Original Article

Prevalence of germline mutations in cancer susceptibility genes in Chinese patients with renal cell carcinoma

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Background: Germline pathogenic variants are estimated to affect 3–5% of patients with renal cell carcinoma (RCC). The identification of patients with hereditary RCC is important for cancer screening and treatment guidance.

Methods: Whole-exome sequencing (WES) (n=69) or gene panel sequencing containing 139 genes (n=54) related to germline cancer predisposition was used to analyze germline mutations in 123 patients with RCC admitted to Department of Urology, The Third Medical Center of Chinese PLA General Hospital. Chi-square test (χ^2) was used to analyze relationship between clinicopathologic parameters and germline mutations.

Results: A total of 13 (10.57%) patients carried pathogenic or likely pathogenic germline mutations in 10 cancer predisposition genes, including *VHL*, *FH*, *FLCN*, *SDHB*, *MUTYH*, *RAD51C*, *NBN*, *RAD50*, *FANCI*, and *FANCM*. A total of 6 of these 10 cancer predisposition genes were associated with maintenance of genomic stability and DNA repair. Patients harboring pathogenic germline mutations tended to have an earlier RCC onset. The prevalence of deleterious mutations was higher in patients with bilateral or multifocal RCC compared to patients without bilateral or multifocal RCC. Patients with non-clear cell RCC (nccRCC) were significantly more likely to have RCC-associated gene mutations.

Conclusions: To our knowledge, this is the first report of pathogenic germline mutations in the *FANCI* and *FANCM* genes and heterozygous germline missense mutation in exon 5 of the *FH* gene c.563A>T:p.N188I in RCC. Young RCC patients, patients with bilateral or multifocal RCC, or patients with nccRCC are more likely to have pathogenic/potentially pathogenic germline mutations.

Keywords: Renal cell carcinoma (RCC); pathogenic germline mutation; *FANCI*; *FANCM*

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Introduction

Renal cell carcinoma (RCC) is the ninth most frequently diagnosed cancer in men (1). RCC comprises several subtypes, including clear cell RCC (ccRCC), papillary RCC type 1 (pRCC T1), pRCC T2, mixed oncocytoma or chromophobe RCC (chRCC), and other rare subtypes (2). Tumors that do not meet the criteria for any established subtype are categorized as unclassified (3). Loss of function

of the von Hippel-Lindau (VHL) protein, mutations in *MET*, *FH*, and *FLCN* have been considered major mutations in ccRCC, pRCC T1, and pRCC T2, and mixed oncocytoma or chRCC, respectively (2). Germline mutations, one of genetic alterations, exist in all cells in body and can be inherited (4,5). Germline mutations are associated with generation of various tumors and drug resistance (6-8). A recent American study found that 16.1% of RCC patients carried germline mutations in a RCC predisposition gene, and some cases of early-onset aggressive RCC without defined pathogenic germline mutations have been observed (9). It seems highly probable that underlying causative germline mutations exist in RCC. Thus, further investigations need to be explored in different patient populations with special molecular and clinical characteristics.

A previous report suggested that patients with renal cancer affected by hereditary factors account for 3–5% of all RCC patients (10), which is likely underestimated (11). Studies have shown that germline mutations in 14 genes (*VHL*, *FH*, *SDHB*, *SDHC*, *SDHD*, *MET*, *FLCN*, *PTEN*, *TSC1*, *TSC2*, *MITF*, *BAP1*, *PBRM1*, and *CDKN2B*) can increase the risk of RCC (12-15). It is particularly relevant to identify Chinese patients with inherited RCC because their clinical implications can differ from those of patients with sporadic RCC (16,17). However, the genetic basis of some inherited renal cancers has not been clearly elucidated.

To explore the genetic basis of inherited renal cancers, we conducted a comprehensive germline analysis of multiple renal cancer predisposition genes in a cohort of Chinese patients. Among the patients in our cohort, 10.57% carried pathogenic/likely pathogenic germline mutations. Most (6/10) of the mutated genes were associated with maintenance of genomic stability and DNA repair. Young RCC patients, patients with bilateral or multifocal RCC, or patients with non-clear cell RCC (nccRCC) are more likely to have pathogenic/likely pathogenic germline mutations. Importantly, this is the first study to report that identical germline mutations of the *FANCI* gene c.158-2A>G and *FANCM* gene c.4515+1G>C exist in patients with RCC and is the first article to show heterozygous germline missense mutation in exon 5 of the *FH* gene c.563A>T:p.N188I in RCC. We present the following article in accordance with the MDAR reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-23-32/rc).

Highlight box

Key findings

- Pathogenic germline mutations in *FANCI* and *FANCM* in RCC; heterozygous germline missense mutation in exon 5 of the FH gene c.563A>T:p.N188I in RCC.

What is known and what is new?

- Previous research found that germline mutations in *VHL*, *FH*, *SDHB*, *SDHC*, *SDHD*, *MET*, *FLCN*, *PTEN*, *TSC1*, *TSC2*, *MITF*, *BAP1*, *PBRM1*, and *CDKN2B* can increase the risk of RCC.
- We found that germline mutations of the *FANCI* gene c.158-2A>G and *FANCM* gene c.4515+1G>C exist in patients with RCC; heterozygous germline missense mutation in exon 5 of the *FH* gene c.563A>T:p.N188I in RCC.

