Comprehensive Review of *BAP1* Tumor Predisposition Syndrome

Research Thesis

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By
Karan Rai

Department of Molecular Genetics, The Ohio State University

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Project Advisors: Robert Pilarski, Department of Internal Medicine, CCC
Dr. Amanda Toland, Department of MVIMG, Internal Medicine, CCC
Dr. Mohamed H. Abdel-Rahman, Department of Ophthalmology and Visual Sciences, Internal Medicine

Abstract

BRCA1-associated protein-1 (*BAP1*) tumor predisposition syndrome (*BAP1*-TPDS) is a recently identified hereditary cancer syndrome and a rapidly developing area of medical research. Germline mutations in this tumor suppressor gene predispose families to the development of various malignancies. The molecular functions of the gene as well as the clinical phenotype of the syndrome are still being clarified. The aim of this study is to conduct a comprehensive review of all published research into *BAP1*-TPDS to more thoroughly delineate the clinical implications of germline *BAP1* mutations. Current evidence suggests that germline *BAP1* mutations predispose families to uveal melanoma, malignant mesothelioma, cutaneous melanoma, renal cell carcinoma, characteristic benign skin lesions, and possibly a range of other cancers as well. Some of these cancers tend to be more aggressive, have a propensity to metastasize, and onset earlier in life in patients with *BAP1* mutations. Survival in these patients is significantly decreased. Although further research is necessary, this information can aid in the management, diagnoses, prognoses, and therapy of these patients and their families, and highlights the importance of genetic counseling.

Keywords

mutation, cancer, genetics, hereditary, germline

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CHAPTER 1: INTRODUCTION

Germline mutations in tumor suppressor genes are of special importance in the medical field as they often characterize hereditary cancer syndromes. The value in understanding the gene function and phenotypic spectrum of these genes lies in the ability to prevent cancer and improve prognoses in known high-risk families by way of additional preventative testing, early detection, and targeted therapy. Germline mutation in *BRCA1-associated protein-1* (*BAP1*) underlies the recently identified tumor predisposition syndrome (*BAP1*-TPDS) OMIM 614327 (1). The major detective work originally identifying this hereditary cancer syndrome came simultaneously from three independent research groups, focused in the different disease areas of uveal melanoma (UM), mesothelioma (MMe), cutaneous melanoma (CM) and skin lesions (2-4). Shortly after this, renal cell carcinoma (RCC) was identified as a major cancer associated with the syndrome (5). An increasing number of patients and families with germline *BAP1* mutations have been reported since.

Despite the rapidly evolving literature, a complete understanding of the gene function and phenotypic spectrum of the gene has not yet been established. Researchers have approached the study of this gene from cancer-specific perspectives, rather than a collective *BAP1* approach. Rather than studying the spectrum of cancers associated with *BAP1*, researchers have reported chance findings of *BAP1* mutations in their field of study. As such, the results of this research are often difficult to use by clinicians. Geneticists and genetic counselors may not be on the lookout for the *BAP1* gene when faced with characteristic *BAP1* families. Further, providers may not have the information necessary to make management recommendations to families when faced with a chance finding of *BAP1* mutation. The aim of this review is to compile all reported research into the *BAP1* tumor predisposition syndrome and summarize the current evidence for the phenotype for the syndrome in addition to other important clinical characteristics. This will

help to establish counseling, testing, and management guidelines. statistics. Since there were a larger number of females reported in the literature, gender distribution statistics were adjusted based on a weighting methodology with the following equation: ((proportion of gender with cancer type in general population) / (proportion of gender in this cohort)) * (proportion of gender with cancer type out of total patients with cancer type in *BAP1* carriers).

CHAPTER 2: METHODOLOGY

A literature review was conducted on all peer-reviewed articles on *BAP1* and its Drosophila homolog, *Calypso* published through January 1, 2015. A search on PubMed was directed with the keywords "BRCA1 associated protein-1," "BAP1," and "Calypso." Unpublished material was not included and selected articles were limited to English language. Seventy seven articles pertaining to the human *BAP1* gene and its association with cancer were obtained. Of these, 25 articles described patients with germline *BAP1* mutations. The articles were collated and data were extracted via an article-by-article systematic review. Online supplemental material was consulted if available. Data extracted from the review included clinical information, molecular testing results, and method of molecular analysis. All reported mutations were reviewed and updated to the current standard nomenclature. All data were analyzed and calculated by the authors to produce relevant statistics.

CHAPTER 3: RESULTS

3.1 Molecular Function of *BAP1*

BAP1 was originally found to be a deubiquitinating protein, specifically in the carboxy-terminus hydrolase subfamily, in 1998 (6). Ubiquitin is a small protein that has been found in almost all tissue types (ubiquitously) attached to proteins as a post-translational modification to mark them for degradation and/or suppress their expression. Deubiquitinases remove ubiquitin

via hydrolysis to return expression of these proteins to normal levels. When tumor suppressors are ubiquitinated their expression levels decrease, often-increasing cell proliferation and decreasing apoptosis rates, among other oncogenetic activities. Therefore, depending on their targets, deubiquitinases can act as tumor suppressors themselves. Although, earlier reports speculated that the *BAP1* tumor suppressor function was through its deubiquitinating activity upon BRCA1, this was refuted and later studies have indicated that it is an independent tumor suppressor (7). Rather, BAP1 was found to interact with the BRCA1/BARD1 tumor suppressor heterodimer via the BRCA1 RING finger domain to regulate DNA damage response and cell cycle activities (8).

BAP1 has also been shown to be a tumor suppressor independently. Nuclear-localized BAP1 is upregulated and inhibits cell proliferation in BRCA1-deficient cells (7). RNAi for *BAP1* results in cell proliferation, supporting that *BAP1* acts as a tumor suppressor in an independent fashion as well (9). Further, studies show that BAP1 acts as a coactivator of transcription by forming complexes with Host Cell Factor 1 (HCFC1) and Yin Yang 1 (YY1), among other coactivators including *OGT*, and *FOXK1/2* (10). *HCFC1* is known to advance the cell cycle at the G1/S phase by forming histone-modifying complexes. Given *BAP1'*s association with *HCF1*, it was suggested that *BAP1* functions as a cell cycle regulator as well, specifically as a cell proliferation activator (11). A similar role for *BAP1* was found with *ASXL1*; forming the Polycomb repressive deubiquitinase (PR-DUB) complex, involved in removing ubiquitin from H2A histones (12). This implies a wider and more nuanced role for *BAP1* in cancer. Further, this suggests that *BAP1* is itself regulated, possibly by a master regulator. BAP1 undergoes autodeubiquitination to avoid sequestration in the cytoplasm and enter the nucleus to regulate other genes, demonstrating that *BAP1* regulates its own expression as well (13).

BAP1 has also been implicated to function in DNA damage repair as it is phosphorylated and unbound from chromatin following UV-induced DNA damage and replicative stress (14). Eletr et al. implied three possible scenarios in its function. The first scenario involved replication or repair machinery being allowed access to DNA to fix the damage. The second scenario involved BAP1 activating transcription of DNA repair genes. Both of these scenarios repair DNA damage to prevent tumor formation. Lastly, it was also thought that BAP1 may be involved in inducing apoptotic signals in cells with severe DNA damage. This allows cells with heavy DNA damage that are susceptible to oncogenesis to undergo cell death before tumor formation. All of these scenarios show *BAP1*'s crucial role in cancer initiation or progression. BAP1 was found to be involved in DNA double stranded break repair in chicken DT40 cell lines through homologous recombination (15). Recently, germline mutations in *BAP1* have been found to result in DNA double stranded break repair deficiencies (16). Thus *BAP1* appears to play a vital and very broad role in cell proliferation and tumor suppression.

3.2 Clinical Findings Reported in Families with Germline *BAP1* Mutations

While the full phenotype of *BAP1* tumor predisposition syndrome has not been fully characterized, increased awareness and study involving the gene has resulted in new data. There have been a total of 51 families with 167 individuals reported to carry *BAP1* mutations, found either by genetic testing or through obligate carrier status (see references below) (Table 1). Of the mutation carriers, 66 are male (40%), 93 are female (56%), and no gender information was reported for 8 patients. The data from reported cases suggests that hereditary cancers which are likely associated with *BAP1* include UM, MMe of the pleura and peritoneum, CM, RCC, as well as characteristic benign cutaneous lesions referred to herein as atypical Spitz tumors (AST). UM is the most common cancer diagnosed in patients with germline *BAP1* mutations with a total of

49 patients (29%) (2-5, 17-26). Fifty of the 51 families presented with one or more of the four main cancers (UM, MMe, CM, and RCC). In the remaining family, the proband with the truncating mutation, c. 214del, p. I72L*6, presented with AST and reported a family history of gastric cancer, but no other individual in the family was tested. It should be noted that not all reported cancers were confirmed by the authors. The Venn diagrams (Figure 1a, 1b, Figure 2) summarize the cancer histories reported in these families. While we tried to clarify the phenotype of *BAP1*-TPDS by comparing the frequency of cancers diagnosed in our cohort with the frequency of those cancers in the general population by the Surveillance, Epidemiology and End Results (SEER) database, this is an imperfect comparison as our cohort consists of patients reported throughout the world while SEER only tracks diagnoses in the United States.

Tumor studies were also commonly conducted in both germline mutation carriers and in sporadic tumors. Biallelic inactivation in germline mutation carriers was commonly reported among tumors thought to be *BAP1* related and is a strong indication that a particular tumor may have been caused by a gene mutation. Further, protein studies on tumors in mutation carriers also indicate lack of BAP1 protein expression, indicating loss of the wild-type allele. This also fits Knudson's two hit model of tumor suppressors. Somatic *BAP1* mutations and lack of BAP1 protein expression have also been observed in sporadic tumors and implicate *BAP1* involvement in the tumor.

