IMPORTANCE Patients with germline mutations in BAP1 may develop several flesh-colored melanocytic BAP1-mutated atypical intradermal tumors (MBAITs). These tumors generally develop earlier than other BAP1-associated tumors, highlighting an important role for dermatologists in identifying and screening patients with a history suggestive of a germline mutation.

OBJECTIVE To describe 8 new families with germline mutations in BAP1 and provide a comprehensive review of reported cases.

DESIGN, SETTINGS AND PARTICIPANTS Patients were identified in an outpatient dermatology clinical setting over a 6-month period (10 mutation carriers from 8 families) and through a literature review using PubMed (205 patients).

EXPOSURES Mutations were identified through next-generation sequencing of saliva or blood samples, and RNA was extracted from fibroblasts cultured from a patient with an intronic variant to determine the impact of the mutation on the coding sequence.

MAIN OUTCOMES AND MEASURES All 215 patients were assessed for personal and/or family history and genotype. These findings were compiled and assessed for any association between genotype and phenotype.

RESULTS Overall, this study included 215 patients (108 women, 91 men, and 16 gender unspecified; median [range] age, 46.5 [10.0-79.0] years). Nine of the 10 patients who were identified in the outpatient dermatology setting were found to have MBAITs on clinical examination. Forty of 53 patients (75%) identified in the literature review who underwent total-body skin examinations (TBSE) were found to have MBAITs, suggesting a high penetrance in patients who have undergone TBSE. The most prevalent malignancies among BAPI mutation carriers were uveal melanoma (n = 60 [28%]), mesothelioma (n = 48 [22%]), cutaneous melanoma (n = 38 [18%]), and renal cell carcinoma (n = 20 [9%]). A total of 71 unique mutations in BAPI have been reported.

CONCLUSIONS AND RELEVANCE Our results indicate that germline mutations in both coding and noncoding regions throughout the BAPI gene can impair protein function, leading to an increased risk for several associated malignancies. Four of the 8 probands we present had no history of BAPI-associated malignancies and were assessed for germline mutations when found to have MBAITs on dermatologic examination. Dermatologists can identify patients with a high likelihood of the BAPI cancer syndrome through personal and family history and TBSE for the presence of possible MBAITs.
The significance of the BAP1 gene was originally established when inactivating BAP1 mutations were found in aggressive uveal melanoma tumor specimens. Subsequent genomic evaluation of families with familial uveal melanoma and mesothelioma led to the identification of a germline tumor predisposition syndrome associated with BAP1 inactivation. The syndrome associated with BAP1 deficiency was later expanded to include a risk for renal cell carcinoma and cutaneous melanoma and continues to be associated with new malignancies.

In addition to malignant tumors, some patients with BAP1 germline mutations were found to develop a range (5-50) of skin-colored, dome-shaped, well-circumscribed papules with characteristic histomorphology, referred to as melanocytic BAP1-mutated atypical intradermal tumors (MBAITs) when first presented in the literature. Dermatologists can play a central role in the diagnosis and management of this syndrome because MBAITs are estimated to have a high penetrance and generally appear earlier than other BAP1-associated tumors. In this study, we describe 8 families with germline BAP1 mutations and discuss the typical age of onset and estimated penetrance of the various associated tumors as well as potential genotypic-phenotypic correlations found in a comprehensive literature review.

Methods

DNA Extraction

Saliva samples were collected after informed consent was acquired, and next-generation sequencing was performed with the Ion PGM platform (ThermoFisher Scientific) as standard of care. For Families 1 and 2, the BAP1 gene was targeted with a customized primer pool designed with an AmpliSeq designer (ThermoFisher Scientific). The GRC37/hg19 reference genome and transcript NM_004656.3 were used. Results were analyzed using IonTorrent Suite 4.4 (ThermoFisher Scientific). Family 3 had blood samples sent to Prevention Genetics while Families 4 through 6 had blood samples sent to Ambry Genetics. Families 7 and 8 had saliva samples sent to Ambry Genetics for diagnosis.

Institutional review board approval was obtained from both the Northwestern and Partners institutional review boards for this study.

