

**Case study**

# Conventional renal cancer in a patient with fumarate hydratase mutation<sup>☆</sup>

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**Summary** Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a tumor predisposition syndrome caused by mutations in the *fumarate hydratase* (*FH*) gene. HLRCC is characterized by uterine and cutaneous leiomyomas, renal cell cancer, and uterine leiomyosarcoma. Typically, renal cell cancers in HLRCC are unilateral and display a papillary type 2 or ductal histology. We describe here a 23-year-old patient carrying a novel *FH* mutation (N330S) with a bilateral renal cell cancer. Carcinoma of the right kidney showed papillary structure, but the left tumor was diagnosed as a conventional (clear cell) renal carcinoma, a type not previously described in HLRCC. The clear cell renal carcinoma also displayed loss of the normal *FH* allele and the FH immunostaining. Our finding extends the number of cases in which HLRCC can be suspected, and the FH immunohistochemistry may serve as a useful tool to screen for HLRCC in young individuals with clear cell renal carcinoma.

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**1. Introduction**

The recently identified tumor predisposition syndrome hereditary leiomyomatosis and renal cell cancer (HLRCC) is characterized by a high penetrance occurrence of cutaneous and uterine leiomyomas. Renal cell cancer (RCC) and uterine leiomyosarcoma (ULMS) are detected in a subset of the

families [1,2]. HLRCC is caused by heterozygous mutations in the *fumarate hydratase* (*FH*, *fumarase*) gene, which encodes one of the tricarboxylic acid cycle's enzymes. Altogether, 47 different *FH* mutations have been identified so far [1,3–10]. Because biallelic inactivation of the gene is observed in most benign and malignant HLRCC tumors, *FH* is suggested to function as a tumor suppressor [1–3,10,11].

At present, 117 families with HLRCC have been reported throughout the world, mainly in Europe and North America [1,3–11]. The prevalence of RCC seems to differ between and within the affected families because only about a fifth of the families (21%, 25/117) display RCC. These families are from the UK (1 patient in 1 family), Poland (1 patient in 1 family), Finland (12 patients in 5 families), and North America

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**Table 1** Immunohistochemical stainings of renal tumors

Antigen	Case right RCC	Case left RCC	FAM-1 <sup>a</sup> M19	FAM-1 <sup>a</sup> M17	FAM-1 <sup>a</sup> M13
FH	+	-	-/+	-	-
vimentin	+	+	+	+	+
EMA	+	+	-	+	+
S-100	+	+	+	+	-
CD10	+	-/+	-	-	-
CK8	+	+	-	-	-
CK7	-	-	-	-	-
AE1/AE3	-	+	+	+	+