3.3 Common *BAP1*-TPDS Tumors

3.31 Uveal Melanoma

UM, the most common ocular malignancy in adults, has been shown to be associated with both germline and somatic *BAP1* mutations (Table 2). Forty nine (29%) of reported *BAP1* carriers have had a diagnosis of UM (2-5, 17-30). Twenty two (45%) were male, 25 (51%) were

female, and 2 patients did not have gender information reported. The incidence of UM in patients with germline *BAP1* is much higher than the 5.1 diagnoses per million people in the general population, suggesting UM is a common feature of *BAP1* (31).

The earliest age of onset in a *BAP1* carrier is reported in a UM at age 16 and there have been a total of 4 UMs diagnosed by age 20 (19, 21, 24, 28). In fact, the median age of onset is earlier in UM patients with germline *BAP1* mutation (51 years, range 16 – 72) compared with the general population (62 years), suggesting that germline *BAP1* mutations predispose to the cancer (32)

Beyond epidemiologic data, there is good molecular evidence that UM is associated with the *BAP1*-TPDS. Genetic analysis of UM tumor tissue (DNA sequencing, microsatellite markers, or SNP analysis) has been performed in at least 7 patients with germline *BAP1* mutations. This work showed loss-of-heterozygosity (LOH) or loss of expression of the wild type allele, adding further support for the inclusion of UM in the phenotypic spectrum of the *BAP1*-TPDS (2, 4, 17, 19, 21, 24).

Somatic mutations of *BAP1* have also been widely reported in UM (Table 3). Seven out of 15 (47%) UM tumors in mouse models were found to have somatic mutations through DNA sequencing (33). Small interfering RNA (siRNA) knockout of *BAP1* in 3 human UM cell lines induced a stem-cell like phenotype in melanoma cells and decreased cell proliferation, suggesting that *BAP1* functions in a manner uncharacteristic of traditional tumor suppressors (34). Further, 153 out of 725 (21%) human UM tumors tested via tumor sequencing showed somatic mutations in *BAP1* (4, 17, 35-39). Koopmans et al. showed these somatic mutations were also correlated with a lack of BAP1 protein expression in the tumor as 30 of 35 (86%) tumors with somatic mutations also lacked BAP1 staining by immunohistochemistry (38). In

addition, a total of 73 out of 174 (42%) other UM tumors have lacked BAP1 expression upon immunohistochemistry (38, 40-42). This makes UM the most firmly established cancer type in *BAP1* tumor predisposition syndrome.

3.32 Malignant Mesothelioma

MMe can be diagnosed in multiple sites, most commonly the pleural lining of the lung, but also in the peritoneum. Both sites have been implicated in germline *BAP1* carriers. A total of 39 patients (23%) with germline mutations have been diagnosed with MMe (2-5, 19, 22, 26, 29, 30, 43, 44). Twenty six patients (67%) were reported to have pleural MMe, 12 patients (31%) had peritoneal MMe, and 1 patient had diagnoses of both pleural and peritoneal MMe. Twelve (31%) of these patients are male and 27 (69%) patients are female. Interestingly, all 13 patients reported to have diagnoses of peritoneal MMe are female, while peritoneal MMe is slightly more commonly diagnosed in males (45).

Another finding in this subset of patients is the high frequency of multiple cancers. Excluding cases of multiple MMe, 14 patients (36%) have been diagnosed with another primary cancer in addition to MMe. Of the second cancers, the co-occurrence of UM and MMe is a strong indicator of the *BAP1*-TPDS. The diagnosis of UM occurred in 5/39 (13%) of the MMe patients with a germline *BAP1* mutation. In addition, Testa et al. found only 2 of 26 tested sporadic MMe patients with asbestos exposure carried germline *BAP1* mutations. Upon further analysis it was determined those were the only two patients in the cohort with prior diagnoses of UM (3).

Similar to the earlier onset of UM, the median age of diagnosis for MMe among the germline *BAP1* mutation carriers was 56 (range 34 – 85) years, which is much earlier than onset in the general population (74 years) (46).

Studies on MMe tumor tissue from patients with germline *BAP1* mutation add further support for the cancer type to be included in the spectrum of cancers associated with *BAP1* mutations. MMe tumors from 7 germline *BAP1* mutation carriers have been shown to have loss of the wild type *BAP1* allele or its expression as seen via tumor DNA sequencing, array-comparative genomic hybridization, and/or absent staining for BAP1 via immunohistochemistry (3, 44).

Asbestos exposure is a strong predisposing factor for MMe development in the general population, and an interaction with germline *BAP1* mutation might result in an additive or synergistic effect. Testa et al. detected asbestos traces in homes of all affected family members in both of the families they report with germline mutations (3). Interestingly, Arzt et al. found no statistically significant effect of asbestos exposure on BAP1 protein expression and any potential mechanism of how asbestos and BAP1 may interact is unknown (47).

Somatic *BAP1* mutations have also been reported in presumably sporadic MMe (Table 3). A total of 162 of 406 (40%) MMe tumors were found to have *BAP1* mutations via tumor DNA sequencing (3, 48-52). Of these, 14 tumors (5%) had biallelic *BAP1* mutations (49). In addition, 156 of 314 (50%) MMe cell lines or tumor tissues lacked BAP1 expression by immunohistochemistry (3, 43, 47-49, 52, 53). Sequencing of MMe cell lines and fluorescence in-situ hybridization (FISH) analysis showed somatic mutations in *BAP1* in 10 of 30 (33%) lines and loss of *BAP1* in 6 of 25 (24%) lines tested, respectively (48, 49).

3.33 Cutaneous Melanoma

CM is a common skin malignancy in the general population. Although *CDKN2A* mutations are a common factor in familial CM, *BAP1* is also a likely contributor to hereditary CM. There are 23 (14%) reported patients with CM diagnoses out of a total of 167 patients with

germline *BAP1* mutations (2, 4, 5, 18, 21). Thirteen (57%) reported patients are male and 10 (43%) are female. The median age of diagnosis in *BAP1* patients is 46 (range 25 – 72) years, much earlier than in the general population which is estimated at a median of 58 years (54). Notably, five (22%) of these patients have had multiple diagnoses of CM with a maximum of 7 melanomas in a single patient (5, 18, 19). Further, 11 (48%) have had CM diagnosed in addition to another cancer (4, 5, 18, 29, 30, 44). Tumors from 4 of these patients show LOH and/or loss of expression of BAP1 via DNA sequencing, array-based comparative genomic hybridization, and negative staining by immunohistochemistry (4, 18, 44). Njauw et al. noted three families carrying germline mutations had diagnoses of nevoid type CM, a particularly rare subtype. Follow-up of these tumors revealed they were characterized by distinctly semitransluscent orange-red pigmentation and had high levels of Ki67 staining. These lesions were distinct from traditional CMs and may be related to AST (18).

Somatic mutations in CM have also been noted in patients without germline mutation, indicating *BAP1* may be involved in the pathogenesis of the malignancy (Table 3). A total of 3 of 60 (5%) tumors were found to have somatic *BAP1* mutations and 11 of 238 (5%) tumors were found to lack BAP1 staining by immunohistochemistry (4, 55, 56). This data indicates *BAP1* may be involved in the pathogenesis of the malignancy.

3.34 Renal Cell Carcinoma

Clear cell RCC is the most common primary malignancy of the kidney in the general population. Recently strong data suggesting a correlation between RCC and *BAP1* has been published (Table 2). Seventeen (10%) *BAP1* patients have been reported with RCC out of 167 total patients with germline *BAP1* mutations (3, 5, 18, 26, 57, 58). Seven (50%) reported patients are male, 7 (50%) female, and 3 reported cases did not include gender. The median age of onset

for RCC among *BAP1* carriers is 47 (range 36-72) years, which is much earlier than in the general population, (64 years) (54).

Tumor tissue studies in these patients also support the inclusion of RCC in the spectrum of tumors caused by *BAP1*. A total of six tumors in five of these germline patients were tested via SNP arrays, tumor DNA sequencing, and/or immunohistochemistry and all were found to have LOH or loss of protein expression in the tumor (5, 57).

Somatic studies on RCC tumors also support an association (Table 3). A total of 249 of 2483 (10%) renal tumors studied by tumor DNA sequencing or whole exome sequencing were found to carry somatic *BAP1* mutations, while 273 out of 2343 (12%) tumors studied by immunohistochemistry had no BAP1 expression

3.35 Cutaneous Melanocytic Lesions

BAP1-TPDS is associated with a distinct subset of benign skin lesions located on the skin of the head and neck, trunk and limbs. There has been a range of names given to these, including melanocytic *BAP1*-mutated atypical intradermal tumors (MBAITs), AST and nevi, Wiesner nevi, and nevoid melanoma-like melanocytic proliferations (NEMMPs), but will be referred to herein as AST as these lesions fit closest clinically and pathologically to this designation, though they constitute a distinct subgroup (18, 59-61). These lesions are well-circumscribed dome shaped, skin-colored or reddish-brown nodules with average size of 5 mm. They widely range in number in patients and in different family members. Morphologically, the lesions are mostly located intradermal with occasional cases of involvement of the junctional epidermis and show cytological features resembling atypical Spitz nevi (62). These lesions are characterized by biallelic inactivation of *BAP1* and frequent $BRAF^{V600E}$ mutation and both can be reliable markers for aiding in the diagnosis (61).

