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Molecular Case Studies

RESEARCH REPORT

Pathogenic ATM and BAP1 germline mutations in a case of early-onset, familial sarcomatoid renal cancer

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Abstract Metastatic renal cell carcinoma (RCC) remains an incurable malignancy, despite recent advances in systemic therapies. Genetic syndromes associated with kidney cancer account for only 5%–8% of all diagnosed kidney malignancies, and genetic predispositions to kidney cancer predisposition are still being studied. Genomic testing for kidney cancer is useful for disease molecular subtyping but provides minimal therapeutic information. Understanding how aberrations drive RCC development and how their contextual influences, such as chromosome loss, genome instability, and DNA methylation changes, may alter therapeutic response is of importance. We report the case of a 36-yr-old female with aggressive, metastatic RCC and a significant family history of cancer, including RCC. This patient harbors a novel, pathogenic, germline ATM mutation along with a rare germline variant of unknown significance in the *BAP1* gene. In addition, somatic loss of heterozygosity (LOH) in *BAP1* and *ATM* genes, somatic mutation and LOH in the *VHL* gene, copy losses in Chromosomes 9p and 14, and genome instability are also noted in the tumor, potentially dictating this patient's aggressive clinical course. Further investigation is warranted to evaluate the association of *ATM* and *BAP1* germline mutations with increased risk of RCC and if these mutations should lead to enhanced and early screening.

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INTRODUCTION

Renal cancer is the ninth most common cancer and is responsible for around 115,000 deaths per year (Næraa et al. 2019). Patients with small, localized kidney cancers have high survival; in contrast, advanced and metastatic kidney cancer has only a 2-yr survival rate of 20% (Capitanio and Montorsi 2016). Many germline predisposition genes associated with familial kidney cancers have been characterized. These include von Hippel–Lindau (VHL), hereditary leiomyomatosis renal cell carcinoma (HLRCC), Birt–Hogg–Dubé (BHD), hereditary papillary renal carcinoma (HPRCC), succinate dehydrogenase (SDH) deficiency, and tuberous

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sclerosis complex (TSC) (Guo et al. 2021). von Hippel–Lindau is an autosomal dominant disease, presenting as a result of mutation in the *VHL* gene (Maher et al. 1991). *VHL* mutation leads to the development of tumors and cysts in the kidney with high penetrance (Gossage et al. 2015). Chromosome 3 translocation has also been linked to hereditary kidney cancer (Woodward et al. 2010). A balanced translocation between the short arm of Chromosome 3 and the long arm of Chromosome 8 is most common in hereditary kidney cancer and leads to overexpression of the vascular endothelial growth factory (VEGF) (Maher 2018). Germline mutation in the *SWI/SNF* chromatin remodeling complex, *PBRM1*, is also tied to a very rare form of hereditary kidney cancer in a dominant transmission pattern (Benusiglio et al. 2015). Hereditary papillary renal cell carcinoma patients are at risk for many microscopic tumors of the kidney, and this syndrome is tied to mutations in the proto-oncogene, tyrosine kinase *MET* (Schmidt et al. 1997). BHD is a syndrome leading to the development of a variety of types of kidney cancer and is tied to mutation in the *FLCN* gene (Nickerson et al. 2002). *FLCN* is in the *LKB1/AMPK* pathway and leads to chronic activation of mTORC (Woodward et al. 2008). Mutations in fumarate hydratase and succinate dehydrogenase can also lead to hereditary kidney cancer (Baysal et al. 2000). Tuberous sclerosis often leads to renal tumors, particularly benign angiomyolipomas, and is tied to mutations in *TSC1* and *TSC2*, which together activate the mTOR pathway (Peron et al. 2016). Other germline kidney cancer–predisposing genes are *BAP1*, *PTEN*, and the *SHD* family of genes (Haas and Nathanson 2014; Linehan et al. 2019). All of these recorded hereditary genetic cases comprise only 5%–8% of kidney cancers (Haas and Nathanson 2014). There are many other families in which multiple individuals develop kidney cancer without any of the known mutations. In fact, 58% of all kidney cancers are in families that have more than one family member with kidney cancer (Linehan 2012). Although environmental factors are no doubt also important, this suggests some unknown genetic basis of renal cancer. Germline sequencing in patients with known RCC revealed that 5.5% of patients have mutations in RCC-associated genes and 10.5% have mutations in other cancer-associated genes (Carlo et al. 2018). Previous work has implicated DNA repair pathway proteins such as ATM with kidney cancer (Guo et al. 2021). *BAP1*, a tumor suppressor gene, is also commonly mutated in advanced kidney cancer and a germline mutation is present in some kidney cancers. This data suggests that germline mutations are heavily associated with the development of kidney cancer, but that all of the potential germline risk factors are unknown. Clearly there is an unmet need to uncover other genetic syndromes within RCC that may result in a hereditary predisposition. This case study reveals inherited germline mutations of *BAP1* and *ATM* in a young patient with aggressive, metastatic RCC.

