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Short Report

A recurrent germline *BAP1* mutation and extension of the *BAP1* tumor predisposition spectrum to include basal cell carcinoma

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We report four previously undescribed families with germline BRCA1-associated protein-1 gene (*BAP1*) mutations and expand the clinical phenotype of this tumor syndrome. The tumor spectrum in these families is predominantly uveal malignant melanoma (UMM), cutaneous malignant melanoma (CMM) and mesothelioma, as previously reported for germline *BAP1* mutations. However, mutation carriers from three new families, and one previously reported family, developed basal cell carcinoma (BCC), thus suggesting inclusion of BCC in the phenotypic spectrum of the *BAP1* tumor syndrome. This notion is supported by the finding of loss of BAP1 protein expression by immunochemistry in two BCCs from individuals with germline BAP1 mutations and no loss of BAP1 staining in 53 of sporadic BCCs consistent with somatic mutations and loss of heterozygosity of the gene in the BCCs occurring in mutation carriers. Lastly, we identify the first reported recurrent mutation in *BAP1* (p.R60X), which occurred in three families from two different continents. In two of the families, the mutation was inherited from a common founder but it arose independently in the third family.

Conflict of interest

The authors have declared no conflicting interests.

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The BRCA1-associated protein-1 gene (*BAP1*) encodes a deubiquitinase, which functions as a tumor suppressor by regulating the cell cycle, cellular differentiation, and cell death (1, 2). BAP1 is also recruited to double-stand DNA break sites and promotes error-free DNA-repair (3). Germline mutations of *BAP1* predispose to uveal malignant melanoma (UMM), cutaneous malignant melanoma (CMM), epithelioid atypical spitzoid tumors, mesothelioma and renal cell cancer (RCC) (4–16). A wide range of other tumor types have been seen in germline *BAP1* mutation carriers but low numbers preclude formal statistical association with the syndrome. However, somatic mutation and/or loss of heterozygosity (LOH) of the locus suggest that some of these other tumor types, particularly cholangiocarcinoma (17) and paraganglioma (16), are probably to be part of the *BAP1* tumor predisposition syndrome (18).

In this study, we report four new families with germline *BAP1* mutations, and identified mutation carriers in three of these families and one previously reported family affected with basal cell carcinoma (BCC). LOH of the *BAP1* wildtype allele was observed in a BCC case, and two cases of BCC from other BAP1 carriers showed no BAP1 immunostaining (consistent with LOH), thus suggesting inclusion of BCC in the phenotypic spectrum of the *BAP1* tumor syndrome. In contrast, 0 of 53 sporadic BCCs showed loss of BAP1 expression by immunochemistry. Lastly, we document the first reported recurrent mutation in *BAP1* (p.R60X), which occurred in three families from two different continents.

Material and methods

Ethics approval

Approval for this project was granted by the Human Research Ethics Committee of the Queensland Institute of Medical Research, and the Committee of Biomedical Research Ethics of the Capital Region of Denmark. Each participant gave their written informed consent.

Patients and families

Details of the tumor types and clinical information for family members are given in Table S1, Supporting Information.

BAP1 mutation detection

Families 1 and 4 (F1, F4) were referred to the genetic clinic of Rigshospitalet, Denmark, for genetic counseling, and screening for a germline *BAP1* mutation was performed. Sanger sequencing of the entire coding region of *BAP1* was carried out according to routine procedures. Family 2 (F2) was referred to the Department of Cancer Genetics at the Royal Prince Alfred Hospital, Sydney, Australia for genetic counseling, and examination of a possible germline *BAP1* mutation was performed. In Family 3 (F3) exome sequencing was performed on selected individuals, as previously described (7). Sanger sequencing of polymerase chain reaction (PCR) products was subsequently used to confirm mutations using the following primers: 5′-tcagaggcttatgctttgctc-3′, 5′-tcctcctgtcttcctccattt-3′. Products were sequenced using BigDye Terminator v3.1 chemistry in an Applied Biosystems 3130xL Genetic Analyser (Life Technologies, Carlsbad, California).

