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-polyposis 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 gastrointestinal tumours, indicating atypical HNPCC. Further reports on families with germline MSH6 mutations have also shown different clinical features compared with traditional HNPCC phenotype, such as later age of cancer diagnosis, lower penetrance, and predominance of endometrial carcinomas. In a recent study by Hendriks et al, the cumulative risks at 70 years for MLH1, MSH2, and MSH6 mutation carriers were similar for all the HNPCC related tumours combined. For women, colorectal cancer risk was significantly lower and endometrial cancer risk significantly higher in MSH6 carriers compared with MLH1 and MSH2 carriers. In contrast to MLH1 and MSH2 mutation carriers, whose colorectal and endometrial tumours very often exhibit high microsatellite instability (MSI-H), tumours from MSH6 carriers more frequently show a lower degree of MSI (MSI-L) or are microsatellite stable (MSS). In breast cancer, MSI has been observed in some sporadic and familial breast tumours and in a few breast tumours of known HNPCC patients. The genetic background in breast cancer families with colorectal and/or endometrial cancer is mostly unknown. Studies on breast cancer risk in HNPCC have also given conflicting results. For example, a statistically significant over-representation of breast cancer was observed in MLH1 mutation positive families and in families with no detectable MLH1 or MSH2 mutations, but the finding was not confirmed in another study. The aforementioned studies have mainly focused on classic HNPCC families, and thus the possible effect of MSH6 may have been overlooked. The MSH6 protein, along with MLH1 and MSH2, is part of the so called BRCA1 associated genome surveillance complex (BASC), which is suggested to serve as a sensor for DNA damage. This large protein complex is comprised of BRCA1 associated DNA repair proteins, all of which possess the ability to bind abnormal DNA structures. Protein–protein interaction between BRCA1, the protein product of a known breast cancer predisposing gene, and the proteins associated with it may indicate that they function in the same DNA damage response pathway. Reduced MSH6 mRNA expression has also been observed in breast tumour derived cell lines. 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, and (b) either endometrial or colorectal carcinoma patient, and (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,
adenocarcinoma of the cervix, or malignant brain tumour. Families were classified according to the presence of breast, colorectal, and endometrial cancer cases in the family (table 1). One family fulfilled the Amsterdam criteria, and 10 families the Revised Bethesda Guidelines for HNPCC.29 30 No BRCA1 or BRCA2 mutations were detected in any of the families, and the presence of the three MLH1 founder mutations that account for the large majority of all MMR mutations in Finland were excluded. For each family, the index patient and, when available, relatives with endometrial or colorectal carcinoma were analysed for germline MSH6 mutations, with the total of 42 samples in the analysis.

**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 unselected31 (also unpublished data). Three of the 245 families fulfilled the Amsterdam criteria, while 15 families fulfilled the Revised Bethesda Guidelines for HNPCC.29 30 Colorectal cancer patient samples were selected from a population based series of 1042 consecutive colorectal cancer cases previously described.15 36

MSI was determined using a polyA marker BAT26, a robust marker for high level MSI detection; 24 of the 245 samples were MSI positive, and 14/245 were shown to harbour a marker for high level MSI detection; 24 of the 245 samples were MSI positive, and 14/245 were shown to harbour a germline mutation in either MLH1 or MSH235 36 (also unpublished data). Three of the 245 families fulfilled the Amsterdam criteria, and 83 families fulfilled the Revised Bethesda Guidelines for HNPCC.29 30 To evaluate the frequency of the observed MSH6 mutation in population controls, blood samples from 268 healthy anonymous blood donors were studied.

All specimens were collected and analysed with informed consent and under protocols approved by ethics committees of the Departments of Obstetrics and Gynecology, and Oncology, Helsinki University Central Hospital, Finland, and the Ministry of Social Affairs and Health in Finland.

### Haplotype analysis

Haplotype analysis was performed in order to evaluate the possible common ancestry of the observed MSH6 mutation. The polymorphic microsatellite markers used were: D2S119, D2S2298, D2S2174, D2S2240, and D2S2378. The analysis was done by end labelling one of the primers with γ-33P-dATP (PerkinElmer Life Sciences) and separating the denatured PCR products in a mildly denaturing 10% polyacrylamide gel.

