Online Mutation Report

No MSH6 germline mutations in breast cancer families with colorectal and/or endometrial cancer


Background: The genetic background in breast cancer families with colorectal and/or endometrial cancer is mostly unknown. The functional connection between MSH6 and the known breast cancer predisposition gene product BRCA1 suggests that the MSH6 gene may also play a role in breast cancer predisposition.

Methods: We analysed 38 breast cancer families with colorectal and/or endometrial cancer for germline mutations in MSH6.

Results: No disease associated mutations were detected among the breast cancer families. However, mutation analysis revealed a Glu995STOP mutation in an atypical HNPCC family. The same mutation was found in a patient with both breast and colorectal carcinoma in our previous study, and haplotype analysis confirmed a common ancestral origin. The Glu995STOP mutation was further examined in an extensive series of 245 colorectal and 142 breast carcinoma patients with a family history of breast, colorectal, and/or endometrial carcinoma, and in 268 healthy population controls, but none was found to carry the mutation.

Conclusions: Our results suggest that MSH6 may not be the underlying gene in breast cancer families with a history of colorectal and/or endometrial cancer. The Glu995STOP founder mutation is not a familial breast cancer predisposition allele and makes only a limited contribution to colorectal cancer burden in Finland.

Inherited mutations in DNA mismatch repair (MMR) genes cause susceptibility to hereditary non-polypsis colorectal cancer (HNPCC), an autosomal dominant cancer predisposition syndrome characterised by early onset colorectal cancer, and frequently showing extracolonic tumours such as cancers of the endometrium, stomach, ovaries, small bowel, ureter, and renal pelvis. The vast majority of the observed germline mutations in HNPCC families are in the MLH1 (~50%) and MSH2 (~40%) genes (www.insight-group.org: International Society for Gastrointestinal Hereditary Tumours). The first germline mutation in MSH6 was found in a patient with three colorectal tumours and a weak family history of colorectal, and/or endometrial carcinoma, and in 268 healthy population controls, but none was found to carry the mutation.

In our previous study, we found a germline MSH6 mutation in a patient with both breast and colorectal carcinoma, and a similar patient has been reported by Plaschke et al. In addition, Hendriks et al have reported on an MSH6 mutation positive breast cancer patient whose tumour showed an MSI-H phenotype and no MSH6 expression. In this study, our aim was to evaluate the role of MSH6 in breast cancer predisposition by analysing 38 breast cancer families with family history of colorectal and/or endometrial cancer for germline MSH6 mutations. In addition, a previously reported putatively breast cancer associated Finnish MSH6 mutation was evaluated in an extensive set of breast and colorectal cancer cases.

Patients and Methods

Breast cancer families

In the study, 38 families originally ascertained from a group of breast cancer patients at the Departments of Oncology or Clinical Genetics, Helsinki University Central Hospital, Finland, fulfilled the following clinical criteria for this study: 
(a) at least one breast cancer case,
(b) either endometrial or colorectal carcinoma patient,
(c) additional endometrial or colorectal carcinoma patient or family history of at least one of the following tumours: stomach, small intestine, bile duct, pancreatic, ovarian, kidney, or ureter carcinoma.
Breast and colorectal carcinoma patients with family history of cancer

The observed MSH6 founder mutation was analysed in 142 breast carcinoma patients who themselves or whose first degree relative had colorectal (n = 70) or endometrial (n = 81) carcinoma, and 245 colorectal carcinoma patients who themselves or whose first degree relative had additional colorectal carcinoma (n = 143), breast (n = 86), or endometrial (n = 23) carcinoma. The 142 breast cancer patients belong to the cohorts of 889 unselected (also unpublished data) and 710 familial breast cancer cases described.22 Briefly, genomic DNA was amplified by PCR, with one of the primers being end labelled with γ-32P-dATP (PerkinElmer Life Sciences, Boston, MA, USA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA, USA). PCR products were then denatured for 10 minutes at 95°C, and heteroduplexes were allowed to form by letting the samples slowly cool to room temperature. Samples were run on mildly denaturing CSGE gels (10% acrylamide, 10% ethylene glycol, 15% formamide) either at 35 W for 3–4 hours or at 3 W overnight. All samples with an abnormal band shift were reamplified and sequenced by direct sequencing using BigDye Terminator Cycle Sequencing kit (version 3.1) and ABI 310 automated sequencer (Applied Biosystems, Foster City, CA, USA).

