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SHORT COMMUNICATION

# Germline *BAP1* mutations misreported as somatic based on tumor-only testing

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**Abstract** We present three unrelated patients with germline mutations in *BAP1* misreported as somatic mutations. All had strong family histories of cancer. One of these patients presented with an invasive breast cancer with the tumor tissue showing partial loss of the mutant rather than the wild type allele, suggesting that the germline BAP1 mutation didn't contribute to breast cancer development in this patient. This data highlights the importance of sequencing matching germline and tumor DNA for proper assessment of somatic versus germline mutation status. In patients with somatic mutations reported from laboratories carrying out tumor-only genomic testing, the possibility that a variant may be a germline mutation should be considered, especially if the personal and/or family history suggests hereditary cancer predisposition. Since tumor-only testing can reveal germline mutations, ethical issues for patients being tested should be considered including proper consent and genetic counseling.

**Keywords** BAP1 · Hereditary cancer predisposition · Familial cancer · Uveal melanoma

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## Introduction

There is increasing interest in genomic-based management of tumors with several clinical laboratories offering molecular genetic testing using extended panels of known cancer genes. Several of these laboratories utilize tumor tissue without matching germline DNA for their assessment. Results are based on in-house developed calling algorithms based on tumor cellularity and minor allele frequency (MAF) of the mutation. The results of the test are reported usually to the requesting oncologist with no direct contribution from medical genetics. Here we present three patients reported with somatic mutations of the *BAP1* gene in various tumors. Based on the strong personal and family histories of cancer we reevaluated them in our laboratory and determined that the reported mutations were germline.

## Subjects and methods

Four patients with reported somatic mutations in *BAP1* were referred to our clinic for further evaluation of their germline *BAP1* mutation status based on their strong family histories of cancer. All patients were reported based on tumor-only testing.

The first patient (FUM153-III.4) was diagnosed by MolecularHealth (Cambridge, MA). The patient presented with an invasive unilateral breast cancer at age 45 with family history of uveal melanoma (UM), mesothelioma (MMe), renal cell carcinoma (RCC) and several other cancers, Fig. 1. A non-synonymous c.604T>C, p.W202R variant of uncertain significance was reported as somatic with a tumor content estimated at 75 %, minor allele frequency of 35.2 % and sequencing allele depth of 500.

The other three patients were diagnosed by Foundation Medicine, (Cambridge, MA). The clinical presentation, family

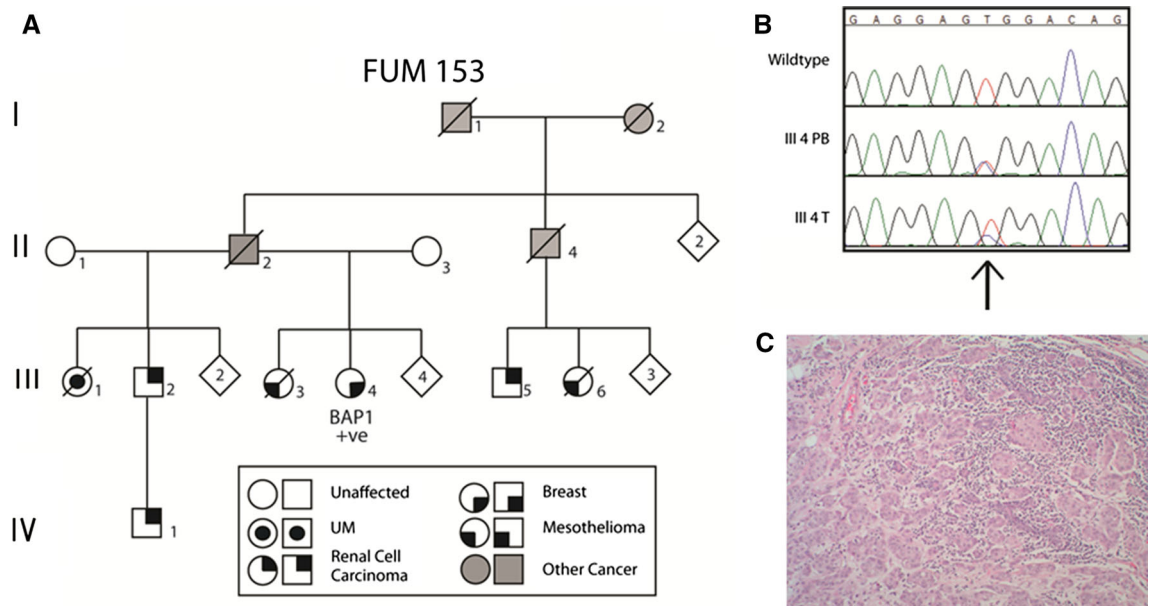


**Fig. 1** Example of a misreported *BAP1* germline mutation. **a** Pedigree of the family showing strong family history of cancers associated with *BAP1*-TPDS including renal cell carcinoma (*III.2*, *III.5*, *IV.1*), uveal melanoma (*III.1*), mesothelioma (*III.3* and *III.6*). The proband (*III.4*) presented with invasive breast cancer. Other cancers reported in the family included liposarcoma (*II.2*), urinary bladder cancer (*I.1*) and unknown cancer site (*I.1* and *II.4*). **b** Sequencing of peripheral blood (PB) germline DNA from *III.4* showed a heterozygous mutation with equal mutant and wild type allele heights. Sequencing of the tumor (*III.4* T) showed allelic imbalance with lower mutant allele height suggesting partial deletion of the mutant allele. **c** Representitive section of the sequenced tumor showing heavy infilteration by mononuclear inflammatory cells

histories and pedigrees of two cases (FUM152-III.2 [1] and FUM103-III.1 [2]) have been previously reported by our group. Briefly, FUM152-III.2 presented with a metastatic UM to the breast with family history of a father with UM and a grandfather with a tumor of unknown origin. FUM103-III.1 presented with metastatic adenocarcinoma likely from a hepatic cholangiocarcinoma [1, 2]. Cancers reported in the family were pancreatic, cutaneous melanoma (CM), ovarian cancer, MMe, non-melanoma skin cancer and a cancer of an unknown origin. The fourth patient presented with metastatic cholangiocarcinoma (61 years) with personal history of CM and family history of CM, basal cell carcinoma, chronic lymphocytic leukemia, lung carcinoma, prostate carcinoma, multiple myeloma, uterine carcinoma, and breast carcinoma in first and second-degree relatives. The laboratory didn't include either the tumor content, depth of sequencing or the MAF in their final report.