### 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,27–29 whereas the latter, with MSH2 and MSH3, preferentially recognises insertion/deletion loops.30–32 The partial functional redundancy of the two complexes may explain both the rarity of germline MSH6 mutations in HNPPC and the atypical HNPPC phenotype common to families with such mutations. Interestingly, in contrast to MSH6, where several disease causing germline mutations have been identified in HNPPC and atypical HNPPC, 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 from appears unlikely. Results from epidemiological studies suggest that breast tumours are not associated with HNPPC syndrome.1 22 24 although Scott et al23 reported a 15 fold excess in lifetime breast cancer risk among MLH1 mutation carriers. As the aforementioned studies have mostly concentrated on classic HNPPC 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 HNPPC. We27 and others30 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.41 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 1](https://www.jmedgenet.com)

<table>
<thead>
<tr>
<th>Family history of breast, colorectal, and endometrial cancer among the families studied</th>
<th>No. of families</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥3 breast cancer patients</td>
<td>28</td>
</tr>
<tr>
<td>with at least one colorectal cancer patient in a family</td>
<td>16</td>
</tr>
<tr>
<td>with at least one endometrial cancer patient in a family</td>
<td>6</td>
</tr>
<tr>
<td>with at least one colorectal and one endometrial cancer patient in a family</td>
<td>6</td>
</tr>
<tr>
<td>2 breast cancer patients</td>
<td>7</td>
</tr>
<tr>
<td>with at least one colorectal cancer patient in a family</td>
<td>4</td>
</tr>
<tr>
<td>with at least one endometrial cancer patient in a family</td>
<td>2</td>
</tr>
<tr>
<td>with at least one colorectal and one endometrial cancer patient in a family</td>
<td>1</td>
</tr>
<tr>
<td>1 breast cancer patient</td>
<td>3</td>
</tr>
<tr>
<td>with at least one colorectal and one endometrial cancer patient in a family</td>
<td>2</td>
</tr>
<tr>
<td>with several primary tumours (at least breast and colorectal/endometrial)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
</tr>
</tbody>
</table>
missense changes (Gly39Glu and Leu396Val) have been previously classified as polymorphisms, and functional analysis of the latter has shown that a plasmid with the Leu396Val gene is able to complement the mismatch repair defect of an msh3 msh6 double mutant strain.4 Intronic variants observed in this study were common and located outside the conserved splice sites, suggesting that they may not have functional significance. It is possible that some mutations may have been missed in this study, as the CSGE method is known to be less than 100% sensitive and we did not look for large genomic rearrangements. In addition, the sample size in the mutation screening is quite small. Despite these limitations, the absence of disease associated mutations suggests that MSH6 germline mutations are not, or only rarely, the cause of breast cancer in families with family history of colorectal and/or endometrial cancer.

In the course of this study, a complete mutation analysis of an additional family in which the colorectal tumour showed no MSH6 expression in immunohistochemical analysis revealed the same Glu995STOP mutation that was seen in our previous study in a patient with both breast and colorectal carcinoma.25 Glu995STOP is a protein truncating 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 dimerisation44 45 (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, fulfill the revised Bethesda Guidelines for HNPCC32; 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.11 15 16 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.

In the other MSH6 positive family (5130), a CHEK2 1100delC mutation has also been identified.27 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 allele46 47 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

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**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>vs vs vs</td>
</tr>
<tr>
<td>Exon 1</td>
<td>186C→A</td>
<td>Arg62Arg</td>
<td>15</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Exon 2</td>
<td>276A→G</td>
<td>Pro92Pro</td>
<td>9</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Exon 3</td>
<td>540T→C</td>
<td>Asp180Asp</td>
<td>15</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Intron 3</td>
<td>628-56C→T</td>
<td></td>
<td>9</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Exon 4</td>
<td>642C→T</td>
<td>Tyr214Tyr</td>
<td>8</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Exon 4</td>
<td>1186C→G</td>
<td>Leu396Val</td>
<td>2</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Exon 5</td>
<td>1300T→A</td>
<td>Thr1102Thr</td>
<td>1</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Intron 5</td>
<td>3438+14A→T</td>
<td></td>
<td>20</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Intron 6</td>
<td>3557-18delT/insT</td>
<td></td>
<td>1</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Intron 7</td>
<td>3646-29delCTAT</td>
<td></td>
<td>35*</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Intron 7</td>
<td>3647-54delT</td>
<td></td>
<td>9*</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>Intron 9</td>
<td>4002-27delT</td>
<td></td>
<td>3</td>
<td>vs vs vs</td>
</tr>
<tr>
<td>3′UTR</td>
<td>4083+49G→C</td>
<td></td>
<td>1</td>
<td>vs vs vs</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).
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 cancer, 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.29 Despite the MSH6 positive breast cancer patient
whose tumour exhibits MSI-H and loss of MSH6 expression,30
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 co-
extistence may be due to chance clustering of two common
cancers, but there may also be genetic factors that predispose
to both cancer types.50 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.12–51 In addition, the studies on
whether the breast cancer risk is increased among HNPCC
families have given contradictory results.18–22 24 Altogether,
germline 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.52 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.53 However, the observation was not confirmed in
a study by Klipivaa et al.44 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 predisposi-
tion 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-
ibility genes are likely to be the cause in such families.

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