Haplotype analysis

Analysis of a possible common founder in these families was carried out using whole-genome single nucleotide polymorphism (SNP) arrays (Illumina HumanOmniExpress-h12v1 Illumina, San Diego, California). The age of the mutation was estimated using

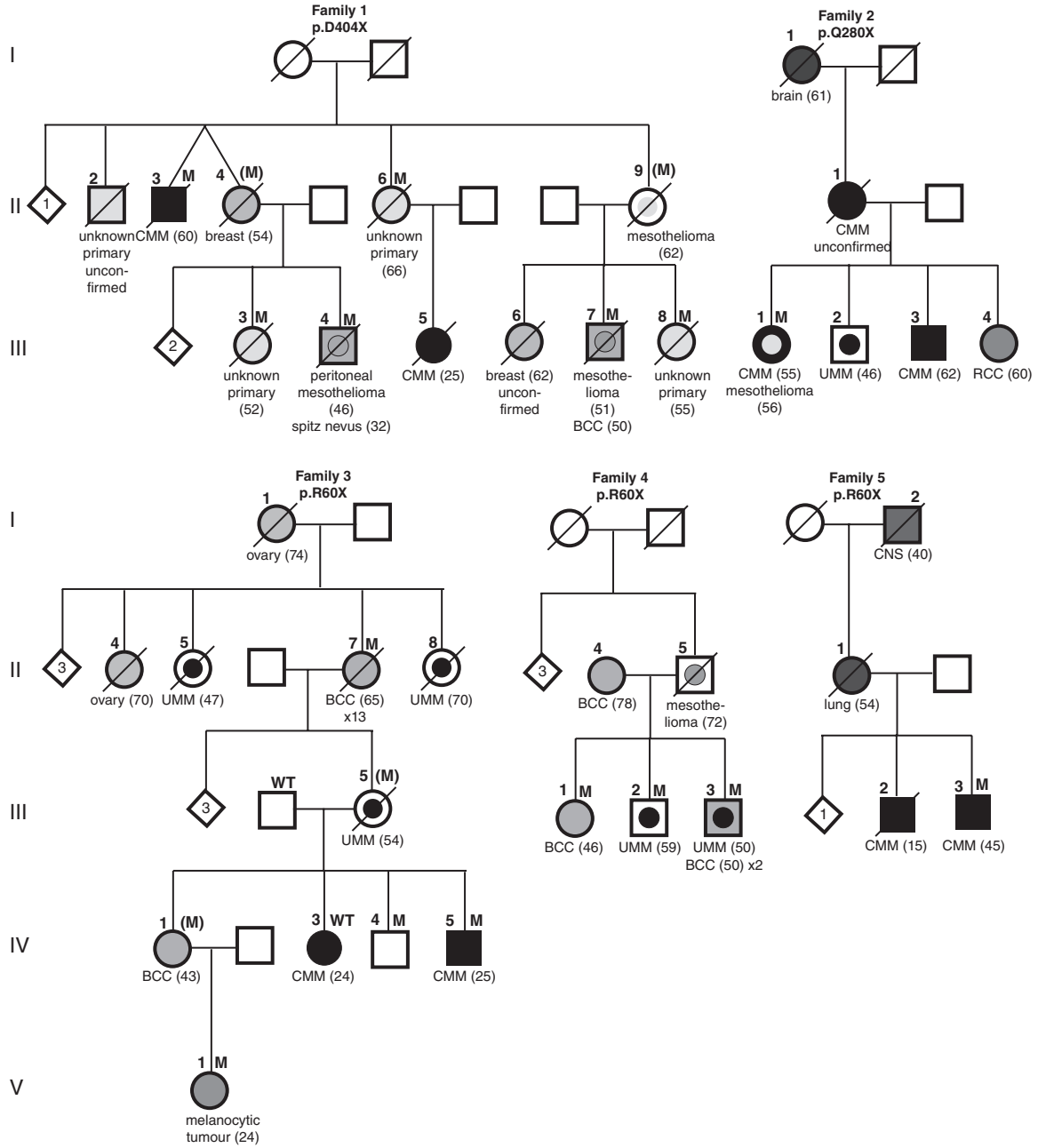

Fig. 1. Pedigrees of five families with *BAP1* mutations. Individuals with cancer are colored by cancer type (see legend). The cancer type is also indicated below the individual, and the age of diagnosis of each tumor is indicated in brackets. *BAP1* mutation carriers are indicated by an M. An M in brackets indicates an obligate carrier.

a statistical model as described by (19). Assuming 1 cM equals 1 Mb and a *de novo* mutation rate of 1.2×10^{-8} per generation and bp, a joint likelihood of the genotype data was calculated taking into account the ancestral haplotype, number of generations, G, because the ancestor, and allele frequencies of the SNPs among the European population. An estimate of G was calculated as the value of G that maximizes the likelihood, and a confidence interval was found by finding the range of values of G that yielded a likelihood of at least a tenth of the maximum likelihood.

