Contents lists available at ScienceDirect

Cancer Genetics

Case Report

Intrahepatic cholangiocarcinoma development in a patient with a novel BAP1 germline mutation and low exposure to asbestos

G Brandi [a,b], M Deserti [b], A Palloni [a], D Turchetti [c], R Zuntini [c,d], F Pedica [e], G Frega [a], S De Lorenzo [a], F Abbati [a], A Rizzo [a], M Di Marco [a,b], F Massari [b], S Tavolari [b,*]

[a] Department of Experimental, Diagnostic and Specialty Medicine, S. Orsola-Malpighi University Hospital, Bologna, Italy
[b] Division of Oncology, S. Orsola-Malpighi University Hospital, Bologna, Italy
[c] Department of Medical and Surgical Sciences: Center for Hereditary Cancer Studies, S. Orsola-Malpighi University Hospital, Bologna, Italy
[d] Medical Genetics Unit, Mother and Child Health Department, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy
[e] Pathology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

ARTICLE INFO

ABSTRACT

BRCA1 associated protein-1 (BAP1) germline mutations define a novel hereditary cancer syndrome, namely *BAP1* tumor predisposition syndrome (*BAP1*-TPDS), characterized by an increased susceptibility to develop different cancer types, including mesothelioma, uveal and cutaneous melanoma, renal cell carcinoma, and basal cell and squamous cell carcinoma. Currently, the role of *BAP1* germline mutations *in intrahepatic cholangiocarcinoma (iCCA) pathogenesis is less known. H*ere we report the first clinical case of a female patient who developed an iCCA when she was 47-years-old and was found to carry a novel germline mutation at a splicing site of exon 4 in *BAP1* gene (NM_004656.4: c.255_255+6del). *An accurate anamnesis revealed the absence of risk factors linked to iCCA development, except for a low occupational exposure to asbestos.* In tumor tissue, *BAP1* sequencing, multiplex ligation-dependent probe amplification and immunoistochemistry showed the loss of heterozygosity and lack of nuclear expression, suggesting that *BAP1* wild-type allele and functional protein were lost in cancer cells, in line with the classical two-hit model of tumor suppressor genes. Further studies are needed to confirm whether iCCA may be included into *BAP1*-TPDS cancer phenotypes and whether minimal asbestos exposure may facilitate the development of this malignancy in individuals carrying *BAP1* germline mutations.

Introduction

Cholangiocarcinoma (CCA) is the second most common primary liver cancer after hepatocellular carcinoma and arises from the epithelial cells of the intrahepatic (iCCA) and extrahepatic (eCCA) biliary tree [1]. During the past three decades, iCCA incidence has been progressively increased worldwide, with an average age of presentation in general population in the seventh decade of life and a slight preponderance in males [2]. Despite several pathological conditions have been clearly established as risk factors for iCCA and eCCA and may explain, at least in part, the increasing worldwide iCCA incidence [2], the role of *inherited* genetic *disorders in the pathogenesis of this disease is less known. Mutations in HFE1* and PKHD1 genes, *inherited* in an autosomal recessive manner, are linked to *hemochromatosis type 1 and* Caroli's disease development, respectively, both reported as risk factors for iCCA [2].

Heterozygous germline mutations in the BRCA1 associated protein-1 (BAP1) gene, codifying a deubiquitinating hydrolase [3], define a novel hereditary cancer syndrome, namely BAP1 tumor predisposition syndrome (BAP1-TPDS), characterized by an increased susceptibility to develop multiple cancer types, including mesothelioma, uveal and cutaneous melanoma, renal cell carcinoma, and basal cell and squamous cell carcinoma [4]. Initially discovered in two families from USA, currently BAP1-TPDS has been described in about one-hundred of families worldwide [5–7]. Similarly to *TP53* germline mutations in the Li-Fraumeni syndrome, BAP1 germline mutations are inherited in autosomal-dominant manner with a very high penetrance, as more than 80% of carriers develop throughout their life at least one of the main cancer types associated to BAP1-TPDS [4]. Genomic analysis of tumors from these individuals typically shows the loss of the remaining BAP1 wild-type allele, suggesting that BAP1 may act as a classical

* Corresponding author.
   E-mail address: simona.tavolari@unibo.it (S. Tavolari).

