)	Controls: No Metastasis (Frequency)	Cases: Metastasis (Frequency)	OR (95% CI)	OR Adjusted (95% CI)†
GNAQ	WT	61 (0.54)	28 (0.54)	33 (0.54)	0.99 (0.47–2.1)	0.67 (0.24–1.9)
	Mutant	52 (0.46)	24 (046)	28 (0.46)		
GNA11	WT	75 (0.65)	40 (0.75)	35 (0.56)	**2.5 (1.1–5.5)**	2.3 (0.75–7.3)
	Mutant	41 (0.35)	13 (0.25)	28 (0.44)		
SF3B1	WT	99 (0.90)	46 (0.90)	53 (0.90)	1.0 (0.30–3.6)	0.56 (0.11–2.9)
	Mutant	11 (0.10)	5 (0.10)	6 (0.10)		
EIF1AX	WT	93 (0.84)	36 (0.71)	57 (0.95)	**0.13‡ (0.034–0.47)**	**0.13‡ (0.024–0.68)**
	Mutant	18 (0.16)	15 (0.29)	3 (0.05)		
BAP1	WT	55 (0.50)	36 (0.73)	19 (0.31)	**6.3 (2.7–14.4)**	**3.6 (1.2–10.2)**
	Mutant	56 (0.50)	13 (0.27)	43 (0.69)		

Values in bold indicate statistically significant association.

* See Supplementary Tables S1 through S3 for detailed list of mutations.

† OR adjusted for tumor diameter, thickness, and location shown to be significant in Table 1. TNM staging was not included in the adjusted model because the staging criterion was based solely on tumor size and location, which were already included in the model as separate variables.

‡ Odds ratio corresponds to a 7.9-fold decreased risk of metastasis in both the unadjusted and adjusted models.

involvement are comparable with that found in a study of 452 UMs by Damato et al.[7] Thus, these data for clinical tumor characteristics provide substantial evidence that our sample set was representative of UM in general.

This study was not designed to address the problem of potential tumor heterogeneity, which can be a function of tumor size. Maat et al.[50] have shown for large tumors that often require enucleation, there is intratumor heterogeneity that can lead to imprecise molecular classification. In contrast, Onken et al.[51] have recently shown by gene expression profiling that there was little or no intratumor heterogeneity in either FNAB or enucleated tumors. This question remains unresolved and it could not be addressed in this study due to the lack of availability of multiple samples from the same tumor. Finally, there was a limitation in the number of available UMs with adequate follow-up time, complete information on metastatic status, and mutation status for the five genes. This led, in some instances, to a broad range in the 95% CIs, resulting in imprecise estimates of odds of metastasis, especially in the case of *EIF1AX*, where the frequency for the replace with mutated allele was small.

In our study, logistic regression analysis indicated that the presence of mutations in *GNA11*, but not *GNAQ*, was associated with the development of metastasis at 48 months (Table 3). This finding of metastasis in many tumors carrying *GNA11* mutations is relevant in light of the finding of Griewank et al.,[52] where three times as many metastatic UMs carried *GNA11* mutations compared with *GNAQ*. This led the authors to suggest that *GNA11*-mutant tumors may have a higher tendency to metastasize than *GNAQ*-mutant tumors. In our study of primary UM, the ratio of *GNA11* to *GNAQ* mutations was 1.3. When combined with the previous observation, it suggests that cells carrying the *GNA11* mutation are selected for in the metastatic process. This is supported in part by the finding that, although there was no significant difference in basal diameter between *GNA11*-WT and *GNA11*-mutant tumors, those carrying the *GNA11* mutant were significantly thicker than *GNA11*-WT tumors ($P = 0.032$, data not shown).