BAP1 immunohistochemistry

Immunohistochemistry (IHC) for BAP1 was performed on 4 µm-thick sections cut from formalin-fixed, paraffin-embedded (FFPE) tissue blocks. IHC was conducted using an automated IHC system (Ventana BenchMark ULTRA, Ventana Medical Systems Inc., Tucson, AZ) utilizing an UltraView universal Alkaline Phosphatase IHC Detection Kit (Ventana, Tuscon, Arizona). Following deparaffinization of FFPE sections, heat-induced epitope retrieval (HIER) was applied using CC2 for 68 min at 95°C. The sections were incubated

with the mouse monoclonal BAP1 (C-4) antibody (Santa Cruz Biotechnology, Santa Cruz, California; 200 µg/ml) at dilution 1:50 for 1 h followed by incubation with hematoxylin II counter stain for 4 min and then with blueing reagent for 4 min. A positive control was included in each IHC round. The antibody reacts with the epitope at amino acids 430–729 of human BAP1. Loss of nuclear staining in tumor cells was taken to indicate inactivation of BAP1 (2, 20). The following tumors were stained for BAP1: from the proband of family 2: atypical Spitzoid nevus/combined nevus, cutaneous melanoma, and peritoneal mesothelioma; from family 3: BCC from IV-1; from family 4: BCCs from II-4 and III-1.

Results

Tumor spectrum in mutation carriers

We report four new families with germline *BAP1* mutations (Fig. 1). The tumor spectrum in these families is predominantly UMM, CMM and mesotheliomas. However, we also identified five mutation carriers with BCC (Family 1, III-7, Family 3, II-7 and IV-1, Family 4, III-1 and III-3). Individual II-7 from family 3 had 13 cutaneous BCCs. In addition, there were multiple primary BCCs in two germline *BAP1* mutation carriers from a published Danish family (7) (Table S1). All BCCs were non-metastatic and most were located on sun-exposed areas. None of the affected had history of occupational exposure to sunlight.

Haplotype analysis

With one exception (rs699469), the carriers in the two Danish families (Family 3 and Family 4) shared an allele for 256 SNPs stretching from rs13073141 (3:51,410,485) to rs6772648 (3:53,244,603), a 1,834,119 bp region spanning *BAP1* (Table S2). These data are consistent with the families sharing a haplotype from a common ancestor and one of the families acquiring a variant in rs699469 after the *BAP1* mutation. Estimates of when the BAP1 mutation arose gave a maximum likelihood for 91 generations ago (90% CI: 20–254). Assessment of 176 'phased' haplotypes from 88 parent-offspring trios in Hapmap indicated that the founder haplotype on which the p.R60X mutation is carried occurs at a frequency of 24%. The mutation arose independently in Family 5, based on the lack of sharing of the same *BAP1* haplotype as the Danish families (Table S2).

Loss of heterozygosity in BCC

LOH analysis was performed on a BCC from individual II-7 in Family 3 and showed clear loss of the wildtype *BAP1* allele (Fig. 2).

Immunohistochemistry

The CMM, mesothelioma and atypical Spitzoid tumor from the proband of Family 2 all had negative staining

