Clinical Characteristics of Uveal Melanoma in Patients With Germline BAP1 Mutations

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IMPORTANCE Somatic mutations in BAP1 (BRCA1-associated protein 1 gene) are frequently identified in uveal melanoma. To date, the role of germline BAP1 mutations in uveal melanoma has not been characterized.

OBJECTIVE To characterize the clinical phenotype of uveal melanoma in patients with germline BAP1 mutations.

DESIGN, SETTING, AND PARTICIPANTS Retrospective cohort study at an academic ophthalmology referral center among 507 patients with uveal melanoma who consented for collection of blood samples. The study dates were June 22, 1992, to December 14, 2010.

MAIN OUTCOMES AND MEASURES Clinical characteristics of uveal melanoma and the development of metastases. BAP1 gene sequencing from blood samples of patients with uveal melanoma was correlated with clinical characteristics.

RESULTS Of 507 blood samples analyzed, 25 patients (4.9%) exhibited 18 BAP1 polymorphisms, of which 9 were novel. Computational analyses predicted that 8 BAP1 mutations in 8 patients (1.6%) were likely to result in damaged BAP1 protein. Five of these 8 mutations were novel. These 8 patients were compared with 482 patients in whom no BAP1 polymorphisms were identified. In univariate analyses, patients with germline BAP1 mutations exhibited larger tumor diameters (mean, 15.9 vs 12.3 mm; P = .004) and higher rates of ciliary body involvement (75.0% vs 21.6%, P = .002) and metastases (71.4% vs 18.0%, P = .003) compared with control subjects. Patients with germline BAP1 mutations exhibited increased frequency of family history of cancer (100% vs 65.9%, P = .06), particularly cutaneous melanoma (62.5% vs 9.9%, P < .001) and ocular melanoma (25.0% vs 1.9%, P = .01). No differences were identified in age at diagnosis, sex, history of other malignant neoplasm, presenting visual acuity, distance of the tumor from the optic nerve or fovea, iris involvement, extrascleral extension, or tumor pigmentation. Germline BAP1 mutations increased risk of metastasis independent of ciliary body involvement (P = .02). Germline BAP1 mutation approached significance as an independent risk factor for metastasis (P = .09).

CONCLUSIONS AND RELEVANCE These data suggest that germline BAP1 mutations occur infrequently in uveal melanoma and are associated with larger tumors and higher rates of ciliary body involvement, 2 known risk factors for metastasis.

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Uveal melanoma is the most common primary intraocular tumor in adults. Monosomy of chromosome 3 has long been associated with a worse prognosis and high risk of metastasis, suggesting that loss of one copy of chromosome 3 unmasks a mutation in the remaining chromosome. Indeed, uveal melanomas with monosomy of chromosome 3 exhibit frequent somatic mutations in \textit{BAP1} (BRCA1-associated protein 1 gene) (OMIM 603089). More recently, germline \textit{BAP1} mutations have been identified in patients with uveal and cutaneous melanoma and are thought to be implicated in a cancer predisposition syndrome. To date, the clinical features of uveal melanoma in patients with germline \textit{BAP1} mutations have not been characterized. We sequenced the \textit{BAP1} gene from blood samples of 507 patients with uveal melanoma and correlated these findings with clinical data.

### Methods

This study was approved by the institutional review board of Massachusetts Eye and Ear Infirmary. Blood samples were obtained from patients with uveal melanoma evaluated at Massachusetts Eye and Ear Infirmary between June 22, 1992, and December 14, 2010. All patients had given written informed consent for their samples to be included in a biorepository for future studies. In total, 507 blood samples were available for this study. All analyses were performed in a Health Insurance Portability and Accountability Act–compliant fashion and in accordance with the Declaration of Helsinki.

**\textit{BAP1} Sequencing**

Germline \textit{BAP1} sequencing was performed as previously reported. Briefly, each blood sample was subjected to DNA extraction and polymerase chain reaction amplification of each \textit{BAP1} exon. The polymerase chain reaction products were size confirmed by gel electrophoresis and submitted to the sequencing core facility at the University of Utah. Sequences were analyzed using a software program (Sequencher 5.0; Gene Codes Corporation).

