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Germline Mutations of Renal Cancer Predisposition Genes and Clinical Relevance in Chinese Patients With Sporadic, Early-Onset Disease

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Abstract

BACKGROUND: An inherited susceptibility to renal cancers is associated with multiple predisposing genes, but most screening tests are limited to patients with a family history. Next-generation sequencing (NGS)–based multigene panels provide an efficient and adaptable tool for investigating pathogenic germline mutations on a larger scale. This study investigated the frequency of pathogenic germline mutations in renal cancer predisposition genes in patients with sporadic, early-onset disease.

METHODS: An NGS-based panel of 23 known and potential renal cancer predisposition genes was used to analyze germline mutations in 190 unrelated Chinese patients under the age of 45 years who presented with renal tumors. The detected variants were filtered for pathogenicity, and then their frequencies were calculated and correlated with clinical features. Germline variants of the fumarate hydratase (*FH*) and BRCA1-associated protein 1 (*BAP1*) genes were comprehensively analyzed because of their aggressive potential.

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AUTHOR CONTRIBUTIONS

Junlong Wu: Data curation, formal analysis, visualization, and writing– original draft. **Hongkai Wang:** Data curation. **Christopher J. Ricketts:** Writing–original draft and visualization. **Youfeng Yang:** Methodology and investigation. **Maria J. Merino:** Methodology and validation. **Hailiang Zhang:** Investigation and resources. **Guohai Shi:** Investigation and resources. **Hualei Gan:** Methodology and validation. **W. Marston Linehan:** Conceptualization, supervision, and writing–review and editing. **Yao Zhu:** Conceptualization, funding acquisition, supervision, and writing–review. **Dingwei Ye:** Conceptualization, funding acquisition, supervision, resources, and writing–review.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

RESULTS: In total, 18 patients (9.5%) had germline mutations in 10 genes. Twelve of these 18 patients had alterations in renal cancer predisposition genes (6.3%), and 6 patients had mutations in potential predisposition genes such as *BRCA1*/*2*. Notably, pathogenic mutation carriers had a significant family history in second-degree relatives in comparison with those without pathogenic mutations ($P < .001$). Variants of unknown clinical significance in *FH* and *BAP1* demonstrated evidence of additional somatic loss in tumors

CONCLUSIONS: In patients with early-onset disease, a multigene panel identified a high pathogenic germline mutation rate in renal cancer predisposition genes. This study emphasizes the importance of screening patients with early-onset disease for mutations in cancer predisposition genes. Germline screening should be encouraged in early-onset patients to provide personalized medicine and improve patient outcomes.

Keywords

BRCA1-associated protein 1 (*BAP1*); cancer predisposition; early onset; fumarate hydratase (*FH*); next-generation sequencing; renal tumor

INTRODUCTION

Renal cell carcinoma (RCC) is a deadly malignancy. In the United States and China, 62,700 and 66,800 new cases of kidney cancer are estimated to have been diagnosed in 2016, and these led to 14,200 and 23,400 deaths, respectively.[1,2] Previous reports have proposed that hereditary kidney cancer accounts for approximately 3% to 5% of all kidney cancers, but this is likely to be an underestimation.[3] It is important to identify patients with inherited diseases because their management and targeted therapies can be different from those for patients with sporadic cancers.

Kidney cancer is composed of several different subtypes, all arising within the same organ, that are classified by histology and present with disparate genetic alterations and clinical features. The genetic basis for several hereditary kidney cancer syndromes associated with various histological subtypes of renal tumors, such as Von Hippel-Lindau (VHL) syndrome, has been elucidated via family studies.[4–6] Currently, there are 14 genes in which germline mutations are associated with a predisposition to renal cancer; however, the genetic basis of some inherited renal cancers remains unclear.[7] A number of genes such as SET domain containing 2 (*SETD2*), lysine demethylase 6A (*KDM6A*), *KDM5C*, neurofibromin 2 (*NF2*), and transcription elongation factor B polypeptide 1 (*TCEB1*)[8–11] have been shown to be somatically mutated at a high frequency in RCC, and germline mutations in these genes may explain some of the cases negative for the 14 genes. In addition, germline mutations in DNA repair genes such as *BRCA1*/*2*[12] and other frequently mutated cancer predisposition genes such as cyclin-dependent kinase inhibitor 2A (*CDKN2A*)[13,14] and tumor protein 53 (*TP53*)[15,16] are associated with a high susceptibility to various cancers and may also play a role in renal tumor development.