What is the implication, and what should change now?

- FANCI and FANCM pathogenic germline mutations might play an important role in tumorigenesis and tumor progression in RCC. Young RCC patients, patients with bilateral or multifocal RCC, or patients with nccRCC should be offered genetic testing for more precise clinical implications.

Methods

Patients

Peripheral blood was obtained from 123 patients with RCC in the Department of Urology, The Third Medical Center, Chinese PLA General Hospital between 1 August 2017 and 31 December 2020. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Chinese PLA General Hospital (No. S2013-065-01) and informed consent was taken from all the patients.

Genomic sequencing

In our cohort, whole-exome sequencing (WES) (n=69) or gene panel sequencing containing 139 genes (n=54) related to germline cancer predisposition was performed to evaluate the pathogenic/likely pathogenic germline mutation rate and identify *de novo* pathogenic/likely pathogenic germline mutations in patients with RCC (Table S1). Germline DNA was obtained from peripheral white blood. Polymerase chain reaction (PCR) was performed to amplify DNA. The enriched DNA, which was converted to sequencing libraries, was sequenced and analyzed using the Illumina Novoseq platform (Illumina, San Diego, CA, USA), as previously described (18).

Table 1 Demographic characteristics

Characteristics	N	%
Total	123	100.0
Age at diagnosis (years)		
Median	54	
Range	19 to 85	
Age 46 or younger	41	33.3
Age 47 or older	82	66.7
Sex		
Female	30	24.4
Male	93	75.6
Race or ethnic background		
Chinese	123	100.0
Family history of RCC		
First-degree relative	1	0.8
First, second, or third-degree relative	2	1.6
More than 1 relative	2	1.6
Family history of malignancy		
First-degree relative	34	27.6
First, second or third-degree relative	39	31.7
More than 1 relative	11	8.9
Tumor histologic subtype		
Clear cell	95	77.2
Papillary	9	7.3
Chromophobe	6	4.9
Translocation-associated	6	4.9
Unclassified	4	3.3
Others	3	2.4
Patient history of prior malignancy		
Yes	7	5.7
No	116	94.3
Bilateral or multifocal RCC at diagnosis		
Yes	16	13.0
No	107	87.0
More than one germline mutation		
Yes	2	1.6
No	121	98.4
Nephrectomy		
Yes	121	98.4
No	2	1.6
Stage at diagnosis		
Stage I	52	42.3
Stage II	5	4.1
Stage III	41	33.3
Stage IV	23	18.7
Unknown	2	1.6
Grade at diagnosis		
Grade I	7	5.7
Grade II	52	42.3
Grade III	34	27.6
Grade IV	5	4.1
Unknown	25	20.3

RCC, renal cell carcinoma.

Statistics

Clinical and pathologic characteristics were compared using chi-square test (χ^2) and P values <0.05 were considered significant. Statistical analyses were performed using SPSS 24.0 software (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

The characteristics and clinical features of the 123 patients are summarized in *Table 1*. In our study, the age at diagnosis varied from 19 to 85 years (median, 54 years). Among the 123 patients, 95 had ccRCC (77.2%) and 28 had nccRCC (22.8%). Overall, 7 patients (5.7%) with a history of a second malignant tumor were identified. Of the 7 patients with secondary malignant tumors, 2 were diagnosed with breast cancer (data not shown). A total of 16 (13.0%) patients with bilateral or multifocal disease were reported and only 2 (1.6%) patients had a family history of RCC (data not shown).

Germline mutations

A total of 13 patients (10.6%) harbored pathogenic or likely pathogenic germline variants in 10 different cancer predisposition genes (*Figure 1A*). Mutations in RCC-associated genes were identified in 9 (7.3%) patients, and