Sample, Messenger RNA, and Complementary DNA Generation

A skin biopsy was performed on the proband from Family 4 after consenting to a human protocol approved by the Partners institutional review board. Dermal fibroblasts were isolated and cultured using methods from Rittié et al. RNA was extracted from the fibroblasts with the RNAeasy mini kit (Qiagen). Complementary DNA (cDNA) was generated with the High-Capacity RNA-to-cDNA kit (Applied Biosystems) according to the manufacturer's instructions.

Polymerase Chain Reaction

Two primers, 5'-TAG CGA ATT CGA GTT GGC ATG AGC AAA GGA TAT GCC ATT GG-3' and 5'-CCG CGG ATC CGA TCA TCC TCC TCG TCA TCC TC-3', were used to amplify BAP1 exons 6 to 12 from the cDNA. Each polymerase chain reaction (PCR) contained 1 μL of cDNA, 2 μL of 10X Ex Taq buffer, 1.6 μL of 2.5 mM dNTP, 0.4 μL of 100 μM of the above 2 primers, 0.1 μL of Ex Taq (TAKARA), and water to a final volume of 20 μL. Cycling conditions included denaturation at 94° C for 4 minutes, followed by 35 cycles at 94° C for 30 seconds, 60° C for 30 seconds, and 72° C for 90 seconds. Polymerase chain reaction products were gel extracted with the QIAEX II Gel Extraction kit (Qiagen), and 1 μL of extracted products were used for PCR and gel purified again to get sufficient DNA for cloning.

Cloning

The PCR products were restriction digested with EcoRI and BamHI, and were cloned into vector CD516B-2. Plasmids were transformed into bacteria by routine molecular methods. Individual bacterial colonies were isolated and DNA was extracted using the QIAprep Spin Miniprep kit (Qiagen) and sequenced at the MGH sequencing core facility.

Case Identification and Statistical Analysis

Cases were identified through a literature review using PubMed, and all reported patients who tested positive for BAP1 mutations, as well as those indicated as obligate carriers, were included for review. Age distributions were compared using the Kruskal-Wallis test, and \( P < .05 \) was considered statistically significant.

Results

Case Presentation

The proband of Family 1 is a teenage female who presented with a dome-shaped papule on her upper arm that demonstrated a central pink structureless area and pigment globules at the lateral edges of the lesion on dermoscopy (Figure 1). Although not specific, these features can be seen in MBAITs. The proband has a family history of mesothelioma in her father in his 50s, renal cancer in her paternal grandfather, cutaneous melanoma in her paternal grandmother, and ovarian cancer in her maternal aunt. The presenting patient and her sister, who was also found to have several pedunculated nevi suspected to be...
MBAITs on clinical examination, were found to have a c.1321C>T, p.Q441X mutation in the BAP1 gene.

The proband of Family 2 is a female in her 30s who presented with a 3 × 2-mm pink papule with scattered foci of brown pigment on her right arm and found to be an MBAIT on histology. The patient’s paternal grandfather had mesothelioma, and her father had Parkinson disease and cutaneous melanoma. The patient was found to have a 1-basepair deletion in the 3′ untranslated region of the BAP1 gene (chr3, g.52435660delC).

The proband of Family 3 is an adolescent female diagnosed with multiple MBAITs starting at a young age. The patient was found to have a deletion in BAP1 (c.1717delC, p.L573fs*3) that has been previously published.1,12-14 None of the patient’s first-degree family members have any history of malignancies, and her mother and sister were found to be negative for mutations in BAP1.

The proband of Family 4 is a female in her 50s with a history of ocular melanoma, temporal lobe meningioma, mammary ductal carcinoma in situ (DCIS), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC) who was found to carry a c.659 + 3A>C intronic variant in BAP1. The patient did not demonstrate any lesions resembling MBAITs on clinical examination. She has a family history of mesothelioma, meningioma, melanoma, BCC, SCC, and other malignancies. The patient’s maternal first cousin once removed also carries the same intronic variant and has a history of 2 Spitz nevi, 1 of which was histologically confirmed to be an MBAIT. Although the mutation lies in a noncoding region, we surmised that the variant could have an impact on splicing. To prove this, we obtained skin fibroblasts from the patient and analyzed the BAP1 RNA transcript. As shown in Figure 2, the patient’s wild-type allele exhibits normal joining of exons 8 and 9 while the mutated transcript shows allele-specific retention of intron 8. This variant causes transcriptional read-through and introduces a putative novel termination codon approximately 120 codons downstream of the exon 8 junction.