"two-hits" tumor suppressor gene [8]. The tumor suppressor function of BAP1 has been ascribed not only to its ability to maintain genome integrity, but also to regulate mitochondrial apoptosis and ferroptosis upon exposure to cellular stress conditions ([3,9,10]). Impairment of these cellular mechanisms makes BAP1 mutant cells more prone to accumulate DNA mutations and may account for the high cancer rate observed in individuals carrying BAP1 germline mutations. To date the whole spectrum of the cancer types associated with BAP1-TPDS still remains to be fully characterized; notwithstanding, it is becoming clear that besides mesothelioma, uveal and cutaneous melanomas, renal cell carcinomas, basal cell and squamous cell carcinomas, other tumors may be linked to this syndrome [4].

As far as CCA is concerned, current epidemiological data are still too scarce to confirm a link between BAP1 germline mutations and the development of this disease. To date the only literature cases include a patient with a metastatic adenocarcinoma from a cancer of unknown primary origin (likely from a CCA) who was found to carry the BAP1 germline truncating mutation c.1182C>G, p.Tyr394* [11], and a patient with uveal melanoma, peritoneal mesothelioma and a primary biliary tract adenocarcinoma developed in short succession who was found to carry the BAP1 germinal missense mutation g.52441252A>G, p.Tyr173Cys [6]. Further clinical evidences are therefore needed to confirm this link.

On this basis, here we report the first clinical case of a female patient carrying a novel BAP1 germline mutation, who developed an iCCA when she was 47-years-old and was found to be exposed to low levels of asbestos fibers.

Materials and methods

Evaluation of asbestos exposure

Information on asbestos exposure of the patient at work, home and in general environment was collected according to the National Registry of Mesotheliomas (*ReNaM)* questionnaire [12], which was administered by a trained interviewer. The questionnaire included the following sections: (1) occupational history, with special sections for industrial activities and occupations with possible asbestos use; (2) residential history, including address and description of dwellings and their neighbourhood environment; (3) description of the dwelling environment, with checklists for the presence/use of asbestos-containing materials. Duration (the difference between the start and end date) and frequency (the proportion of time) of occupational, domestic and environmental asbestos exposure was estimated from the questionnaire by an expert. A combined exposure index (intensity $\times$ frequency $\times$ duration) was computed for each type of exposure, and all exposures were summed to calculate a cumulative exposure index. Asbestos exposure was classified as follows: a) high: subject working in an industrial activity using asbestos as raw material, insulator, shipyard worker, or docker; b) low: subject never working in any high-exposure activity, but with at least one job that was assessed as entailing occupational exposure or with at least one identified circumstance of non-occupational exposure; c) no exposure: subject with a complete history without any identified exposure circumstance; d) unknown exposure: subject with incomplete history.

Genomic DNA extraction and BAP1 sequencing

Genomic DNA was extracted from peripheral blood lymphocytes and from formalin-fixed, paraffin-embedded (FFPE) iCCA tumor tissue using the QIAamp® DNA Blood Maxi Kit (QIAGEN, CA, USA) and the QIAamp® DNA FFPE Tissue Kit (QIAGEN, CA, USA), respectively, according to the manufacturer's protocol. PCR amplification of BAP1 exons and splicing junctions was performed

under standard conditions and purified PCR products were sequenced with Big Dye v1.1 (Life Technologies, CA, USA) according to manufacturer's instructions. The reaction products were analyzed on 3730 DNA Analyzer (Life Technologies, Carlsbad, CA, USA). The primers were designed using the reference sequences (NM_004656.4) on GRCh38.p13 (Supplementary Table 1). Nomenclature of variants was defined according with Human Genome Variation Society (HGVS).

BAP1 deletion/duplication analysis

BAP1 deletions and duplications were assessed in peripheral blood lymphocytes and FFPE iCCA tumor tissue DNA by Multiplex Ligation-dependent Probe Amplification analysis (MLPA) (SALSA MLPA P417-B2 BAP1 probemix, MRC−Holland, Amsterdam, the Netherlands), according to the manufacturer's protocol. The reaction products were analyzed on 3500 Dx DNA Analyzer (Life Technologies, Carlsbad, CA, USA) and Coffalyser Software v.140721.1958 (MRC Holland) was used for the quantitative analysis of the electropherograms.

BAP1 immunohistochemistry

FFPE tumor tissue from resected iCCA was collected from the patient. Immunohistochemistry (IHC) was carried out using Novolink Polymer Detection System (Leica Mycrosystems, Germany) according to the manufacturer's instructions. Briefly, 3-μm-thick sections were cut from FFPE block, deparaffinized, rehydrated and subjected to antigen retrieval by heating for 30 min at 99 °C in citrate buffer pH 6.0. Endogenous peroxidase activity and non-specific binding sites were blocked by 5 min of incubation in peroxidase block and protein block, respectively. Tumor sections were then incubated overnight at 4 °C with BAP1 rabbit polyclonal antibody (GeneTex, Inc. San Antonio, TX, USA). At the end of the incubation, immune complexes were incubated in post-primary antibody for 30 min at room temperature, and then in Novolink polymer tertiary antibody for a further 30 min at room temperature. Finally, sections were developed in 3,3′-diaminobenzidine and counterstained with hematoxylin.