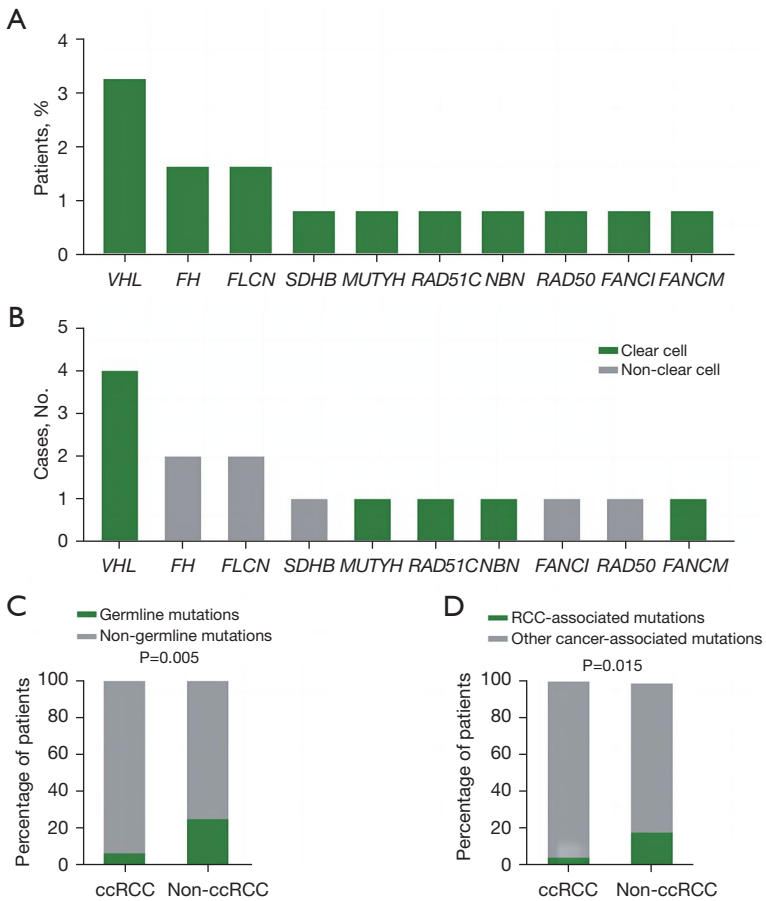

Figure 1 Frequency of germline variants in the cohort of 123 patients with RCC. (A) Percentage of patients with germline variants in RCC predisposition genes including *VHL*, *FH*, *FLCN*, *SDHB*, *MUTYH*, *RAD51C*, *NBN*, *RAD50*, *FANCI*, and *FANCM*. One patient harbored VHL and FANCM mutations simultaneously, and another patient harbored *VHL* and *RAD51C* mutations simultaneously. (B) Number of pathogenic mutations by histologic subtype. (C,D) Percentage of germline mutations in ccRCC and nccRCC. ccRCC, clear cell renal cell carcinoma; nccRCC, non-clear cell renal cell carcinoma; RCC, renal cell carcinoma.

mutations in genes not clearly associated with RCC were identified in 6 (4.9%) patients (*Figure 1B*). A total of 6 of 95 (6.3%) ccRCC patients had pathogenic/likely pathogenic germline mutations, and 7 of 28 (25.0%) nccRCC patients had pathogenic/likely pathogenic germline mutations (*Figure 1C*, *Table 2*). Among the 95 ccRCC patients, 4 (4.2%) patients harbored pathogenic/likely pathogenic germline mutations in RCC-associated genes (*Figure 1D*). Among the 28 patients with nccRCC, 5 (17.9%) harbored pathogenic/likely pathogenic germline mutations in RCC-associated genes (*Figure 1D*). For RCC-associated genes (*Figure 1B*, Table S2), 4 deleterious *VHL* mutations were detected in 4 individuals (3.3%), including 75.0% (n=3) known deleterious missense mutations and 25.0% (n=1) nonsense mutations. We detected 2 deleterious *FLCN* mutations in 2 participants (1.6%), including 1 frameshift in/del mutation and 1 deleterious missense mutation. We detected 2 deleterious *FH* mutations in 2 participants (1.6%), both of which were deleterious missense mutations. We also found 1 *SDHB* deleterious missense mutation in 1 participant (0.8%). For other cancer-associated genes (*Figure 1B*, Table S2), 1 *MUTYH* deleterious missense mutation, 1 *RAD51C* deleterious missense mutation, 1 *NBN* likely pathogenic missense mutation, 1 *RAD50* frameshift mutation, 1 *FANCI* deleterious missense mutation, and 1 *FANCM* deleterious missense mutation were identified. The *VHL*, *FH*, *FLCN*, *SDHB*, *MUTYH*, *NBN*, and *RAD51C* pathogenic or likely pathogenic mutations in

Table 2 Clinicopathologic parameters associated with germline mutations

Clinicopathologic parameters	With germline mutations	Without germline mutations	P value
Age at diagnosis, n			<0.001
≤46 years	11	30	
>46 years	2	80	
Subtype, n			0.005
Clear cell	6	89	
Non-clear cell	7	21	
Stage, n			0.396
Stage I & stage II	8	53	
Stage III & stage IV	5	55	
Grade			0.678
Grade 1 & grade 2	6	53	
Grade 3 & grade 4	3	36	
Family history of malignancy, n			0.369
Yes	6	39	
No	7	77	
Bilateral or multifocal RCC, n			0.004
Yes	5	11	
No	8	99	

RCC, renal cell carcinoma.

RCC have been reported before, whereas the *FANCI* and *FANCM* pathogenic germline mutations in RCC were reported for the first time in this study. Interestingly, 2 patients in our cohort had simultaneous occurrences of 2 causative mutations (Table S2). Patients harboring *VHL* and *FANCM* mutations (nonsense and missense, respectively) simultaneously experienced stage I ccRCC at 29 years of age, without a family history of malignant diseases. Similar characteristics were observed in a patient harboring *VHL* and *RAD51C* mutations (deletions and missense mutations, respectively). He was diagnosed with stage I ccRCC at 44 years of age, with a family history of malignant cancers. Compared with the phenotypes of patients with only 1 mutation in *VHL*, no apparent phenotypic differences were found between these 2 patients. The only difference was that patients with only *VHL* mutations had a single lesion, whereas the patients with both *VHL* and *FANCM/RAD51C* mutations had multifocal RCC. In these 2 cases, it seemed that additional *FANCM* or *RAD51C* mutations did not affect the age of RCC onset. Thus, to draw a decisive conclusion, further investigations are required to demonstrate the precise role of *FANCM* and *RAD51C* in tumorigenesis and tumor progression of RCC.