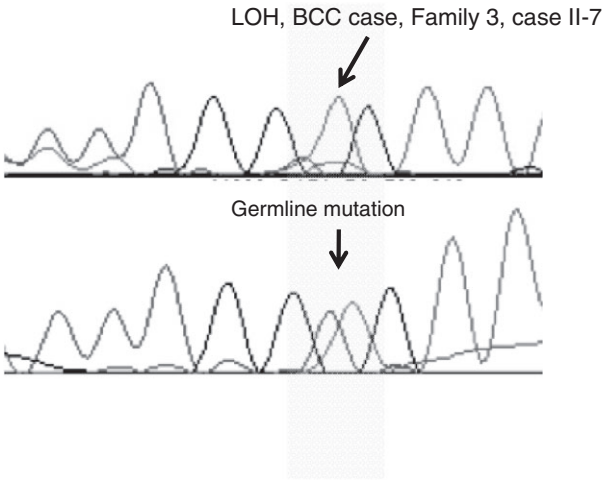

Fig. 2. Loss of heterozygosity analysis performed on BCC from individual (Family 3, II-7). A distinct loss of the wildtype allele in the BCC is observed.

for BAP1 (Fig. 3). IHC of three BCCs from individuals Family 3: IV-1; Family 4: II-4 and III-1, showed loss of BAP1 immunoreactivity, consistent with LOH in these tumors (Fig. S1). It is unknown if II-4 from family 4 is a mutation carrier. IHC analysis of 53 sporadic BCCs did not show loss of BAP1 protein expression in any case (Fig. S1).

Discussion

Germline *BAP1* mutations predispose to a variety of cancers with a high degree of inter- and intrafamilial variability, which could indicate the presence of modifier genes or environmental components which influence the specific cancer risk in a mutation carrier. Alternatively, the phenotype could be more mutation-specific. But, as documented by Cheung et al. (21), almost all published germline *BAP1* mutations are predicted to result in a truncated protein, and no specific genotype–phenotype pattern has been identified to date. Here, we provide clinical details of three families with the same *BAP1* mutation. The phenotype differs in these families, with CMM, UMM and BCC each in two families and mesothelioma in only one family.

We document a family with at least three cases of metastatic carcinoma from unknown primary tumors, where extensive up-to-date IHC analyses, radiological investigations and clinicopathologic correlation failed to reveal the primary tumor site in each case. This could however be caused by very dedifferentiated tumors, and in fact, two of these cancers of unknown primaries were most likely a cholangiocarcinoma and a papillary meningioma (see Appendix S1), both of which have been documented in other *BAP1* mutation carriers (5, 8, 12). One person from this family had a very unusual clinical presentation of peritoneal mesothelioma (anemia, enlarged retroperitoneal lymph nodes, apart from ascites), and the diagnosis was in fact made post-mortem, and only in retrospect because of the knowledge of a