### Computational Analyses

Known single-nucleotide polymorphisms (SNPs) were identified and confirmed using University of California Santa Cruz Genome Browser (http://genome.ucsc.edu/), National Center for Biotechnology Information’s SNP database (http://www.ncbi.nlm.nih.gov/), and Ensembl (http://uswest.ensembl.org/index.html). Novel SNPs were confirmed by sequencing in forward and reverse directions and by cross-checking position number with the above databases and the Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/).

For each \textit{BAP1} polymorphism identified, the resulting codon change was determined, and the mutation was characterized as missense (synonymous or nonsynonymous), insertion, deletion, insertion deletion, or nonsense. For nonsynonymous missense mutations, previously validated tools were used to predict whether the amino acid change resulting from the mutation was likely to impair protein function: these included PolyPhen (http://genetics.bwh.harvard.edu/pph/data/) and Sorting Intolerant From Tolerant (SIFT) (http://sift.jcvi.org/). For synonymous mutations, potential splice site changes were identified (http://www.fruitfly.org).

### Clinical Review

The medical record of each patient was reviewed, and the following clinical data were collected: age at the time of diagnosis, sex, history of other malignant neoplasm, family history of cancer, and visual acuity (VA) at the time of diagnosis. In reviewing history of other malignant neoplasm and family history of cancer, nonmelanoma skin cancers were not considered. Patients in whom the history included only an unknown cancer or unknown skin cancer were excluded from analysis. Snellen VAs were converted to logarithm of the minimum angle of resolution (logMAR) equivalents, with counting fingers corresponding to a logMAR acuity of 2 (Snellen 20/2000) and hand motions corresponding to a logMAR acuity of 3 (Snellen 20/20 000). Patients with light perception or no light perception (1 patient each in the control group) were excluded from VA analyses.

Clinical characteristics of the primary tumor, which had been assessed by a single examiner (E.S.G.), were reviewed. These included distance of the tumor from the fovea (in disc diameters), distance of the tumor from the optic nerve (in disc diameters), largest tumor diameter (in millimeters), presence of ciliary body involvement, presence of iris involvement, presence of extrascleral extension, and pigmentation. Pigmentation was graded as absent, mild, moderate, or heavy and was determined based on clinical examination by a single examiner (E.S.G.), who has been using this system for more than 30 years. This pigmentation system has been used in previously published studies.

The medical records were also reviewed for the development of metastasis identified through follow-up at Massachusetts Eye and Ear Infirmary or through annual contact with the referring ophthalmologist. In addition, vital status was ascertained using http://www.ancestry.com, as well as through searches of the National Death Index and the Social Security Death Index, maintained by the US National Center for Health Statistics. The Social Security Death Index was used to screen for patient deaths not identified through the patient’s clinical records, and the National...
Death Index was used to confirm deaths and identify cause. Patients in whom death from uveal melanoma was identified were also classified as having developed metastases.

**Study Groups**
Patients with BAP1 mutations likely to impact BAP1 protein function were compared with control subjects. To ensure that the scope of the definition of the BAP1 mutation and control groups did not markedly alter the results, we conducted the same analysis using alternative definitions of these groups, as listed in eTable 1 in the Supplement. Analysis 1A is presented herein. Analyses 1B, 2A, 2B, 3A, and 3B yielded similar conclusions and are reported as supplemental data (eTables 2 through 6 in the Supplement).

**Statistical Analysis**
Clinical characteristics of patients in the BAP1 mutation group were compared with those of control patients. Age at diagnosis, largest tumor diameter, distance from the optic nerve or fovea, and VA did not pass the D’Agostino and Pearson omnibus test for normality of variables. Therefore, the Kolmogorov-Smirnov test was used to analyze these variables. The Mann-Whitney test was used for the nonparametric ranked variable of pigmentation grade. The Fisher exact test was used to analyze the binary variables of sex, history of other malignant neoplasm, family history of cancer, family history of cutaneous melanoma, family history of ocular melanoma, presence of ciliary body involvement, presence of iris involvement, presence of extrascleral extension, and development of metastases. Logistic regression analyses were performed for multivariable analyses of germline BAP1 mutation, ciliary body involvement, and tumor diameter, with the outcome measure being metastasis.