Clinical characteristics associated with germline mutations

Our cohort comprised 6 patients with ccRCC, 2 patients with pRCC, 2 with chRCC, 1 with microphthalmia (MiT) family translocation RCC, and 2 with unclassified RCC who carried pathogenic/likely pathogenic germline mutations (*Figure 2*, Table S2). The proposed renal cancer predisposition gene *FH* pathogenic germline mutations were identified in the 2 patients with pRCC. There were 2 patients with chRCC who harbored mutations in either *FLCN* or *SDHB*. The mean age of onset of RCC in germline mutation carriers was 37.2 years. Of these 13 patients, 2 (15.4%) experienced metastasis. A positive family history of cancer was reported in 6 patients (Table S2). The

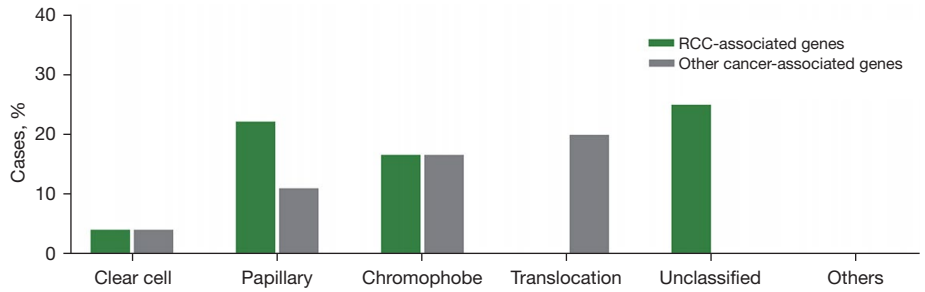

Figure 2 Number of pathogenic mutations by disease. RCC, renal cell carcinoma.

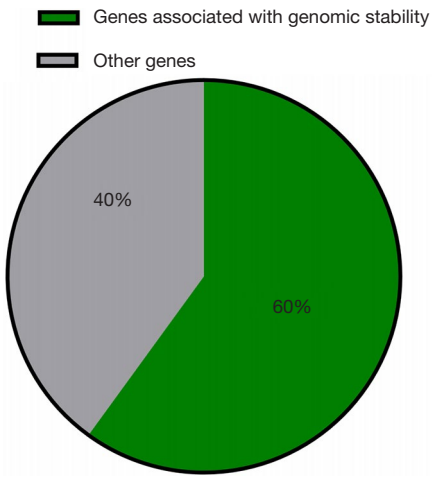

Figure 3 Percentage of genes associated with genomic stability or DNA repair.

prevalence of deleterious germline mutations is associated with the age at diagnosis. The probability of germline mutations occurring in patients aged 46 years or younger remained higher than that in patients older than 46 years [11 (26.8%) of 41 *vs.* 2 (2.4%) of 82, P<0.001] (*Table 2*). We also found that the prevalence of germline mutations was associated with the histologic subtype. Patients with nccRCC were more likely to carry germline mutations [7 (25%) of 28 *vs.* 6 (6.3%) of 95, P=0.005] (*Figure 1C*, *Table 2*). Furthermore, we also found that patients with nccRCC were more likely to have RCC-associated gene mutations [5 (17.9%) of 28 *vs.* 4 (4.2%) of 95, P=0.015] (*Figure 1D*). Compared to patients without bilateral or multifocal RCC, those with bilateral or multifocal RCC were more likely to harbor deleterious mutations [5 (38.5%) of 13 *vs.* 11 (10.0%) of 110, P=0.004] (*Table 2*). However, a family history of cancer was not associated with more mutations (*Table 2*). Additionally, a higher stage or grade was not associated with more deleterious pathogenic germline mutations in our study.

Genes involved in the maintenance of genomic stability or DNA repair were frequently mutated

In our cohort, 13 (13/123, 10.6%) patients harbored deleterious germline mutations in cancer predisposition genes. Some 6 of 10 cancer predisposition genes were associated with maintenance of genomic stability and DNA repair (*Figure 3*). Deleterious germline mutations of 3 Fanconi anemia (FA)-related genes (*FANCI*, *FANCM*, and *RAD51C*) were detected. The protein products of these genes function cooperatively with other proteins associated with DNA repair processes to maintain genome homeostasis, and loss of function of these genes leads to FA (19). In addition, deleterious germline mutations in *RAD50* and *NBN* have been detected: *RAD50* and *NBN*, members of the MRE11/RAD50/NBN double-strand break repair complex, are thought to repair DNA double-strand breaks and activate DNA damage-induced checkpoints (20,21). Loss of function of these genes is associated with rare chromosomal instability disorders (20,21). Additionally, a deleterious germline mutation in *MUTYH* was detected. *MUTYH*, a gene encoding a DNA glycosylase, plays an important role in oxidative DNA damage repair. Inactivation of *MUTYH* leads to G:C>T:A transversion, which increases the risk of colorectal cancer (22).