CASE REPORT

A 36-yr-old female presented to the emergency department with severe right flank pain and gross hematuria (Fig. 1). She reported dysuria, urinary frequency, dizziness, and lightheadedness. Imaging revealed a right upper pole necrotic mass ~7.5×7.5×8.5 cm. Robotic assisted right nephrectomy occurred on October 1, 2019 with pathology demonstrating pT3 clear cell renal carcinoma, World Health Organization/International Society of Urological Pathology (WHO/ISUP) nuclear grade 4, in concordance with the previously assessed tumor biopsy findings. The tumor was 5.5 cm in size and invading the perirenal adipose tissue. It consisted of 10% sarcomatoid/rhabdoid component; 0/1 lymph nodes were involved. The International Metastatic RCC Database Consortium (IMDC) risk category is intermediate at time of presentation. She was diagnosed with WHO/ISUP grade 4 clear RCC with rhabdoid and sarcomatoid features and with extension into the perirenal soft tissue (pT3a, pN0, cM0) (Fig. 2). Hematoxylin and eosin (H&E) staining of the nephrectomy specimen showed both

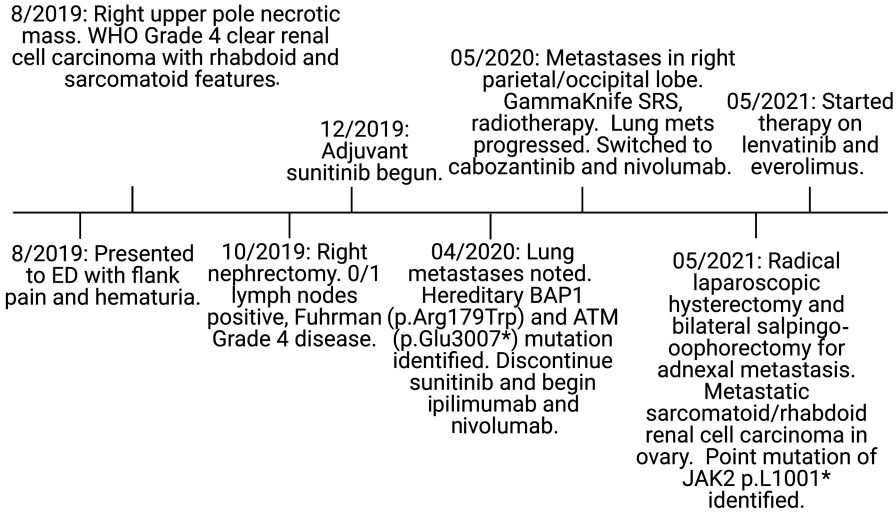

Figure 1. Disease progression from diagnosis to metastasis. A 36-year-old female presented to the emergency department with flank pain and hematuria and was diagnosed with clear cell renal cell carcinoma (ccRCC) in October of 2019. Lung metastases were noted, as well as hereditary mutations in *BAP1* and *ATM* in April of 2020.

low- and high-grade areas (Fig. 2A). Carbonic anhydrase 9 (CA9), a characteristic immunohistochemical marker of clear cell RCC (ccRCC) that is not expressed in normal renal tissue, was strongly positive (Fig. 2B). BAP1 expression is extremely low in the tumor region as assessed by immunohistochemistry (Fig. 2C). Adjuvant sunitinib was begun and 4 mo later, lung metastases were noted on imaging performed on April 1, 2020.