**Results**
Of 507 blood samples analyzed, 25 patients (4.9%) exhibited 18 BAP1 polymorphisms. Three patients had insertions, 1 patient had an insertion/deletion, 2 patients had nonsense mutations, 6 patients had nonsynonymous missense mutations,

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Mutation Summary in cDNA Format</th>
<th>Corresponding Protein</th>
<th>SNP ID</th>
<th>Minor Allele Frequency in Genomic DNA Format</th>
<th>Mutation Type</th>
<th>Location of Mutation in Protein Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.294C&gt;T p.Ser98Ser rs140641333 SNP</td>
<td>A = 0.0004</td>
<td>Synonymous missense</td>
<td>Ubiquitin carboxy-terminal hydrolase domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2, 3</td>
<td>c.501G&gt;A p.Ala167Ala rs148631953 SNP</td>
<td>T = 0.0010</td>
<td>Synonymous missense</td>
<td>Ubiquitin carboxy-terminal hydrolase domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>c.510_511insT p.Val171Cysfs*12 Novel</td>
<td>NA</td>
<td>Insertion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>c.639_640insT p.Ile214Tyrfs Novel</td>
<td>NA</td>
<td>Insertion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6, 7, 8, 9</td>
<td>c.1002A&gt;G p.Leu334Leu rs28997577 SNP</td>
<td>C = 0.0084</td>
<td>Synonymous missense</td>
<td>BARD1-binding domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10, 11, 12, 13</td>
<td>c.1026C&gt;T p.Ser342Ser rs71651686 SNP</td>
<td>A = 0.0016</td>
<td>Synonymous missense</td>
<td>BARD1-binding domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>c.1153C&gt;T p.Arg385*</td>
<td>COSM48859 somatic SNV; patient 3382 in Njauw et al., 2012</td>
<td>NA</td>
<td>Nonsense</td>
<td>Truncation just after the host cell factor C1-binding domain</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>c.1327A&gt;T p.Asn443Tyr Novel</td>
<td>NA</td>
<td>Nonsynonymous missense</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>c.1345G&gt;A p.Ala449Thr Novel</td>
<td>NA</td>
<td>Nonsynonymous missense</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>c.1413T&gt;G p.Ala471Ala rs34736117 SNP</td>
<td>C = 0.0262</td>
<td>Synonymous missense</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>c.1445C&gt;T p.Ser482Leu Novel</td>
<td>NA</td>
<td>Nonsynonymous missense</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>c.1721C&gt;T p.Ala574Val Novel</td>
<td>NA</td>
<td>Nonsynonymous missense</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>c.1745_1746delinsTTT p.Ser582Phefs Novel</td>
<td>NA</td>
<td>Insertion/deletion</td>
<td>Frameshift before the BRCA1-binding domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>c.1786A&gt;G p.Ser596Gly rs79014342 SNP</td>
<td>C = 0.0270</td>
<td>Nonsynonymous missense</td>
<td>BRCA1-binding domain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>c.1899_1900insGCTGG p.Ala634Glyfs*5</td>
<td>Patient 2734 in Njauw et al., 2012</td>
<td>NA</td>
<td>Insertion</td>
<td>Frameshift beginning in the BRCA1-binding domain</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>c.1975A&gt;T p.Lys659*</td>
<td>Patient ID 3101 in Njauw et al., 2012</td>
<td>NA</td>
<td>Nonsense</td>
<td>Truncation within the nuclear localization signal domain</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>c.2044C&gt;T p.Leu682Leu Novel</td>
<td>NA</td>
<td>Synonymous missense</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BAP1, BRCA1-associated protein 1 gene; cDNA, complementary DNA; NA, not applicable; SNP, single-nucleotide polymorphism; SNV, single nucleotide variant.
and 13 patients had synonymous missense mutations (Table 1). Of the 18 unique BAP1 mutations identified, 9 were novel.

Of 6 nonsynonymous missense mutations, SIFT and PolyPhen analyses revealed that 2 (in patients 16 and 19) resulted in amino acid changes likely to impair protein function. Patient 16 exhibited a mutation causing an arginine to tyrosine change at position 443, which by SIFT analysis exhibited a 99.4% chance of being damaging to protein function. No other nonsynonymous mutations were predicted to be possibly damaging to protein function. Of 13 synonymous missense mutations, computational analysis identified that 2 SNPs identified in 3 patients (patients 2, 3, and 25) had a low likelihood (<50%) of introducing splice site changes.