The proband of Family 5 is a male in his 50s with history of meningioma in his 30s, lentigo maligna and BCC in his 40s, as well as renal cell cancer, prostate cancer, peritoneal mesothelioma, and 2 MBAITs in his 50s found to carry a deletion of exon 3 in the BAP1 gene. During initial consultation, he was found to have skin phototype II, some solar lentigines in photoexposed areas and a moderate density of clinically atypical nevi. He has a family history of liver, bladder, and breast cancer, as well as glioma and several cancers of unknown type.

The proband of Family 6 is a female with a history of multiple MBAITs starting in her 20s who was found to have a c.1416delG, p.S473Vfs*9 mutation in BAP1. On dermatologic examination, the patient had a moderate density of benign appearing nevi as well as an 8-mm mutilobulated pink plaque histologically diagnosed as an MBAIT. The patient has...
a family history of 2 melanomas in her mother, a benign brain tumor in her maternal aunt, breast cancer in her maternal grandmother, and mesothelioma in her paternal grandfather. All other family members, including over 30 first-degree relatives and a brother in his 30s were reported to be unaffected, although their surveillance status was unknown to the patient.

The proband of Family 7 is a female in her 40s diagnosed with a T2b BAP1-associated nevoid melanoma of the right parietal scalp and several MBAITs. She demonstrated several flesh-colored pedunculated lesions with varying degrees of peripheral pigmentation thought to be additional MBAITs on clinical examination. She was found to carry a c.771_772insTACTA, p.A258Yfs*2 mutation in BAP1. She has a family history of uveal and cutaneous melanoma and breast cancer in her paternal grandmother, BCC, SCC, thyroid, prostate, and colon cancer in her father, glioblastoma multiforme in her paternal uncle, glioblastoma multiforme in her maternal aunt, breast cancer in her maternal grandmother, BCC, SCC, thyroid, prostate, and colon cancer in her maternal grandmother, and mesothelioma in her paternal grandmother, BCC, SCC, thyroid, prostate, and colon cancer in her maternal grandmother.

The proband of Family 8 is a male with a c.79dupG, p.V27fs BAPI mutation who was diagnosed with grade II peritoneal mesothelioma in his 30s. The patient’s 2 brothers, who are also in their 30s, are unaffected. He has a family history of leukemia, breast, and brain cancer, as well other unknown malignancies on his maternal side. The patient was found to have a nevus of the iris on ophthalmologic examination, as well as several uniform hyperpigmented papules, some of which were exophytic, on dermatologic examination.

Penetrance
The prevalence and median age of onset of each associated neoplasms in our series and in the literature are shown in the Table. There was a significant difference in age of onset between different types of BAPI-associated tumors (P < .001) with MBAITs demonstrating the earliest age of onset (Figure 3). Of the 10 patients that we present with BAPI mutations confirmed with genetic testing, 90% (9 of 10) were found to have suspected MBAITs on clinical examination. The median (range) age of presentation of these lesions in our series was 31 (10-56) years. In their review, Rai et al13 reported that only 43 of 174 individuals reported with the BAPI syndrome were assessed via TBSE, with 31 (72%) of these patients presenting with MBAITs.13 With the addition of the patients in our series with both clinically suspected and confirmed lesions, 75% (40 of 53) of patients with the BAPI syndrome assessed via TBSE were found to have MBAITs.

In a review of the relevant literature, a total of 215 patients from 87 families have been reported with the addition of our 8 families.1-10,12-14,18-38 Sixty of these patients developed uveal melanoma (28%), 48 patients developed mesothelioma (22%), 38 developed cutaneous melanoma (18%), and 20 developed renal cell carcinoma (9%). Other reported malignancies seen in patients found to have germ-line mutations in BAPI included basal cell carcinoma, meningioma, breast cancer, lung adenocarcinoma, pancreatic cancer, and thyroid cancer.