RESULTS AND DISCUSSION

Identification of mismatches that have occurred during replication or recombination of homologous but non-identical DNA sequences is accomplished by two heterodimeric complexes, MSH3 and MSH7. The former, comprising of MSH2 and MSH6, is primarily responsible for the recognition of single nucleotide mismatches and small insertion/deletion loops,37 whereas the latter, with MSH2 and MSH7, preferentially recognises insertion/deletion loops.38 The partial functional redundancy of the two complexes may explain both the rarity of germline MSH6 mutations in HNPCC and the atypical HNPCC phenotype common to families with such mutations. Interestingly, in contrast to MSH6, where several disease causing germline mutations have been identified in HNPCC and atypical HNPCC, such mutations are thus far lacking for MSH3.

The role of germline mutations in MMR genes in breast cancer predisposition has remained controversial, though major contribution appears unlikely. Results from epidemiological studies suggest that breast tumours are not associated with HNPCC syndrome,1 12 14 although Scott et al reported a 15 fold excess in lifetime breast cancer risk among MLH1 mutation carriers. As the aforementioned studies have mostly concentrated on classic HNPCC families with either MLH1, MSH2, or unknown mutation status, it may be that the effect of MSH6 has been overlooked as germline mutations in MSH6 predispose mainly to atypical HNPCC. We27 and others39 have also reported on MSH6 mutation carriers with both breast and colorectal carcinoma. Colorectal cancer is a common feature in breast cancer families, and 16% of Finnish breast cancer families include also colorectal cancer patients.40 The underlying predisposition gene in such families is unknown. The aim of this study was to formally analyse the role of MSH6 in breast cancer families who also have endometrial and/or colorectal cancer patients.

Altogether, eight germline alterations in the coding and seven in the non-coding region were observed in the 38 families (table 2). Six of the coding variants were silent substitutions, five of which have been reported as polymorphisms (www.insight-group.org; International Society for Gastrointestinal Hereditary Tumours). In addition, both

<table>
<thead>
<tr>
<th>Table 1 Family history of breast, colorectal, and endometrial cancer among the families studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breast, colorectal, and endometrial cancer patients in the families</strong></td>
</tr>
<tr>
<td><strong>No. of families</strong></td>
</tr>
<tr>
<td>≥3 breast cancer patients</td>
</tr>
<tr>
<td>with at least one colorectal cancer patient in a family</td>
</tr>
<tr>
<td>with at least one endometrial cancer patient in a family</td>
</tr>
<tr>
<td>with at least one colorectal and one endometrial cancer patient in a family</td>
</tr>
<tr>
<td>2 breast cancer patients</td>
</tr>
<tr>
<td>with at least one colorectal cancer patient in a family</td>
</tr>
<tr>
<td>with at least one endometrial cancer patient in a family</td>
</tr>
<tr>
<td>with at least one colorectal and one endometrial cancer patient in a family</td>
</tr>
<tr>
<td>1 breast cancer patient</td>
</tr>
<tr>
<td>with at least one colorectal and one endometrial cancer patient in a family</td>
</tr>
<tr>
<td>with several primary tumours (at least breast and colorectal/endometrial)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
MSH6 and breast cancer predisposition

Leu396Val gene is able to complement the mismatch repair previously classified as polymorphisms, and functional missense changes (Gly39Glu and Leu396Val) have been colorectal carcinoma. Glu995STOP is a protein truncating our previous study in a patient with both breast and revealed the same Glu995STOP mutation that was seen in an additional family in which the colorectal tumour showed suggests that MSH6 sample size in the mutation screening is quite small. Despite not look for large genomic rearrangements. In addition, the method is known to be less than 100% sensitive and we did not have functional significance. It is possible that some variants observed in this study were common and located history of colorectal and/or endometrial cancer.