In the primary analysis (analysis 1A, eTable 1 in the Supplement), the BAP1 mutation group was defined as patients with BAP1 mutations likely to be damaging to protein function, including insertions, deletions, insertions/deletions, nonsense mutations, nonsynonymous mutations likely to be damaging to protein function (>75%), and synonymous mutations likely to result in a splice site change (>75%). In total, 8 patients (1.6%) were included in this group (patients 4, 5, 14, 16, 19, 21, 23, and 24 in Table 1). Two had nonsynonymous missense mutations (patients 16 and 19), 3 had insertions (patients 4, 5, and 23), 1 had an insertion/deletion (patient 21), and 2 had nonsense mutations (patients 14 and 24). Three of these 8 germline mutations were previously described,10 and 5 are novel. Seventeen patients with BAP1 SNPs unlikely to affect protein function (based on computational tools) were excluded from analysis. The excluded group included a small percentage (3.4%) of the total population studied. The 482 patients with no BAP1 polymorphisms composed the control group. The results of this analysis (analysis 1A) are presented herein. Analyses 1B, 2A, 2B, 3A, and 3B, in which the definitions of the BAP1 mutation and control groups were modified slightly (as listed in eTable 1 in the Supplement), yielded similar overall conclusions and are presented in the supplemental data (eTables 2 through 6 in the Supplement).

The mean age at diagnosis in the germline BAP1 mutation group was 56.8 years vs 58.5 years in the control group (P = .44).

Patients with germline BAP1 mutations were not significantly more likely to have a prior diagnosis of another malignant neoplasm (25.0% vs 15.1%, P = .35) (Table 2). Two patients with germline BAP1 mutations had a history of another malignant neoplasm. Patient 4 had a history of colon cancer (at age 53 years), prostate cancer (at age 63 years), and kidney...
Uveal Melanoma in Patients With Germline BAP1 Mutations

Table 4. Family History of Cancer Among Patients With Germline BAP1 Mutations

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Family History Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Cutaneous melanoma in sister, colon cancer in father, stomach/liver cancer in nephew</td>
</tr>
<tr>
<td>5</td>
<td>Breast cancer in mother, skin cancer (unknown type) in mother</td>
</tr>
<tr>
<td>14</td>
<td>Ocular melanoma in brother, ocular melanoma in brother, ocular melanoma in maternal aunt, ocular melanoma in niece, cutaneous melanoma in nephew, bladder cancer in brother, breast cancer in mother, breast cancer in sister, breast cancer in niece, cholangiocarcinoma in nephew</td>
</tr>
<tr>
<td>16</td>
<td>Nonocular/noncutaneous cancer with unknown details</td>
</tr>
<tr>
<td>19</td>
<td>Lung cancer in father, lung cancer in 2 sisters, cutaneous melanoma in mother</td>
</tr>
<tr>
<td>21</td>
<td>Cutaneous melanoma in daughter, nonocular/noncutaneous cancer with unknown details in father</td>
</tr>
<tr>
<td>23</td>
<td>Cutaneous melanoma in father, bladder cancer in father</td>
</tr>
<tr>
<td>24</td>
<td>Ocular melanoma in cousin, kidney cancer in 2 maternal uncles, bone cancer in maternal aunt, bone cancer in maternal uncle</td>
</tr>
</tbody>
</table>

There was increased frequency of family history of cancer among patients with germline BAP1 mutations (100% vs 65.9%, \( P = .06 \)) (Table 2). In addition, there were increased frequencies of family history of cutaneous melanoma (62.5% vs 9.9%, \( P = .001 \)) and family history of ocular melanoma (25.0% vs 1.9%, \( P = .01 \)) in patients with germline BAP1 mutations. Details of family history of cancer among patients with germline BAP1 mutations are listed in Table 4.