BAP1 was considered positive when a nuclear positivity was observed in tumor cells; negative staining was defined as complete absence of nuclear staining in neoplastic cells in presence of a positive internal control (such as stromal cells or inflammatory cells). Cytoplasmic staining with negative nuclear staining was considered negative, as previously reported [13].

Results

Case presentation

A 47-year-old woman presented with a significant weight loss in April 2017. A contrast enhanced computed tomography (ceCT) scan of thorax, abdomen and pelvis revealed a heterogeneously enhancing 56 $\times$ 43 mm mass in the V segment of the liver with portal vein thrombosis. In June 2017, the patient underwent laparoscopic resection of the V liver segment and hepatic pedicle lymphadenectomy (Fig. 1, red circle), with pathological diagnosis of iCCA pT3N0M0, stage IIIA, according to the 8th edition of the AJCC staging system [14]. An accurate anamnesis revealed the absence of risk factors linked to iCCA development [2] except for a low occupational exposure to asbestos, assessed by a standardized questionnaire [12], during her job in industrial kitchens (about 15 years). Given the presence of unresectable portal vein thrombosis, surgical resection of iCCA in the V liver segment was considered not radical (R2) and the patient underwent four cycles of gemcitabine/cisplatin chemotherapy (ABC-02 regimen) from July to September 2017. Due to a significant progression of neoplastic
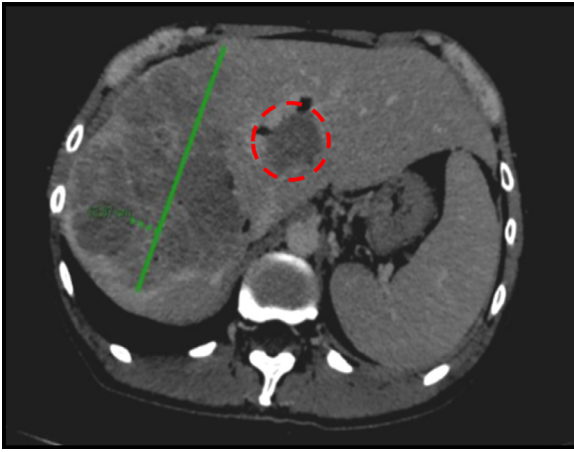
Fig. 1. Arterial phase ceCT scan *of* patient's *abdomen* showing *laparoscopic resection of* primary *iCCA in the V segment of the liver* (red circle) and subsequent multiple confluent iCCA lesions affecting the right liver lobe (green line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

thrombosis, a second-line chemotherapy with 5-FU/LV plus oxaliplatin (Folfox 4) was administered from October 2017 to January 2018. The ceCT scan after 6 cycles of chemotherapy showed a further progression of the disease, with the appearance of multiple confluent iCCA lesions affecting the right liver lobe (*Fig. 1, green line*). At the same time, the patient reported an acute-onset cervical spine pain. A cervical MRI revealed a neoplastic mass replacing the C3 vertebral body and right posterior elements, without spinal cord compression; palliative radiotherapy (35 Gy in 14 fractions) was administered with transient clinical benefit. Laboratory data showed preserved hepatic function and hypercalcemia. In May 2018, the patient started a third-line chemotherapy with 5-FU/LV plus irinotecan (Folfiri), that was definitively interrupted due to intrahepatic progressive disease and appearance of peritoneal carcinosis in August 2018. Therefore treatment with only best supportive care was continued until patient death in October 2018.

BAP1 mutation analysis, multiplex ligation-dependent probe amplification and immunohistochemistry

Since multiple relatives were reported to have developed cancer, the patient was referred to genetic counselling. Family pedigree (*Fig. 2*), based on patient report, showed several first and second-