This study represents the first comprehensive germline analysis of multiple renal cancer predisposition genes within a large cohort of Chinese patients selected by the age of onset (<45 years old), regardless of their family history. Germline mutations were demonstrated in

9.5% of the patients. Germline mutations in fumarate hydratase (*FH*) and BRCA1-associated protein 1 (*BAP1*), which have been associated with aggressive forms of type 2 papillary renal cell carcinoma (PRCC) and clear cell renal cell carcinoma (ccRCC), respectively, were further investigated because of the potential clinical importance of these diagnoses.[17,18]

MATERIALS AND METHODS

Patient and Sample Selection

After approval by the scientific and ethics committee of the Fudan University Shanghai Cancer Center, we enrolled consecutive patients from July 2006 to June 2014. All the patients recruited to this study met the following 2 criteria: 1) an age at diagnosis younger than 45 years and 2) a renal tumor histologically classified as ccRCC, PRCC, chromophobe renal cell carcinoma (ChRCC), or angiomyolipoma (AML).

We identified 190 patients who provided informed consent. Pedigrees and medical records were collected at study entry via questionnaires. We also reviewed the pathology reports of the patients and their blood relatives and other medical reports when available.

Gene Selection

In total, 23 genes were selected for testing as either known or candidate renal cancer predisposition genes. Fourteen genes had been previously reported to be associated with hereditary renal cancers of a variety of histologic types. The remaining 9 candidate genes were selected because of either a high frequency of somatic mutations in RCC or an association with a predisposition to various cancers, which suggested that germline mutations could result in a predisposition to renal cancer. All the candidate genes are summarized in Supporting Table 1.

Next-Generation Sequencing (NGS), Bioinformatics, and Variant Filtering

Genomic DNA extraction, NGS, bioinformatics analysis, and variant filtering and annotation are described in detail in the supporting information. All NGS data have been deposited in the Sequence Read Archive with the identifier SRP143503.

An in silico evaluation of missense mutations was performed with SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), Provean (http://provean.jcvi.org/), and mCSM (http://bleoberis.bioc.cam.ac.uk/mcsm/). Protein structure modeling was performed with PyMol 1.7.1, and protein structure models were downloaded from the Protein Data Bank Web site (http://www.rcsb.org/pdb/).

Immunohistochemical Analysis

An immunohistochemical analysis was performed according to previously reported procedures, and it is described in the supporting information.[19]

Statistical Analysis

Sex, smoking status, pathogenic mutation carrier status, family history (first- or second-degree relatives), and personal history were considered binary variables and are presented as

proportions. Age at diagnosis and body mass index were viewed as continuous variables and are reported as medians and ranges. Continuous data were compared with the Student *t* test and are presented as means and standard deviations. The Fisher exact test was used to analyze categorical variables. Linear regression analysis was used to explore linear correlations. All *P* values were 2-tailed, and *P* values less than .05 were considered statistically significant. Statistical analysis was performed with SPSS 22.0, and data were visualized with Origin Pro 9.0.

RESULTS

Clinical Characteristics

In total, 190 unrelated Chinese patients younger than 45 years old who had been diagnosed with renal tumors were selected. The patient demographic and clinical characteristics are presented in Table 1. The median age of onset was 36 years, and the age of onset ranged from 17 to 42 years. The 190 renal tumor cases were histologically segregated into 128 ccRCCs, 11 PRCCs, 21 ChRCCs, and 29 AMLs, and a single patient had both ccRCC and PRCC. Forty-one patients had a family history of cancer, and 16 patients had a personal history of another type of tumor. The median body mass index was 23.19 kg/m^2, and 19.5% of the patients were current or former smokers at the time of diagnosis.

Targeted NGS and Pathogenic Variant Detection

Targeted NGS of 14 known renal cancer predisposition genes (*VHL*, tuberous sclerosis 1 [*TSC1*], *TSC2*, phosphatase and tensin homolog [*PTEN*], *MET*, *FH*, succinate dehydrogenase complex B [*SDHB*], *SDHC*, *SDHD*, folliculin [*FLCN*], *BAP1*, melanogenesis-associated transcription factor [*MITF*], HNF1 homeobox B [*HNF1B*], and polybromo 1 [*PBRM1*]) and 9 potential predisposition genes (*BRCA1*, *BRCA2*, *KDM5C*, *KDM6A*, *NF2*, *SETD2*, *CDKN2A*, *TCEB1*, and *TP53*) was performed on germline DNA for all 190 patients (Supporting Table 1). The quality of the NGS results, including the number of reads, sample coverage, and sequencing depth, is presented in Supporting Table 2. In total, we identified approximately 59,000 germline alterations (46,374 single-nucleotide variants [SNVs] and 12,447 insertions or deletions [indels]) in the 23 selected target genes within the 190 patients. A systematic procedure for variant filtration was performed, and 169 rare germline variants met the selection criteria for further analysis (Fig. 1).