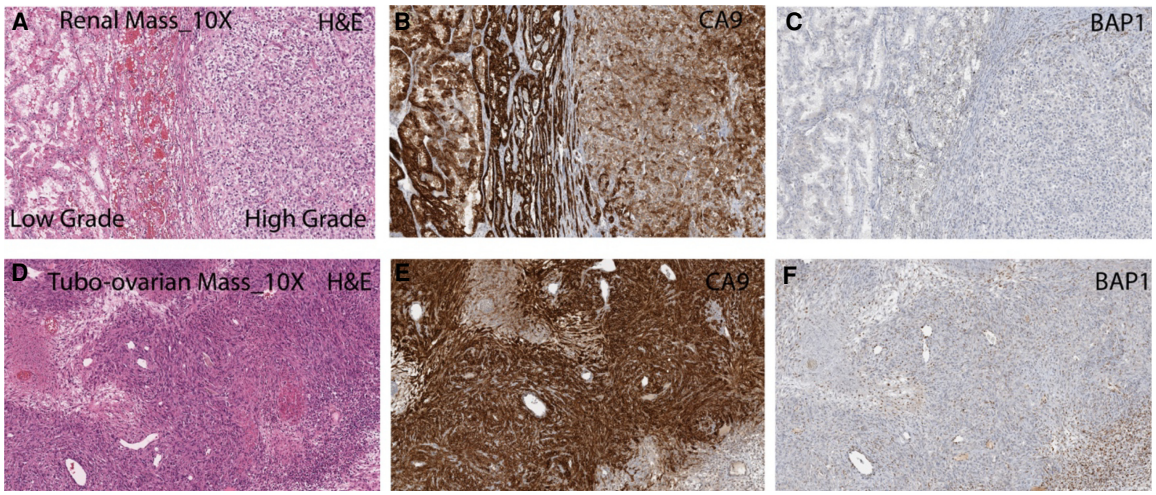

Figure 2. BAP1 staining in primary kidney tumor correlates with grade. (*A*) Photomicrograph of the right renal mass exhibiting high-grade and low-grade areas of clear cell renal cell carcinoma (hematoxylin and eosin [H&E]; 200×). The corresponding areas showing (*B*) strong brown membranous staining with carbonic anhydrase IX (CA9; 200×) immunohistochemistry and (*C*) loss of BRCA1-associated protein 1(BAP1; 200×) immunoexpression in tumor epithelia. (*D*) The tissue section from the tubo-ovarian metastatic mass shows sarcomatoid differentiation with focal areas of necrosis (H&E; 200×). The corresponding areas (*E*) exhibiting strong membranous immunoexpression of carbonic anhydrase IX (CA9; 200×) with loss of BRCA1-associated protein 1(BAP1; 200×) immunoexpression in tumor epithelia (*F*).