degree relatives (mainly on father side) affected by different cancer types diagnosed at a relatively early age (range: 43–63 years), with a distribution pattern consistent with autosomal dominant inheritance. When considering the tumor types, the co-occurrence of kidney cancer and pleural mesothelioma in the patient's brother, along with the report of uveal melanoma in an uncle, led us to hypothesize that the cause could reside in a *BAP1* gene defect. Indeed uveal melanoma, mesothelioma and clear cell renal cancer, together with cutaneous melanoma, atypical Spitz tumor and basal cell carcinoma, are confirmed BAP1-TPDS tumours and testing for *BAP1* is suggested when two or more of these tumours occur in one patient and/or in first or second-degree relatives [4]. Therefore, germline BAP1 mutation analysis was performed. As shown in Fig. 3A, compared to an iCCA patient harbouring BAP1 wild-type used as control (upper panel), a novel heterozygous BAP1 germline mutation was detected in our iCCA patient (lower panel). Notably, the mutation occurred at a donor splice site of BAP1 gene and was due to a deletion involving the last nucleotide of exon 4 and the first six nucleotides of intron 4 (NM_004656.4: c.255_255+6del). This mutation was predicted to create a *BAP1* truncated protein of 132 aminoacids, with a substitution of 47 aminoacids starting from the Gln85 of the BAP1 ubiquitin carboxyl hydrolase (UCH) domain onwards (p.Gln85?). *Unfortunately, no other relatives of the patient were available for genetic testing, therefore we were unable to assess segregation of the variant in the family. Further BAP1* sequencing on resected iCCA of the patient revealed *BAP1* loss of heterozygosity (LOH) (Fig. 3B); in particular MLPA analysis on peripheral blood and iCCA tumor tissue proved whole gene deletion (Fig. 3C, D). Further IHC analysis showed the complete loss of BAP1 protein expression in the nucleus and a faint positivity in the cytoplasm of tumor cells (Fig. 4, panels a, b). Conversely, a concomitant nuclear/cytoplasmic BAP1 staining was observed in the tumor tissue of the iCCA patient harbouring BAP1 wild-type, used as control (Fig. 4, panels c, d). *Overall* these findings suggest that in our patient, besides a BAP1 germline mutation, a further somatic loss of *BAP1* wild-type allele occurred in tumor cells.

Discussion

BAP1 somatic inactivating mutations occur with a relatively high frequency in iCCA (16–32%) and have been reported to correlate with poor overall survival and relapse-free survival [15–17]. Conversely, the incidence of BAP1 germline mutations and their contribution to cholangiocarcinogenesis still remain to be fully clarified. The current few available studies suggest a possible predisposition to develop this malignancy in individuals carrying
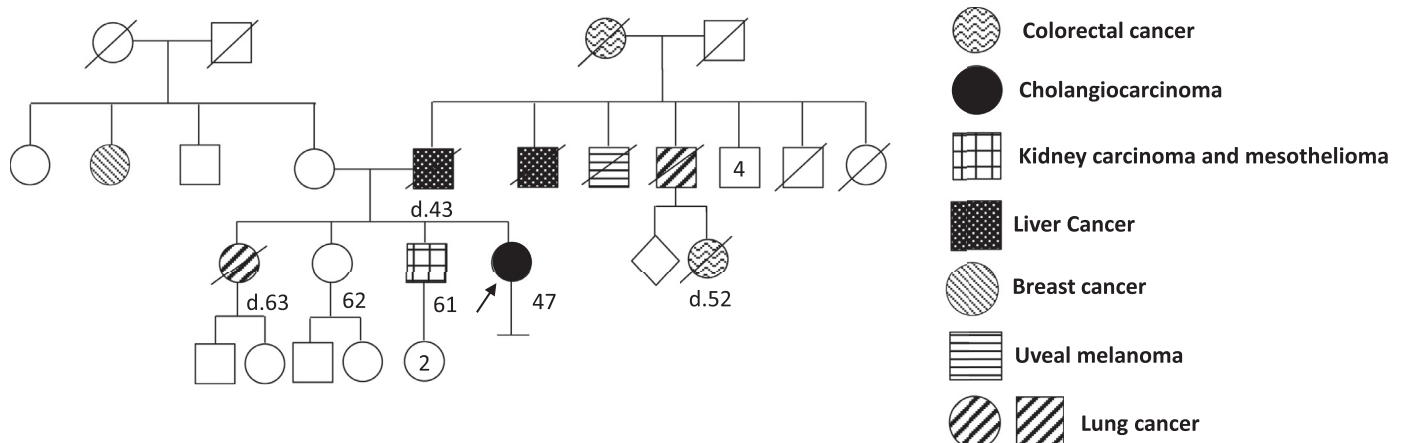