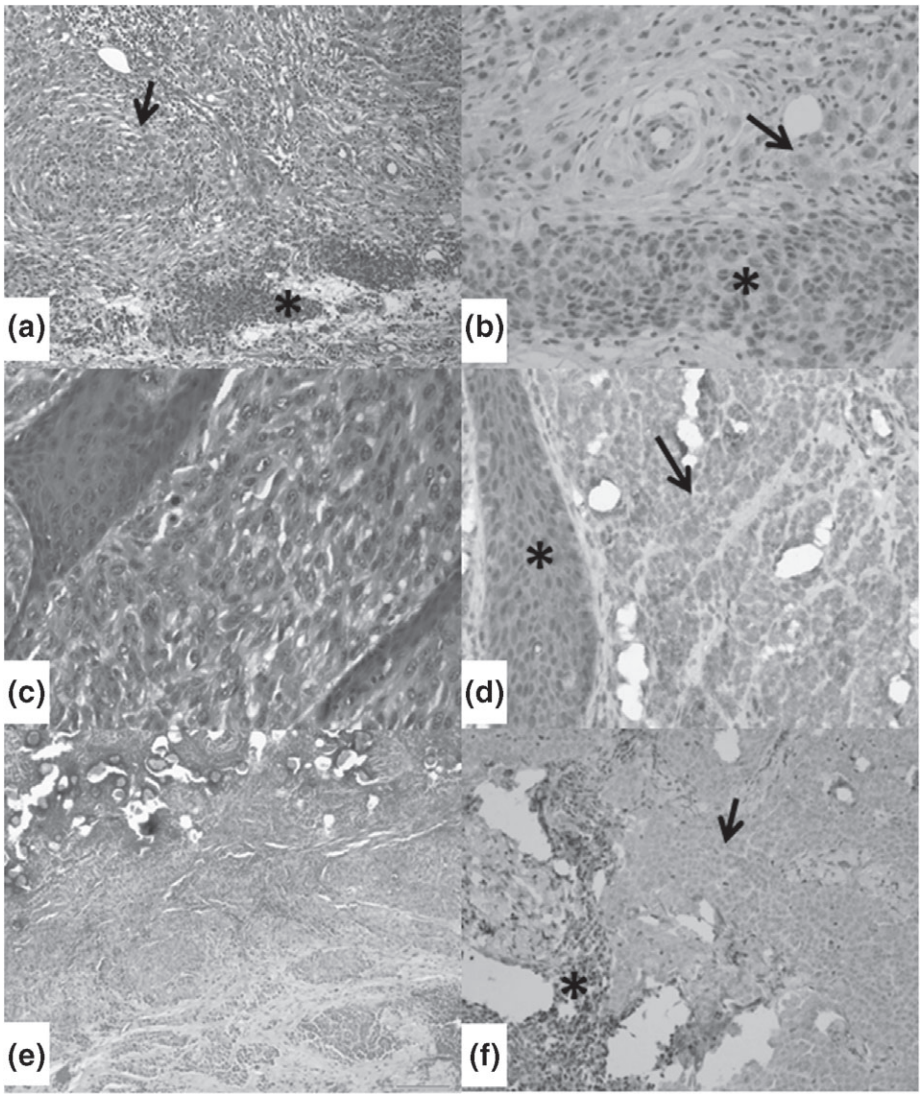

Fig. 3. Hematoxylin & eosin (H&E) and BAP-1 staining of a combined nevus (**a**, **b**), primary cutaneous melanoma (**c**, **d**) and mesothelioma (**e**, **f**) from patient (family 2, III-1). (**a**). H&E stain showing a combined nevus formed by large epithelioid Spitz nevus cells (arrow) and small banal dermal nevus cells (*). (**b**) BAP1 immunostain showing BAP1 loss in the Spitz nevus cells (arrow) with preserved BAP1 staining in the dermal nevus cells (*). (**c**) H&E stain of primary cutaneous melanoma showing large pleomorphic melanoma cells between the epithelium of some skin appendigeal structures. (**d**) BAP1 stain showing BAP1 loss in the melanoma cells (arrow) and preserved BAP1 staining in the skin appendigeal structures and blood vessels (*). (**e**) H&E stain of mesothelioma (some psammomatous foci of calcification are present within the tumor). (**f**) BAP1 immunostain showing BAP1 loss within the tumor (arrow) and preserved BAP1 staining in peritumoral lymphocytes (*).

germline *BAP1* mutation in the family and reconsideration of this diagnostic possibility. There have previously been single reports of germline *BAP1* mutation carriers with metastatic carcinomas of unknown primary sites (8, 12, 14) but this is the first family reported with multiple individuals with unknown primary cancers; this is important information for clinicians who should consider the possibility of a germline *BAP1* mutation when such a family is encountered. The frequency of germline *BAP1* mutations in individuals with metastatic carcinoma of unknown primary site is currently unknown and warrants further investigation, but suspicion should be focused in the first instance on the types of cancers that have been documented in *BAP1* mutation carriers.

BCCs are the most mutated type of human cancer (22) but have not previously been linked to *BAP1* germline mutation. We document seven Danish carriers with BCC, and one of whom had 13 tumors. We did not observe BCCs in the non-Danish families, possibly indicating polymorphisms in the Danish population which combined with BAP1 loss, promote BCC carcinogenesis. The age-standardized incidence of BCC in Denmark is quite high. In 2012 it was 187 and 176 per 100,000 in men and women, respectively, and since 2005 has increased with 70% in men and 74% in women (23). However, we observed BCC before the age of 70 years in all seven carriers, and four carriers had multiple BCCs. There was LOH of the wildtype allele in the one BCC analysed, and loss of BAP1 immunoreactivity, consistent with LOH in

the two BCC from known BAP1 mutation carriers, and in a BCC from a person with unknown carrier status. We thus propose that *BAP1* mutation carriers have an increased risk of BCC.

