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SHORT REPORT

Germline *fumarate hydratase* mutations in patients with ovarian mucinous cystadenoma

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Germline mutations in the *fumarate hydratase* (*FH*) gene were recently shown to predispose to the dominantly inherited syndrome, hereditary leiomyomatosis and renal cell cancer (HLRCC). HLRCC is characterized by benign leiomyomas of the skin and the uterus, renal cell carcinoma, and uterine leiomyosarcoma. The aim of this study was to identify new families with *FH* mutations, and to further examine the tumor spectrum associated with *FH* mutations. *FH* germline mutations were screened from 89 patients with RCC, skin leiomyomas or ovarian tumors. Subsequently, 13 ovarian and 48 bladder carcinomas were analyzed for somatic *FH* mutations. Two patients diagnosed with ovarian mucinous cystadenoma (two out of 33, 6%) were found to be *FH* germline mutation carriers. One of the changes was a novel mutation (Ala231Thr) and the other one (435insAAA) was previously described in FH deficiency families. These results suggest that benign ovarian tumors may be associated with HLRCC.
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Introduction

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a recently described dominantly inherited cancer syndrome characterized by predisposition to cutaneous and uterine leiomyomas, renal cell cancer (RCC), and uterine leiomyosarcomas (ULMS) (HLRCC, OMIM 605839). HLRCC is caused by heterozygous germline mutations in *fumarate hydratase (FH, fumarase)*.[1,2] Leiomyomas of the skin and the uterus are the most common feature of the syndrome with nearly complete penetrance.[3] RCC and ULMS have been reported in a subset of mutation-positive families. In addition, other tumor types including breast carcinoma, prostate cancer, bladder carcinoma and skin leiomyosarcoma have been observed in HLRCC families.[1–11] Homozygous germline mutations in *FH* also cause a recessive syndrome called FH deficiency. FH deficiency is an inborn error characterized by severe neurological symptoms and developmental delay (OMIM 606812).[12,13]

To identify new families with *FH* mutations and to further examine the tumor spectrum associated with *FH* mutations, we examined a series of Polish patients displaying papillary RCC, single-skin leiomyomas, ovarian cystadenomas or carcinomas for *FH* germline mutations. The rationale for including ovarian tumors in the present study was the recent report of an excess of uterine leiomyomas and ovarian cystadenomas in Polish families with hereditary ovarian cancer unassociated with BRCA1

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mutations.[14] Subsequently, a set of mucinous cystadenocarcinomas was studied for somatic *FH* mutations.

Although we have previously screened multiple different tumor types for somatic *FH* mutations, we took the opportunity to examine here also 48 bladder cancers; the rationale for this was recent detection of loss of the wild-type allele in a bladder cancer of a Finnish *FH* mutation carrier, and a report from UK on a bladder cancer case in an *FH* mutation-positive individual.[3,9] Although previous work on identification of somatic *FH* mutations has been largely though not completely negative, the example of frequent mutations of *LKB1* in lung adenocarcinomas but few other tumor types emphasizes that thorough evaluation of a large variety of tumor types is warranted when a role of a hereditary cancer gene in somatic tumorigenesis is examined.[15,16]

Materials and methods

Patient material

For germline mutation analysis, blood samples from 89 probands were collected. For analysis of somatic *FH* mutations, ovarian carcinomas and bladder carcinomas were collected. Details of the patient material are summarized in Table 1. Samples were collected following informed consent. DNA extractions from blood or tumor samples were performed using standard procedures.

Mutation analysis

Blood samples and ovarian tumors were analyzed for *FH* mutations by genomic sequencing. Polymerase chain reaction (PCR) conditions and the oligonucleotide primers used were published earlier by Kiuru *et al*.[17] The PCR products were purified using ExoSAP-IT PCR purification

Table 1 Description of the patient material included in the *FH* mutation analysis

Samples	*N*	*Tissue*	*Population*	*FH mutation, N (%)*
Tumors in probands	89			
Papillary renal cell cancer	24	Blood	Polish	0
Skin leiomyomas	5	Blood	Polish	0
Ovarian tumors[a]	60			
Mucinous cystadenoma	33	Blood	Polish	2 (6)
Serous cystadenoma	5	Blood	Polish	0
Mucinous cystadenocarcinoma	22	Blood	Polish	0
Tumor samples	61			
Ovarian tumors	13			
Mucinous cystadenocarcinoma	13	Tumor	Finnish	0
Bladder carcinomas	48	Tumor	Danish	0

[a]Nine of the patients with mucinous cystadenoma were selected based on their family history of ovarian ($n=4$) or breast ($n=5$) cancer.

