Germline *SDHB* Mutations and Familial Renal Cell Carcinoma

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Familial renal cell carcinoma (RCC) is a heterogeneous disorder that is most commonly caused by germline mutations in the *VHL*, *MET*, and *FLCN* genes or by constitutional chromosome 3 translocations. However, for many patients with familial RCC, the genetic basis of the disease is undefined. We investigated whether germline mutations in fumarate hydratase (*FH*) or succinate dehydrogenase subunit genes (*SDHB*, *SDHC*, *SDHD*) were associated with RCC susceptibility in 68 patients with no clinical evidence of an RCC susceptibility syndrome. No mutations in *FH*, *SDHC*, or *SDHD* were identified in probands, but 3 of the 68 (4.4%) probands had a germline *SDHB* mutation. Patients with a germline *SDHB* mutation presented with familial RCC (n = 1) or bilateral RCC (n = 2) and no personal or family history of pheochromocytoma or head and neck paraganglioma. Age at diagnosis of RCC in *SDHB* mutation carriers ranged from 24 to 73 years. These findings 1) demonstrate that patients with suspected inherited RCC should be examined for germline *SDHB* mutations, 2) suggest that all identified *SDHB* mutation carriers should be offered surveillance for RCC, and 3) provide a further link between familial RCC and activation of hypoxic-gene response pathways.

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Renal cell carcinomas (RCC) account for approximately 2% of all cancers in the West, and they are histologically heterogeneous, with most (~80%) classified as clear cell, that is, conventional RCC (cRCC). Among the non–clear cell types, papillary (chromophilic) and chromophobic tumors are the most frequent (1). Although only 2–3% of all cases of RCC are familial, the identification of familial cases is important so that surveillance can be offered to patients and at-risk relatives and morbidity and mortality reduced (2,3). In addition, information on the molecular basis of familial RCC (eg, in von Hippel–Lindau disease) has provided the scientific basis for the introduction of novel therapeutic strategies for sporadic RCC (4).

Familial RCC may be caused by mutations in the *VHL*, *MET*, or *FLCN* genes (mutation of *FLCN* causes Birt–Hogg–Dube syndrome) or by constitutional translocations (particularly involving chromosome 3) (5–7). However, in many cases, the genetic basis of familial RCC is unknown (8,9). Germline mutations in *FH*, the gene encoding fumarate hydratase, an enzyme in the Krebs cycle, causes a hereditary leiomyomatosis syndrome that is characterized by cutaneous leiomyomas and, in women, multiple early onset uterine leiomyomas (fibroids). In men and women with these mutations, type 2 papillary RCC may also occur (10,11). Germline mutations affecting another Krebs cycle enzyme, succinate dehydrogenase (SDH), can also cause an inherited tumor susceptibility syndrome. Thus, inherited mutations in *SDHB*, *SDHC*, and *SDHD* genes (encoding subunits of SDH) are associated with a high risk of head and neck paragangliomas and/or adrenal and extra-adrenal pheochromocytoma (12–15). Very rarely, germline mutations in *SDHB* have been found in patients with RCC and a personal or family history of pheochromocytoma and/or paraganglioma (16). Similarly, germline mutations in *SDHB*, *SDHC*, and *SDHD* have been detected in patients with a combination of gastrointestinal stromal tumors and paraganglioma (17).

We investigated whether 68 patients with features of nonsyndromic inherited RCC susceptibility might harbor mutations in *FH*, *SDHB*, *SDHC*, or *SDHD*. Twenty-nine of the probands had familial RCC, and 39 were without demonstrable family history of RCC (21 of the latter group had early multicentric RCC and 18 had early-onset [age at diagnosis <40 years] disease). All patients studied had 1) no clinical evidence of an RCC susceptibility syndrome and 2) normal cytogenetic analysis and normal *VHL* and *FLCN* mutation analysis. All subjects gave consent for genetic studies, and the investigations were approved by the South Birmingham Research Ethics committee. To detect mutations, the *FH*, *SDHB*, *SDHC*, and *SDHD* genes were sequenced as described previously (18). No mutations in *FH*, *SDHC*, or *SDHD* were detected. However, 3 of the 68 (4.4%, upper limit of calculated population proportion 95% confidence interval = 9.29%) probands (3.4% of probands with familial disease and 9.5% of those with multicentric RCC) had a germline *SDHB* mutation.