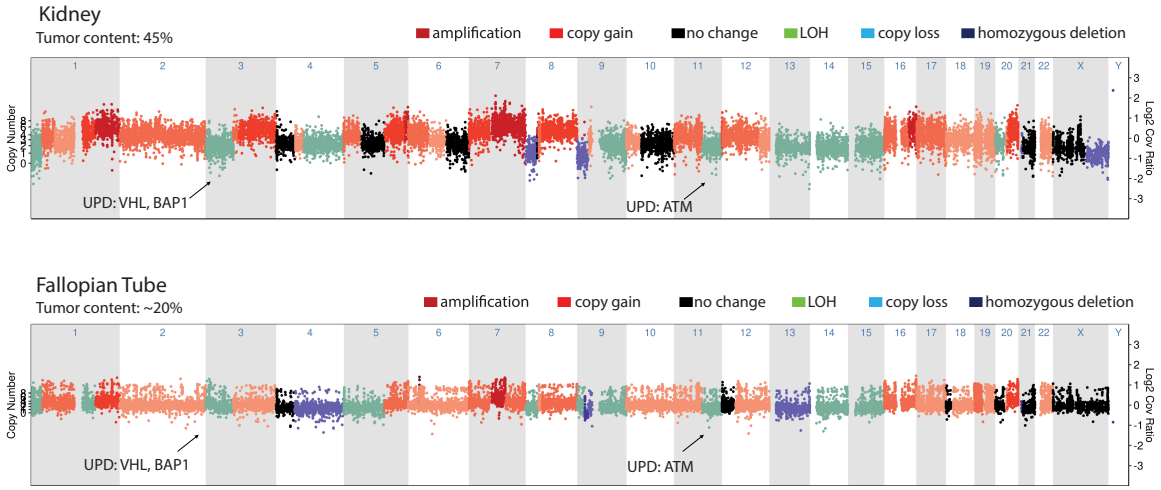

Figure 3. Chromosome plot of the primary renal tumor compared to the fallopian tube metastasis. The kidney tumor and the fallopian tube metastatic tumor have very similar genomic landscapes. They both have uniparental disomy of the regions encompassing VHL, BAP1, and ATM.

Sequencing of the primary right kidney tumor through MI-ONCOSEQ found a JAK2p.L1001* stop-gain mutation. All sequencing abnormalities are described in Supplemental Table 1. MI-ONCOSEQ uses a targeted panel focusing on protein-coding exons in a 1700-gene set. *VHL* had a somatic indel, p.Asp150IfsTer9, with an acquired loss of homozygosity by uniparental disomy in the tumor. Sequencing also revealed an acquired uniparental disomy of Chromosome 3p, which contains *VHL* and *BAP1* and uniparental disomy of Chr 11q, which carries *ATM* (Fig. 3). Outlier gene expression was seen of *CA9* and *PAX8*. *CA9* is not expressed in normal kidney tissue, but is expressed in most ccRCCs, secondary to a functional *VHL* loss in these tumors. Germline testing showed a hereditary BAP1 mutation of unknown significance and a hereditary *ATM* mutation (Tables 1 and 2; Supplemental Table S1). Somatic mutations in the renal tumor are cataloged in (Tables 1 and 2; Supplemental Table S1). Germline mutation in *FANCD2*, a key protein in the DNA damage repair pathway, was also noted. The patient was initially started on adjuvant treatment with sunitinib, but new lung metastases were seen ~4 mo after beginning treatment. Lung biopsy was consistent with metastatic ccRCC. Patient was started on ipilimumab and nivolumab. Within months of therapy, imaging revealed brain metastases in the right parietal/occipital lobe, which were treated with GammaKnife radiosurgery and steroids. She also received palliative radiotherapy to a right calvarial lesion. Progression-free survival on the ipilimumab and nivolumab was only 25 d before progression of disease in lung, neck, and scalp. Lung metastases progressed, and she was switched to cabozantinib and nivolumab. After severe hand-foot syndrome on 60 mg of cabozantinib, the dose was decreased to 40 mg with good tolerability. Progression-free survival on the cabozantinib and nivolumab was 8 mo before imaging showed slight progression of lung nodules, and an adnexal mass was noted to be suspicious for metastatic disease. Pulmonary embolism was also noted in the same time frame.

She underwent a robotic-assisted modified radical laparoscopic hysterectomy and bilateral salpingo-oophorectomy for adnexal metastasis in May of 2021. The uterus showed no endometrial hyperplasia or malignancy, whereas the right fallopian tube and ovary showed metastatic sarcomatoid/rhabdoid RCC in ovary, 9 cm, with 20% punctate geographic necrosis (Fig. 2D–F). She was subsequently treated with lenvatinib and everolimus. Based on immunohistochemical assessment, the adnexal metastasis was strongly positive for *CA9*. Sequencing showed a somatic point mutation of *JAK2 p.L1001** gain of a stop codon