The prevalence of AST in *BAP1*-TPDS is unclear as in the majority of reported patients, these lesions were not carefully assessed. Out of 43 germline *BAP1* patients where these lesions were assessed there have been a total of 31 patients (72%) with AST (4, 18, 19, 21, 25, 27, 30, 44, 59). At least 11/31 (35%) of these patients had multiple lesions, with a range of 2 to more than 50 (4, 5, 18, 27, 29, 44). The median age of diagnosis of AST in germline *BAP1* carriers was 42 years old. However, the median age of onset may actually be much younger due to the difficulty of diagnosis. Although their natural history is unknown, Busam et al. found AST to be present since childhood in one germline *BAP1* mutation carrier (27, 63). Fourteen/31 (45%) of these patients have been diagnosed with cancer in addition to AST (4, 5, 18, 21, 29, 30, 44). Loss-of heterozygosity in the tumor was confirmed in 22 neoplasms in 3 germline mutation carriers from one family (4).

Lesions with similar morphological and molecular alterations were also reported in patients with no germline *BAP1* mutation (4, 56, 61, 63-67). Given the unique clinical, morphological and molecular characterization of these subtype of AST and the high frequency of these lesions in patients with germline *BAP1* mutation it is highly recommended that ASTs in particular those with a prominent epithelioid component be screened for BAP1 status by immunohistochemistry. If BAP1 loss is detected, referral for genetic counseling and germline BAP1 testing should be considered (55).

3.4 Uncommon *BAP1*-TPDS Tumors

3.41 Basal Cell Carcinoma

Eleven germline *BAP1* mutation carriers from seven unrelated families had BCC (7%) (Table 2) (3, 24, 29, 30). Seven of these 11 (64%) patients presented with more than one tumor, with one patient presenting with 13 tumors. Immunostaining revealed complete or partial loss of

BAP1 protein in all of the 19 tested tumors from two patients with germline *BAP1* mutations but in none of the 22 tumors from individuals with no germline *BAP1* mutation (29). Thus, although there is a high incidence of BCC in the general population and the strong association with sun exposure makes it difficult to assess a possible association, the biallelic inactivation of *BAP1* in BCC tumors from a subset of patients with germline *BAP1* mutation suggests that BCC may be a feature of the *BAP1*-TPDS phenotype.

3.42 Breast Cancer

BAP1's role in hereditary breast cancer has been suspected, given its interaction with the breast cancer tumor suppressor *BRCA1*. However, data are somewhat conflicting as to whether breast cancer is part of the *BAP1* tumor predisposition syndrome (Table 1). There are a total of 9/93 (10%) of the female patients with *BAP1* germline mutations with diagnoses of breast cancer, including a newly tested member of a previously reported family, (FUM104, IV.1) (3, 5, 18, 19, 26, 30). One of these patients has had bilateral breast cancer (5). This is slightly lower than the approximately 12.3% risk of developing breast cancer for women in the general population (54). However, since this is compared to a quoted lifetime risk, the proportion of *BAP1* carriers with breast cancer may also grow as germline *BAP1* mutation carriers from our cohort may very well develop breast cancer in the future. The median age of onset based on ages reported for 5 of these patients is 58 years (range 37 – 85), while 2 of these patients (including the patient with bilateral breast cancer) are only reported as "early onset". Molecular studies on tumor tissue were performed on two of these patients using tumor DNA sequencing and immunohistochemistry. These tests showed loss of the wild type allele and loss of BAP1 staining indicating biallelic inactivation (5). However, germline *BAP1* mutations are not common in breast cancer despite family histories consistent with a TPDS. For example, studies have shown

only two synonymous, non-truncating germline *BAP1* variants and no truncating mutations in a total of 330 breast cancer patients with high predisposition to breast cancer, including 143 patients who tested negative for mutations in the traditional breast cancer predisposition genes, *BRCA1* and *2* (5, 68, 69). Somatic studies have also shown a lack of association between *BAP1* and breast cancer (70) (Table 3). Je et al. found no somatic *BAP1* mutations in breast cancers from 45 Korean patients without germline mutations. Thus, while rates of breast cancer may be elevated in patients with germline *BAP1* mutation, the finding of *BAP1* mutation in breast cancer is rare and more research must be conducted to clarify the association of *BAP1* mutation and breast cancer. It should be noted that *BAP1* has been recently added to some of the multigene panels offered by several clinical laboratories including those for breast cancers.

3.43 Lung Carcinoma

There have been 6 reported cases (4%) of lung adenocarcinoma in germline *BAP1* mutation carriers and no reports of small cell or squamous cell carcinoma (2, 18, 19, 24). There were no reports on the patients' smoking status. Tumor testing revealed LOH in one of these tumors as well as lack of BAP1 staining by immunohistochemistry (2). Somatic studies of *BAP1* in unselected lung cancer found a low mutation rate as Jensen et al. found 1 somatic mutation of 44 (2%) small-cell lung cancer tissues tested, and 1 somatic mutation of 33 (3%) non-small cell lung cancers tested (6). However, lung cancer cell lines in mice were found to have an increase in tumorigenicity after *BAP1* knockout (7). Further, immunostaining showed a high rate of *BAP1* loss in lung adenocarcinoma compared with squamous cell carcinoma (78% vs 46%) and there was a significant association between *BAP1* loss and histological type as well as tumor aggressiveness (71). This suggests that in lung cancer, mechanisms other than direct gene

mutation play a crucial role in BAP1 protein loss. Further studies are needed to assess the possible association of *BAP1*-TPDS with lung adenocarcinoma.

3.44 Cholangiocarcinoma

Cholangiocarcinoma is a rare, particularly aggressive form of cancer and evidence for its association with *BAP1* is growing (Table 2). There have been only four patients (2%) with germline mutations diagnosed with cholangiocarcinoma (18, 25, 26, 30). However, low survival rates may mean patients diagnosed with cholangiocarcinoma are not tested, possibly deflating carrier statistics. Unfortunately, tumor studies were only done for one of these patients, which showed that the metastatic cholangiocarcinoma retained the wild type allele as seen by tumor DNA sequencing, but the tumor showed loss of nuclear localization of BAP1 (26). Among presumably sporadic patients, there have been a total of 32 cholangiocarcinoma tumors that were found to have somatic *BAP1* mutations out of a total of 283 tumors studied (11%) via tumor DNA and Next-Generation Sequencing (72-74). This may indicate that *BAP1* is involved in the tumorigenesis of cholangiocarcinoma (72-76) (Table 3).

3.45 Meningioma

Two patients presenting with meningioma were found to carry *BAP1* mutations and for one of these patients a second-degree relative also had a meningioma, but was not tested for *BAP1* (2, 29). Tumor studies were conducted for one of these patients and biallelic inactivation of *BAP1* was confirmed through lack of BAP1 staining via immunohistochemistry. Though malignant meningiomas occur infrequently, pathological examination of a metastatic tumor from the other patient with a germline *BAP1* mutation suggested that the primary tumor was a papillary meningioma (30).

3.46 Other Tumors

A number of other tumor types that have been reported in germline *BAP1* mutation carriers; however there is a limited amount of data supporting their inclusion in *BAP1*-TPDS and continued research is needed (Table 1).

There have been 3 reported diagnoses (2%) of non-specific abdominal carcinomas among germline *BAP1* mutation carriers, one of which was felt to be an ovarian cancer by the authors (2, 24, 26). Three patients (3% of females) with germline *BAP1* mutations have been diagnosed with ovarian cancer, including a newly tested patient from a family previously reported by our group (FUM104, III.8) (2, 3, 26). There have been single patients with germline *BAP1* mutations with diagnoses of cervical cancer, unspecified pancreatic cancer, squamous cell carcinoma, unspecified thyroid cancer, and urothelial (transitional cell) carcinoma; however no tumor tissue was available to confirm *BAP1* involvement (3, 5, 25). The mutation carrier diagnosed with urothelial (transitional cell) carcinoma was also diagnosed with UM with liver metastasis (25). There have been a few germline mutation carriers diagnosed with a range of sarcomas including leiomyosarcoma, malignant fibrous histiocytoma, spinal bone cancer, and spindle cell type soft tissue sarcoma (3, 19, 26). No tumor studies were conducted on the histiocytoma; however metastatic tissue from the same patient did not show somatic loss of the wild type allele in the tissue (19). Two germline mutation carriers presented with neuroendocrine cancers, including paraganglioma of the pericardium. Tumor studies from the paraganglioma tissue showed LOH via tumor DNA Sanger sequencing (19).

One patient with a germline *BAP1* mutation was diagnosed with a colorectal cancer, but no tumor was available for study (26). Somatic studies of *BAP1* have also been done on unselected colorectal cancer cases, with inconsistent results: 1/45 tumors showed a somatic

mutation while 5/252 tumors showed loss of BAP1 staining and 127/260 tumors showed low staining (70, 77).

There has been one patient with a germline *BAP1* mutation that was diagnosed with a prostate cancer, though no tumor testing was done (21). In a cohort of Korean patients, Je et al. found no somatic *BAP1* mutations in 45 prostate tumors using tumor DNA sequencing (70).

Many of these tumor types occur frequently in the general population and it is possible they arose coincidentally in germline *BAP1* mutation carriers. As such it is difficult to be certain these cancers are a feature of the *BAP1* phenotype.

3.5 Germline BAP1 Mutation and Tumor Aggressiveness

There is evidence that patients with germline *BAP1* mutations tend to have more aggressive cancers with higher tumor staging and a greater likelihood of metastasis. Germline mutation results in higher rates of metastasis in UM, especially to the liver, with Njauw et al. finding 4 of 50 metastatic UM patients carried germline mutations as compared to 0 of 50 in a non-metastatic cohort (18).