Harbour et al.[27] and Furney et al.[29] both reported a significant association between the presence of *SF3B1* mutations and significantly improved survival in Kaplan-Meier

Table 4. Multivariate Logistic Regression Analysis of Association of Chromosome (Chr) 3 Status and *EIF1AX* or *BAP1* Mutations With Metastatic Status

		Case/Control Status		Logistic Regression	
Variables	Total (Frequency)	Controls: No Metastasis (Frequency)	Cases: Metastasis (Frequency)	OR (95% CI)	OR Adjusted (95% CI)*
Chr3-*EIF1AX*				***P* = 0.001**	***P* = 0.021**
Disomy, mutant†	13 (0.12)	12 (0.24)	1 (0.02)	Reference	Reference
Disomy, WT	27 (0.24)	17 (0.33)	10 (0.17)	7.1 (0.79–62.7)	10.1 (0.90–114)
Monosomy, mutant	5 (0.04)	3 (0.06)	2 (0.03)	8.0 (0.53–121)	**38.2 (1.2–1225)**
Monosomy, WT	66 (0.60)	19 (0.37)	47 (0.78)	**29.7 (3.6–244)**	**31.4 (3.0–332)**
Chr3-*BAP1*				***P* < 0.001**	***P* = 0.015**
Disomy, WT	36 (0.32)	27 (0.55)	9 (0.15)	Reference	Reference
Disomy, mutant	3 (0.03)	2 (0.04)	1 (0.02)	1.5 (0.12–18.6)	1.0 (0.072–14.3)
Monosomy, WT	19 (0.17)	9 (0.18)	10 (0.16)	**3.3 (1.0–10.8)**	4.1 (0.91–18.4)
Monosomy, mutant	53 (0.48)	11 (0.22)	42 (0.68)	**11.5 (4.2–31.3)**	**7.6 (2.1–27.3)**

Values in bold indicate statistically significant association.

* Odds ratio for chromosome 3 status and gene mutation combination adjusted for tumor diameter, thickness, and location.

† Reference category is Chr3-disomy, EIF1AX-mutant corresponding to the two lower-risk alleles.

Table 5. Logistic Regression Analysis of Association of the Joint Effects of Chromosome 3 Status Combined With *BAP1* and *EIF1AX* Allele Status With Metastasis

	Chromosome 3 Disomy				Chromosome 3 Monosomy			
	BAP1 WT		*BAP1* Mutant		*BAP1* WT		*BAP1* Mutant	
	EIF1AX Mutant	*EIF1AX* WT	*EIF1AX* Mutant	*EIF1AX* WT	*EIF1AX* Mutant	*EIF1AX* WT	*EIF1AX* Mutant	*EIF1AX* WT
No.	13	23	0	3	2	17	2	48
Metastasis no/yes	12/1	15/8	—	2/1	1/1	8/9	1/1	10/38
Logistic regression								
OR (95% CI)*	Reference	6.4 (0.70–58.5)	—	6.0 (0.26–140)	12.0 (0.38–375)	**13.5 (1.4–128)**	12.0 (0.38–375)	**45.6 (5.3–394)**
OR (95% CI)†	Reference	10.6 (0.92–123)	—	5.4 (0.19–153)	49.6 (1.2–2042)	**19.1 (1.5–251)**	13.4 (0.004–42892)	**37.5 (3.4–414)**

Values in bold indicate statistically significant association.

* Odds ratio and 95% CI intervals for joint effects model.

† Odds ratio and 95% CI intervals adjusted for tumor thickness, diameter, and location.

survival analysis, and Martin et al.[28] also reported a nonsignificant trend toward better prognosis. However, this association was not observed in our study, possibly due to differences in study design and methodology.

Mutations in *EIF1AX* were shown to play a protective role in UM metastasis and, even after adjusting for the effect of other known risk factors, there was an 8-fold decreased risk of metastasis, which confirmed the trend noted by Martin et al.[28]

Mutations in the chromosome-3p–linked tumor suppressor protein, *BAP1* have been shown to be associated with metastasizing UM.[23] Our study identified 52 different *BAP1* mutations in 56 tumors. Forty-three (77%) of the tumors carrying *BAP1* mutations metastasized, and of these, all but one was chromosome 3 monosomy, which is consistent with recessive *BAP1* mutations being uncovered by the loss of one copy of chromosome 3. Nineteen of the 72 tumors with monosomy-3 did not carry a *BAP1* sequence mutation, of which 10 developed metastases. For the nine UMs that did not metastasize within 48 months, it remains to be determined whether there are other, as yet unidentified, genes on chromosome 3 critical to the metastatic process that need to be lost in monosomy-3/*BAP1*-WT UM.