Genotypic-Phenotypic Associations
With the addition of our families, a total of 71 unique mutations in BAPI were identified10,12-14,18-38 (Figure 4). Six mutations were found to occur in multiple families (Table 1 in the Supplement). A majority of these variants (5 of 6) have not been described among the general population while 1 variant (c.20S0C>T, p.Q684X) was identified in 0.0008% of the general population.40 A majority of the mutations reported (46 of 71) were predicted to result in protein truncation owing to frameshift or nonsense mutations. Ten mutations were reported to affect splicing, and 15 mutations were missense point mutations.

Of all patients included, 37% (79 of 215) were found to have mutations expected to impact the first 240 amino acids of the
protein, which represent the catalytic UCH (ubiquitin carboxy-terminal hydrolase) domain. Fifty percent of all patients diagnosed with melanoma (19 of 38) and 75% of patients diagnosed with more than 1 melanoma (6 of 8) were found to have mutations that affected this domain (eTable 2 in the Supplement). Three patients from 2 different families were found to have multiple basal cell carcinomas, and all carried mutations affecting the UCH domain.

Forty-eight patients were diagnosed with mesothelioma (eTable 3 in the Supplement). Because the median age of onset of mesothelioma is 56 years in patients with the BAP1 syndrome, we compared the 48 patients with mesothelioma to patients with the BAP1 syndrome 56 years or older who did not develop mesothelioma (n = 29). Eight of these 29 patients (28%) had missense mutations, 19 (66%) had mutations expected to be truncating, and 2 (7%) had mutations that affected splicing with unknown effect on protein structure (eTable 4 in the Supplement). Of the 48 patients diagnosed with mesothelioma, 46 (96%) carried truncating mutations. The 2 patients with nontruncating missense mutations were diagnosed with mesothelioma at ages much older than the median (71 and 72 years).

### Figure 4. Diagram of the BAP1 Protein With Reported Mutations

REPORTED MUTATIONS THAT AFFECT CODING REGIONS OF THE BAPI GENE ARE SHOWN WITH AN APPROXIMATION OF THEIR RELATIVE LOCATION OF IMPACT ON THE RESULTING PROTEIN. 39 MUTATIONS REPORTED IN MULTIPLE FAMILIES ARE BOLDED. BARD1 INDICATES BARD1 BINDING DOMAIN; BRCA1, BRCA1 BINDING DOMAIN; HBM, HCF BINDING MOTIF; NLS, NUCLEAR LOCALIZATION SIGNAL; UCH, UBIQUITIN CARBOXY-TERMINAL HYDROLASE.

### Table. Median Age of Onset and Prevalence of Characteristic Tumors in 215 Patients With BAP1 Syndrome

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Cases, No.</th>
<th>Estimated Penetrance, %a</th>
<th>Median Age of Diagnosis in the Literature, y</th>
<th>Median Age of Diagnosis in Our Series, y</th>
<th>Median Age of Diagnosis in General Population, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uveal melanoma</td>
<td>60</td>
<td>28.0</td>
<td>53</td>
<td>59</td>
<td>6115</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>48</td>
<td>22.0</td>
<td>56</td>
<td>46</td>
<td>7416</td>
</tr>
<tr>
<td>Cutaneous melanoma</td>
<td>38</td>
<td>18.0</td>
<td>41</td>
<td>43</td>
<td>6115</td>
</tr>
<tr>
<td>MBAITs</td>
<td>36</td>
<td>17.0</td>
<td>32</td>
<td>31</td>
<td>2416</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>20</td>
<td>9.0</td>
<td>47</td>
<td>51</td>
<td>6415</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>14</td>
<td>6.5</td>
<td>52</td>
<td>41</td>
<td>7515</td>
</tr>
</tbody>
</table>

**Abbreviation:** MBAITs, melanocytic BAPI-associated intradermal tumors.

*The estimated penetrance is based on the prevalence of each tumor reported in BAPI patients identified in the literature and in our series.