Tumors with somatic *BAP1* mutations have also demonstrated larger tumor sizes, more aggressive disease with poorer oncological outcomes, and higher rates of metastasis (58, 78-80). Somatic *BAP1* mutations are observed more frequently in the more aggressive “class 2” UM tumors (84%) as compared with the less aggressive “class 1” tumors (4%) (17). It was suggested that the single “class 1” tumor with a *BAP1* somatic mutation in this study was in transition to becoming a “class 2” tumor, implicating *BAP1* mutation as a precursor event to “class 2” tumor status. The five “class 2” tumors not found to have somatic mutations had low levels of *BAP1* mRNA expression, suggesting epigenetic inactivation. Somatic *BAP1* mutations may also define a separate class of MMe tumors. de Reynies et al. found that somatic *BAP1* mutations in MMe

were strongly connected with a new subset of epithelioid MMe defined as “class 1” by molecular profile, which showed better prognoses and higher mutation rates than “class 2” MMe. In their cohort of 104 tumors, 87% of class 1 MMe harbored somatic *BAP1* mutations whereas only 37% of class 2 MMe had *BAP1* mutations (50). These findings stand in contrast to UM, tumors in which the more aggressive tumors, designated “class 2” by gene expression profile had high frequency of somatic *BAP1* mutation, epithelioid cell type, and worse disease prognosis. Interestingly, *BAP1* mutations are characteristic of epithelioid morphology in both UM and MMe. *BAP1* mutations seem to characterize different classes of tumors in CM as well. Murali et al. found that absent staining for BAP1 by immunohistochemistry was seen more frequently in desmoplastic melanomas (22%) than in other CM subtypes (3%) (55). It is possible that *BAP1* loss is a feature inherent to desmoplastic melanomas specifically (81). Similar findings were reported in RCC as Pena-Llopis et al. showed RCC tissues rarely harbored coexisting somatic *BAP1* and *PBRM1* mutations, but rather there was strong histological distinction in the tumors depending on the mutant gene (82). RCC tumor staging in patients with germline *BAP1* mutations was variable: 3 tumors were Fuhrman grade III, 4 tumors were grade II, 3 tumors were grade I-II, and 1 was grade I (5, 57).Characterization of separate classes of UM, MMe, CM and RCC tumors by mutational profile could provide valuable diagnostic and prognostic indicators for clinicians.

Studies with regard to survival have also pointed to shorter survival in patients with tumors exhibiting somatic *BAP1* mutations or lack of BAP1 expression. Tissue microarray (TMA) studies of primary UM showed mean survival in patients that did not have BAP1 protein expression was 4.74 years as compared to 9.97 years in patients with UM tumors with BAP1 protein expression (41). In CM, tumors with low BAP1 expression tends to have poorer

outcomes; however BAP1 has also been seen to play a survival role in melanoma cells as BAP1 depletion reduces proliferation and increases apoptosis rates while resuming metabolism of survivin (81). Similarly, Hakimi et al. found an average of 31.2 months survival in RCC patients with somatic *BAP1* mutations as compared to 78.2 months survival in patients whose tumor tested wild type for *BAP1* in a cohort of 421 patients (83). Kapur et al. determined similar survival rates with an average of 1.9 years survival in RCC patients with tumors with somatic *BAP1* mutations versus 5.4 years survival in patients with tumors with *PBRM1* mutations in a cohort of 327 patients (84). A second, smaller cohort of 145 patients in the study showed 4.6 years survival in *BAP1* mutant tumors versus 10.6 years survival in *PBRM1* mutant tumors.

In contrast to the data that germline *BAP1* mutation portends a worse prognosis in UM, CM, and RCC, survival in patients with germline *BAP1* mutations developing MMe suggest that these patients have longer overall survival as compared with sporadic MMe patients. Baumann et al. found 7-fold longer survival in a cohort of 23 mesothelioma patients with germline *BAP1* mutations. Patients with germline *BAP1* mutation and peritoneal mesothelioma also exhibited improved survival as compared to patients with pleural mesothelioma (85). Long term survival and well-differentiated tumor histology were also observed in one of our patients with MMe (FUM064, III.12). Similar findings were reported in two patients from another family. The mutations reported in the two families with well-differentiated MMe were truncating, c. 758_759insA, p. Gln253fs*31 and c. 2050C>T, p. Gln684*. The c. 2050C>T, p. Gln684* mutation was also carried by several other family members with aggressive cancers including UM, bone cancer, and abdominal cancer. The molecular mechanism of such variation in aggressiveness between MMe and other cancers such as UM, CM, and RCC is not clear. It has been suggested that these cancer cells are more susceptible to therapy.

3.6 Penetrance of *BAP1*-TPDS

Current evidence suggests that the penetrance of *BAP1* mutations is high with 153/167 (92%) germline BAP1 mutation carriers affected with cancer (5, 18, 19, 21, 26). Although ages for unaffected individuals were not reported, estimates based on the pedigrees placed an average unaffected age in the late 50's. However, it should be noted that several reported unaffected carriers are young and therefore may develop cancers in the future. One *BAP1* patient (FUM036, III.9) previously reported to be unaffected by our group subsequently developed MMe at age 60 and passed away (2). Additionally, two newly tested members of families previously reported by our group were found to carry the family mutations and remain unaffected at ages 47 and 35, respectively (FUM103, III.3; FUM152, III.2) (26, 28).

It is likely, however, that these penetrance data are inflated by test bias since the patients and family members who get tested are generally those affected by cancer. Out of the 51 reported families, in 25 only the affected proband was tested, and only 17 families had more than 3 individuals tested. This selection bias inflates penetrance data and the true penetrance for *BAP1* mutations may well lie lower than that calculated from current reported cases.

The average age of cancer onset in *BAP1* mutation carriers is 50 years with a range of 16 to 85 years. The earliest reported age was for UM (21). Benign cutaneous lesions, which are characterized by somatic *BAP1* loss, were present since childhood in at least one patient (27, 63). The natural history of these lesions is unknown, however. Aside from MMe, the other BAP1 tumors all exhibit a significantly earlier age of onset as compared to tumors arising in the general population, supporting *BAP1*'s role in cancer predisposition (Table 2).

3.7 Genotype-Phenotype Correlation

Out of 51 families, 44 had unique mutations, two families had the c. 2050C>T, p. Gln684* mutation, 2 families had the c. 1882_1885delTCAC, p.Ser628Profs*8 mutation, while three families had the c. 178C>T, p. Arg60*mutation. Discussions between the authors concluded the families carrying the c. 2050C>T, p. Gln684*and c. 1882_1885delTCAC, p. Ser628Profs*8 mutations were unrelated. Discussions between the authors and further testing suggested that the two families from Denmark carrying the c. 178C>T, p. Arg60*mutation were related, whereas the family carrying the mutation from the United States was not (3, 18, 26, 30). This could indicate a possible founder mutation or mutational hotspots.

The vast majority (34/48, 71%) of the reported mutations were truncating, 9 (19%) were missense mutations thought to be pathogenic, and 8 (17%) were splice-site variations. Five of the splice site variations also caused protein truncation. Thus 18% of the reported germline mutations are missense mutations and 76% cause protein truncation. All the truncating mutations were proximal to the location of the nuclear localizing regions of BAP1.

With the exception of one patient, all reported pathogenic mutations in *BAP1*, including truncating, missense, and splice site mutations, were associated with at least one of the four cancers, UM, CM, RCC, and/or MMe, in the family (Figure 3). All four cancers were observed with all different classes of mutations. Taken together, the available data suggest no clear genotype-phenotype correlation between type or location of the mutation and the type of cancer in the patients.

Aoude et al. reported an interesting family with seven individuals with UM and a splice site variant in *BAP1*, c.581-2A>G. The variant was predicted to cause splicing out of exon 8 leading to a premature truncation, but the observed splice product was smaller than would be predicted. In addition, one of the four UM patients who were tested was negative for the variant.

Although the authors concluded that the *BAP1* c.581-2A>G variant was deleterious and the non-carrier member was a sporadic case of UM occurring within a hereditary family, it should be noted that a genome wide linkage analysis of the same family found linkage to chromosomal region 9q21 suggesting the existence of another candidate gene in that family (86). Hence, another plausible explanation of their observation is that the c.581-2A>G variant was unrelated or modulates the effect of an undiscovered gene predisposing to cancer in this family.

CHAPTER 4: DISCUSSION

4.1 Genetic Counseling and Patient Management

Since 125 of 167 (75%) reported patients with *BAP1* mutations were diagnosed with UM, MMe, CM, RCC, and/or BAP1 deficient atypical Spitz tumors, and 93% of these families had at least two of these tumors in first or second degree relatives, we feel that genetic assessment and testing for *BAP1* mutations should be considered in patients with personal or family histories of two or more of these tumors in first or second degree relatives (with the exclusion of families with only multiple CM cases). Though a recent article recommended *BAP1* testing in families with 2 or more cases of CM diagnosed before age 75 in first or second degree relatives, we feel this is overly broad given the high frequency of CM in the general population and the low likelihood that these families carry *BAP1* mutations (87). Further, Harbour et al. suggested testing guidelines be extended to include family histories of one UM and two or more primary cancers of any type (88). Once again, we feel this recommendation is overly broad as there is not sufficient evidence at this time supporting the involvement of other cancers of any type. Only 1 out of 44 *BAP1* mutation families with reports of family history presented with UM and other cancers that weren't the common *BAP1* tumors. Since only one family presented with only 2

cases of CM and no history of the other *BAP1* cancers, 91% of reported families would have been detected with our suggested criteria.