Fig. 2. Pedigree of the patient's family. Type of malignancies are shown in the legend. Numbers near the symbols represent the age at pedigree collection or at death (d). Black arrow indicates the proband.
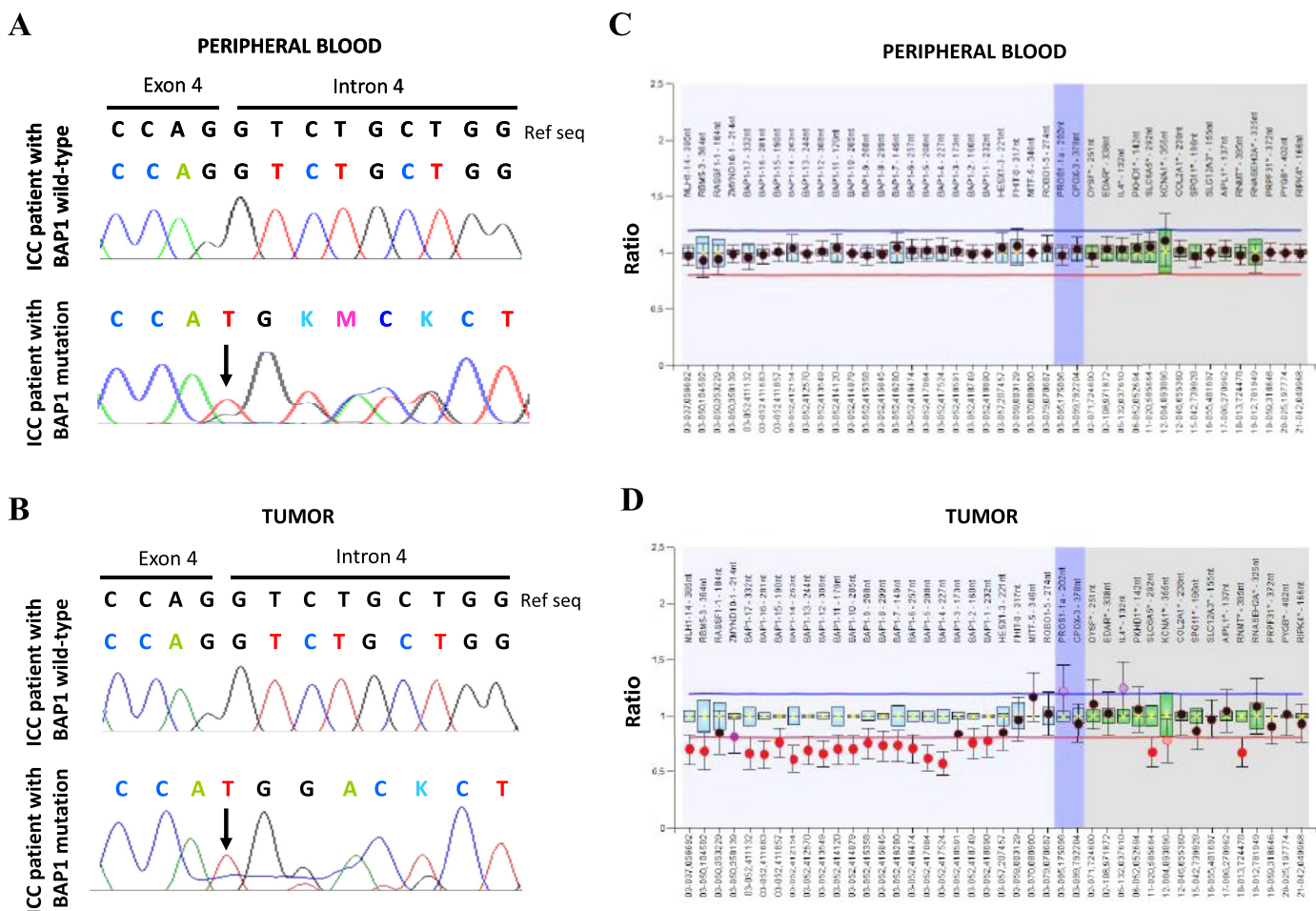
Fig. 3. A) DNA Sanger sequencing from the peripheral blood of an iCCA patient harbouring BAP1 wild-type used as control (upper panel), and from the peripheral blood lymphocytes of our patient (lower panel). Black arrow shows a heterozygous BAP1 germline mutation found in our patient. The mutation occurred at a donor splice site and was due to a deletion involving the last nucleotide of exon 4 and the first six nucleotides of intron 4 of BAP1 gene (NM_004656.4: c.255_255+6del); B) DNA Sanger sequencing from the tumor tissue of an iCCA patient harbouring BAP1 wild-type used as control (upper panel) and from the resected tumor tissue of our patient (lower panel). Black arrow shows LOH; C, D) *BAP1* MLPA analysis in the peripheral blood and in the tumor tissue of the iCCA patient harbouring the germline *BAP1* mutation. Ref seq: reference sequence.