Novel pathogenic germline mutation in RCC

In this study, *FANCI* and *FANCM* pathogenic/likely pathogenic germline mutations in patients with RCC were reported for the first time. Patient RCC88 diagnosed with ccRCC carried a *FANCM* germline mutation annotated as pathogenic in ClinVar (23). This revealed a heterozygous

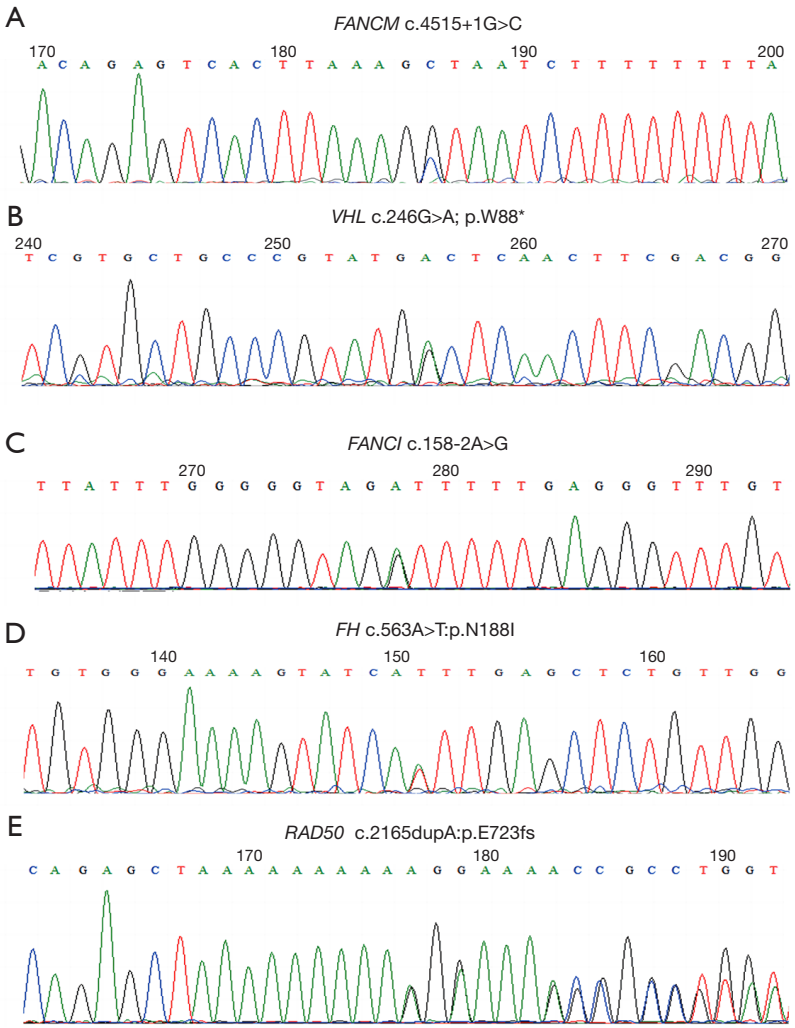

Figure 4 Variants of *FANCM*, *VHL*, *FANCI*, *FH*, and *RAD50* in patients with RCC. Sanger sequencing chromatograms for *FANCM*-positive, *VHL*-positive, *FANCI*-positive, *FH*-positive, and *RAD50*-positive cases. Genetic testing of peripheral blood collected from patient RCC88 identified (A) a *FANCM* germline mutation (c.4515+1G>C) and (B) a *VHL* germline mutation (c.264G>A; p.W88*). Genetic testing of peripheral blood collected from patient RCC63, RCC121, and RCC93 identified (C) a *FANCI* germline mutation (c.158-2A>G), (D) an *FH* germline mutation (c.563A>T:p.N188I), and (E) a *RAD50* germline mutation (c.2165dupA; p. E723fs), respectively. RCC, renal cell carcinoma.

NM_020937:c.4515+1G>C germline *FANCM* missense mutation, resulting in inactivation of *FANCM* (Table S3). This intronic variant is a nucleotide substitution located 1 nucleotide downstream of coding exon 17. Interestingly, patient RCC88 simultaneously harbored a pathogenic *VHL* germline mutation. Subsequent Sanger sequencing of DNA samples collected from the blood of patient RCC88 confirmed the presence of *FANCM* and *VHL* germline mutations (*Figure 4A,4B*). Interestingly, genetic testing of DNA samples collected from the proband's parents and brother revealed that none of them were carriers of *FANCM* or *VHL* mutations (Figure S1), suggesting that the *de novo* germline mutation occurs during formation of the sperm or egg from an unaffected parent. In addition, patient RCC88 did not have a family history of cancer. Patient RCC63, diagnosed with unclarified RCC, carried a heterozygous NM_001113378: c.158-2A>G germline *FANCI* missense mutation, which is predicted to inactivate the *FANCI* gene (Table S3). This intronic variant is a nucleotide substitution located 2 nucleotides upstream of coding exon 4.

Subsequent Sanger sequencing of DNA samples collected from the blood of patient RCC63 confirmed *FANCI* germline mutations (*Figure 4C*). Annotated as pathogenic in ClinVar, the *FANCI* variant was considered pathogenic according to the American College of Medical Genetics and Genomics (ACMG) criteria (24). The patient's father was diagnosed with gastric cancer. In addition, we report a case of aggressive hereditary leiomyomatosis and RCC (HLRCC) in a 32-year-old woman who presented with a novel heterozygous germline missense mutation in exon 5 of *FH* (c.563A>T:p.N188I) (*Figure 4D*, Table S3). The patient's mother was diagnosed with endometrial carcinoma. Consistent with a recent study, we also found a pathogenic *RAD50* germline mutation (c.2165dupA; p.E723fs) in a 24-year-old RCC patient with no family history of cancer (*Figure 4E*, Table S3) (25).