**Discussion**

The histopathology of melanocytic BAPI-mutated atypical intradermal tumors is highly characteristic and shows a central population of epithelioid melanocytes, often with spitzoid cytometry, in the dermis flanked by more conventional nevomelanocytes laterally. Commonly, MBAITs are pink or flesh-colored dome-shaped papules with a structureless central area and some lateral focal pigment globules on dermoscopy; MBAITs arise from conventional nevi, most commonly with initiating oncogenic mutations in BRAF, which are represented by the lateral pigment globules that may be seen on dermoscopy. Biallelic inactivation of BAPI leads to the proliferation of a second subclone of larger epithelioid melanocytes which typically lack pigment and may result in a central pink or flesh-colored structureless area that can be seen on both dermoscopy and clinical examination. Polymorphous vessels may be seen in this central structureless area.

Immunohistochemistry can be a very helpful adjunctive study for the assessment of MBAITs. Because BAPI is a nuclear protein, cells with 2 wild-type copies of the gene should...
demonstrate strong nuclear positivity. Cells with monoallelic inactivation, whether somatic or germline, will show nuclear staining but may additionally have cytoplasmic staining if the non-wild-type allele involves a mutation, such as a truncating mutation, that affects the nuclear localization sequence (NLS) at the C terminus of the protein. Cells with biallelic inactivation of BAP1 will demonstrate complete loss of nuclear staining with or without some cytoplasmic staining, again depending on whether mutations in either of the 2 alleles impact the NLS.

In their review, Rai et al.13 found that 11 of the 31 patients (35%) who underwent full TBSEs had multiple MBAITs on clinical examination. Additionally, a majority of patients with the BAPI syndrome reported to undergo TBSEs were found to have MBAITs (40 of 53 [75%]). Hence, we suspect that the penetrance for MBAITs is likely higher than that reported in the literature and in fact may be the most penetrant of the various related tumors in these patients. This apparent penetrance, however, is limited by clinically directed screening of patients with the BAPI syndrome. The true prevalence could only be determined with certainty through prospective research-driven assessment.

In addition to the relatively high estimated penetrance of MBAITs, the other significant feature of these lesions is the relatively young age of onset compared with other associated tumors (Figure 3). Wiesner et al.2 noted that BAPI-deficient melanocytic tumors in patients with germline mutations in BAPI often begin to develop during the second decade of life and progressively increase in number as the patient ages.2 The median age of onset of MBAITs in patients with germline BAPI mutations is 32. However, these tumors may have been present earlier in some patients and not found until they were diagnosed with a BAPI-associated malignancy and underwent TBSEs later in life. Additionally, 4 of our 8 probands presented only with MBAITs in their 30s or earlier, which then led to the diagnosis of a germline syndrome. The diagnosis of an MBAIT is not possible on clinical examination alone without histologic confirmation, yet the presence of multiple lesions with characteristic features, as well as a positive pertinent clinical or family history, may be an indication for a dermatologist to discuss genetic testing. Alternatively, a dermatologist familiar with the syndrome may be alerted to the possibility of a germline mutation based on the histologic features of a biopsied lesion, even if the lesion was not suspected to be an MBAIT on clinical examination. Dermatologists therefore play an important role in recognizing patients who may carry germline mutations in BAPI and referring them to undergo genetic testing as well as screening for the other associated tumors.

Although no clear associations between genotype and phenotype have been identified in the BAPI syndrome, we found that almost all patients who develop mesothelioma carry germline truncating mutations of the BAPI gene. All truncating mutations that have been identified in patients with the BAPI syndrome occur before the nuclear localization sequence, which can result in cytoplasmic retention of the BAPI protein. Aberrant BAPI protein has been shown to form amloid aggregates that can accumulate in the cytoplasm of cells.11 We hypothesize that these aggregates may contribute to the development of mesothelioma by contributing to chronic inflammation and cytotoxic effects. The chronic inflammation induced by asbestos fibers in asbestos-related sporadic mesothelioma has been shown to play a central role in carcinogenesis and to lead the generation of reactive oxygen and nitrogen species that damage DNA.42-44 We postulate that a similar mechanism may be responsible, at least in part, for carcinogenesis in cells with cytoplasmic aggregates of BAPI protein. Accordingly, a majority of BAPI patients who were found to develop mesothelioma were not exposed to asbestos. Also, interestingly, overall survival for BAPI-associated mesothelioma is better than for non–BAPI-associated mesothelioma, while patients with BAPI-associated uveal melanoma and renal cell carcinoma have a worse prognosis than non–BAPI-associated cases.45