BAP1 germline mutations ([6,11]), but further clinical evidences are needed.

In the present study we report the clinical case of a female patient who developed an iCCA when she was 47-years-old and who was found to carry a novel heterozygous germline mutation in the UCH domain of BAP1 gene (NM_004656.4: c.255_255+6del). It is well recognized that missense and truncating mutations occurring in the UCH and NLS (nuclear localization signal) domains of BAP1 mature protein may lead to cancer development, as they can affect BAP1 deubiquitinating activity and nuclear localization, both required for the tumor suppressor function of this gene [18]. More recently, it has been shown that also mutations affecting the splice sites of BAP1 pre-mRNA may contribute to tumorigenesis by disruption of normal splicing and formation of aberrant mRNA transcripts [19]. Proper pre-mRNA splicing is indeed an essential step for normal protein translation and function and requires an accurate recognition by the spliceosome machinery of consensus sequences that define the exon-intron boundaries. Among these, the most evolutionary conserved consensus sequences are the donor splice site (mainly composed of an intronic GU dinucleotide preceded by an exonic AG dinucleotide) located at the 5′ region of the intron, and the acceptor splice site located at the 3′ end of the intron [20]. Mutations occurring in these conserved sequences have been reported to result in errors during the splicing process, due to an improper intron removal and exon joining, thus leading to al-

terations of the open reading frame [21]. Notably, the heterozygous germline mutation identified in our iCCA patient was found to occur in the conserved consensus sequence of the donor splice site involving the 3′ end of exon 4 and the 5′ region of intron 4 of BAP1 gene. This mutation was predicted to create a *BAP1* truncated protein of 132 aminoacids, with a substitution of 47 aminoacids starting from the Gln85 of the BAP1 ubiquitin carboxyl hydrolase (UCH) domain onwards (p.Gln85?). Although the functional role of this novel mutation is unknown, the critical role of the UCH domain in driving BAP1 tumor suppressor function has been well established [18]. It is therefore conceivable that this genetic event may result in a truncated non-functioning BAP1 protein, as suggested by the lack of nuclear translocation. This observation is in line with previous studies showing that BAP1 inactivating mutations invariably associated with the lack of nuclear localization and/or presence of cytoplasmic staining [22]. In addition, *BAP1* LOH in the tumor tissue of our patient indicate that, besides this germline mutation, a further genetic event occurred at somatic level in tumor cells, in line with the classical Knudson two-hit model of tumor suppressor genes [8]; this phenomenon may account for the faint *BAP1* cytoplasmic positivity in tumor cells.

Previous studies have also shown that BAP1 heterozygous germline mutations may increase cancer risk after minimal exposure to asbestos fibers in animal models [23]. In particular, it has been reported that BAP1 (+/-) mice have a significantly higher
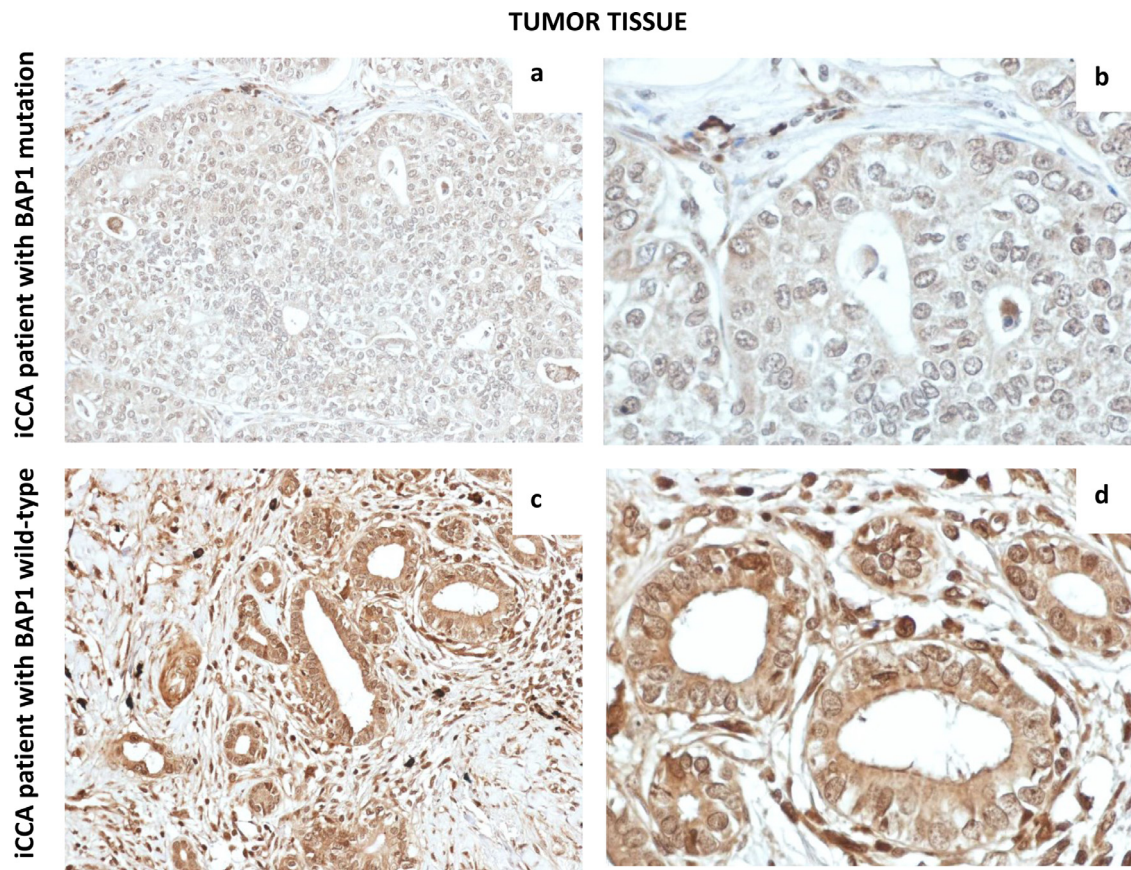
TUMOR TISSUE