The 169 selected variants were further filtered for known or potentially damaging mutations likely to result in a loss of function according to the literature and databases. This identified 21 mutations in 20 different patients, including 3 nonsense mutations, 2 same missense mutations, 7 splicing mutations (4 SNVs and 3 indels), 8 frameshift mutations (with 1 mutation observed in 2 patients), and 1 in-frame insertion. Sanger sequencing confirmed 19 of these mutations, with 2 mutations in *TSC2* failing to be confirmed. Thus, mutations were identified in 18 of 190 patients (9.5%; 95% confidence interval [CI], 5.3%-13.7%), including 12 patients with mutations in 7 of the known renal cancer predisposition genes (6.3%; 95% CI, 2.8%-9.8%) and 6 patients with mutations in 3 of the proposed predisposition genes associated with other cancer types (3.2%; 95% CI, 0.7%-5.7%; Supporting Fig. 1 and Supporting Table 3). Among early-onset RCC patients, 15 of 161

(9.3%) carried deleterious mutations, whereas the pathogenic mutation carrier rate in AML patients was 10.3% (3 of 29). Notably, 1 patient had pathogenic mutations in 2 renal cancer predisposition genes, namely a *BAP1* splice mutation and a *TSC2* frameshift mutation (Supporting Fig. 1 and Table 2).

Clinical Characteristics Associated With Pathogenic Germline Mutations

Patients with pathogenic germline mutations included 11 patients with ccRCC, 3 patients with ChRCC, 3 patients with AML, and 1 patient with PRCC (Table 3). The 3 patients with ChRCC all had mutations in the proposed renal cancer predisposition genes; they included 1 with a novel *CDKN2A* mutation and 2 with the same frameshift mutation of *BRCA2* (p.Thr1282fs). Two of the 3 patients with AML had mutations in either *TSC1* or *TSC2*. The average age of onset for germline-damaging mutation carriers was 35.1 years. Five of the 18 patients (27.8%) demonstrated recurrence or metastasis, and 8 of the 18 patients (44.4%) were deceased. Two patients died within 1 year of the diagnosis, 5 patients died within 1 to 5 years of the diagnosis, and 1 died 63 months after the diagnosis. Two patients had previously had other tumors, including an ovarian teratoma in a *TSC2* patient and breast cancer in a *BRCA1* patient. Three patients had first-degree relatives with cancer, and 5 patients had second-degree relatives with a history of cancer (Table 3).

Notably, 1 patient with a germline *VHL* nonsense mutation (p.Gln96*) had a first-degree relative with RCC. Further investigation demonstrated a pedigree consistent with VHL syndrome. The family history was consistent with maternal inheritance, and the proband demonstrated classic clinical features of VHL syndrome– related ccRCC. Details are described in the supporting information and Supporting Figure 2.

A comparison of clinical variables was performed between the 18 patients with pathogenic germline mutations and the remaining 172 individuals. No significant differences in sex, RCC histologic type distribution, personal history of cancer, overall and first-degree relative history, smoking status, body mass index, or age at diagnosis were observed between the 2 groups (Supporting Table 4). Notably, pathogenic mutation carriers had a significantly higher rate of second-degree relatives with any type of tumor than those without pathogenic mutations ($P < .001$), and this could be consistent with a lower rate of penetrance for these mutations, which could cause generations to be skipped (Supporting Table 4).

Somatic Sequencing Analysis

Fresh tumor tissue was available for 13 of the 18 pathogenic germline mutation carriers. Targeted NGS of the 23 selected genes was performed with tumor DNA to investigate loss of heterozygosity (LOH) or second hits in the identified predisposition genes and to highlight any additional somatic mutations.

Among the 13 tumors, 2 AMLs demonstrated LOH of germline mutations in either *TSC1* or *BRCA1*, 2 ccRCCs demonstrated LOH of germline mutations in either *VHL* or *BAP1*, and 1 ccRCC with a germline *TSC1* mutation demonstrated a second somatically gained *TSC1* mutation (Supporting Table 5). The 3 ChRCCs analyzed did not demonstrate any LOH, but 1 gained 2 heterozygous *TSC2* splicing mutations, and another gained a heterozygous *TP53* mutation. Five of the 7 ccRCCs gained somatic *VHL* mutations, and 1 AML with a germline

BRCA1 mutation gained a *TSC2* mutation (Supporting Table 5). Tumors were not investigated for promoter hypermethylation or focal deletions outside the gene region containing the germline mutation, which could provide further evidence of somatically gained second hits.