Patients with germline BAP1 mutations exhibited larger tumor diameters than those in the control group (mean, 15.9 vs 12.3 mm; \( P = .004 \)) (Table 2). Ciliary body involvement was more frequently noted in patients with BAP1 mutations (75.0% vs 21.6%, \( P = .002 \)). Metastatic disease developed more frequently in patients with germline BAP1 mutations compared with the control group (71.4% vs 18.0%, \( P = .003 \)). No differences were identified in initial VA, distance of the tumor from the optic nerve or fovea, iris involvement, extraciliary extension, or tumor pigmentation. Similar conclusions were drawn when analyses 1B, 2A, 2B, 3A, and 3B (eTable 2 through 6 in the Supplement).

Given that germline BAP1 mutations were associated in univariate analyses not only with metastasis but also with 2 known risk factors for metastasis (larger tumor diameter and ciliary body involvement), multivariable logistic analyses were performed for germline BAP1 mutation, tumor diameter, and ciliary body involvement as predictors of metastasis (Table 5). These revealed that tumor diameter was the strongest risk factor for metastasis and was independent of BAP1 mutation and ciliary body involvement in increasing risk of metastasis (\( P < .001 \)). Germline BAP1 mutations increased risk of metastasis independent of ciliary body involvement (\( P = .02 \)). Multivariable analysis did not identify germline BAP1 mutation as a risk factor for metastasis independent of tumor diameter (\( P = .09 \)). Germline BAP1 mutation approached significance as an independent risk factor for metastasis (\( P = .09 \)).

Discussion

The natural history of uveal melanoma is characterized by frequent development of metastases. Several features of the primary tumor are associated with poor prognosis, including location in the ciliary body, diffuse configuration, larger size, extraciliary extension, and histopathologic features. Genetic factors have also been implicated, most commonly monosomy of chromosome 3 within the primary tumor, which is reported in up to 50% of primary uveal melanomas and is considered a poor prognostic factor. More recently, uveal melanomas were shown to segregate by microarray gene expression profiles into the following 2 distinct tumor classes: class 1 tumors, which have a low risk of metastasis, and class 2 tumors, which are associated with higher rate of metastasis (the majority of which exhibit monosomy of chromosome 3).

The aggressive nature of tumors with monosomy of chromosome 3 suggests that loss of 1 copy of chromosome 3 unveils a deleterious gene on the remaining chromosome. Indeed, Harbour et al demonstrated that primary uveal melanomas exhibit a high frequency (47.4%) of somatic mutations in the BAP1 gene, located at chromosome 3p21.1. BAP1 protein is a ubiquitin hydrolase and tumor suppressor. Various studies have implicated the protein in chromatin remodeling, DNA double-stranded break repair, and cell cycle control.

In this study, we sought to characterize the uveal melanoma phenotype in patients bearing germline BAP1 mutations. BAP1 polymorphisms identified from blood of patients with uveal...
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BAP1 mutations were subjected to previously validated tools to identify which polymorphisms were likely to impair protein function. Overall, 18 BAP1 polymorphisms were identified in 25 patients. Of these 18 polymorphisms, 9 were novel. Previously validated computational analysis tools predicted that 8 BAP1 mutations in 8 patients were likely to be damaging to protein function. These 8 patients comprised the BAP1 mutation group. Five of these 8 mutations were novel (Table 1). Patients with no BAP1 polymorphisms comprised the control group.

We noted a slightly lower rate (1.6%) of deleterious germline BAP1 mutations in uveal melanoma than the 3% to 4% reported in other studies5-12 with smaller sample sizes. However, the present study reflects the prevalence of germline BAP1 mutations among an unselected overall clinic population of patients with uveal melanoma rather than a subset of patients selected for family history of melanoma or metastatic disease as in other studies. The low rate of germline BAP1 mutations contrasts with the much higher rates (approximately 47%) of somatic BAP1 mutations reported in primary tumors4-32 and an even higher rate (81%) in metastatic lesions from uveal melanoma.33

Germline BAP1 mutations have been identified in patients with uveal and cutaneous melanomas and are thought to be implicated in a hereditary cancer predisposition syndrome involving not only melanomas but also other tumors, including mesothelioma, renal cell carcinoma, and others.5-9 Consistent with this finding, we noted that patients with uveal melanoma with germline BAP1 mutations exhibited increased likelihood of family history of cancer, as well as increased frequency of family history of cutaneous melanoma or ocular melanoma. Despite the tendency of patients with cancer predisposition syndromes to manifest tumors at a younger age, we noted no difference in age at diagnosis between patients with germline BAP1 mutations and those without. Similarly, we noted no increased rate of history of other malignant neoplasms in patients with germline BAP1 mutations.