rarely, the cause of breast cancer in families with family MSH6 – CHEK2 – A

A Co ca 34 y Br ca 34 y Ve ca 34 y

B Vi ca 34 y Co ca 34 y

Table 2 Germline MSH6 alterations in breast cancer families

<table>
<thead>
<tr>
<th>Location</th>
<th>Nucleotide change</th>
<th>Effect on protein</th>
<th>Families with the variant</th>
<th>Variant previously described (reference no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>116G→A</td>
<td>Gly39Glu</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Exon 1</td>
<td>186C→A</td>
<td>Arg62Lys</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>276A→G</td>
<td>Pro93Ala</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>540G→T</td>
<td>Asp180Asp</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Intron 3</td>
<td>628-56C→T</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Exon 4</td>
<td>642C→T</td>
<td>Tyr214Phe</td>
<td>8*</td>
<td></td>
</tr>
<tr>
<td>Exon 4</td>
<td>1186C→G</td>
<td>Leu396Val</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Exon 4</td>
<td>1875C→T</td>
<td>Ser625Ser</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Exon 5</td>
<td>330T→A</td>
<td>Thr1102Thr</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Intron 5</td>
<td>3438+1A→T</td>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Intron 6</td>
<td>3557-18del/T/ins1T*</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Intron 7</td>
<td>3646-29delCTAT</td>
<td></td>
<td>35*</td>
<td></td>
</tr>
<tr>
<td>Intron 7</td>
<td>3646-29delCTAT</td>
<td></td>
<td>35*</td>
<td></td>
</tr>
<tr>
<td>Intron 9</td>
<td>4002-27delT</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3′UTR</td>
<td>4083+49G→A</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Also homozygotes for the rare allele; †microsatellite with 12-14xT (12/13, n=25; 13/14, n=13); ‡microsatellite with 17-18xT, all samples heterozygotes (17/18).

In the other MSH6 positive family (5130), a CHEK2 1100delC mutation has also been identified. Both mutations were found in the index patient diagnosed with both breast and colorectal carcinoma at the age of 34 years, and in her mother, who had been diagnosed with benign meningioma. As the CHEK2 1100delC is now known to act as a low penetrance breast cancer allele and MSH6 is known to predispose to colorectal cancer, it appears likely that the breast carcinoma from the index patient was caused by the CHEK2 mutation and the colorectal tumour by the MSH6 mutation deleting 365 amino acids from the C terminal end of the protein, including domains that are conserved in many MutS homologues and participate in, for example, DNA binding, ATPase activity, and protein dimerisation (www.ensembl.org/homo_sapiens/). The mutation positive families that are not known to be related originate from the same geographical region of Southern Finland, and haplotype analysis confirmed the common ancestral origin of the mutation (fig 1). Both families show atypical HNPCC, with predominance of endometrial carcinoma. Both families do, however, fulfil the revised Bethesda Guidelines for HNPCC: family 5130 with the presence of a colorectal cancer patient diagnosed before 50 years of age, and family 2342 with the presence of both colorectal and endometrial carcinoma in the same patient.

Recurrent founder mutations provide another approach to evaluate the presence of susceptibility gene mutations in more extensive sets of patients and families, and have been useful in cancer susceptibility gene studies in Finland. To further analyse the importance of this particular Glu995STOP mutation among Finnish breast and colorectal carcinoma patients with family history of cancer, we studied its frequency in 245 colorectal cancer patients who themselves or whose first degree relative had been diagnosed with additional colorectal carcinoma or breast or endometrial carcinoma, and in 142 breast cancer patients who themselves or whose first degree relative had colorectal or endometrial carcinoma. Of these 387 patients, 72 (18.6%; 26 colorectal and 46 breast cancer patients) were from the same geographical region as the mutation carriers. None was found to carry the mutation. The mutation was also absent in 268 anonymous Finnish blood donors. This suggests that the Glu995STOP is a new Finnish MSH6 founder mutation that may have some relevance in a geographically restricted area, but its contribution to colorectal cancer burden is limited. The results also indicate that the Glu995STOP is not a familial breast cancer predisposition allele.