The mutations in these three patients were as follows. Case subject 1: A germline heterozygous nonsense mutation, p.Arg46Stop (c.136C>T, Figure 1) in exon 2 was detected in a 58-year-old male proband who had been diagnosed with right-sided clear-cell RCC at age 24 years. His father (now deceased) and paternal uncle had also developed clear cell RCC, at ages 24 and 73 years, respectively. The presence of the c.136C>T mutation in the affected uncle was confirmed subsequently. Case subject 2: A germline heterozygous missense mutation, p.Arg46Gln (c.137G>A, Figure 1) was identified in a 44-year-old woman who presented with bilateral RCC at age 30 years. Case subject 3: A novel germline heterozygous missense mutation,

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See "Notes" following "References."

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CONTEXT AND CAVEATS

Prior knowledge

Germline mutations in *SDHB*, a gene encoding a subunit of the mitochondrial enzyme succinate dehydrogenase (SDH), have been found in patients with renal cell carcinoma (RCC) who had a personal or family history of pheochromocytoma or paraganglioma. It was not known whether mutations would be found in genes encoding subunits of SDH in people with evidence of a familial history of RCC but with no family history of these other cancers.

Study design

People for whom there was evidence of increased susceptibility to RCC (ie, family history or early age of diagnosis), but for whom there was no evidence of family history of pheochromocytoma or paraganglioma, were tested for germline mutations in genes encoding three subunits of SDH.

Contribution

Some people with evidence of inherited susceptibility to RCC but for whom there was no other evidence of another cancer susceptibility syndrome harbored germline mutations in *SDHB*.

Implication

Inherited mutations in *SDHB* may predispose individuals to RCC.

Limitations

The added risk of RCC conferred by mutations in *SDHB* and the benefit of screening for these mutations in patients with evidence of familial RCC remain to be determined.

From the Editors

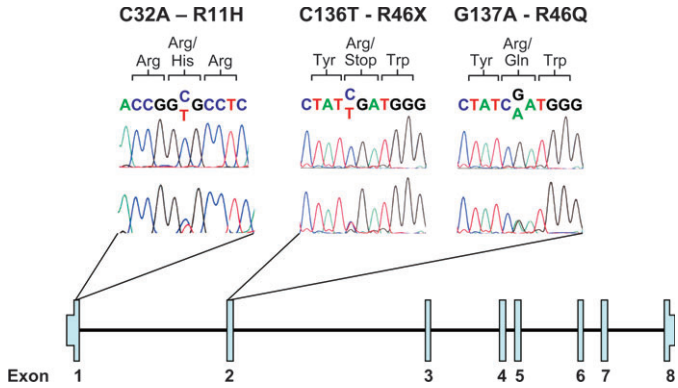

Figure 1. *SDHB* mutations in probands with familial or bilateral renal cell carcinoma. The mutations detected in the probands are shown in the diagram above with electropherograms of the wild-type sequence above and the mutation below. The nucleotide and amino acid changes are shown (the nucleotide number is based on the A of the ATG start codon being nucleotide 1).