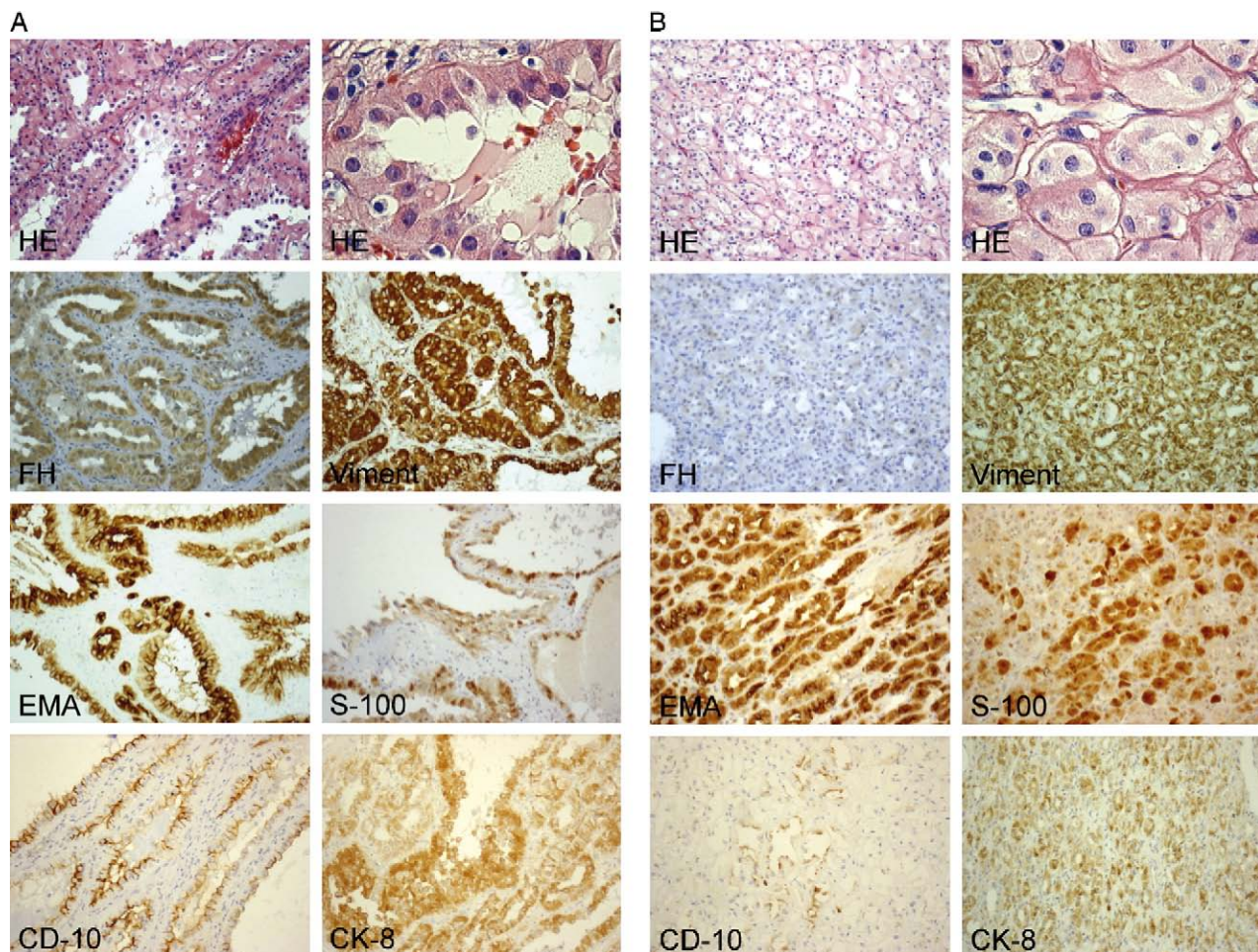
Symbols: +, positive staining; -, negative staining; -/+, light staining.

<sup>a</sup> FAM-1 is a Finnish HLRCC family previously published by Launonen et al [1].

(33 patients in 18 families) [1,3,4,7-9,12]. Although the prevalence of the malignant tumors (RCC, ULMS) varies between and within the families with HLRCC, no clear correlation between the type or site of the *FH* mutation and the phenotype has been found. Identical mutations can be seen in the families with and without RCC. Therefore,

existence of other genetic factors modifying the risk for RCC (and ULMS) has been widely discussed [2,7,13,14]. In addition, the lower prevalence of RCC in HLRCC compared with other hereditary RCC syndromes such as von Hippel-Lindau (VHL) disease and hereditary papillary renal cancer support the hypothesis [7].

HLRCC-associated RCCs are usually solitary, unilateral, and aggressive. The typical histological features are a papillary type 2 structure, a high Fuhrman grade (from 3 to 4), tall cells with an abundant cytoplasm, and large nuclei with prominent eosinophilic owl-eye-like nucleoli [1,3]. Originally, the specific histology of RCCs led to the identification of the syndrome. The 47 HLRCC-associated RCC cases reported to date have included also 4 collecting duct carcinomas and 1 oncocytic tumor [4,7,9], but the most common type of RCC, a conventional (clear cell) carcinoma (CRCC), has to our knowledge not yet been reported in the context of HLRCC. The histology of the RCCs has played a key role in the diagnosis of HLRCC. In individuals with CRCC, HLRCC is typically not suspected because that tumor type has not been associated with *FH* germline mutations.