Families in which a germline *BAP1* mutation is found should receive counseling regarding cancer risk management options and risks to family members. Testing of at-risk family members is indicated since the syndrome follows an autosomal dominant inheritance pattern and first degree relatives have a 50% risk of inheriting the mutation. Although evidence-based management recommendations have not been established, the cancer risks in these families cannot be ignored. As such, regular examinations to facilitate early diagnosis, and thereby improved prognosis, are necessary. Although the recommended ages to begin screening given below are based on currently reported ages of diagnoses, if an individual family has a member diagnosed at an even earlier age for a specific cancer, screening for that cancer should begin for other members of that family about five years before that age of diagnosis.

Yearly ophthalmic screenings with dilated examination and ophthalmic imaging are recommended due to the high risk for development of UM. As the earliest reported case of UM in a *BAP1* mutation carrier was diagnosed at age 16, we suggest that these ophthalmic screenings begin at age 11 and should be performed by an ocular oncologist. Any nevi detected should be monitored with imaging at least every 6 months and considered for early treatment, given the association of *BAP1* mutation with elevated metastatic risk in UM. For patients with UM and germline *BAP1* mutation, we recommend screening for metastasis similar to what is currently recommended for high-risk class 2 patients. This includes screening every 3-6 months with liver-directed imaging (e.g., abdominal ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI) which is most sensitive) since metastatic spread from UM is most

frequent to the liver. Additionally, we recommend pulmonary imaging by CT or chest X-ray every 6-12 months to detect pulmonary metastasis.

Unfortunately, screening for MMe is difficult. According to the National Comprehensive Cancer Network (NCCN) guidelines, there is no data indicating that screening improves survival in MMe patients with asbestos exposure (89). Currently, it is unknown whether screening could impact patients with germline *BAP1* mutation, particularly since nearly 1/4 of identified germline carriers developed MMe and since they may have lower grade disease. Interestingly, Faig et al. report that survival is improving for peritoneal MMe, and possibly for pleural MMe patients to a lesser extent, and suggest that newer therapies play a role in this improvement (90).

The radiological features of MMe include peritoneal/pleural thickening and effusion. Importantly, biomarker tests are in development to improve early detection for MMe (91, 92).

Thus, although this field is in flux, we recommend that the risk for developing MMe should be discussed with patients so they are on the lookout for symptoms and they should have a yearly physical examination whether they have symptoms or not. It has been suggested that MRI exams can aid in diagnosing peritoneal MMe and it is possible that patients followed for our RCC screening guidelines can also be screened for any peritoneal MMe development simultaneously (93). CT and chest X-rays have also been used in screening programs for MMe, but as a word of caution, given the potential role of *BAP1* in DNA repair pathways, frequent radiation-based imaging modalities should be avoided when possible in these patients (15, 16).

BAP1 mutation carriers are at increased risk for the development of both CM as well as atypical melanocytic nevi. Thus we recommend yearly full body dermatological screenings beginning at age 20, which is five years before the earliest reported case of CM in a *BAP1* mutation carrier. This parallels recommendations for carriers of germline mutations in the

CDKN2A gene made by The International Melanoma Genetics Consortium. Carriers are also instructed to conduct self-skin examinations following the ABCDE characteristics of melanoma and to use sun protection (94, 95).

Atypical Spitz tumors with BAP1 loss are characteristic in families with germline *BAP1* mutations and can be used as a diagnostic marker. As diagnosis of the AST found in *BAP1*-TPDS cannot be made through clinical features alone, patients found to harbor atypical Spitz tumors upon dermatological screenings should receive immunohistochemical staining for BAP1 and *BRAF* mutation testing through pathological examination, especially if the lesions carry a prominent epithelial component (55). Patients with atypical Spitz tumors with BAP1 loss should be referred for genetic counseling and germline *BAP1* testing.

Consensus screening recommendations for RCC in at-risk patients in the general population have not been established. However, germline mutations in the *VHL* gene have been found to contribute risk for development of RCC in patients with von Hippel-Lindau (VHL) disease. To manage risk for development of RCC in VHL, it has been recommended that patients undergo yearly abdominal ultrasound examinations as well as abdominal magnetic resonance imaging (MRI) every 2 years (96). Until further data are available to direct management, we recommend considering this screening protocol for patients with *BAP1* mutations. Since the earliest reported RCC in *BAP1*-TPDS patients was at age 36 years we recommend starting the screening at age 31 years.

4.2 Potential Adjuvant Therapy

Since *BAP1* research is still in its early stages and clinical applications are only recently being explored, there are currently no FDA-approved targeted treatments for *BAP1*-driven malignancies. Intriguingly, however, preliminary attempts at discovering therapies have shown

modest success in UM. Landreville et al. performed *in silico* and *in vitro* screens for treating class 2 UM with *BAP1* loss and found histone deacetylase (HDAC) inhibitors to have anti-tumor activity. Specifically, *BAP1*-depleted UM cell lines were found to halt proliferation and tumor growth when exposed to the HDAC inhibitors valproic acid (VPA), trichostatin A (TSA), and LBH-589. TSA and LBH-589 also increased apoptosis rates, while VPA-treated cells returned to a class 1 (less aggressive) gene expression profile, regaining melanocytic differentiation (97). Tumors that may respond to such therapy may be recognized through detection of diminished BAP1 protein or RNA levels in tumor tissue. Currently a phase II clinical trial (NCT01587352) is on-going utilizing vorinostat in treating patients with metastatic UM. It will be interesting to see the impact of this agent in the adjuvant setting. A recent United States provisional patent submission (62/014,594, SK2014-029) by the Memorial Sloan Kettering Cancer Center aims at adapting an EZH2 inhibitor therapy, which is being used in Phase I/II trials for non-Hodgkins lymphoma patients, towards patients with *BAP1*-deficient tumors. The rationale for this use lies in BAP1's function in forming the PR-DUB complex with ASXL1 and deubiquitinating H2A. EZH2 is upregulated when H2A is aberrantly hyperubiquitinated, as is the case in *BAP1* mutation (98). EZH2 inhibitor therapy may restore EZH2 expression to normal levels. No data for the use of this therapy in *BAP1*-driven tumors have been published, however.

4.3 Conclusions

UM, MMe, CM, RCC, and atypical Spitz tumors are clearly established as part of the phenotypic spectrum associated with germline *BAP1* mutations. Although other cancers, in particular breast cancer, lung adenocarcinoma, and cholangiocarcinoma might be associated at low frequency, further studies are needed to fully define the clinical phenotype of the *BAP1*-TPDS. Although UM, CM, and RCC tumors in patients with germline *BAP1* mutations tend to

be more aggressive and have poorer oncologic outcomes, for unknown reasons patients with diagnoses of MMe tend to have longer survival. There appears to be no genotype-phenotype correlation with this syndrome, as mutations in all domains of the *BAP1* gene have been seen in patients. Penetrance for the gene appears to be high, but ascertainment bias makes it difficult currently to establish accurate estimates of cancer risk. Nonetheless, increased screening is indicated, particularly for skin and eye cancers.

CHAPTER 5: TABLES AND FIGURES

5.1 Table 1

Table 1: Personal and family cancer histories of all reported families with *BAP1* mutations.

Family/ Case	**Cancers in patients with *BAP1* mutation (Age of diagnosis)**	**Mutation**	**Mutation Classification**	**Other Reported Cancers/Features in untested family members**	**Reference**
MM087	UM (53)	c. 1318_1319insA p. Glu402fs*2	Frameshift - Truncating	N/A	Harbour et al., 2010 (17)
SP-002	MMe (55), UM, leiomyosarcoma	c. 1717delC p. Leu573Trpfs*3	Frameshift - Truncating	N/A	Testa et al., 2011 (3)
SP-008	MMe (63), UM	c. 1882_1885delTCAC p. Ser628Profs*8	Frameshift - Truncating	N/A	Testa et al., 2011 (3)
Family L	MMe (50, 59, 63), UM, Non-melanoma Skin Ca, Pancreatic Ca	c. 2050C>T p. Gln684*	Truncating	MMe, UM, Prostate	Testa et al., 2011 (3) Carbone at al., 2012 (99)
Family W	MMe (36, 44, 50, 58, 58), Ovarian Ca (59), RCC (57), Breast Ca (37)	c. 438-2 A>G p. Pro147fs*48	Splice Site – Frameshift - Truncating	None	Testa et al., 2011 (3) Carbone at al., 2012 (99)
Family 1	UM (72), CM, AST (36, 42)	c. 1305delG p. Gln436Asnfs*135	Frameshift - Truncating	AST, Cervical Ca, Multiple Myeloma	Weisner et al., 2011 (4) Weisner et al., 2012 (61)
Family 2	UM (44), CM (38, 39, 50), AST (31, 40, 62), Peritoneal MMe	c. 2057-2A>G p. Met687Glufs*28	Splice Site – Frameshift - Truncating	AST	Weisner et al, 2011 (4) Weisner et al., 2012 (61)
Family FUM036	UM (50, 52), CM (72), MMe (55, 75), Meningioma, Lung Ca (56), Neuroendocrine Ca (52), Abdominal Adenocarcinoma/Ovarian Ca? (69)	c. 799C>T p. Gln267*	Truncating	UM, CM x2, MMe x2, RCC, Meningioma, Testicular Ca, Abdominal Ca x3, Esophagus Ca, Adrenal Gland Ca, CaSU	Abdel-Rahman et al., 2011 (2)