Table 1. Germline, kidney, and adnexal mutations

Germline	Kidney	Adnexal metastasis
AKT1 p.R406C	*AKT1* p.R406C (germline)	*AKT1* p.R406C (germline)
ATM p.E3007*	*ATM* p.E3007* (germline)	*ATM* p.E3007* (germline)
BAP1 p.R179W	*BAP1* p.R179W (germline)	*BAP1* p.R179W (germline)
EXT2 p.Y615Ter	*EXT2* p.Y615Ter *(germline)	*EXT2* p.Y615Ter *(germline)
FANCD2 p.N545S	*FANCD2* p.N545S (germline)	*FANCD2* p.N545S (germline)
	BAZ1A p.A74Serfs*30	*AFF2* p.S101Y
	BTG1 p.P160R	*DSP* p.K2706del
	CDK15 p.S3P	*EXT1* p.M100V
	DSP p.K2706del	*FLT3* p.I538V
	EXT1 p.M100V	*JAK2* p.L1001*
	FANCM p.M1397V	*KMT2D* p.T1246M
	GNA13 p.F245L	*KMT2E* p.M1115L
	JAK2 p.L1001*	*LRP5* p.M827I
	LRP5 p.M827I	*MAP3K14* p.H401Q
	MAP3K14 p.H401Q	*NF1* p.F23L
	NF1 p.F231L	*PPP2R2B* p.S19delinsRAAA
	NR3C1 p.G91V	*RYK* p.R4fs
	NTRK3 p.P738H	*SKOR1* p.R799M
	NUP214 p.T14494R	*VHL* p.N150llefsTer9
	TENM2 p.R1017W	
	TRIO p.M1000I	
	VHL p.N150llefsTer9	
	WNK1 p.E1114D	
	XPC p.G217C	

(Tables 1 and 3), identical to the one observed in the primary tumor. This *JAK2*-truncating mutation is associated with resistance to PD1 blockade (Carlo et al. 2018). A somatic indel was also seen in *VHL*, *p.Asp150lfsTer9*, with loss of homozygosity by uniparental disomy (Fig. 3). Sequencing also revealed uniparental disomy of Chromosome 3p (*VHL*, *BAP1*) and uniparental disomy of Chr 11q (*ATM*). These mutations were consistent with those noted in original right kidney primary tumor. However, the fallopian tube metastasis had fewer overall somatic mutations than the kidney cancer, which could be due to the lower tumor content noted for this specimen. Again, germline variants of *ATM p.Glu3007** stop-gain, with loss of heterozygosity in the tumor, were identified. This *ATM* variant may lead to eligibility in PARP inhibitors trials. In addition, the *BAP1 p.Arg179Trp* was again identified, highlighting the need for genetic counseling and the potential role of *BAP1* in her disease. Copy-number assessments on specimens from both sites showed overall a heavy copy-

Table 2. Sequencing quality assessment

Sample site	Sample quality	Sequencing quality	Library quality	Sample identity (SNP fingerprinting)
Fallopian tube	20% tumor content	Splice junction coverage < 40,000	Pass	Pass
Right kidney	Pass	Pass	Pass	Pass

(SNP) Single-nucleotide polymorphism.

Table 3. Detailed mutation information

Gene	Site	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP/ dbVar ID	Allelic fraction
BAP1	Germline	Chr 3: 52407219	NM_004656:c.535C > T	p.Arg179Trp	Missense	Substitution	N/A	Normal allelic fraction: 50.20%; tumor allelic fraction: 78.90%
ATM	Germline	Chr 11: 108365356	NM_000051:c.9019G > T	p.Glu3007Ter	Missense	Substitution	N/A	Normal allelic fraction: 54.10%; tumor allelic fraction: 74.70%
EXT2	Germline	Chr 11: 44232436	NM_000401:c.1845T > A	p.Tyr615Ter	Missense	Substitution	N/A	Normal allelic fraction: 52.20%; tumor allelic fraction: 54.10%

number burden involving almost all chromosomes, indicating high genome instability. In addition, loss of Chr 9p and LOH of Chr 14 are notable as these events along with genome instability have been previously associated with poor prognosis (Turajlic et al. 2018) (Fig. 3). Two-month-interval brain magnetic resonance imaging (MRI) after starting everolimus and levatinib showed interval development of four supratentorial enhancing lesions consistent with brain metastasis. Computed tomography (CT) chest/abdomen/pelvis showed interval worsening of mediastinal and bilateral hilar lymphadenopathy. Malignant lymphadenopathy completely surrounded the distal trachea and both mainstem bronchi.