Evaluation of Variants of Unknown Clinical Significance (VUSs)

Eighty-nine variants were classified as VUSs, and they included 77 missense single-nucleotide polymorphisms and 12 indels within the splicing regions of 3 genes (*MET*, *FH*, and *PBRM1*) at either the +3/+4 of the donor site or the –3/–4 of the acceptor site (Supporting Table 6). Among these VUSs, 57 were in known renal cancer predisposition genes, and 32 were in the proposed predisposition genes. On average, each patient carried 0.47 VUSs (range, 0–4), and the number of VUSs detected per gene was linearly correlated with the length sequenced; this demonstrated a relatively clear but not statistically significant relation ($P = .078$; Supporting Fig. 3A,B).

Evaluation of Germline *FH* and *BAP1* VUSs

This study identified 6 different *FH* missense mutations in 7 RCC patients, including 2 patients with 1 known pathogenic mutation (p.Arg101Gln) and 5 VUSs (Fig. 2A). An in silico analysis of the 7 missense VUSs highlighted the 5 *FH* VUSs as demonstrating strong evidence for pathogenicity; all were predicted to be deleterious by SIFT, PolyPhen-2, Provean, and mCSM in highly conserved amino acids (Fig. 2B). Recent studies have demonstrated that FH-deficient tumors often lose FH expression and acquire high succination levels on immunohistochemical staining. Four of 5 tumors demonstrated a loss of FH staining in comparison with normal tissue (Fig. 2C). Accordingly, these 4 tumors showed high succination levels (Fig. 2C). Notably, 1 tumor maintained robust FH staining and low succination levels. That tumor was derived from a patient with a pathogenic mutation in *BAP1* and a VUS in *FH* (Fig. 2C). Most *FH* VUS tumors were classified as ccRCCs, and the tumor from the patient with the p.Asn154His mutation demonstrated the classic histology associated with FH-deficient tumors hereditary leiomyomatosis and renal cell carcinoma (HLRCC). In addition, we found 1 novel VUS in the *BAP1* gene. A comprehensive analysis of this novel VUS is presented in the supporting information.

DISCUSSION

Currently, most patients are screened for mutations of renal cancer predisposition genes only if they present with a significant family history, other known syndromic clinical features, or bilateral/multifocal disease. This process has identified many predisposition genes such as *VHL*, *MET*, and *FLCN*, but it is dependent on a relatively high rate of penetrance for tumors and large families. RCC and AML predisposition genes with less penetrance make identifying familial cases much harder, especially in China, where the 1-child policy has produced small family units. The strict criteria for genetic screening also represent an economic concern because individual testing of a large number of genes within a broadly selected population could be prohibitively expensive. Fortunately, NGS-based screening using multigene panels provides clinicians with a great tool for broad genetic analysis, although the benefits of these comprehensive testing strategies have been debated because of

potential problems with interpretation. Even with broad, economically viable NGS-based screening, some patient selection criteria are necessary, and a young age of onset has been demonstrated to be an important criterion.[20,21] This has been previously recommended in guidelines for genetic testing schemata.[22,23]

This study identified 18 of 190 Chinese patients with early-onset disease and pathogenic germline mutations (9.5%; 95% CI, 5.3%-13.7%). This revealed a higher frequency of pathogenic germline mutations than was previously predicted for patients with sporadic disease, and it highlighted the importance of screening these patients with early-onset disease. Germline mutations were found in several genes that are not standardly defined as RCC or AML predisposition genes, including *BRCA1* and *BRCA2*, which are commonly associated with breast, ovarian, and prostate cancers. Although previous studies have proposed that germline *BRCA1* mutations could be associated with an inherited predisposition to RCC, further investigation of these genes as RCC predisposition genes is necessary.[24] In addition, our study identified VUSs in *FH* and *BAP1* and demonstrated evidence of pathogenicity. Identifying pathogenic variants of these genes is particularly important because they are associated with aggressive disease and a poor prognosis. This highlights the importance of being able to evaluate novel mutations.[25,26]

The expected association between a pathogenic germline mutation status and a first-degree relative with a history of cancer was not observed, but an association was observed between a pathogenic germline mutation status and a second-degree relative with a history of cancer. This may be a unique feature for young Chinese patients within this period, partly because of the typical 1-child policy in China. Patients younger than 35 years seldom have brothers or sisters, and their children are still very young, whereas their fathers and mothers may have many brothers or sisters.