2734	UM (58)	c. 1899_1900ins5 p. Ala634Glyfs*5	Frameshift - Truncating	CM, Bladder Ca	Njauw et al., 2012 (18)
3101	UM (53)	c. 1975A>G p. Lys659*	Truncating	UM, RCC	Njauw et al., 2012 (18)
3123	UM (37)	c. 1831_1834del4 p. Glu611Argfs*5	Frameshift - Truncating	Uterine Ca	Njauw et al., 2012 (18)
Fam562	CM (31, 37, 37, 34, 35, 35, 38, 45), UM (62), RCC (46), Lung Ca (49), AST x11	c. 706_707insG p. Asp236Glyfs*7	Frameshift - Truncating	None	Njauw et al., 2012 (18)
Fam714	CM (45), AST	c. 178C>T p. Arg60*	Truncating	CM, Lung Ca, CNS Tumor	Njauw et al., 2012 (18) Wadt et al., 2014 (30)
Fam729	UM (51, 55, 57, 59), CM (36, 60), Lung Ca (57), AST, Cholangiocarcinoma (47), Breast Ca x2	c. 1153C>T p. Arg385*	Truncating	Breast Ca	Njauw et al., 2012 (18)
Family 3	MMe (34, 44), Peritoneal MMe (34, 63), AST	c. 79delG p. Val27Cysfs*45	Frameshift - Truncating	MMe	Weisner et al., 2012 (44)
	RCC (70)	c. 121G>A p. Gly41Ser	Truncating	RCC x3, Parotid Gland Carcinoma, Breast Ca x2, Lung Ca, Sarcoma x2, Adenocortical Carcinoma	Pena-Llopis et al., 2012 (82)
	UM (18, 46, 62), CM (27, 27, 33), MMe (47), Peritoneal MMe (84), Lung Ca (46), Paraganglioma (42), Breast Ca (75), MFH (45)	c. 1708C>G p. Leu570Val	Missense (Cryptic Splice Site Donor)	Prolactinoma	Wadt et al., 2012 (100)
	UM	c. 1480_1481delGA p. D494fs	Frameshift - Truncating	N/A	Aoude et al., 2012 (20)
	UM	c. 1806G>C p. E602D	Missense	N/A	Aoude et al., 2012 (20)
	UM (16, 39, 44), CM,	c. 75insG	Frameshift -	None	Hoiom et al., 2013 (21)

	Prostate Ca (67), AST	p. Lys25fs*43	Truncating		
	AST (21)	c. 214del p. I72L*6	Frameshift - Truncating	Gastric Ca	Busam et al., 2013 (27)
	UM (57), Peritoneal MMe (44, 56)	c. 758_759insA p. Gln253fs*31	Frameshift - Truncating	Peritoneal MMe	Ribeiro et al., 2013 (22)
A	Adenocarcinoma of Unknown Primitive Tumor, Breast Ca x3, RCC (37, 39, 40, 47, 47), Cervical Ca, CaSU	c. 277A>G p. Thr93Ala	Missense (Splice Site Donor)	Esophagus Ca x2, Head and Neck Carcinoma, Lung Ca, Breast Ca	Popova et al., 2013 (5)
B	UM (35), RCC (36)	c. 629dupT p. Met211Hisfs*32	Frameshift - Truncating	RCC x2, UM x2, Bone Ca	Popova et al., 2013 (5)
C	UM (52, 53), MMe (41, 59), RCC (50), Digestive Tract Ca (45)	c. 1654delG p. Asp552Ilefs*19	Frameshift - Truncating	MMe, CaSU	Popova et al., 2013 (5)
D	UM (48), CM (34), BCC (51)	c. 437+1G>A	Splice Site	Lung Ca, RCC, UM x2	Popova et al., 2013 (5)
E	UM (44), Thyroid Ca (34)	c. 219delT p. Asp73Glufs*5	Frameshift - Truncating	MMe x2, Thyroid Ca, Bladder Ca	Popova et al., 2013 (5)
F	MMe (44)	c. 670dupC p. His224Profs*19	Frameshift - Truncating	MMe	Popova et al., 2013 (5)
G	UM (57), CM (29, 31, 34, 49), RCC (36)	c. 37+1delG	Splice Site	MMe	Popova et al., 2013 (5)
H	MMe (62)	c. 1647delT p. Val550Serfs*21	Frameshift - Truncating	MMe	Popova et al., 2013 (5)
I	UM (44)	c. 1846delG p. Val616*	Truncating	CM	Popova et al., 2013 (5)
J	UM (53), RCC (53)	c. 78_79delG p. Val27Alafs*41	Frameshift - Truncating	RCC, MMe	Popova et al., 2013 (5)
K	CM (47, 52), BCC (50)	c. 660-11T>A	Splice Site	CM x2, MMe, RCC, UM	Popova et al., 2013 (5) de la Fouchardier et al., 2014 (29)
L	CM (47)	c. 588G>A	Splice Site -	UM, Prostate Ca	Popova et al., 2013 (5)

		p. Trp196*	Truncating		
NCI-1326	RCC (40, 44, 46, 54, 57)	c. 41T>A p. L14H	Missense	RCC	Farley et al., 2013 (57)
	UM (40)	c. 723T>G p. 241*	Truncating	UM, CM x3, MMe, Lung Ca x3, Colon Ca, Ovarian Ca, Gastric Ca x2	Cheung et al., 2013 (23)
	UM (20, 57, 69), CM (35), Lung Ca (59), Abdominal Ca (64), BCC x3	c. 581-2A>G	Splice Site	UM x4, Breast Ca x2, Colon Ca, Non-melanoma Skin Ca, Lung Ca, Neuroendocrine Rectal Ca	Aoude et al., 2013 (24)
F4	RCC (56)	c. 1946G>A p. C649Y	Missense	N/A	Gossage et al., 2014 (58)
A8	RCC (72)	c. 851A>G p. E284G	Missense	N/A	Gossage et al., 2014 (58)
	UM (45, 56), Cholangiocarcinoma (71), Urothelial Carcinoma (48)	c. 299T>C p. L100P	Missense	RCC, Brain Ca, Leukemia, Uterine Ca	Maerker et al., 2014 (25)
FUM064	UM (41, 49), Peritoneal MMe (48), Abdominal Ca (57), Bone Ca (64), Soft Tissue Carcinoma (42)	c. 2050C>T p. Gln684*	Truncating	UM, MMe x2, RCC, CaSU, Pancreatic Ca, Papillary Thyroid Ca, Colorectal Ca, Breast Ca	Pilarski et al., 2014 (26)
FUM103	Cholangiocarcinoma	c. 1182C>G p. Tyr394*	Truncating	Pancreactic Ca, Ovarian Ca, MMe, CaSU x2, Non-melanoma Skin Ca	Pilarski et al., 2014 (26)
FUM104	UM (49, 67), RCC (47, 49), MMe (44), Peritoneal MMe (85), Breast Ca (58, 85), Colon Ca (71), Ovarian Ca (34)	c. 1882_1885delTCA p. Ser628Profs*8	Frameshift - Truncating	RCC x3, MMe x2, Lung Ca x2, Breast Ca x2, Hematological Ca, Bladder Ca, Pancreatic Ca	Pilarski et al., 2014 (26)
Family 1	CM (60), Breast Ca (54), CaSU (pathologies point to Cholangiocarcinoma and Meningioma) (52, 55,	c. 1209_1210dupT p. D404*	Truncating	CaSU, CM, Breast Ca	Wadt et al., 2014 (30)

	66), MMe (51, 62), Peritoneal MMe (46), Basal Cell Carcinoma (50), AST (32)				
Family 2	CM (55), Peritoneal MMe (56), AST (45)	c. 838C>T p. Q280*	Truncating	UM, CM x2, RCC, Brain Ca	Wadt et al., 2014 (30)
Family 3	UM (54), BCC (43, 43, 65, 65-80), CM (25), AST (24)	c. 178C>T p. R60*	Truncating	Ovarian Ca, Granulosa Cell Tumor, UM x2, CM	Wadt et al., 2014 (30)
Family 4	UM (50, 59), BCC (46, 50)	c. 178C>T p. R60*	Truncating	BCC, MMe	Wadt et al., 2014 (30)
Family A	Peritoneal MMe (63), MMe (79), Mucoepidermoid carcinoma (36)	c. 46_47insA p. Thr16fs*52	Frameshift - Truncating	Hepatic Carcinoma, MMe	Betti et al., 2015 (43)
FUM152	UM (18)	c. 1717delC p. L573fs*3	Frameshift - Truncating	UM, CaSU	Cebulla et al., 2015 (28)
FUM124	UM (60), CM (72), MMe (71), BCC (56, 65, 68)	c. 539T>C p. Leu180Pro	Missense	Breast Ca x2, BCC x6, SCC, Non-melanoma Skin Ca, CM x2, Prostate Ca, Uterine Ca, Liposarcoma, Melanoma (meningeal), Cervical Ca, CaSU x3, MMe, GI Tract Cancer	Previously Unpublished OSU Family
FUM128	Peritoneal MMe (60)	c. 256-4_256-2del	Splice Site – Frameshift - Truncating	UM, MMe x3, Pancreatic Ca, Bladder Ca, Abdominal Ca, Ovarian Ca	Previously Unpublished OSU Family

UM, uveal melanoma; MMe, malignant mesothelioma; CM, cutaneous melanoma; RCC, renal cell carcinoma; AST, typical Spitz tumors; BCC, basal cell carcinoma; CaSU, cancer site unknown; CNS, central nervous system; MFH, malignant fibrocystic histiocytoma

5.2 Table 2

Table 2: Summary of cancers reported more than once in patients with germline *BAP1* mutations.