In conclusion, *BAP1* is being increasingly recognized as a broad spectrum tumor suppressor gene and the list of cancers associated with *BAP1* mutations is growing. It is critical that clinicians and pathologists are aware of the various cancer associations to increase the chance of early recognition of mutation carriers and to facilitate appropriate counseling and screening of patients and their families, who will benefit from genetic counseling, close clinical follow up and regular dermatologic and ophthalmologic evaluations.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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References

1. Carbone N, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G BAP1 and cancer. Nat Rev Cancer 2013: 13: 153–159.
2. Murali R, Wiesner T, Scolyer R. Tumours associated with BAP1 mutations. Pathology 2013: 45: 116–126.
3. Yu H, Pak H, Hammond-Martel I et al. Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. Proc Natl Acad Sci U S A 2014: 111: 285–290.
4. Testa JR, Cheung M, Pei J et al. Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 2011: 43: 1022–1025.
5. Njauw CNJ, Kim I, Piris A et al. Germline BAP1 inactivation is preferentially associated with metastatic ocular melanoma and cutaneous-ocular melanoma families. PLoS One 2012: 7: e35295.
6. Höiom V, Edsgärd D, Helgadottir H et al. Hereditary uveal melanoma: a report of a germline mutation in BAP1. Genes Chromosomes Cancer 2013: 52: 378–384.
7. Aoude LG, Wadt K, Bojesen A et al. A BAP1 mutation in a Danish family predisposes to uveal melanoma and other cancers. PLoS One 2013: 8: e72144.
8. Abdel-Rahman MH, Pilarski R, Cebulla CM et al. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. J Med Genet 2011: 48: 856–859.
9. Harbour JW, Onken MD, Roberson EDO et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. Science 2010: 330: 1410–1413.
10. Maerker D, Zeschnigk M, Nelles J et al. BAP1 germline mutation in two first grade family members with uveal melanoma. Br J Ophthalmol 2014: 98: 224–227.
11. Wiesner T, Obenauf A, Murali R et al. Germline mutations in BAP1 predispose to melanocytic tumors. Nat Genet 2011: 43: 1018–21.
12. Pilarski R, Cebulla CM, Massengill JB et al. Expanding the clinical phenotype of hereditary BAP1 cancer predisposition syndrome, reporting three new cases. Genes Chromosomes Cancer 2014: 53: 177–182.
13. Ribeiro C, Campelos S, Moura CS, Machado JC, Justino A, Parente B Well-differentiated papillary mesothelioma: clustering in a Portuguese family with a germline BAP1 mutation. Ann Oncol 2013: 24: 2147–2150.
14. Popova T, Hebert L, Jacquemin V et al. Germline BAP1 mutations predispose to renal cell carcinomas. Am J Hum Genet 2013: 92: 974–980.
15. Farley MN, Schmidt LS, Mester JL et al. A novel germline mutation in BAP1 predisposes to familial clear-cell renal cell carcinoma. Mol Cancer Res 2013: 11: 1061–1071.
16. Wadt K, Choi J, Chung J-Y et al. A cryptic BAP1 splice mutation in a family with uveal and cutaneous melanoma, and paraganglioma. Pigment Cell Melanoma Res 2012: 25: 815–818.
17. Chan-On W, Nairismägi M-L, Ong CK et al. Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. Nat Genet 2013: 45: 1474–1478.
18. Wiesner T, Fried I, Ulz P et al. Toward an improved definition of the tumor spectrum associated with BAP1 germline mutations. J Clin Oncol 2012: 30: e337–e340.
19. Neuhausen SL, Mazoyer S, Friedman L et al. Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. Am J Hum Genet 1996: 58: 271–280.
20. Murali R, Wilmott JS, Jakrot V et al. BAP1 expression in cutaneous melanoma: a pilot study. Pathology 2013: 45 (6): 606–609.
21. Cheung M, Talarchek J, Schindeler K et al. Further evidence for germline BAP1 mutations predisposing to melanoma and malignant mesothelioma. Cancer Genet 2013: 206: 206–210.
22. Jayaraman SS, Rayhan DJ, Hazany S, Kolodney MS Mutational landscape of basal cell carcinomas by whole-exome sequencing. J Invest Dermatol 2014: 134: 213–220.
23. Danish Cancer Registry. Retrieved Aug 1, 2014 from (http://www.ssi.dk/Sundhedsdataogit/Registre/Cancerregisteret.aspx).