Discussion

Patients with renal cancer affected by hereditary factors account for 3–5% of all RCC patients. Pathogenic germline mutations in cancer predisposition genes are associated with tumorigenesis and development of RCC (26). VHL disease is an autosomal dominant disorder caused by germline mutations in the *VHL* gene. Patients with V*HL* disease are particularly prone to renal tumors when a stochastic secondary inactivation of the other *VHL* allele occurs (27). Similar to those with VHL disease, patients with *TSC1/2*, FH and *SDHB/C/D* germline mutations are more likely to have RCC (28-30). Germline mutation in *MET* can promote hereditary pRCC initiation and progression (31,32). The identification of RCC patients with certain pathogenic germline mutations has important clinical implications, guiding systemic therapy and clinical trial eligibility (16,17,33). Although most studies have investigated the prevalence of germline mutations among patients with RCC and some cancer-predisposition genes involved in the tumorigenesis and tumor progression of RCC have been identified (9,25,34), the genetic basis of some inherited renal cancers has not been clearly elucidated. Here, we conducted a study using WES or gene panel sequencing of 139 genes to evaluate the pathogenic/likely pathogenic germline mutation rate and identify *de novo* pathogenic/likely pathogenic germline mutations in patients with RCC.

Patients with nccRCC or multifocal RCC, multifocal disease at diagnosis, are at higher risk for inherited syndromes and are significantly more likely to have pathogenic germline mutations (9). Similarly, we showed that patients with bilateral or multifocal nccRCC are more likely to have pathogenic/likely pathogenic germline mutations. Early-onset cancer is a hallmark of an inherited cancer predisposition (35,36), and studies have shown that patients with mutations in RCC-associated genes, such as *VHL*, *FH*, *FLCN*, and *SDHB*, are at risk for the development of early-onset RCC (37-39). Loss of function of *MUTYH* accounts for 3% of early-onset CRC (40,41). Mutations in MRE11-RAD50-NBS1 (MRN) complex components, *FANCM*, or *FANCI* are associated with early-onset cancers (42-44). We also found that patients with early onset are more likely to carry pathogenic mutations (*Table 2*). However, Carlo *et al.* suggested that age at diagnosis was not related to the possibility of germline mutations in patients with advanced RCC (9). These seemingly contradictory findings may be due to Carlo *et al.* only including patients with advanced tumor stages in their study.

Genome instability is a hallmark of cancer cells and can be accelerated by defects in cellular responses to DNA damage (45,46). In our study, we found that 6 of 10 cancer predisposition genes were associated with maintenance of genomic stability and DNA repair (*Figure 3*). Deleterious germline mutations of 3 FA-related genes were reported. FA is considered a genetic disease associated with a predisposition to non-hematological and hematological malignancies (47,48). The proteins encoded by these genes comprise the DNA damage response (DDR) system and play vital roles in various cellular processes. Increasing evidence suggests that the defective function of these proteins can be associated with genomic instability, increasing cancer risk (49). Thus, further studies are required to elucidate the role of each component in tumorigenesis and tumor progression in RCC. RAD50 and NBN are components of the MRN complex and are involved in the repair of DNA double-strand breaks. As one of the first sensors and responders to DNA damage, the MRN complex plays a vital role in DDR (45,50). Mutations in the MRN complex are also associated with an increased risk of cancer, suggesting that the complex functions as a tumor suppressor (51-54). However, some studies have shown that the complete knockout of any component of the murine MRN complex can impair early embryonic development (55-57), suggesting that some functions of the MRN complex must be preserved in human disease-associated alleles.

In our study, we found that 2 patients with RCC harbored *FANCI* and *FANCM* pathogenic germline mutations. To our knowledge, this is the first report of

germline mutations in these pathogenic genes in RCC. As highly conserved DNA remodeling enzymes (58-60), *FANCI* and *FANCM* can activate the FA DNA repair pathway to maintain genomic stability. Patients with FA, characterized by the clinically and genetically heterogeneous syndrome of bone marrow failure, are predisposed with a predisposition to cancers (61-64). Studies have shown that the loss of function of some FA genes (such as *BRCA1* and *BRCA2*) by germline inactivation can result in familial breast cancer predisposition syndromes (65-70). In addition, pathogenic FA germline mutations can occur in colorectal cancer, pancreatic cancer, and leukemia (71-73). As members of the FA complementation group, *FANCI* and *FANCM* play important roles in the initiation of various cancers. Wang *et al.* found FANCM mutation in a patient with four primary cancer including renal cancer (74). Mutations in these two genes are associated with renal ectopia malformations or dysplasia (19,75) Thus, we suspected that *FANCI* and *FANCM* pathogenic germline mutations might play an important role in tumorigenesis and tumor progression in RCC and further studies are needed to confirm this hypothesis.