Tumor Type	Frequency in germline *BAP1* patients	Lifetime risk of diagnosis in general population (percent) SEER[+] (54)	Median age of onset in germline *BAP1* patients (range)	Median age of onset in general population (54)[#]	Sex ratio M:F	Biallelic inactivation shown in tumor[*]
Uveal Melanoma	49/167 (2-5, 17-30)	0.00051% (31)	51 (16-72)	62 (32)	22:25	Yes, 7 (2, 4, 17, 19, 21, 24)
Mesothelioma	39/167 (2-5, 19, 22, 26, 29, 30, 43, 44)	0.13%	56 (34-85)	74 (46)	12:26	Yes, 7 (3, 44)
Cutaneous Melanoma	23/167 (2, 4, 5, 18, 19, 21, 24, 29, 30, 44)	3.25%	46 (25-72)	58	12:10	Yes, 4 (4, 18, 44)
Renal Cell Carcinoma	17/167 (3, 5, 18, 26, 57, 58, 82)	1.60%	47 (36-72)	64	7:7	Yes, 5 (5, 57)
Basal Cell Carcinoma	11/167 (3, 5, 24, 29, 30)	-	50 (42-65)	-	4:3	Yes, 3 (30)
Breast Cancer	9/93 (3, 5, 18, 19, 26, 30)	12.33%	58 (37-85)	61	9	Yes, 2 (5)
Lung Cancer	6/167 (2, 18, 19, 24)	6.99%	56 (46-59)	70	3:3	Yes, 1 (2)
Cholangiocarcinoma	4/167 (18, 25, 26, 30)	0.89%	66 (47-71)	50 (101)	2:2	N/A
Ovarian Cancer	3/93 (2, 3)	1.12%	59 (34-69)	63	3	N/A
Meningioma	2/167 (2, 30)	-	52	65 (102)	0:2	Yes, 1 (2)
Abdominal Cancer	2/167 (24, 26)	0.86%	64 (57-64)	71	0:2	N/A

*In patients with *BAP1* germline mutation.

[+]Age-adjusted SEER data from 2007-2011.

[#]Age-adjusted SEER data from 2011.

-SEER does not track epidemiological data for basal cell carcinoma and age of onset is generally not estimated in the literature.

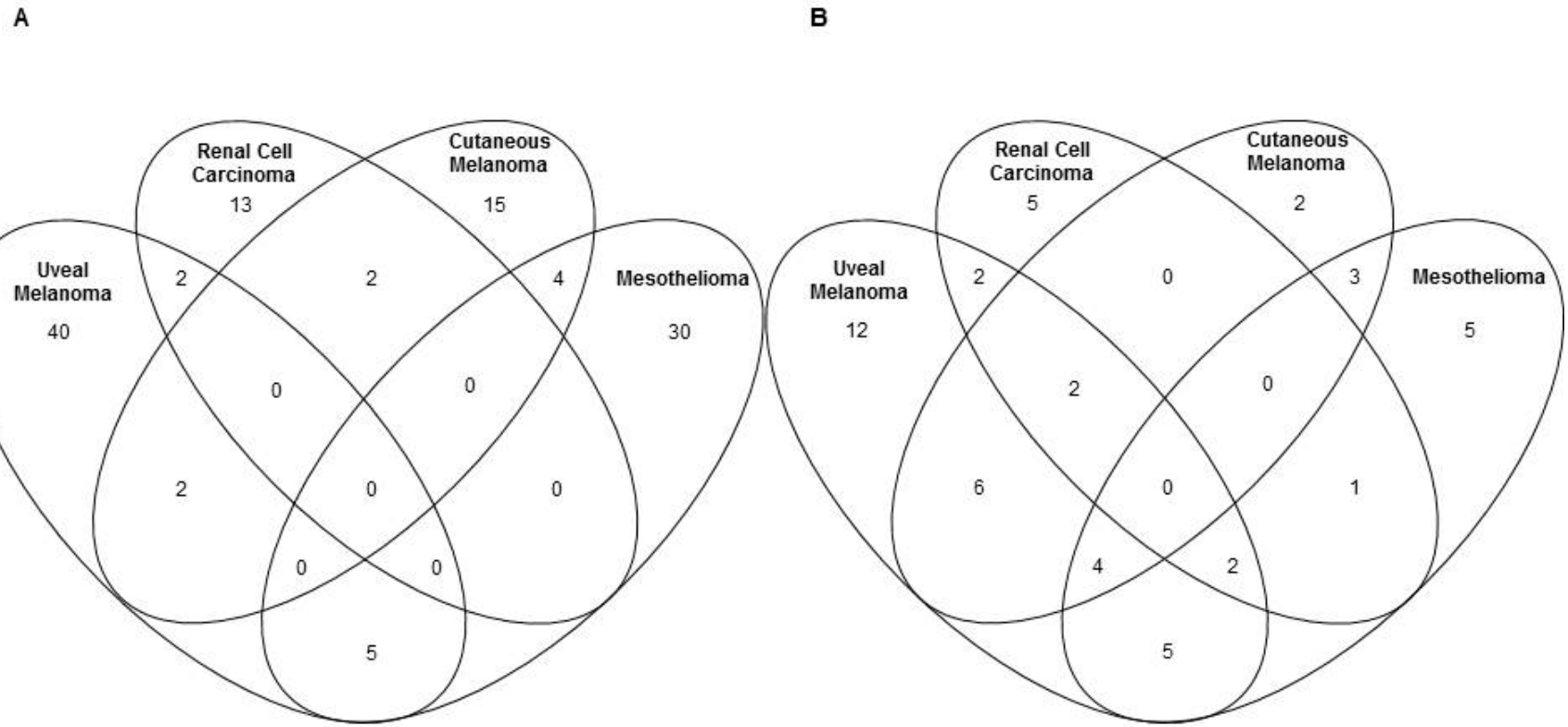
5.3 Table 3

Table 3: Studies demonstrating somatic *BAP1* mutations and/or loss of expression in tumor cells.

Tumor Type	Cell Lines DNA Sequencing	Cell Lines Fluorescence In-Situ Hybridization (FISH)	Cell Lines IHC	IHC	Tumor DNA Sequencing	Array Comparative Genomic Hybridization	COSMIC Gene Expression (103)	COSMIC Point Mutations (103)
Uveal Melanoma				103/209 (38, 40-42)	153/725 (4, 17, 35-39)			75/198
Renal Cell Carcinoma				273/2343 (79, 82, 104, 105)	249/2483 (58, 78, 80, 82-84, 106-109)		7/503	117/1673
Cutaneous Melanoma				11/238 (55, 56)	3/60 (4)		2/336	34/904
Mesothelioma	10/30 (48, 49)	6/25 (48)	8/13 (3, 49)	148/301 (43, 47-49, 52, 53)	162/406 (3, 48-52)			81/260
Atypical Spitz Tumors				71/208 (56, 61, 63-65, 67)	15/104 (4, 63, 64, 66, 67)	29/436 (67)		
Bladder Cancer					8/54 (110)			
Breast Cancer					0/45 (70)		73/989	11/1653
Cholangiocarcinoma					44/502 (72-76)		13/579	12/1154
Gallbladder Cancer					2/42 (72, 74)			
Gastric Cancer					0/45 (70)		8/285	11/420
Lung Cancer					2/77 (6)		51/865	13/1741
Colorectal Cancer				5/252 (77)	1/45 (70)			
Pancreatic Cancer					1/23 (111)		2/70	5/1734
Prostate Cancer					0/45 (70)		2/198	5/631
Thymic Cancer					9/106 (112, 113)		2/494	2/529

