

## SHORT REPORT

# No germline *FH* mutations in familial breast cancer patients

Maija Kiuru<sup>1,4</sup>, Rainer Lehtonen<sup>1,4</sup>, Hannaleena Eerola<sup>2,3</sup>, Kristiina Aittomäki<sup>1</sup>, Carl Blomqvist<sup>3</sup>, Heli Nevanlinna<sup>2</sup>, Lauri A Aaltonen<sup>1</sup> and Virpi Launonen<sup>\*1</sup>

<sup>1</sup>Department of Medical Genetics, Biomedicum Helsinki, University of Helsinki, P.O. Box 63 (Haartmaninkatu 8), FIN-00014 Helsinki, Helsinki, Finland; <sup>2</sup>Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Haartmaninkatu 8, FIN-00029 HUS, Helsinki, Finland; <sup>3</sup>Department of Oncology, Helsinki University Central Hospital, Haartmaninkatu 4, FIN-00029 HUS, Helsinki, Finland

*Fumarate hydratase (FH)* was recently identified as the predisposing gene for a tumor predisposition syndrome, hereditary leiomyomatosis and renal cell cancer (HLRCC) (MIM 605839). In HLRCC, individuals with a germline heterozygous mutation in the *FH* gene typically develop benign leiomyomas of the skin and the uterus (fibroids, myomas). In a subset of the families, predisposition to renal cell carcinoma and uterine leiomyosarcoma occurs. Other malignancies including breast cancer have also been detected in patients with a germline *FH* mutation. To examine whether *FH* could be involved in predisposition to breast cancer, we analyzed germline *FH* mutations from 85 Finnish breast cancer patients. Most of the cases were selected based on positive family or personal history for malignancies associated with HLRCC. No mutations were found. These results show that *FH* is not a major predisposing gene for familial breast cancer.

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## Introduction

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant tumor susceptibility syndrome caused by heterozygous germline mutations in the *FH* gene.<sup>1,2</sup> HLRCC is characterized by benign leiomyomas of the skin and the uterus, uterine leiomyosarcoma and renal cell cancer (RCC).<sup>1,3–6</sup> At present, there are over 100 HLRCC families reported mainly in Europe and North America.<sup>1,4–9</sup>

Benign tumors, leiomyomas of the skin and the uterus are the most common lesions in HLRCC families. In the

Finnish families, these tumors have been detected in six out of seven families (86%). RCC has been detected more frequently in the mutation-positive families in Finland (five out of seven families, 71%) than in North America (five out of 35 families, 14%) or in the UK (one out of 35 families, 3%). The five Finnish HLRCC families include altogether 12 RCC cases. Uterine leiomyosarcoma has been detected in five patients in three Finnish HLRCC families (43%). Altogether three Finnish HLRCC patients carrying *FH* mutation in two families have been diagnosed with breast cancer. The first breast cancer detected in HLRCC patient displayed lobular histology, a histology which comprises 15% of unselected breast carcinomas. In addition, one case of prostate carcinoma, one case of multiple myeloma, and one case of Hodgkin's lymphoma have been detected.<sup>1,10</sup> In HLRCC families from other countries, in addition to RCC, only leiomyosarcoma of the skin has been reported in one North American patient.<sup>6</sup>

\*Correspondence: Dr V Launonen, Department of Medical Genetics, Biomedicum Helsinki, University of Helsinki, P.O. Box 63 (Haartmaninkatu 8), FIN-00014 Helsinki, Helsinki, Finland. Tel: +358 9 1911; FAX: +358 9 19125105; E-mail: virpi.launonen@helsinki.fi

<sup>4</sup>These authors contributed equally to this work.

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Recently, germline *FH* mutations were examined in prostate cancer families in two different studies.<sup>11,12</sup> In addition to prostate cancer being detected in one of the *FH* mutation carriers, positive linkage in prostate cancer families has previously been reported to 1q42–q43, where the *FH* gene is localized.<sup>13</sup> Negative results of these studies provided evidence that mutations in *FH* do not confer susceptibility to familial prostate cancer.

The susceptibility genes for familial breast cancer are still largely unknown. The major known high-penetrance predisposition genes *BRCA1* and *BRCA2* account for a large majority of families with multiple cases of early-onset breast cancer and ovarian cancer, but only a small fraction of familial breast cancer without these characteristics.<sup>14,15</sup> Genetic linkage studies have suggested breast cancer loci on chromosomes 2q32, 6q25, 8p21, and 13q22.<sup>16–19</sup> So far, the putative susceptibility genes in these regions have not been identified.

In the current study, we analyzed 85 Finnish breast cancer cases for *FH* mutations, the rationale of the work being occurrence of three breast cancers in Finnish HLRCC patients carrying germline *FH* mutation. The patient material was mainly selected based on positive family or personal history for malignancies detected in *FH* mutation-positive individuals in HLRCC families.