When we evaluated the joint effects of chromosome 3 status with mutations in *BAP1* and/or *EIF1AX*, it was difficult to provide precise estimates for the various multilocus genotype combinations, in part because the protective *EIF1AX* mutant allele was relatively rare. The OR for monsomy-3 tumors, without accounting for any other tumor characteristic of gene mutation, was 6.2 (95% CI 2.6–14.4, Table 1). In the joint effects model combining chromosome 3, *EIF1AX*, and *BAP1* status, considerably more informative ORs were provided. We showed that the lowest risk of metastasis is associated with disomy-3/*BAP1*-WT/*EIF1AX*-mutant tumors, which served as the reference category. The tumors with disomy-3 and no mutations in EIF1AX or BAP1 had a 10-fold increased risk of metastasis at 48 months with respect to the reference category. Tumors with chromosome 3 monosomy and *EIF1AX*-WT alleles, irrespective of *BAP1* status, were at a greater than 13-fold risk of developing metastases at 48 months, and if a *BAP1* mutation was present, the risk increased to greater than 35-fold.

In conclusion, the aim of this study was to determine the contribution of gene mutation profiles to metastatic outcome in UM. We have shown that although the most significant prognostic indicators remain the classic predictors, chromosome 3 status and tumor size and location, combining these factors with *BAP1* and *EIF1AX* mutation status, adds considerably more information and significance to the stratification of individuals with respect to prognostic outcome.

Acknowledgments

The authors thank Kim Moran for her technical assistance and Lindsey Mighion for her service in reviewing patient charts.

Supported by development funds of Genetic Diagnostic Laboratory, Perelman School of Medicine, University of Pennsylvania (AG) and the Eye Tumor Research Foundation, Philadelphia, Pennsylvania, United States (CLS).

Disclosure: **K.G. Ewens**, None; **P.A. Kanetsky**, None; **J. Richards-Yutz**, None; **J. Purrazzella**, None; **C.L. Shields**, None; **T. Ganguly**, None; **A. Ganguly**, None

References

1. Kujala E, Mäkitie T, Kivelä T. Very long-term prognosis of patients with malignant uveal melanoma. *Invest Ophthalmol Vis Sci*. 2003;44:4651–4659.
2. Lorigan JG, Wallace S, Mavligit GM. The prevalence and location of metastases from ocular melanoma: imaging study in 110 patients. *AJR Am J Roentgenol*. 1991;157:1279–1281.
3. Paul EV, Parnell BL, Fraker M. Prognosis of malignant melanomas of the choroid and ciliary body. *Int Ophthalmol Clin*. 1962;2:387–402.
4. Aalto Y, Eriksson L, Seregard S, Larsson O, Knuutila S. Concomitant loss of chromosome 3 and whole arm losses and gains of chromosome 1, 6, or 8 in metastasizing primary uveal melanoma. *Invest Ophthalmol Vis Sci*. 2001;42:313–317.
5. Damato B, Coupland SE. A reappraisal of the significance of largest basal diameter of posterior uveal melanoma. *Eye*. 2009; 23:2152–2162.
6. Damato B, Dopierala J, Klaasen A, van Dijk M, Sibbring J, Coupland SE. Multiplex ligation-dependent probe amplification of uveal melanoma: correlation with metastatic death. *Invest Ophthalmol Vis Sci*. 2009;50:3048–3055.
7. Damato B, Dopierala JA, Coupland SE. Genotypic profiling of 452 choroidal melanomas with multiplex ligation-dependent probe amplification. *Clin Cancer Res*. 2010;16:6083–6092.
8. Damato B, Duke C, Coupland SE, et al. Cytogenetics of uveal melanoma: a 7-year clinical experience. *Ophthalmology*. 2007; 114:1925–1931.
9. Damato B, Eleuteri A, Taktak AFG, Coupland SE. Estimating prognosis for survival after treatment of choroidal melanoma. *Prog Retin Eye Res*. 2011;30:285–295.
10. Kilic E, Naus NC, van Gils W, et al. Concurrent loss of chromosome arm 1p and chromosome 3 predicts a decreased disease-free survival in uveal melanoma patients. *Invest Ophthalmol Vis Sci*. 2005;46:2253–2257.