Figure 1 Pedigrees of the families with the germline MSH6 Glu995STOP mutation: (A) 5130, (B) 2342. Probands are indicated by an arrow. Tumour site(s) and age at diagnosis are indicated: Br, breast; Co, colon; En, endometrium; Ki, kidney; Men, meningioma; Pr, prostate; Ve, ventricle. *Diagnosis could not be verified from medical documents; the age presented is the age at death. Haplotypes are shown beneath the individuals, with the shadowed haplotype referring to a shared disease haplotype.

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mutation. This is further supported by the MSH6 immuno-

histochemical staining and MSI analyses on the colorectal

and breast tumours: while the colorectal tumour shows no

MSH6 expression and exhibits MSI-H, the breast tumour is

MSS and shows only slightly reduced MSH6 expression as

compared with adjacent normal tissue. The index patients’

sister was diagnosed with breast cancer at the age of

38 years, but she was not found to carry either of the

mutations and her tumour is most likely sporadic.

Alternatively, there may also be a currently unknown genetic

factor or an undetected BRCA1/2 mutation underlying the

early onset breast cancer cases in this family. In another

MSH6 mutation carrier affected with both colorectal and

breast carcinoma, the breast tumour did not exhibit MSI or

loss of MSH6 expression, and it was suggested that also that

breast tumour is most likely not due to mismatch repair

deficiency.40 Despite the MSH6 positive breast cancer patient

whose tumour exhibits MSI-H and loss of MSH6 expression,

in most published cases the mutation status of the breast

cancer patient is unknown or the patient with breast cancer

has not been a mutation carrier.5 11 12 48 49 Therefore, many

breast cancer cases among MSH6 families may be sporadic or
due to other breast cancer predisposition alleles.

Both breast and colorectal carcinomas are common

tumours that often occur together in families. This
coexistence may be due to chance clustering of two common
cancers, but there may also be genetic factors that predispose
to both cancer types.52 Mutations in LKB1 that predispose to

Peutz-Jeghers syndrome have been associated with an

increased risk for both breast and colorectal cancer.11

Elevated colorectal cancer risk has also been reported for

BRCA1 and BRCA2 mutation carriers, although the results

have been inconsistent.18–24 In addition, the studies on

whether the breast cancer risk is increased among HNPCC

families have given contradictory results.18–20 Altogether,

germine mutations in all of these genes may explain only a

small fraction of families with both of these tumour types.

The presence of families with a strong family history of both

breast and colorectal carcinomas and no mutations in the

known predisposition genes have led to the suggestion that

there may be a novel gene(s) associated, when mutated, to an

increased risk for both cancer types.50 Recently, the

CHEK2 1100delC mutation was found to be associated with families

with both breast and colorectal cancer, and was suggested to

underlie the hereditary breast and colorectal cancer syn-
drome HBOC.51 However, the observation was not confirmed in

a study by Kilkivaa et al.47 Furthermore, no statistically

significant increase in the 1100delC mutation frequency has

been observed either in familial or in sporadic colorectal

cancer,54 or in patients with multiple adenomas,55 suggest-
ing that its effect on colorectal cancer is very low or non-
existent.

The absence of germline MSH6 mutations in our set of

breast cancer families and patients studied here do not

exclude the possibility that MSH6 mutations may be

associated with an increased breast cancer risk in the context of

MSH6 mutation carrier HNPCC families. However, our

results suggest that MSH6 is not a breast cancer predispo-
sition gene that would manifest itself in breast cancer patients

and families with the family history of colorectal and/or

endometrial cancer. Other breast/colorectal cancer suscept-
bility genes are likely to be the cause in such families.

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