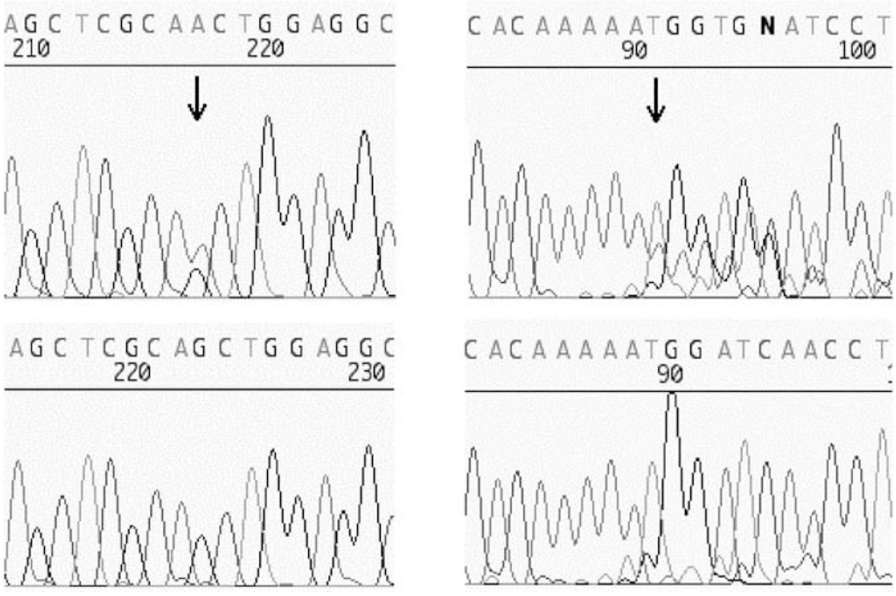

Figure 1 Sequence chromatograms of germline mutations in the *FH* gene detected in patients with ovarian mucinous cystadenoma: a missense substitution 691G>A (Ala231Thr) and a three-base pair insertion at codon 435 (435insAAA). Mutations are marked by arrows.

kit (USB Corporation, Cleveland, Ohio, USA) and the sequencing reactions were performed using the Big Dye Terminator v.3.1 kit (Applied Biosystems, Foster City, CA, USA). Electrophoresis was performed on an ABI3730 Automatic DNA sequencer (Applied Biosystems). Bladder tumors were analyzed by denaturing high-performance liquid chromatography (DHPLC) as described earlier.[18] Bladder tumor samples showing heteroduplex peaks in DHPLC analyses were reanalyzed by direct sequencing. One hundred and fifty healthy Polish individuals were used as controls.

Results and discussion

In the *FH* germline mutation, analysis of 89 probands with P-RCC, skin leiomyomas or ovarian tumors, two mutations were found. Neither of the mutations was found in control samples. Both mutation-positive patients were diagnosed with ovarian mucinous cystadenoma (two out of 33, 6%) (Table 1 and Figure 1). One of the changes was a novel missense substitution 691G>A (Ala231Thr) and the other was a previously reported three-base pair insertion at codon 435 (435insAAA). Unfortunately, ovarian tumor tissues of mutation carriers were not available, and thus, possible biallelic inactivation of *FH* gene in the tumors could not be examined.

The patient with a missense mutation Ala231Thr was diagnosed with mucinous cystadenoma of the left ovary and cysta endometrialis of the right ovary at the age of 25 years. The patient had no features of HLRCC. The skin was evaluated for cutaneous leiomyomas by a dermatologist, and abdominal ultrasound showed normal kidneys. There was no evidence of family history of HLRCC, but one case of breast cancer and one case of leukemia were observed in her second-degree relatives. The absence of HLRCC phenotype might be due to the patient's relatively young age. The risk of skin and uterine leiomyomas in *FH* mutation carriers have been proposed to be 28 and 9% at the age of 25 years, respectively. Moreover, the mean age at diagnosis of uterine leiomyomas has been estimated to be 31 years (median, 30 years; range, 19–47 years).[3]

The 435insAAA carrier was diagnosed with mucinous cystadenoma at the age of 49 years. The patient was also diagnosed with small multiple uterine myomas, but no skin lesions, evaluated by a dermatologist, were observed. Abdominal ultrasound revealed no kidney lesions. Her father was also found to be a mutation carrier but without HLRCC phenotype. A family history of cancer was also negative in this family. However, her *FH* mutation negative mother and her first-degree relative were diagnosed with ovarian cancer. 435insAAA mutation has been previously reported in four different FH deficiency families.[12,13] Three other missense mutations, K187R, R190C and R190H, have been previously detected in both tumor predisposition and FH deficiency families, and also, phenotypic similarities have been observed between heterozygous parents of FH deficiency patients and HLRCC patients.[2]

After detecting germline mutations in patients with ovarian mucinous cystadenoma, we investigated the role of *FH* somatic mutations in a set of ovarian mucinous cystadenocarcinomas. No mutations were found in 13 samples. In addition, as bladder carcinoma has recently been linked to *FH* mutation-positive families and this tumor type has not been previously analyzed, we studied 48 bladder carcinomas for *FH* mutations with negative results.[3,9]

Taken together, the present study revealed for the first time that germline *FH* mutations might predispose to benign ovarian lesions. Recently, also other benign tumors including atypical uterine leiomyomas, kidney cysts, and adrenal gland adenomas have been reported in mutation carriers.[9] Further studies and increased clinical awareness should clarify which tumor types are indeed causally associated with FH germline mutations.

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