p.Arg11His (c.32G>A, Figure 1) was identified in a 46-year-old man who presented with bilateral eosinophilic chromophobe RCC at age 38 years. p.Arg11 and p.Arg46 are conserved in *SDHB* orthologues from chimpanzee, horse, dog, chicken, mouse, rat, African clawed frog, puffer fish, and drosophila. There was no family history of RCC for case subject 2 or 3, and no other family members were available for mutation testing. None of the four mutation carriers reported a personal or family history of pheochromocytoma or head and neck paragangliomas, although two of the mutations identified (c.136C>T→p.Arg46Stop and c.137G>A→Arg46Gln) have been described previously in individuals with pheochromocytoma and/or head and neck paragangliomas (19–21). None of the three mutations were present in the National Center for Biotechnology Information Single-Nucleotide Polymorphism database (http://www.ncbi.nlm.nih.gov/projects/SNP/) or detected in 600 anonymized laboratory control chromosomes from UK population.

Our results show, to our knowledge for the first time, that germline *SDHB* mutations may be present in patients with familial or multicentric RCC without any personal or family history of pheochromocytoma or paraganglioma. Previously, three patients with early-onset RCC were reported in two families with germline *SDHB* mutations (16). Two of these patients had a personal and family history of paraganglioma, and the mother of the third patient was diagnosed with malignant paraganglioma. Subsequently, there were reports of two patients with RCC and a personal or family history of pheochromocytoma/paraganglioma who had germline *SDHB* mutations (22,23). However, *SDHB* mutations have not been considered to be a cause of familial RCC and the risk of RCC was not considered to be elevated is in those with *SDHB* mutations.

There are limitations to our study. The study group analyzed was not based on referrals to a specialized center and may not exactly reflect population-based cases of familial RCC. In addition, gene sequencing will not detect all mutations, such as exon deletions or mutations deep within an intron.

Our findings suggest that individuals presenting with features of inherited RCC susceptibility should be screened for germline *SDHB* mutations because surveillance for *SDHB*-related tumors can then be offered to mutation-positive patients and relatives. Many centers undertake routine annual abdominal MRI screening for adrenal and extra-adrenal pheochromocytoma in *SDHB* mutation carriers, and screening for RCC should be considered for inclusion in such surveillance programs. Although histopathologic analysis can inform genetic testing priorities in familial RCC (eg, germline *VHL* mutations are invariably associated with clear-cell RCC and *MET* mutations with papillary RCC), there does not appear to be such a close correlation between germline *SDHB* mutations and histopathologic subtypes of RCC. Although most *SDHB*-associated RCCs are clear-cell RCC, other types of disease may occur (23). All three RCC-associated *SDHB* mutations that we detected were located in the first 50 amino acids. However, it was not possible to deduce clear genotype–phenotype correlations because two of the mutations that we detected have previously been detected in families with pheochromocytoma/paraganglioma without RCC, and RCC has also been associated with *SDHB* mutations closer to the 3′ end of the gene (eg, c.847-50delTCTC) (16). More extensive analysis of cohorts of patients with inherited RCC and screening for RCC in *SDHB* patient cohorts will provide information on genotype–phenotype correlations that might influence clinical management.

Pheochromocytomas in patients who carry *VHL* or *SDHB* mutations show evidence of increased expression of HIF-1 and HIF-2 (24). In addition, *SDHB* mutations and missense *VHL* mutations have been

suggested to predispose to pheochromocytoma development by inhibiting normal developmental apoptosis of sympathoadrenal cells via a HIF-independent pathway (25). Genotype-phenotype correlations in VHL disease have closely linked loss of pVHL's ability to inhibit HIF to risk of RCC (26,27), and HIF-2 overexpression can overcome pVHL tumor suppressor activity (28). In contrast to what is observed for *VHL* (5), somatic mutations of *SDHB* appear to be rare in sporadic RCC (18). Further study of the relationship between *SDHB* mutation spectrum, RCC risk, and the functional effect of specific *SDHB* mutations on HIF-1 and HIF-2 expression could provide further insight into the pathogenesis of RCC in individuals with *SDHB* mutations and the role of HIF-dependent and HIF-independent pathways in familial pheochromocytoma and RCC.

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Notes

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