**Fig. 1** Hematoxylin-eosin and positive immunohistochemical stainings of the 2 renal cancers. A, Right-side tumor. B, Left-side tumor. Original magnification of the photos is  $\times 100$ , and the HE staining is, in addition, shown with  $\times 400$  magnification. HE indicates hematoxylin-eosin; Vimentin, vimentin; EMA, epithelial membrane antigen; CK-8, cytokeratin 8.

## 2. Case

The case reported here is a female patient who was admitted to a hospital (L'Hospitalet, Barcelona, Spain) at age 23 because of unusual uterine bleeding. An ultrasound revealed a myomatous uterus, and at the same time, a bilateral renal mass was incidentally observed. A computed tomography confirmed the presence of lesions suggestive of bilateral renal cancer. The mother of the patient had been diagnosed with multiple uterine leiomyomas at age 33. An abdominal ultrasound at age 54 revealed no kidney lesions.

### 2.1. Pathological findings

A partial nephrectomy was performed for the right kidney tumor. The maximum diameter of the lesion was 2.3 cm. The histology revealed a well-circumscribed tumor covered by a collagen capsule. The structure was mainly cystic with some papillary and trabecular areas. The epithelium was eosinophilic cylindrical Delahunt type 2, nuclear grade Fuhrman 2. Foamy macrophages were seen inside the cysts. No psammoma bodies were seen. The immunohistochemistry was positive for FH (1:1000; Nordic Immunology, Tillburg, The Netherlands), vimentin (1:500; Dako, Glostrup, Denmark), CD10 (1:50; Novocastra, Newcastle, UK), EMA (1:500; Dako), S-100 (1:2000; Dako), and CK8 (1:100; Enzo Life Sciences, Farmingdale, NY) and negative for AE1/AE3 (1:700; Dako) and CK7 (1:300; Novocastra) (Table 1 and Fig. 1).

The left kidney tumor was removed via a radical nephrectomy. The maximum diameter of the tumor was 13 cm. The cut surface showed solid and cystic areas. No vascular invasion was seen. This RCC displayed clear cell histology and was encased by a collagen capsule. The structure was tubular with few cysts. The epithelium was clear with a moderate cytoplasm. The Fuhrman nuclear grade was 2. The immunohistochemistry was positive for vimentin, AE1/AE3, EMA, CK8, S-100, and CD10 (light staining) and negative for CK7 and FH (Table 1 and Fig. 1).

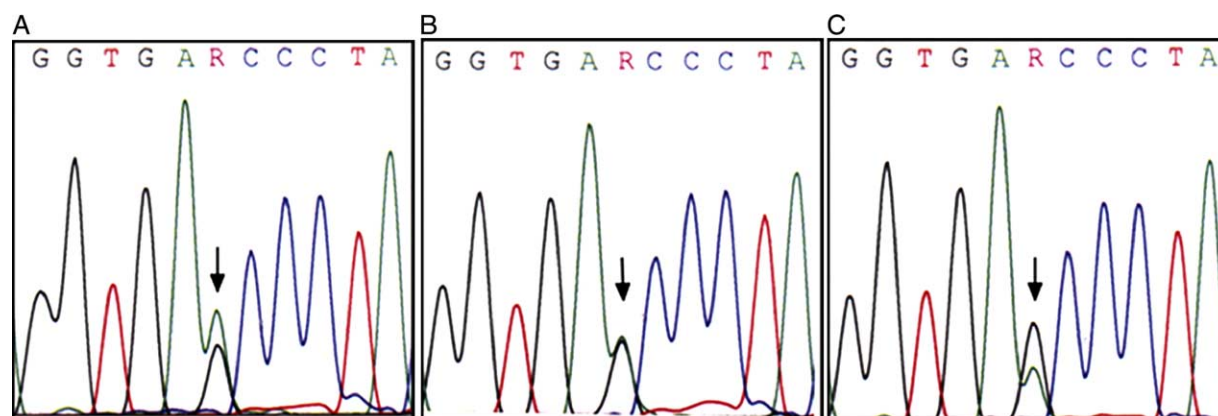
A uterine surgery performed on the patient at age 25 revealed 2 atypical leiomyomas, 8.5 and 2.8 cm in diameter. There was no tumor cell necrosis in the studied specimens, and the mitotic index was less than 10mf/10hpf.