11. Prescher G, Bornfeld N, Hirche H, et al. Prognostic implications of monosomy 3 in uveal melanoma. *Lancet*. 1996;347: 1222–1225.
12. Shields CL, Furuta M, Thangappan A, et al. Metastasis of uveal melanoma millimeter-by-millimeter in 8033 consecutive eyes. *Arch Ophthalmol*. 2009;127:989–998.
13. Sisley K, Rennie IG, Parsons MA, et al. Abnormalities of chromosomes 3 and 8 in posterior uveal melanoma correlate with prognosis. *Genes Chromosomes Cancer*. 1997;19:22–28.
14. White VA, Chambers JD, Courtright PD, Chang WY, Horsman DE. Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma. *Cancer*. 1998;83:354–359.
15. Ewens KG, Kanetsky PA, Richards-Yutz J, et al. Genomic profile of 320 uveal melanoma cases: chromosome 8p-loss and metastatic outcome. *Invest Ophthalmol Vis Sci*. 2013;54: 5721–5729.
16. Malignant melanoma of the uvea. In: Edge SB, Byrd DR, Compton CC, et al, eds. *AJCC Cancer Staging Manual*. 7th ed. New York: Springer; 2010:547–559.
17. Kujala E, Damato B, Coupland SE, et al. Staging of ciliary body and choroidal melanomas based on anatomic extent. *J Clin Oncol*. 2013;31:2825–2831.
18. 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.
19. Onken MD, Worley LA, Ehlers JP, Harbour JW. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. *Cancer Res*. 2004;64:7205–7209.
20. Onken MD, Worley LA, Tuscan MD, Harbour JW. An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. *J Mol Diagn*. 2010;12:461–468.
21. van Gils W, Lodder EM, Mensink HW, et al. Gene expression profiling in uveal melanoma: two regions on 3p related to prognosis. *Invest Ophthalmol Vis Sci*. 2008;49:4254–4262.
22. Worley LA, Onken MD, Person E, et al. Transcriptomic versus chromosomal prognostic markers and clinical outcome in uveal melanoma. *Clin Cancer Res*. 2007;13:1466–1471.
23. Harbour JW, Onken MD, Roberson EDO, et al. Frequent mutation of *BAP1* in metastasizing uveal melanomas. *Science*. 2010;330:1410–1413.
24. Onken MD, Worley LA, Long MD, et al. Oncogenic mutations in *GNAQ* occur early in uveal melanoma. *Invest Ophthalmol Vis Sci*. 2008;49:5230–5234.
25. 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.
26. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in *GNA11* in uveal melanoma. *N Engl J Med*. 2010;363:2191–2199.
27. Harbour JW, Roberson EDO, Anbunathan H, Onken MD, Worley LA, Bowcock AM. Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nat Genet*. 2013;45:133–135.
28. Martin M, Maszhofer 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.
29. Furney SJ, Pedersen M, Gentien D, et al. *SF3B1* mutations are associated with alternative splicing in uveal melanoma. *Cancer Discov*. 2013;3:1122–1129.
30. Pópulo H, Vinagre J, Lopes JM, Soares P. Analysis of *GNAQ* mutations, proliferation and MAPK pathway activation in uveal melanomas. *Br J Ophthalmol*. 2011;95:715–719.
31. Daniels AB, Lee J-E, MacConaill LE, et al. High throughput mass spectrometry-based mutation profiling of primary uveal melanoma. *Invest Ophthalmol Vis Sci*. 2012;53:6991–6996.
32. Kong Y, Krauthammer M, Halaban R. Rare SF3B1 R625 mutations in cutaneous melanoma. *Melanoma Res*. 2014;24: 332–334.