There were some limitations in our study. Larger cohorts will need to be studied to confirm the frequency of *FANCI* and *FANCM* pathogenic germline mutations in RCC and basic experiments are required to validate the biological role of *FANCI* and *FANCM* pathogenic germline mutations in RCC.

Conclusions

Considering our results and the evidence in the literature, the proportion of patients with RCC in our study who carried pathogenic germline mutations may be underestimated. Genetic testing of all patients with nccRCC, patients with bilateral or multifocal RCC, especially those aged 46 years or younger might help identify individual patients for whom targeted therapies are indicated. Patients with *FANCI* and *FANCM* pathogenic germline mutations may play an important role in tumorigenesis and tumor progression in RCC.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-23-32/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-23-32/coif). Xu Zhang serves as an unpaid editorial board member of *Translational Andrology and Urology* from April 2022 to March 2023. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Chinese PLA General Hospital (No. S2013-065-01) and informed consent was taken from all the patients.

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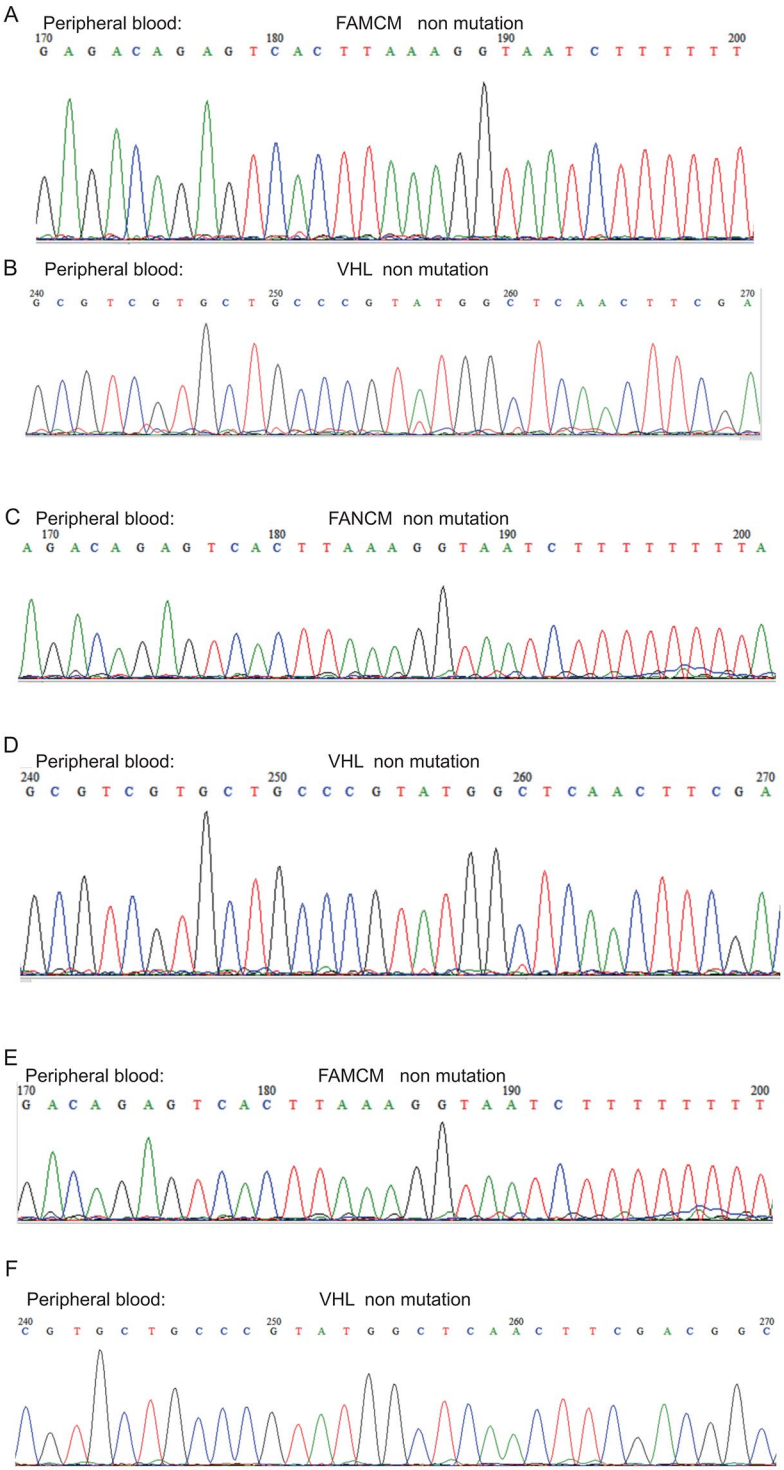

Figure S1 Sanger sequencing chromatograms of *FANCM* and *VHL* for patient RCC88's parents and brother. Genetic testing of DNA samples collected from RCC88's father (A,B), mother (C,D), and brother (E,F) revealed that none of them harbored *FANCM* or *VHL* mutations. RCC, renal cell carcinoma.