### 2.2. Genetic findings

The *FH* mutation status of the patient was analyzed by a direct sequencing of the *FH* gene from blood-extracted DNA. The primer sequences, the polymerase chain reaction (PCR) conditions, and the sequencing method have been previously described by Kiuru et al [11]. The analysis revealed a novel *FH* missense mutation N330S (AAC > AGC) in exon 7, changing amino acid asparagine to serine. The alteration was found to be inherited from the mother, as evidence for a somatic biallelic inactivation of the gene was searched for from the 2 renal cancers. In accordance with the findings in the immunohistochemistry, biallelic inactivation of *FH* was not seen in the right RCC after sequencing of the whole gene (Fig. 2). In the CRCC, loss of the wild-type allele was detected. CRCC is a common tumor in *VHL* disease caused by inherited mutations in the *VHL* gene located at 3p26-p25. Moreover, somatic *VHL* mutations and loss of heterozygosity (LOH) have been seen in up to 60% of sporadic CRCCs [15]. Therefore, the CRCC tumor was further analyzed with 2 microsatellite markers (D3S1038 and D3S3659) for loss of chromosome 3p and by sequencing the sample for *VHL* mutations. (Primer sequences and PCR conditions are available upon request.) The informative marker D3S3659 showed no loss in 3p. *VHL* mutation analysis was also negative. (Data not shown).

## 3. Discussion

We report here a female patient who was diagnosed at age 23 as having bilateral RCC. Sequencing of the *FH* gene from normal DNA showed the patient and her mother to be carriers of a novel *FH* missense mutation N330S. The patient's right RCC showed papillary structure and large cells with an



**Fig. 2** A, Sequence from *FH* exon 7 of the patient's normal tissue showing germline mutation N330S (AAC > AGC). B, Tissue from the right-side renal tumor displayed no loss of *FH* wild-type allele. C, LOH of *FH* was seen in left-side renal tumor. Normal tissue in the tumor sample presents a low wild-type allele peak.

abundant eosinophilic cytoplasm, resembling an HLRCC. A low Fuhrman grade, medium-sized nuclei, and small nucleoli were also seen; features less frequently detected in association with *FH* mutations. The left RCC displayed a clear cell, solid, and acinar pattern with a low Fuhrman grade. The nuclei were small, and the nucleoli were inconspicuous. Thus, no typical histological characteristics of HLRCC were seen. The immunohistochemical profile of the 2 studied RCCs differed from 3 previously characterized HLRCC-associated RCCs (from a Finnish HLRCC family FAM-1 reported by Launonen et al [1]) with positive CK8 and CD10 staining. In addition, the staining for AE1/AE3 was positive in the left RCC as in the 3 controls but negative in the right-side tumor. The CRCC lacked *FH* protein expression and displayed loss of the normal *FH* allele. The role of *VHL* in the tumorigenesis of CRCC was excluded by *VHL* mutation analysis and LOH analysis of chromosome 3p.

In addition to RCC, the patient was diagnosed with 2 atypical uterine leiomyomas. Nuclear atypia can be present in the ULMS, but no other characteristics of ULMS were observed in the studied tumors. HLRCC-associated ULMS has been found only in Finnish families (with 71-fold risk) [10], whereas atypical uterine leiomyomas have been reported previously in Finnish and North American families [7,10].

In studies by Kiuru et al [11] and Morris et al [16], altogether, 60 nonfamilial CRCCs have been screened for *FH* mutations with negative results. To our knowledge, this is the first report showing early-onset CRCC arising through a germline mutation in *FH*. Considering that the CRCC is by far the most common type of renal cancer, this finding has implications in differential diagnostics of RCC of young age at onset. Although *FH* mutations should be vigorously searched for in consenting patients showing strong HLRCC features, HLRCC can be considered as one diagnostic alternative in the young-age-at-onset RCC regardless of the histological findings. The *FH* immunohistochemistry may serve as a useful low-cost screening method to identify HLRCC.

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