Patients with germline BAP1 mutations exhibited larger basal tumor diameter and higher rate of ciliary body involvement, 2 clinical factors correlated with poor prognosis.9,22 In univariate analyses, germline BAP1 mutations were associated with a 4-fold increased risk of metastasis. We report herein the first description to date of germline BAP1 mutations correlating with poor prognosis. Bivariable analysis revealed that germline BAP1 mutations increased risk of metastasis independent of ciliary body involvement (P = .02). Bivariable analysis did not identify germline BAP1 mutation as a risk factor for metastasis independent of tumor diameter (P = .09). When adjusting for the effects of ciliary body involvement and largest tumor diameter, multivariable analysis did not identify germline BAP1 mutations as an independent risk factor for metastasis (P = .09). Given the small size of the germline BAP1 mutation group in this study, our analyses may have been underpowered to adequately detect an association. Further studies with more patients are necessary to determine whether germline BAP1 mutations independently alter risk of metastasis.

To ensure that minor differences in our definition of clinically significant (ie, deleterious) BAP1 mutations (BAP1 mutation group) or of the control group did not significantly alter the results of this study, the analyses reported above were repeated with slightly modified definitions of each group (analyses 1B, 2A, 2B, 3A, and 3B; eTable 1 in the Supplement). All 5 supplemental analyses produced similar overall conclusions as the primary analysis reported herein (eTables 2 through 6 in the Supplement), suggesting that correlation of BAP1 with various clinical characteristics is maintained, despite small, reasonable modifications in definitions of the BAP1 mutation and control groups.

The association of germline BAP1 mutations in this study with known risk factors for metastasis is consistent with recent reports that somatic BAP1 mutations or loss of BAP1 expression in the primary tumor correlates with decreased survival.32-34 Prior studies4-13 have demonstrated that somatic BAP1 mutations are present in high frequency in class 2 uveal melanomas but rarely in class 1 uveal melanomas or benign melanocytic lesions. These findings, along with the strong association of somatic mutations32-34 and now possibly also germline BAP1 mutations with poor prognostic features, suggest that loss of BAP1 may be a later event in the pathogenesis of uveal melanoma. In contrast, the mutually exclusive GNAQ (OMIM 600998) and GNA11 (OMIM 139313) somatic mutations frequently present in uveal melanomas are likely earlier events driving oncogenesis because they are present in nevi as well and do not correlate with class 1 or class 2 status, other prognostic features, or survival.35,36

There were several limitations of this study. The germline BAP1 mutation group was small (8 patients) owing to the low frequency of these mutations in the population with uveal melanoma. Another limitation of this study is that sequencing was performed only for BAP1 exons. Polymorphisms in the promoter region that affect gene expression, as well as those in introns that may affect splicing or gene regulation, were not investigated. Similarly, mechanisms of epigenetic regulation of BAP1 were not evaluated. To our knowledge, there are no reports to date of deleterious germline BAP1 promoter or intronic polymorphisms in uveal melanoma. The impact on protein function of BAP1 gene polymorphisms was predicted by previously validated computational tools rather than being biochemically demonstrated. Finally, correlation of germline BAP1 sequencing with molecular or histologic features of the primary tumors was not performed because most blood samples used in this study were collected well before fine-needle aspiration biopsy of primary tumors became common practice.

Conclusions

In conclusion, we demonstrate that germline BAP1 mutations are present infrequently in uveal melanoma and report 9 novel germline BAP1 mutations in patients with uveal melanoma, 5 of which are likely to be deleterious to protein function. Germline BAP1 mutations in this study were associated with larger tumor diameter and increased risk of ciliary body involvement, 2 known risk factors for metastasis. In this study, germline BAP1 mutations approached but did not reach significance as an independent risk factor for metastasis.
their studies are necessary to determine the clinical significance of germline BAP1 mutations in patients with uveal melanoma. Currently, molecular prognostic testing of the primary tumor remains more valuable in the clinical setting. Germline BAP1 mutation testing may be relevant in certain individuals for family counseling.

REFERENCES