No indication of the potential germline origin of the mutation was reported by either laboratory.

Direct sequencing and genotyping were carried out on DNA extracted from peripheral blood leukocytes in all patients and from tumor tissues on FUM103-III.1 and FUM153-III.4 according to our previously published protocol [3]. For FUM153-III.4 a high density of tumor infiltrating inflammatory mononuclear cells was observed so tumor tissue was dissected to at least 75 % tumor, Fig. 1. Tumor tissue was not available for FUM152-III.2 for resequencing.

## Results

Sequencing confirmed the germline origin for three (FUM152-III.2, FUM103-III.1, and FUM153-III.4) out of the four tested patients, Table 1. Similar allele heights of both mutant and wild type alleles were observed in both tumor and germline DNA in FUM103-III.1 [2].

In FUM153-III.4 direct sequencing of the DNA extracted from peripheral blood leukocytes showed a heterozygous mutation with equal heights of both mutant and wild type alleles. In the tumor tissue the wild type allele was more predominant suggesting partial deletion of the mutant allele in the tumor, Fig. 1. Genotyping with microsatellite markers spanning the *BAP1* gene did not show somatic large deletion (data not shown). The mutation is novel and hasn't been previously reported. The mutation is located in the ubiquitin carboxyl-terminal hydrolase domain of *BAP1* and predicted to be pathogenic by both SIFT [4], Mutation Taster [5], and PolyPhen [6] software.

## Discussion

We and others have characterized a novel hereditary cancer predisposition syndrome caused by germline mutation in *BAP1* [3, 7–9]. Identifying patients with this syndrome is crucial for proper management of the patients and family

**Table 1** Summary of the reported mutations in the patients included in the study

| Patient | Mutation | Germline WT:MT alleles ratio | Somatic WT:MT alleles ratio |
|---|---|---|---|
| FUM152-III.2 [1] | c.1717_1717 delC, p.L573fs*3 | 1:1 | ND |
| FUM103-III.1 [2] | c.1182C>G, p.Tyr394* | 1:1 | 1:1 |
| FUM153-III.4 | c.604T>C, p.W202R | 1:1 | 3:1 |

*WT* wild type, *MT* mutant

members [9]. For instance, an unaffected subject in the FUM152 family tested positive for the family mutation. Ophthalmological examination identified a choroidal nevus that is currently monitored for progression. Somatic mutations in *BAP1* have been reported with high frequency in several cancers, most notably UM (23.8 %), MMe (17 %), cholangiocarcinoma (14.2 %) and RCC (9.2 %) [10], and rarely (0–2 %) in other cancers [10]. This would indicate that tumor testing of many of these patients could be positive for mutation in *BAP1*. Testing of matching germline DNA will identify those patients with germline mutation. Given the high frequency of copy number variation or large chromosomal deletions in chromosome 3 in many of these tumors assessment of minor allele frequency and tumor content may not be sufficient to differentiate between somatic and germline origin of the mutation with tumor only testing. Testing the tumor tissue of FUM153-III.4 is an example of such challenge. The clinical laboratory reported a MAF of 35 % of the mutant allele which was rather similar to our observation by direct sequencing. However, our assessment of both germline and tumor DNAs showed that the lost allele fraction in the tumor is the mutant rather than the wild type. Reevaluation of slides prepared from the same tissue block used for clinical testing identified strong mononuclear inflammatory infiltrate within the tumor which supports that non-tumor cells are the ones contributing most the mutant allele observed by sequencing, Fig. 1. We didn't see evidence of large deletions in *BAP1* using microsatellite markers spanning the gene. The lack of evidence of biallelic inactivation in breast cancer tumor tissue suggests that the germline *BAP1* mutation didn't contribute to breast cancer development in this patient.

Characterization of the germline origin of the mutation in BAP1 will have significant impact on the management including screening for additional cancers in the patients and their family members. We have proposed a screening guideline for common cancers associated with *BAP1*-TPDS [9].

Personal and family history could be an important guide for selection of patients with reported somatic *BAP1* mutation for further germline testing. Based on reported families about 90 % of patients with germline *BAP1* mutation will have family history suggestive of *BAP1*-TPDS [9]. However, it should be noted that a subset of these patients will have no personal or family history suggestive of the syndrome [9].

Another important aspect raised by our finding is the ethical issues involving tumor-only testing. These patients are usually not consented or counseled for receiving germline genetic testing results. Counseling of the patients by the provider for the possibility of identifying germline genetic results through testing their tumor should be carried out prior to testing.

In conclusion, sequencing of matching germline and tumor DNA is crucial for proper assessment of somatic versus germline mutation status. In patients with reported somatic mutations from laboratories carrying out tumor-only genomic testing, consideration of the possibility that a variant may be a germline mutation is warranted especially if personal and/or family history suggest hereditary cancer predisposition.

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**Compliance with ethical standards**

**Conflicts of interest** No conflicts of interest exist for any author.

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