33. Ellis MJ, Ding L, Shen D, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. *Nature*. 2012; 486:353–360.
34. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic *SF3B1* mutation in myelodysplasia with ring sideroblasts. *N Engl J Med*. 2011;365:1384–1395.
35. Wang L, Lawrence MS, Wan Y, et al. *SF3B1* and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med*. 2011;365:2497–2506.
36. Abdel-Rahman MH, Pilarski R, Cebulla CM, et al. Germline *BAP1* mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. *J Med Genet*. 2011;48:856–859.
37. Bott M, Brevet M, Taylor BS, et al. The nuclear deubiquitinase *BAP1* is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat Genet*. 2011;43:668–672.
38. Carbone M, Ferris L, Baumann F, et al. *BAP1* cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MBAITs. *J Transl Med*. 2012;10:179–185.
39. Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. *BAP1* and cancer. *Nat Rev Cancer*. 2013;13:153–159.
40. Jensen DE, Rauscher FJ. *BAP1*, a candidate tumor suppressor protein that interacts with BRCA1. *Ann N Y Acad Sci*. 1999; 886:191–194.
41. Murali R, Wiesner T, Scolyer RA. Tumours associated with *BAP1* mutations. *Pathology*. 2013;45:116–126.
42. Pena-Llopis S, Vega-Rubin-de-Celis S, Liao A, et al. *BAP1* loss defines a new class of renal cell carcinoma. *Nat Genet*. 2012; 44:751–759.
43. Tang J, Xi S, Wang G, et al. Prognostic significance of BRCA1-associated protein 1 in colorectal cancer. *Med Oncol*. 2013;30: 1–8.
44. Testa JR, Cheung M, Pei J, et al. Germline *BAP1* mutations predispose to malignant mesothelioma. *Nat Genet*. 2011;43: 1022–1025.
45. Wiesner T, Obenauf AC, Murali R, et al. Germline mutations in BAP1 predispose to melanocytic tumors. *Nat Genet*. 2011;43: 1018–1021.
46. Cheung M, Talarchek J, Schindeler K, et al. Further evidence for germline BAP1 mutations predisposing to melanoma and malignant mesothelioma. *Cancer Genet*. 2013;206:206–210.
47. Shields CL, Ganguly A, Materin MA, et al. Chromosome 3 analysis of uveal melanoma using fine-needle aspiration biopsy at the time of plaque radiotherapy in 140 consecutive cases: the Deborah Iverson, MD, Lectureship. *Arch Ophthalmol*. 2007;125:1017–1024.
48. Chen Z, Moran K, Richards-Yutz J, et al. Enhanced sensitivity for detection of low-level germline mosaic RB1 mutations in sporadic retinoblastoma cases using deep semiconductor sequencing. *Hum Mutat*. 2014;35:384–391.
49. Seattle SeqAnnotation 137. Available at: http://snp.gs.washington.edu/SeattleSeqAnnotation137/. Accessed February 2013.
50. Maat W, Jordanova ES, van Zelderen-Bhola SL, et al. The heterogeneous distribution of monosomy 3 in uveal melanomas: implications for prognostication based on fine-needle aspiration biopsies. *Arch Path Lab Med*. 2007;131:91–96.
51. Onken MD, Worley LA, Davila RM, Char DH, Harbour JW. Prognostic testing in uveal melanoma by transcriptomic profiling of fine needle biopsy specimens. *J Mol Diagn*. 2006;8:567–573.
52. Griewank KG, van de Nes J, Schilling B, et al. Genetic and clinico-pathologic analysis of metastatic uveal melanoma. *Mod Pathol*. 2014;27:175–183.

Investigative Ophthalmology & Visual Science