Identifying pathogenic germline mutations within the RCC or AML predisposition genes in this panel has clinical implications for both the probands and their relatives. Many hereditary kidney cancer syndromes have been well studied. Now, there are specific management regimes and targeted therapies based on the specific pathways altered by gene mutations. For localized *VHL*-associated and Birt-Hogg-Dubé–associated renal tumors, the recommended disease management includes active surveillance until the largest diameter reaches 3 cm because of the low metastatic rate of tumors smaller than this size, the high likelihood of more tumors developing, and the requirement for multiple nephron-sparing surgeries in these patients. However, active surveillance is never recommended for *FH*-associated and *BAP1*-associated tumors.[4] For *VHL*-associated advanced ccRCC, the Food and Drug Administration has approved 5 drugs (sunitinib, sorafenib, bevacizumab, pazopanib, and axitinib) that target the hypoxia-inducible factor–vascular endothelial growth factor pathway and 2 drugs (everolimus and temsirolimus) that target the mammalian target of rapamycin pathway on the basis of the VHL pathway deficiency. Kidney cancers or AMLs associated with *TSC1*/*2* or *PTEN* germline mutations may specifically benefit from mammalian target of rapamycin inhibitors because of the dysregulation of that pathway by these mutations.[27,28] Currently, there is no approved targeted therapy for *FH*-deficient renal cancers, but phase 1/2 studies investigating the effects of several promising targeted agents, including bevacizumab, erlotinib, and vandetanib, are underway.[29] In addition, olaparib has been

proven to be effective in patients with mutations in DNA repair genes, such as *BRCA1*/*2*, in various cancers,[30,31] and this indicates that the same agent might be beneficial for patients with renal tumors with *BRCA1*/*2* mutations. The screening of germline-mutated patients and their affected relatives for renal cancers and additional syndrome-specific features is also extremely beneficial because it can result in the early detection of tumors and other events, which allows for prompt and effective early intervention. This could be particularly beneficial for patients with germline *FH* or *BAP1* mutations.

This study has several limitations. Only patients from a single cancer center were included, and the age of onset threshold was below the average age of onset for some inherited syndromes such as Birt-Hogg-Dubé syndrome (50.7 years).[32] The study used a conservative method to filter out pathogenic variants to avoid overestimating and falsely interpreting the extent of germline mutations, but this may have resulted in false-negatives in some patients. The issue of correctly evaluating VUSs is an ongoing problem in this field of genetic research. This analysis evaluated only SNVs and small indels and no other potential sources of pathogenic variations, and this could have led to a conservative estimate of the mutation frequency. Despite these limitations, this study provides novel insight into the evaluation of the genetic basis of early-onset renal cancers in the era of multigene panel testing. Knowing the genetic basis of a patient's disease makes personalized treatment and consultation possible for clinicians and aids in the management of the family and the individual. As technologies advance and costs decrease, increasing numbers of patients could be referred for NGS-based screening until all patients can be screened without the necessity for selection criteria. NGS platforms also easily allow for the expansion of screening to include new genes; for instance, since this screening platform was designed, germline mutations of *CDKN2B* have been associated with RCC predisposition, and they can be added to the next iteration of this screening platform.[33]