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Table S1 Genes related to germline cancer predisposition

AKT1	*BRIP1*	*CYLD*	*FANCC*	*HRAS*	*MTAP*	*PMS2*	*SDHAF2*	*TEK*
AKT2	*BTK*	*DDR2*	*FANCG*	*IDH1*	*MUTYH*	*PRKAR1A*	*SDHB*	*TERC*
ALK	*CBL*	*DICER1*	*FANCI*	*IDH2*	*MYC*	*PTCH1*	*SDHC*	*TERT*
APC	*CDC73*	*DNMT3A*	*FANCL*	*JAK1*	*MYD88*	*PTEN*	*SDHD*	*TP53*
AR	*CDH1*	*EGFR*	*FANCM*	*JAK2*	*NBN*	*PTPN11*	*SF3B1*	*TSC1*
ARAF	*CDK4*	*EP300*	*FAS*	*KIT*	*NF1*	*RAD50*	*SH2D1A*	*TSC2*
ATM	*CDKN1B*	*EPCAM*	*FBXW7*	*KRAS*	*NF2*	*RAD51*	*SHOC2*	*U2AF1*
AXIN2	*CDKN2A*	*ERBB2*	*FGFR1*	*MAP2K1*	*NOTCH2*	*RAD51C*	*SLX4*	*VHL*
B2M	*CEBPA*	*ERBB3*	*FH*	*MAP2K2*	*NPM1*	*RAF1*	*SMAD4*	*WT1*
BAP1	*CFTR*	*ERCC2*	*FLCN*	*MEN1*	*NRAS*	*RB1*	*SMARCA4*	*XIAP*
BCL10	*CHEK2*	*ERCC3*	*FLT3*	*MET*	*NSD1*	*RECQL4*	*SMARCB1*	*XPO1*
BLM	*CREBBP*	*ERCC4*	*FLT4*	*MLH1*	*NTHL1*	*RET*	*SMO*	
BMPR1A	*CSF3R*	*ERCC5*	*GATA2*	*MRE11*	*PALB2*	*RIT1*	*SOS1*	
BRAF	*CTLA4*	*ETV6*	*GNAS*	*MSH2*	*PHOX2B*	*RTEL1*	*STAT3*	
BRCA1	*CTNNB1*	*EZH2*	*H3F3A*	*MSH3*	*PIK3CA*	*RUNX1*	*STK11*	
BRCA2	*CXCR4*	*FANCA*	*HNF1A*	*MSH6*	*PLCG2*	*SDHA*	*SUFU*	

Table S2 Clinical characteristics of patients with RCC and pathogenic mutations

Patient ID	Sex	Age at diagnosis (years)	Mutation gene	Histology	Family history	Recurrence or metastasis
RCC15	Male	21	*VHL*	ccRCC	Maternal aunt: VHL	0
RCC16	Male	44	*VHL*	ccRCC	0	0
RCC19	Male	44	*VHL, RAD51C*	ccRCC	Mother: glioma	0
RCC40	Male	36	*MUTYH*	ccRCC	0	0
RCC63	Male	39	*FANCI*	Unclassified	Father: gastric cancer	0
RCC68	Male	56	*FLCN*	Unclassified	Father: gastric cancer	0
RCC88	Male	29	*VHL, FANCM*	ccRCC	0	1: metastasis
RCC93	Male	24	*RAD50*	MiT family translocation RCC	0	0
RCC96	Female	36	*FLCN*	chRCC	0	0
RCC109	Male	62	*NBN*	ccRCC	0	0
RCC115	Male	37	*SDHB*	chRCC	0	0
RCC121	Female	32	*FH*	pRCC	Mother: endometrial carcinoma	1: metastasis
RCC122	Male	24	*FH*	pRCC	Father: RCC	0

RCC, renal cell carcinoma; ccRCC, clear cell renal cell carcinoma; VHL, von Hippel-Lindau; MiT, microphthalmia; chRCC, chromophobe renal cell carcinoma; pRCC, papillary renal cell carcinoma.

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Table S3 Detail on pathogenic mutations

Study ID	Gene 1	Variant 1	Protein 1	Transcript NM_#S	Pathogenicity
RCC15	*VHL*	c.340G>C	p.Gly114Arg	NM_000551	P
RCC16	*VHL*	c.345C>G	p.His115Gln	NM_000551	P
RCC19	*VHL*	c.517_527delGAGAATTACAG	p.Glu173fs	NM_000551	P
RCC19	*RAD51C*	c.904+2T>C	#	NM_058216	P
RCC40	*MUTYH*	c.1214C>T	p.Pro405Leu	NM_001128425	P
RCC63	*FANCI*	c.158-2A>G	#	NM_001113378	P
RCC68	*FLCN*	c.1379_1380delTC	p.Leu460fs	NM_001353229	P
RCC88	*VHL*	c.264G>A	p.W88*	NM_000551	P
RCC88	*FANCM*	c.4515+1G>C	#	NM_020937	LP
RCC93	*RAD50*	c.2165dupA	p.E723fs	NM_005732	P
RCC96	*FLCN*	c.1285dupC	p.H429fs	NM_001353229	P
RCC109	*NBN*	c.235-1G>C	#	NM_002485	LP
RCC115	*SDHB*	c.725G>A	p.R242H	NM_003000	P
RCC121	*FH*	c.A563T	p.N188I	NM_000143	P
RCC122	*FH*	c.191dupA	p.N64fs	NM_000143	P

#, frameshift mutation; *, nonsense mutation; RCC, renal cell carcinoma; P, pathogenic; LP, likely pathogenic.

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