## Materials and methods

DNA samples from 85 *BRCA1* or *BRCA2* mutation-negative breast cancer patients collected at the Helsinki University Central Hospital (Table 1) were included to this study. 75 cases were selected based on family or personal history of renal cell cancer, sarcoma, uterine cancer (including uterine sarcoma), lobular breast cancer, prostate cancer, or multiple myeloma. 26 of these families had one such malignancy, 32 families had two, 12 families had three, and five families had four or more such cancers. In addition, 10 breast cancer patients not fulfilling the above-defined cancer history were included in this study because the experiments were performed on a 96-well plate format and space allowed inclusion of these samples. These 10 patients had at least one first-degree relative affected with breast or other cancer, or were themselves affected with a second cancer. The collection of the breast cancer study material and *BRCA1* and *BRCA2* mutation analyses have been described previously.<sup>14,20–22</sup>

Mutation screening of samples was performed by denaturing high-performance liquid chromatography (DHPLC) method and direct sequencing as described by Lehtonen *et al.*<sup>23</sup> The PCR reactions, conditions, and oligonucleotide primers used were identical to the study of Kiuru *et al.*<sup>7</sup> The potential effect of intronic and silent changes to splicing was predicted by computational

**Table 1** Description of the breast cancer families

No. of families	Family type
85	Breast cancer families
75	Selected breast cancer families
26	Included (one) cancer case <sup>a</sup>
32	Included (two) cancer cases <sup>a</sup>
12	Included (three) cancer cases <sup>a</sup>
5	Included (four) cancer cases <sup>a</sup>
10	Unselected breast cancer families

<sup>a</sup>Cancer types seen in HLRCC patients: RCC, sarcoma, multiple myeloma, lobular breast, prostate, and uterine cancer.

methods using NetGene2 splice site prediction web server (<http://genome.cbs.dtu.dk/services/NetGene2/>).

## Results and discussion

The recent finding of germline mutation in the *FH* gene to cause cancer predisposition, and occurrence of breast cancer in individuals with *FH* mutation, led us to study Finnish breast carcinoma cases for germline mutations in the *FH* gene. Most (75 out of 85, 88%) of the studied cases were selected based on positive family or personal history for tumors seen in HLRCC (Table 1). Mutation analysis revealed no disease-causing changes. Three previously detected polymorphisms<sup>23</sup> (unpublished data) were found: 798 G>A (Pro266Pro) (two individuals, of which one heterozygous and one homozygous), IVS2+61T>A (one individual), and IVS3+32A>G (one individual) (Table 2). The changes were not predicted to have an effect on splicing tested *in silico* by NetGene2 program.

So far, patients with breast cancer have only been detected in the Finnish HLRCC families, accounting for 6% of mutation-positive individuals (three out of 49). Interestingly, two of these patients had also been diagnosed with uterine leiomyosarcoma. However, breast cancer being the most common cancer type in females, it is conceivable that these cases detected in the Finnish families are actually sporadic ones. The role of somatic *FH* inactivation in unselected breast cancers with lobular or other histology breast cancer has previously been evaluated in two separate studies also with negative results.<sup>7,23</sup>

In HLRCC families, RCC appears to be more common in the Finnish HLRCC families (71%) than in the mutation-positive families in the United States (14%) and in the UK (3%). Uterine leiomyosarcoma and breast cancer have only been reported in the Finnish HLRCC families.<sup>10</sup> This may partly be due to the differences in primary identification criteria of HLRCC families. The Finnish HLRCC families are mainly recruited according to the history of RCC and/or uterine leiomyosarcoma (five out of seven families), whereas the families from the United States and UK are

**Table 2** FH polymorphisms detected in breast cancer cases

Variant	Nucleotide change	Family	Cancer history Index case	Family members (no. of cases)
Exon 6	798G/A (heterozygous)	1009	Lobular breast	Breast (3), Rectum (1), lymphoma (1), lung (1), brain (1), skin (1)
Exon 6	798G/A (homozygous)	2221	Breast, sarcoma (body), skin	—
Intron 3	IVS3+61T>A	7154	Breast	Leiomyosarcoma (colon)(1), adrenal gland (1), pancreas (1), bone (1), other GI (1)
Intron 4	IVS4+32A>G	153	Breast	Breast (4), stomach (1), pancreas (1), lung (3), prostate (1), ovarian (1), eye (1), skin (1), colon (1), melanoma (1), unknown cancer (1)

collected based on benign leiomyomas. FH mutation data appear not to explain the difference in the occurrence of RCC/uterine leiomyosarcoma between different families or populations. Thus, one explanation could be a modifier locus common in the Finnish population, increasing cancer risk in these families.

In this study, we examined FH germline mutations in 85 Finnish patients with breast carcinoma, but found no disease-causing mutations. The negative result is unlikely to be due to technical reasons as the sensitivity of DHPLC has been shown to vary between 93 and 100%, consistently exceeding 96% in many data sets.<sup>24</sup> These results make it unlikely that FH is a major susceptibility gene for breast cancer.

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