The expanding use of NGS-based screening platforms in the clinic will undoubtedly raise the question of how patients with renal tumors with different germline mutations should be managed and treated, and this is likely to become the next important topic for clinicians. Global collaborative efforts and innovative research approaches and trials will be needed to answer these questions and improve interpretation of the immense amount of data. This study demonstrates the importance of broadening the screening inclusion criteria for RCC and AML susceptibility gene mutations to identify patients who lack an obvious family history. Several patients with germline mutations that would alter their management and treatment and could influence their outcomes and the outcomes of mutation-carrying relatives were identified. Germline mutation screening represents an achievable aspect of personalized or precision medicine and should improve patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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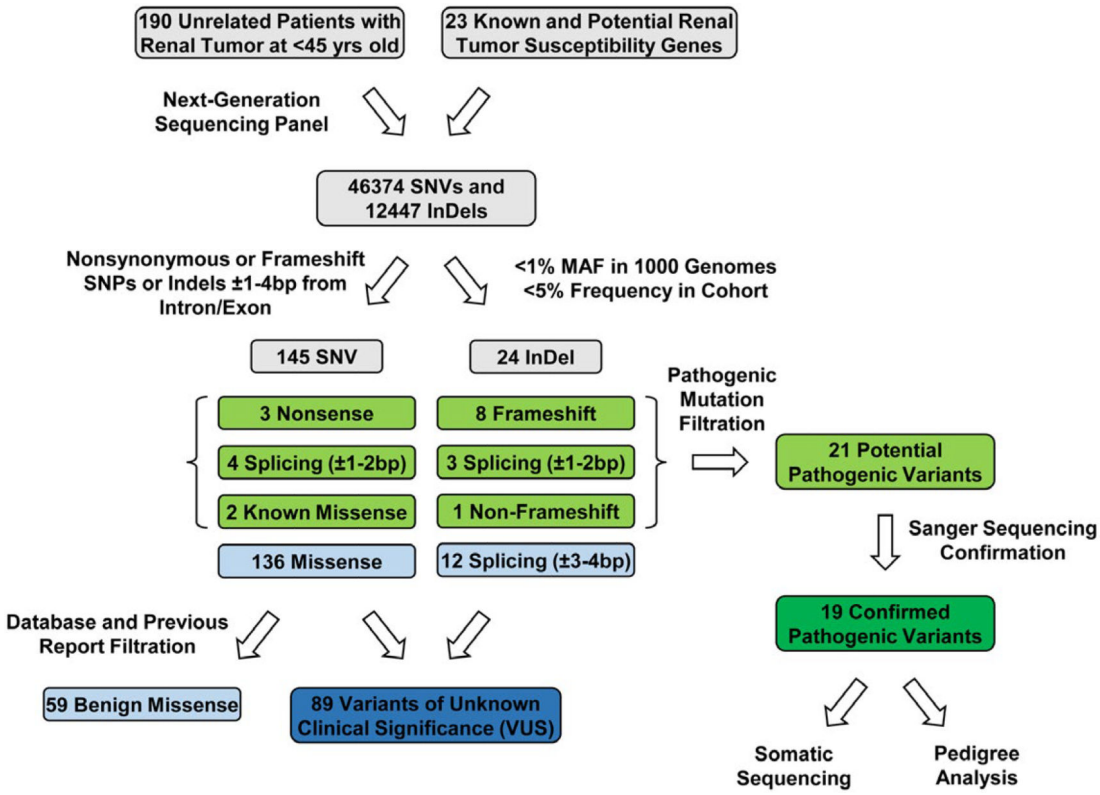

Figure 1.

Flow chart of the study design and the filtration of pathogenic variants and variants of unknown clinical significance. Indel indicates insertion or deletion; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; SNV, single-nucleotide variant.