Additionally, patients who develop multiple cutaneous melanomas or BCCs have been found to be more likely to carry mutations that occur in the UCH catalytic domain of the protein, the area responsible for the deubiquinating function of BAPI. Mutations in the UCH domain have been shown to lead to an inability to recruit BAPI to sites of DNA damage, where it plays a role in repair through homologous recombination.46-48

Both the nuclear localization sequence at the C terminus of the protein and the deubiquinating catalytic domain are necessary for BAPI to act as a tumor suppressor. This observation, however, is of unknown significance and based on a limited sample size. Also, importantly, our findings in Family 4 in this study demonstrate that mutations in noncoding regions of the gene can also result in the BAPI cancer syndrome. Hence, intronic variants, which may not be reported by all commercial assays, cannot be overlooked.

Limitations
This study was based on a relatively small sample size (n = 215) and includes retrospective data about personal and family history of malignancies. A majority of the data was collected from the text and tables of published manuscripts, as well as from published supplemental material. Patients identified may have developed subsequent tumors after the studies included for review were published. Some studies did not consistently report age at presentation and/or malignancy diagnosis and did not indicate whether skin examinations were performed to assess for the presence of MBAITs. Some patients were unable to recall which specific malignancies they had a family history of and there may have been a slight recall bias related to tumors associated with the BAPI syndrome. While the findings identified in this analysis are suggestive, prospective studies of the growing cohort of patients identified with the BAPI syndrome are needed to further assess penetrance and genotype-phenotypic correlation.

Conclusions
The BAPI syndrome demonstrates autosomal dominant inheritance, so all at-risk family members should be tested. We suggest that affected patients undergo TBSEs every 6 months.
and are referred to ophthalmology for uveal melanoma screening. All patients with the BAP1 syndrome identified by dermatologists should be referred to genetics and may be followed by nephrology or pulmonology at specialized centers with knowledge of the BAP1 syndrome. Screening recommendations such as those put forth by Pilarski et al and those recommended by a consensus panel of mesothelioma experts in 2015 are of value for physicians treating patients with the BAP1 syndrome, yet prospective studies of these families are necessary to generate evidence-based screening protocols.

REFERENCES
Chemical peels are one of the most commonly performed cosmetic procedures and have long been used for the treatment of disorders of pigmentation, acne, and fine rhytides. The procedure involves applying an exfoliating agent—such as an α-hydroxyacid (AHA)—to the skin to destroy the epidermis and/or dermis layer, which allows the skin to rejuvenate.1

Chemical peeling is a centuries-old practice, rooted in ancient Egyptian medicine and described in the Ebers Papyrus in 1550 BCE.2 The Egyptians, and in particular Queen Cleopatra, were famously known to bathe in sour milk to improve the look and texture of the skin.3 Today, we know that sour milk contains lactic acid, an naturally occurring AHA. Other ancient civilizations, such as the Greeks and Romans, used corrosive agents, such as lime, to achieve the same results.1

It was not until the 19th century that the cosmetic and dermatologic significance of chemical peels was identified. Ferdinand Ritter von Hebra, known as the father of modern dermatology, started performing the procedure therapeutically to lighten freckles and treat melasma and Addison disease. He used combinations of iodine, lead, croton oil, and even hydrochloric acid as exfoliating agents.2,3 Later on, German dermatologists Saafeld and Unna described the properties of phenol to treat freckles, wrinkles, and acne scars. Unna also pioneered work on salicylic acid, which was later discovered to be a β-hydroxy acid, another popular family of peeling agents used today.3