Fig. 4. *BAP1* IHC analysis in the resected iCCA of our patient (panels a, b), showing a negative nuclear staining and a faint positivity in the cytoplasm in tumor cells, and in the tumor tissue of an iCCA patient harbouring BAP1 wild-type, used as control (panels c, d), showing a concomitant nuclear and cytoplasmic BAP1 expression. Panels a, c: magnification 10X; Panels b, d: magnification 40X.

incidence of mesothelioma after exposure to very low doses of asbestos compared to wild-type [23]. Furthermore, in BAP1 (+/-) mice mesotheliomas have been found to arise faster than in wild-type, showing an increased invasiveness and proliferation rate [24]. Drawing parallels to humans these findings suggest that, compared to wild-type subjects, individuals carrying BAP1 germline mutations may be more susceptible to asbestos carcinogenesis, even when exposed to low levels of fibers. Notably, asbestos exposure has been recently demonstrated to represent a strong risk factor not only for malignant mesothelioma, but also for iCCA development ([25,26]). In line with these observations, our patient carrying a BAP1 heterozygous germline mutation was found to be exposed to low levels of asbestos and to develop an iCCA at a younger age than general population.

In summary, in this case report we identified a novel BAP1 heterozygous germline mutation occurring at a splice site of exon 4 (NM_004656.4: c.255_255+6del). Moreover here we reported the first clinical evidence about a possible link between BAP1 germline mutations and iCCA development in a patient exposed to low levels of asbestos fibers. However, further studies are needed to confirm whether iCCA may be included into *BAP1*-TPDS cancer phenotypes and whether minimal asbestos exposure may facilitate the development of this malignancy in individuals carrying *BAP1* germline mutations.

Ethics statement

The present study has been approved by the local ethical committee of S.Orsola-Malpighi University Hospital. Written informed consent for the study and publication of this case report was ob-

tained from the patient i*n accordance* with institutional guidelines, including the Declaration of Helsinki (and subsequent modifications).

Declaration of Competing Interest

The authors declare that there are no competing interests.

Acknowledgements

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2020.10.001.

References

[1] Brandi G, Venturi M, Pantaleo MA, Ercolani G. GICO. Cholangiocarcinoma: current opinion on clinical practice diagnostic and therapeutic algorithms: a review of the literature and a long-standing experience of a referral center. Dig Liver Dis 2016;48:231–41.

[2] Khan SA, Tavolari S, Brandi G. Cholangiocarcinoma: epidemiology and risk factors. Liver Int 2019;39:19–31.

[3] Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. BAP1 and cancer. Nat Rev Cancer 2013;13:153–9.

[4] Rai K, Pilarski R, Cebulla CM, Abdel-Rahman MH. Comprehensive review of BAP1 tumor predisposition syndrome with report of two new cases. Clin Genet 2016;89:285–94.

[5] Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, et al. Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 2011;43:1022–5.

[6] Klebe S, Driml J, Nasu M, Pastorino S, Zangiabadi A, Henderson D, et al. BAP1 hereditary cancer predisposition syndrome: a case report and review of literature. Biomark Res 2015;3:14.