A family history of RCC was noted, as her father was diagnosed at age 59 and died of rapidly progressive disease without receiving systemic therapy. Her mother had a basal cell carcinoma, her maternal grandfather multiple myeloma at 73 yr of age, and her maternal uncle had brain cancer. The patient had a benign bone tumor in her right knee as a teenager. Genetic testing at time of diagnosis revealed a germline pathogenic mutation in the *ATM* gene, *c.9019G* to T (*p.Glu3007**) as well as a variant of unknown significance in the gene *BAP1*, *c.535C* to T (*p.Arg179Trp*). The substitution in ATM induces a nonsense variant, which truncates the final 49-amino acid residues. *ATM* plays a critical role in DNA repair and response to double-strand breaks (Cremona and Behrens 2014). Twenty percent of metastatic kidney cancers have a mutation in a DNA damage repair protein pathway, and many tumors have *ATM* mutations (Ged et al. 2020). *ATM* expression has been reported to be lower in ccRCC than in adjacent normal tissue, and *ATM* is decreased in tissue from a higher-grade tumor. Low *ATM* tumors are associated with a lower survival rate, suggesting that *ATM* expression level could be a factor in ccRCC prognosis (Ren et al. 2019). However, there is no clear association of germline *ATM* with hereditary kidney cancer. *ATM* is associated with increased risk of breast cancer, pancreatic cancer, and prostate cancer, and germline DNA damage repair pathway mutations are seen in 7.3% of kidney cancers (Truong et al. 2021). The germline *BAP1*, *c.535C* to T (*p.Arg179Trp*) mutation is a recurrent somatic mutation in four cases in COSMIC; in RCC, biliary tract cancer, and lung cancer. *BAP1* is a tumor suppressor gene involved in DNA damage repair, cell cycle, and cellular differentiation (Peña-Llopis et al. 2012). *BAP1* mutation is associated with a syndrome of uveal melanoma, mesothelioma, and ccRCC (Rai et al. 2016). RCC with *BAP1* mutation has been reported to have a rapidly progressing aggressive course (Carlo et al. 2019).The germline *BAP1* and *ATM*, coupled with the hereditary predisposition of ccRCC noted, make this case noteworthy.

DISCUSSION

The patient had a strong family history of cancer. Her mother had basal cell carcinoma, her grandfather developed multiple myeloma, and an uncle had brain cancer. Most significantly,

her father was diagnosed with aggressive RCC and died from it at the age of 61. Combined with her aggressive disease at a young age, this warranted germline genetic testing. Genetic testing revealed a novel pathogenic *ATM* mutation that has not been previously associated with RCC and a novel *BAP1* mutation. The *ATM* mutation is associated with increased risk of breast, prostate, and pancreatic cancer (Cremona and Behrens 2014). *ATM* is a serine/threonine protein kinase that is recruited by double stranded breaks in the DNA. DNA damage repair pathways have been implicated as a frequent pathway mutated in ccRCC. A recent survey of 229 patients revealed that 19% of patients had somatic deleterious DNA damage repair gene alterations, and the most frequently altered genes were *CHEK2* (some germline and some somatic) and *ATM* (all somatic) (Burma et al. 2001). It initiates activation of the DNA damage checkpoint which leads to cell cycle arrest (Ged et al. 2020). This patient also has a germline mutation in *FANCD2*, a crucial protein in the DNA damage response. This is the first report of this specific germline *ATM* mutation in a patient with RCC. Further study will be needed to determine c.9019G to T (p.Glu3007*) pathogenicity in renal cancer, and to determine if it leads to higher risk and the potential need for increased surveillance in family members who inherit this gene.