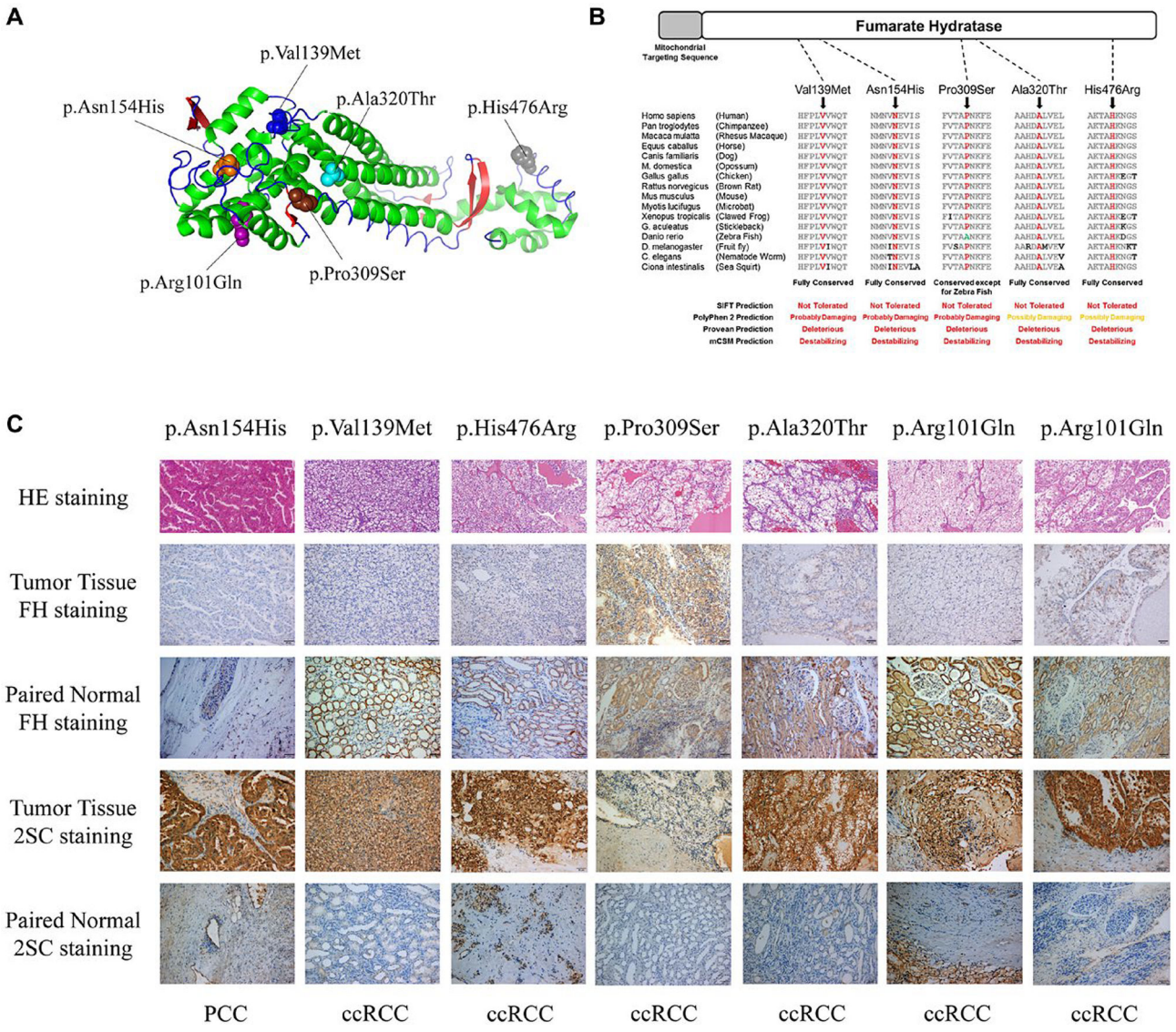

Figure 2.

Analysis of novel *FH* variants of unknown significance. (A) Structure model representing the FH protein (3e04-China A) and showing the detected mutated amino acid sites differentiated by color (purple, p.Arg101; blue, p.Val139; orange, p.Asn154; brown, p.Pro309; cyan, p.Ala320; and gray, p.His476). (B) FH amino acid conservation across species assessed by multiple sequence alignment and in silico analysis using SIFT, PolyPhen-2, Provean, and mCSM to predict the effects of the 5 missense *FH* VUSs identified. (C) H & E staining, FH immunohistochemical staining, and protein succination level (2SC) staining of *FH* VUS–associated tumors and paired normal tissues (×200). Germline *FH* VUS mutations and tumor histologies are shown. 2SC indicates *S*-(2-succinyl)cysteine; ccRCC, clear cell renal cell carcinoma; FH, fumarate hydratase; PCC, papillary renal cell carcinoma; VUS, variant of unknown clinical significance.

TABLE 1.

Demographic and Clinical Characteristics of Patients With Renal Cell Carcinoma

Characteristic	Value
Sex, No. (%)	
Male	117 (61.6)
Female	73 (38.4)
Age, median (range), y	36 (17–42)
BMI, median (range), kg/m^2	23.19 (13.39–46.30)
Pathogenic mutation carrier, No. (%)	18 (9.5)
Smoking status, No. (%)	37 (19.5)
Family history, No. (%)	41 (21.6)
First-degree relative	32 (16.8)
Second-degree relative	14 (7.4)
Personal history, No. (%)	16 (8.4)
Histological types, No. (%)[a]	
Clear cell	129 (67.9)
Papillary	12 (6.3)
Chromophobe	21 (11.1)
Angiomyolipoma	29 (15.3)

Abbreviation: BMI, body mass index.

[a]One patient in the cohort had both clear cell renal cell carcinoma and papillary renal cell carcinoma.

TABLE 2.