[7] Carbone M, Flores EG, Emi M, Johnson TA, Tsunoda T, Behner D, et al. Combined genetic and genealogic studies uncover a large BAP1 cancer syndrome kindred tracing back nine generations to a common ancestor from the 1700s. PLoS Genet. 2015;11:e1005633.

[8] Abdel-Rahman MH, Pilarski R, Cebulla CM, Massengill JB, Christopher BN, Boru G. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. J Med Genet 2011;48:856–9.

[9] Bononi A, Giorgi C, Patergnani S, Larson D, Verbruggen K, Tanji M, et al. BAP1 regulates IP3R3-mediated Ca(2+) flux to mitochondria suppressing cell transformation. Nature 2017;546:549–53.

[10] Zhang Y., Shi J., Liu X., Feng L., Gong Z., Koppula P., et al. BAP1 links metabolic regulation of ferroptosis to tumor suppression. Nat Cell Biol 2018;20;1181–1192.

[11] Pilarski R., Cebulla C.M., Massengill J.B., Rai K., Rich T., Strong L., et al. Expanding the clinical phenotype of hereditary BAP1 cancer predisposition syndrome, reporting three new cases. Genes Chromosomes Cancer 2014:53;177–182.

[12] Magnani C, Agudo A, González CA, Andrion A, Calleja A, Chellini E. Multicentric study on malignant pleural mesothelioma and non-occupational exposure to asbestos. Br J Cancer 2000;83:104–11.

[13] Andrici J, Goeppert B, Sioson L, Clarkson A, Renner M, Stenzinger A, Tayao M, et al. Loss of BAP1 Expression Occurs Frequently in Intrahepatic. Cholangiocarcinoma.Medicine (Baltimore). 2016;95:e2491.

[14] Amin MB, Edge S, Greene F, editors. AJCC cancer staging manual. 8th editor. Cham, Switzterland: Springer International Publishing; 2017.

[15] Jiao Y, Pawlik TM, Anders RA, Selaru FM, Streppel MM, Lucas DJ, et al. Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas. Nat Genet 2013;45:1470–3.

[16] Farshidfar F, Zheng S, Gingras MC, Newton Y, Shih J, Robertson AG, et al. Integrative Genomic Analysis of Cholangiocarcinoma Identifies Distinct IDH-Mutant Molecular Profiles. Cell Rep 2017;18:2780–94.

[17] Chen XX, Yin Y, Cheng JW, Huang A, Hu B, Zhang X. BAP1 acts as a tumor suppressor in intrahepatic cholangiocarcinoma by modulating the ERK1/2 and JNK/c-Jun pathways. Cell Death Dis 2018;9:1036.

[18] Ventii KH, Devi NS, Friedrich KL, Chernova TA, Tighiouart M, Van Meir EG, et al. BRCA1-associated protein-1 is a tumor suppressor that requires deubiquitinating activity and nuclear localization. Cancer Res 2008;68:6953–62.

[19] Morrison A, Chekaluk Y, Bacares R, et al. BAP1 missense mutation c.2054 A>T (p.E685V) completely disrupts normal splicing through creation of a novel 5′ splice site in a human mesothelioma cell line. PLoS ONE 2015;10:e0119224.

[20] Baralle D, Baralle M. Splicing in action: assessing disease causing sequence changes. J Med Genet 2005;42:737–48.

[21] Abramowicz A, Gos M. Splicing mutations in human genetic disorders: examples, detection, and confirmation. J Appl Genet 2019;60:231.

[22] Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Luk H. High Incidence of Somatic BAP1 alterations in sporadic malignant mesothelioma. J Thorac Oncol 2015;10:565–76.

[23] Napolitano A, Pellegrini L, Dey A, Larson D, Tanji M, Flores EG, et al. Minimal asbestos exposure in germline BAP1 heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. Oncogene 2016;35:1996–2002.

[24] Xu J, Kadariya Y, Cheung M, Pei J, Talarchek J, Sementino E, et al. Germline mutation of Bap1 accelerates development of asbestos-induced malignant mesothelioma. Cancer Res 2014;74:4388–97.

[25] Brandi G, Di Girolamo S, Farioli A, de Rosa F, Curti S, Pinna AD. Asbestos: a hidden player behind the cholangiocarcinoma increase? Findings from a case–control analysis. Cancer Causes Control 2013;24:911–18.

[26] Farioli A, Straif K, Brandi G, Curti S, Kjaerheim K, Martinsen JI, et al. Occupational exposure to asbestos and risk of cholangiocarcinoma: a population-based case-control study in four Nordic countries. Occup Environ Med 2018;75:191–8.