Integrative clinical sequencing of the patient's tumor DNA and germline DNA performed at the University of Michigan Clinical Sequencing Program MIONCOSEQ revealed the driver germline aberrations and the large number of somatic driver insults accumulated in the tumor genome. Besides the ccRCC signature Chromosome 3p loss, losses of 14 and 9p were of interest because of their prognostic association. So, we predict that either Chr 3q, 5q, or 8q may be involved in the Chr 3 translocation in this patient, which, however, can be tested only with whole-genome sequencing data that is currently not available. The high copy-number burden we observed indicates the presence of genome instability in case, which is again associated with poor prognosis.

MIONCOSEQ analysis also revealed several mutations including a mutation in BRCA associated protein 1 (*BAP1*) (Robinson et al. 2017). *BAP1* is a tumor suppressor gene that is commonly mutated in ccRCC. *BAP1* germline mutations have been found to segregate with RCC and lead to a substantially increased risk of RCC (Creighton et al. 2013; Popova et al. 2013). *BAP1* encodes a nuclear deubiquitinase and requires two hits to eliminate its tumor suppressor function (Dey et al. 2012). *BAP1* mutations are associated with high grade clear cell RCC, with renal tumors exhibiting rhabdoid morphology and poor patient outcomes (Peña-Llopis et al. 2012). Low expression of *BAP1* is also associated with worse survival. Preclinical studies have suggested that *BAP1* mutation may be predictive of sensitivity to both mTOR inhibitors and radiotherapy (Lim et al. 2016). *BAP1* mutations are associated with a high Fuhrman grade, coagulative necrosis, and poor outcomes (Brugarolas 2013). This patient has Fuhrman grade 4 disease. Recent reports have generated a description of a hereditary *BAP1* tumor syndrome, which involves uveal melanoma, cutaneous melanoma, and mesothelioma (Kobrinski et al. 2019). In addition, *BAP1* mutation in ccRCC has been found to be increased in metastatic cases compared to nonmetastatic patients (Meng et al. 2020). Somatic *BAP1* mutation at a different highly conserved catalytic residue has been associated in a family with early-onset ccRCC with high Fuhrman grade (Farley et al. 2013). *BAP1* and *PBRM1* loss is associated with rhabdoid features, and high tumor grade (Peña-Llopis et al. 2012). The variant in this case is reported in ClinVar and is recorded as a variant of uncertain significance by commercial genetics companies Invitae and Ambry. Invitae has seen this particular variant five times, with mixed clinical information. Ambry has seen this variant less than five times. This particular amino acid change from arginine to tryptophan at position 179 is a moderate physicochemical change, and this region is well-conserved, suggesting that this mutation is functional. Interestingly, *BAP1* anticorrelates with the other commonly mutated ccRCC gene *PBRM1*. *BAP1* mutation is associated with significantly worse overall survival than *PBRM1* mutation ccRCC. Together, this data suggests that

BAP1 and *PBRM1* mutations like denote two different molecular subtypes with different gene expression patterns and outcomes. Specifically, germline *BAP1* mutation *c.277*A to G (*p.Thr93Ala*) has been associated with RCC predisposition (Creighton et al. 2013). The variant c.41T to A, p.L14H has also been associated with RCC. The mutation present in the case here has not been reported to be associated with renal cell carcinoma and is currently considered a variant of unknown significance. The patient's family history of RCC, her aggressive clinical course, and negative *BAP1* immunohistochemical (IHC) staining data demonstrates that the *BAP1* variant reported here is pathogenic and should therefore no longer be considered a variant of unknown significance. Interestingly, in uveal melanoma, concurrent mutation in *BAP1* and *ATM* is correlated with increased tumor stage and increased metastasis risk (Jha et al. 2020). The combination of the *ATM* and *BAP1* mutations is rare, and the double germline occurrence of these mutations is even rarer. The patient's RCC was resistant to contemporary therapies besides the Jak2 mutation noted, which is known to be associated with sarcomatoid RCC (PMID: 30576871) and also may predict for resistance to immune checkpoint inhibitor therapies (Carlo et al. 2018).