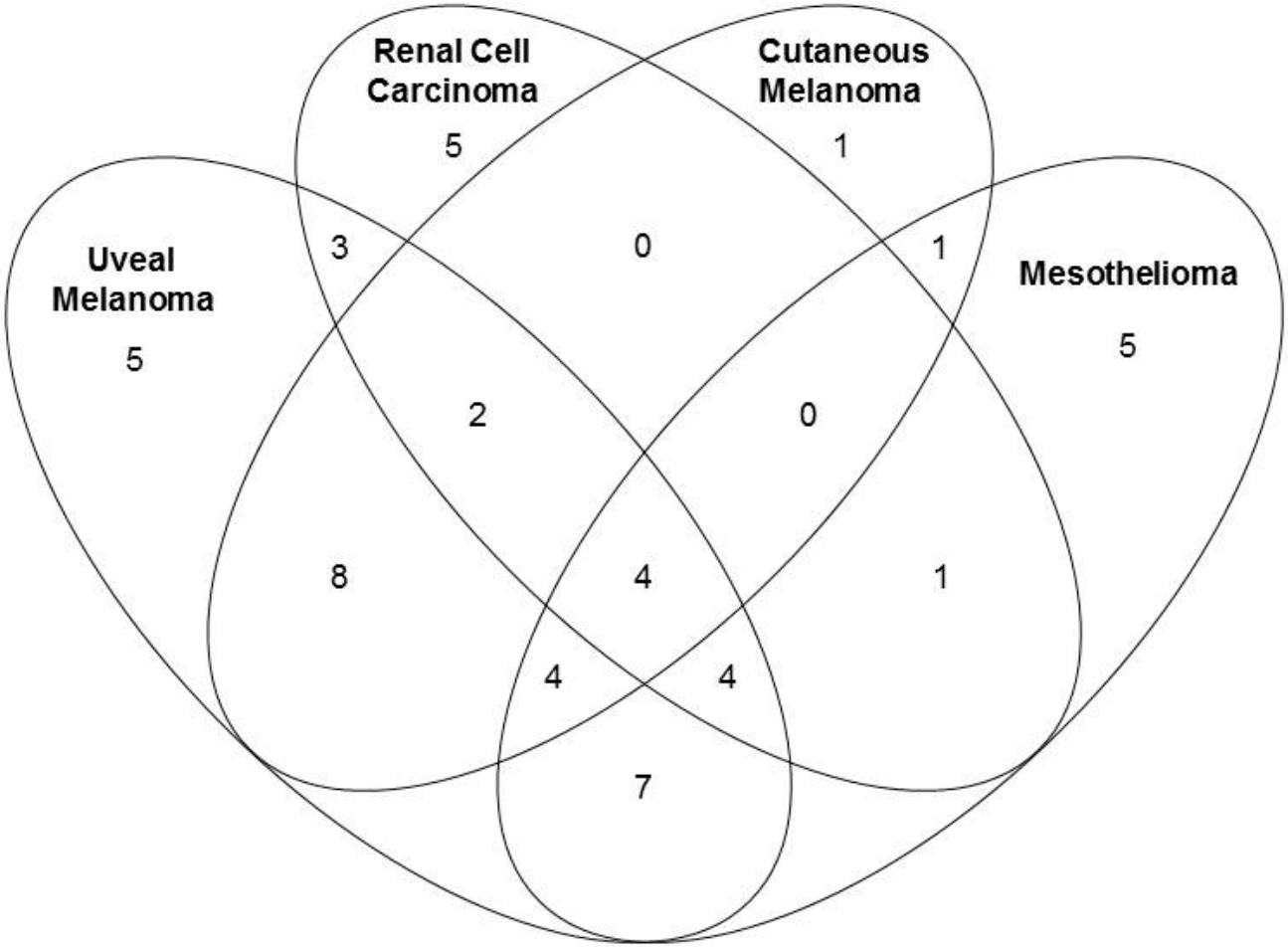
5.4 Figure 1

Figure 1. Venn diagram of the cancers identified in BAP1 patients and families
A) Individuals with *BAP1* mutations diagnosed with UM, RCC, CM, and/or MMe. Twenty eight individuals were only diagnosed with another cancer uncertain to be *BAP1* related. Twelve individuals were diagnosed with atypical Spitz tumors only. Fourteen individuals were unaffected.
B) Families with *BAP1* mutations with mutation-positive members diagnosed with UM, RCC, CM, and MMe. One family presented with only atypical Spitz tumors and another cancer uncertain to be *BAP1* related. One family did not have any members with UM, RCC, CM, or MMe tested.



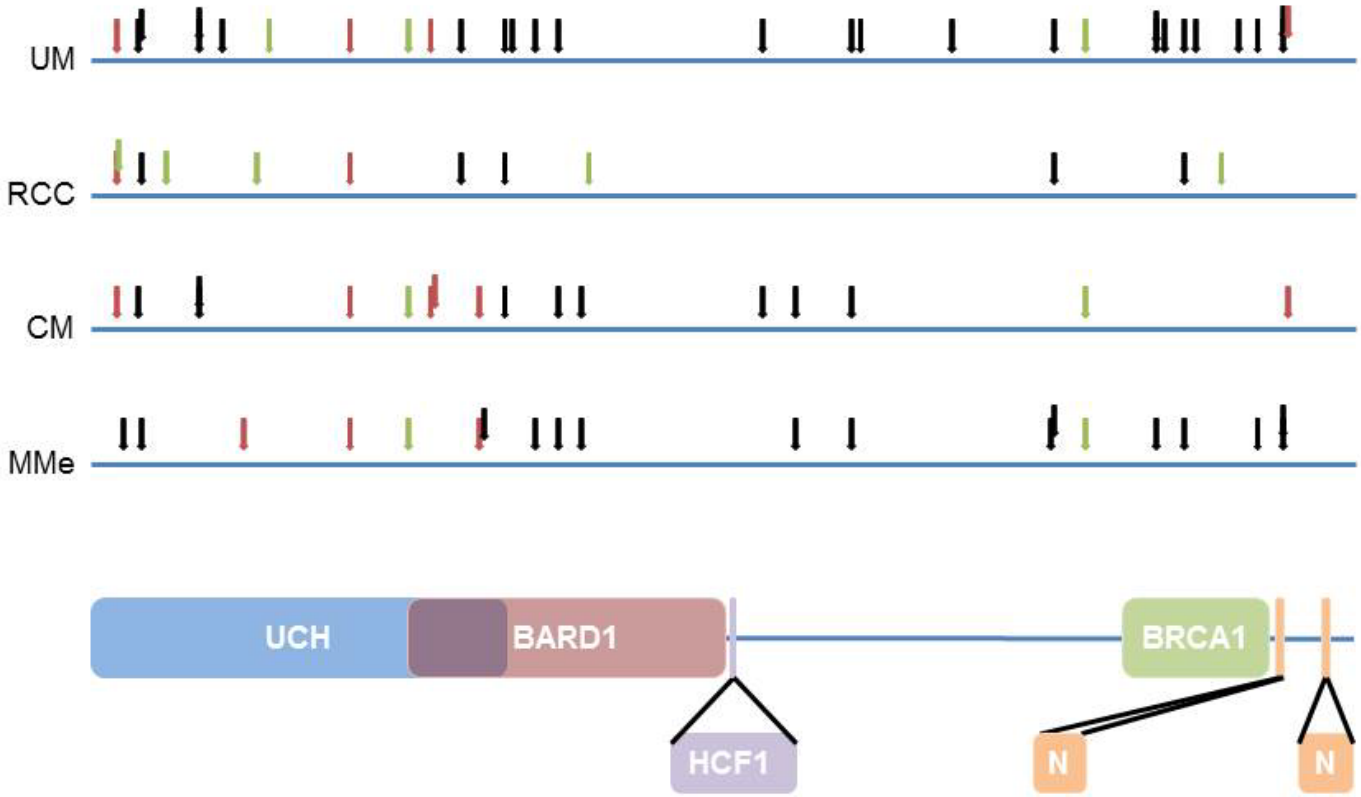
5.5 Figure 2

Figure 2: Families with germline *BAP1* mutations with reported family histories of UM, RCC, CM, and MMe without being proven mutation carriers. One family is reported with only atypical Spitz tumors and another cancer uncertain to be *BAP1* related.



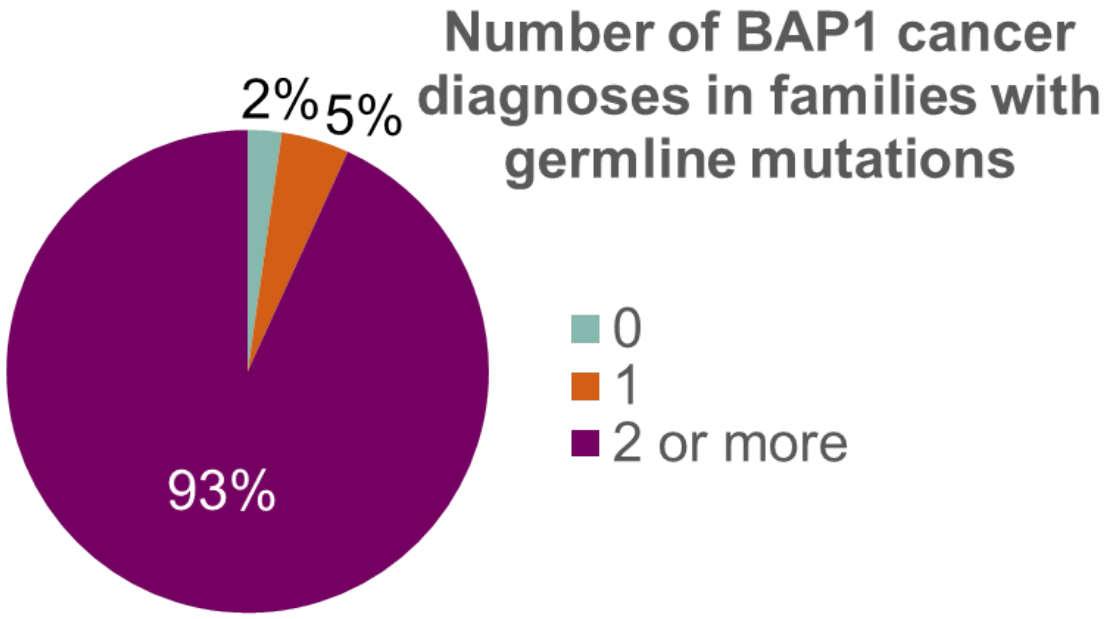
5.6 Figure 3

Figure 3: Gene location and mutation type of the reported germline pathogenic variants in *BAP1* in the four main cancers associated with *BAP1*-TPDS. No genotype/phenotype correlation was observed. One family mutation (c. 214del, p. I72L*6) was found in a family not reporting a UM, RCC, CM, or MMe. One family mutation (c. 1182C>G, p. Tyr394*) was not tested in a member with a personal history of UM, RCC, CM, or MMe.
UCH – Ubiquitin C-terminal hydrolase, BARD1 – BARD1 binding domain, HCF1 – HCF1 binding motif, BRCA1 – BRCA1 binding domain, N – Nuclear localization signal.
[Red arrow – splice-site mutation; Black arrow – truncating mutation; Green arrow – missense mutation]



5.7 Figure 4

Figure 4: Percentage of germline *BAP1* mutation families with reported family history of UM, MMe, CM, or RCC.



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