Details of Presumed Pathogenic Mutations Detected via Next-Generation Sequencing

Gene	Function	Transcript ID	Nucleotide Changes	Amino Acid Changes	Reference	Patient No.
VHL	stop_gained	NM_000551.3	c.286C>T	p.Gin96*	Novel	KB09029
TSC1	frameshift_variant	NM_000368.4	c.1907_908delAG	p.Glu636Glyfs*51	rs118203599	KB127
	frameshift_variant	NM_000368.4	c.3422delC	p.Ser1141Phefs*27	Novel	KB65
TSC2	frameshift_variant	NM_000548.3	c.5049_5050insGG	p.Ser1684Profs*21	Novel	KB09390
	frameshift_variant	NM_000548.3	c.2831_2832insT	p.Lys945Glnfs*15	Novel	KB10714
	splice_donor_variant	NM_000548.3	c.5051_c.5084+16delCCCTGCAGTGCAGGAAAGGTAGGGCCGGGTGGGG	—	Novel	KB121063
FH	missense_variant	NM_000143.3	c.302G>A	p.Arg101Gln	rs75086406[a]	KB11068, KB11153
FLCN	inframe_insertion	NM_44997.5	c.1145_1147dupACC	p.Asp382_Leu383inshlis	Novel	KB121215
BAP1	frameshift_variant	NM_004656.3	c.1214delA	p.Glu405Glyfs*25	Novel	KB09729
	splice_acceptor_variant	NM_004656.3	c.38–1G>A	—	Novel	KB09155
	splice_donor_variant	NM_004656.3	c.68+2T>C	—	Novel	KB09390
BRCA1	stop_gained	NM_007300.3	c.178C>T	p.Gln60*	rs80357471	KB130150
BRCA2	frameshift_variant	NM_000059.3	c.3846_3847delTG	p.Val1283Lysfs*2	rs80359405[a]	KB10789, KB140137
	splice_acceptor_variant	NM_000059.3	c.68–1_c.68–2delAG	—	Novel	KB140129
	stop_gained	NM_000059.3	c.961C>T	p.Gln321*	rs80359234	KB11070
CDKN2A	splice_donor_variant	NM_058197.4	c.425+2T>G	—	Novel	KB121224
PBRM1	splice_donor_variant	NM_018313.4	c.996+1 G>T	—	Novel	KB130267

Abbreviations: BAP1, BRCA1-associated protein 1; CDKN2A, cyclin-dependent kinase inhibitor 2A; FH, fumarate hydratase; FLCN, folliculin; PRBM1, polybromo 1; TSC, tuberous sclerosis; VHL, Von Hippei-Lindau.

[a]Two different patients carried the same deleterious germline mutation.

TABLE 3.

Clinical Characteristics of Patients With RCC and Pathogenic Mutations

Patient ID	Sex	Age at Diagnosis, y	Mutation Gene	Histology	Personal History	Family History	Recurrence or Metastasis	Survival From Diagnosis, mo
KB121215	Female	41	*FLCN*	PCC	0	0	0	42, alive
KB11153	Male	41	*FH*	ccRCC	0	0	Invading surrounding tissues	35, deceased
KB10789	Female	21	*BRCA2*	Chro	0	0	0	68, alive
KB65	Male	27	*TSC1*	ccRCC	0	0	Lung	93, alive
KB10714	Female	40	*TSC2*	AML	Ovarian teratoma	0	Recurrence	58, deceased
KB09729	Male	29	*BAP1*	ccRCC	0	0	0	80, alive
KB140137	Female	32	*BRCA2*	Chro	0	0	0	23, alive
KB11070	Male	37	*BRCA2*	ccRCC	0	0	0	61, alive
KB127	Male	35	*TSC1*	AML	0	0	0	26,deceased
KB09155	Female	36	*BAP1*	ccRCC	0	Grandfather: pelvic cancer Mother: hepatocellular carcinoma	0	85, alive
KB130267	Male	39	*PBRM1*	ccRCC	0	0	0	32, alive
KB09029	Male	36	*VHL*	ccRCC	0	Mother: RCC Maternal grandfather: brain tumor Maternal uncle: pancreatic cancer Maternal aunt: brain tumor	0	88, alive
KB11068	Male	41	*FH*	ccRCC	0	0	Bone	34,deceased
KB140129	Male	33	*BRCA2*	ccRCC	0	Maternal uncle: gastric cancer	0	6,deceased
KB121063	Male	33	*TSC2*	ccRCC	0	Grandfather: lung cancer Father: gastric cancer	0	13, deceased
KB121224	Male	39	*CDKN2A*	Chro	0	0	0	42, alive
KB130150	Female	37	*BRCA1*	AML	Breast cancer	0	0	10, deceased
KB09390	Female	34	*BAR1* and *TSC2*	ccRCC	0	Maternal grandfather: malignant tumor (not clear)	Brain, bone, and pleural	63,deceased

Abbreviations: AML, angiomyolipoma; BAP1, BRCA1-associated protein 1; ccRCC, clear cell renal cell carcinoma; CDKN2A, cyclin-dependent kinase Inhibitor 2A; FH, fumarate hydratase; PCC, papillary renal cell carcinoma; PRBM1, polybromo 1; RCC, renal cell carcinoma; TSC, tuberous sclerosis; VHL, Von Hippel-Lindau; Chro, Chromophobe renal cell carcinoma.