Currently, germline *BAP1*-mutated individuals are recommended to undergo abdominal and respiratory physical examinations starting at 30-yr-old. Ultrasound or MRI surveillance should be considered every 2 years between ages 30 and 55. At age 55, consider a chest/abdomen CT or MRI chest/abdomen with IV contrast every 2 yr (Kobrinski et al. 2019). An annual ophthalmic exam is recommended starting at age 16 consisting of direct and indirect ophthalmoscopy, fundus photography, and ocular ultrasound. Biannual full-skin examinations with dermatologic examination is recommended starting at 18. As for targeted treatment in *BAP1*-mutated RCC, histone deacetylase inhibitors could be useful in reversing the H2A hyperubiquitination caused by *BAP1* loss (Landreville et al. 2012). *BAP1* also increases expression of *EZH2*, so EZH2 inhibitors are another potential targeted therapy to consider (LaFave et al. 2015). Another study showed that the PARP inhibitor rucaparib seems to be selective for *BAP1* mutant cell lines (Louie and Kurzrock 2020). *JAK2* activation up-regulates PD-L1 and can lead to immune evasion (Gupta et al. 2019). A subset of renal tumors with a sarcomatoid transformation exhibit constitutive PD-L1 overexpression, and these patients should be evaluated for enhanced response to immunotherapy (Gupta et al. 2019). Prioritizing studying the variant identified in this patient, and potential molecular vulnerabilities could open up new classes of targeted therapies.

Conclusion

There is a sizeable proportion of inherited kidney cancer whose genetic origins are unknown. This case suggests pathogenesis of a novel germline *ATM* mutation and a novel germline *BAP1* mutation. *BAP1* protein loss of expression in the tumor area as assessed by immunohistochemistry analysis suggests that this *BAP1* mutation has functional consequences. The patient also presents with a *JAK2* mutation that may predispose to resistance to PD1 blockade. Future work is needed to evaluate whether these individual *ATM* and *BAP1* mutations lead to hereditary predisposition to renal cancer and predict resistance to systemic therapies.

METHODS

IHC CA9 (carbonic anhydrase IX) rabbit polyclonal primary antibody (Cat No. NB100-417, Novus Biologicals) and BAP1 (BRCA1-associated protein 1) mouse monoclonal primary antibody (Cat No. sc-28382, Santa Cruz Biotechnology) was performed on 4 μ formalin-fixed, paraffin-embedded (FFPE) tissue sections. IHC was carried out on the Benchmark XT automated slide staining system using known positive and negative control tissues. For CA9, a

strong intensity membranous brown chromogenic staining was taken as positivity. For BAP1, loss of nuclear positivity in the cancer cells was recorded and accompanying non–tumor epithelial cells (immune and stromal cells) exhibiting BAP1 nuclear positivity act as positive internal control. Tumor and normal OncoSeq exome capture libraries and tumor whole transcriptome capture libraries were analyzed for both patients. Quality control for tumor content, sample quality, sequencing quality, library quality, and sample identify with SNP fingerprinting were performed and described in Table 2.

ADDITIONAL INFORMATION

Data Deposition and Access

The novel variants reported have been deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession numbers SCV002107489 for NM_000051.4 (ATM):c.9019G>T(p.Glu3007Ter) and SCV002107403 for NM_004656.4(BAP1):c.535C>T (p.Arg179Trp). All sequencing information is in the supplement of this report.

Ethics Statement

We confirm that we obtained written informed consent for research and publication from this patient, including clinical information, sequencing information, and pathological images prior to the submission of this manuscript from the participant. Human investigations were approved by a local Human Investigations Committee and all regulatory and HIPPA guidelines were followed.

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Author Contributions

D.Z., D.J., and U.V. treated the patient. C.K.-S., R.Ma., Y.Z., R.Me., and S.M.D. performed pathological analysis. H.N.B. and U.V. wrote and edited this manuscript.

Competing Interest Statement

The authors have declared no competing interest.

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