Letter to the Editor

Frequency and nature of hMSH6 germline mutations in Polish patients with colorectal, endometrial and ovarian cancers

To the Editor:

It is estimated, that somewhere between 5% and 10% of human cancers are a result of a constitutional mutation in a single, highly penetrant gene (1). According to the literature, hMSH6 mutation carriers are affected most frequently by cancers of the colorectum, endometrium or ovaries and hMSH6 families often do not fulfill the HNPCC diagnostic criteria (2,3). Currently, there is no information about hMSH6 mutations in the Polish population, except for one reported alteration identified in a previous study (4).

Three cancer types were investigated: colorectal cancers – CRC(489 cases), endometrial cancers – EC(153 cases) and ovarian cancers – OC(179 cases). Each of group was further stratified using the following criteria:

(1) Patients with at least one first-degree relative affected by CRC, EC or OC(83 CRC, 23 EC, 4 OC); in this group MSH2/MLH1 constitutional mutations were excluded by DHPLC/sequencing in cases matching criteria of suspected HNPCC (5).

(2) Patients without a first-degree relative affected by CRC, EC or OC(156 CRC, 30 EC, 47 OC).

(3) Twenty-eight unselected OC of endometrioid type; this group was created, because in initial phase of our studies MSH6 mutation was identified in proband with such rare subtype of OC (4).

(4) Unselected cases analyzed to estimate the frequency of sequence variants detected in the three previous groups(250 CRC, 100 EC, 100 OC).

DNA from 300 anonymous newborns was analyzed to differentiate between mutations and polymorphisms.

Material was obtained from the outpatient clinics of the Hereditary Cancer Center in Szczecin, the University Hospitals (SPSK2 and SPSK1) of Szczecin and the Regional Oncology Hospital of Szczecin.

Material (paraffin-embedded tumor tissue and/or genomic DNA) was analyzed by using immunohistochemistry (IHC), DNA sequencing, MLPA, DHPLC and ASA-PCR methods.

The detected variants/mutations and tumor spectrum among relatives of the patients are summarized in Table 1. In families with aggregation of CRC, EC and/or OC (group 1) five new variants were detected among 110 (4.5%) analyzed cases. In families without above aggregations (group 2) two new variants were found among 233 cases (0.8%). One of them was detected in a proband with CRC diagnosed at age 43. The second variant was found in woman with EC diagnosed at age 75 and bilateral breast cancers at ages 57 and 73. Among OC of endometrioid type (group 3) only one, previously reported missense change was identified (15).

None of changes was found among series of unselected CRC, EC or OC (group 4) or in the control group.

In the present study, eight different variants/mutations were detected including seven changes that have not been reported previously. All of them appear to be pathogenic (Table 2).

According to the literature, in families with aggregations of CRC, EC and OC the frequency of hMSH6 mutations is somewhere between 1% and 4.6% (6–11). We obtained similar results where five mutations were found among 110 families(4.5%). The three remaining families with hMSH6 alterations did not display such a phenotype: two probands were affected by multiple primary tumors and one by early onset CRC, all without familial CRC, EC or OC aggregations.
In the present study we analyzed 11 patients with multiple primary cancers, with the resulting frequency of detected mutations being 18% (2/11). The literature data reported on correlations between multiple primary tumors and hMSH6 abnormalities are not consistent. No mutations were detected in a group of 20 cases with primary CRC and EC without CRC, EC and OC among their relatives (12), but in the group of 78 patients affected by CRC and EC, the frequency of hMSH6 mutations was 7.7%, although the high frequency of changes in hMSH6 was the result of a founder effect (13).

The third hMSH6 mutation phenotype, which was identified, is a sporadic, early onset (before age of 50) CRC. From 26 such cases analyzed, one hMSH6 mutation was found, resulting in a frequency of 3.8%. This frequency is similar to the frequency of hMSH6 mutations identified in other reports where single hMSH6 mutations carriers were found in a series of 83 and 149 of early onset sporadic CRCs, respectively (3,14).

We were not able to show that endometrioid subtype is a characteristic feature of hMSH6-based OC.

In summary, the hMSH6 gene should be investigated for the occurrence of germline abnormalities in probands affected by CRC, EC or OC who have a family history of these tumors. Additionally, hMSH6 examination should be considered for probands with multiple primary tumors (one of them is CRC, EC or OC), or probands with sporadic, early-onset CRC.

Table 1. hMSH6 germline alterations and pedigree data on the families with detected abnormalities

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>Position of sequence alteration</th>
<th>Consequence</th>
<th>Tumor spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.456 + 2 T &gt; A</td>
<td>r.spl?</td>
<td>col39</td>
</tr>
<tr>
<td>1</td>
<td>c.1676 G &gt; A</td>
<td>p.Cys559Tyr</td>
<td>EC68</td>
</tr>
<tr>
<td>2</td>
<td>c.1857 A &gt; C</td>
<td>p.Glu619Asp</td>
<td>br57/73, EC75</td>
</tr>
<tr>
<td>1</td>
<td>c.2339 C &gt; G</td>
<td>p.Ala780Gly</td>
<td>EC47</td>
</tr>
<tr>
<td>3</td>
<td>c.2561 A &gt; T</td>
<td>p.Lys854Met</td>
<td>mm44, ov49E</td>
</tr>
<tr>
<td>1</td>
<td>c.2823-2825 delTGC</td>
<td>p.Ala942del</td>
<td>col43</td>
</tr>
<tr>
<td>1</td>
<td>c.3311-3312 delTT</td>
<td>p.Phe1104fs</td>
<td>ov49E</td>
</tr>
</tbody>
</table>

EC, endometrial cancer; ovE, ovarian cancer of endometrioid type; col, colon; br, breast; mm, melanoma malignum; cx, cervix; lu, lung; leu, leukemia; liv, liver; gal.bl, gall bladder; ic, intra-abdominal cancer; rec, rectum; fgt, female genital tract; sb, small bowel; csu, cancer site unknown; st, stomach; F, father; M, mother; B, brother; S, sister; D, daughter.

Confirmed variant/mutation carriers are bold.

Table 2. Characteristics of hMSH6 alterations

<table>
<thead>
<tr>
<th>Mutation/variant</th>
<th>Cosegregation with disease</th>
<th>Presence in control group</th>
<th>Location in functional domain</th>
<th>Change polarity</th>
<th>Conserved aminoacidsa</th>
<th>Scores with ESEfinder (SF2,ASF/SC35/SRp40/SRp55)b</th>
<th>Tumor spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>r.spl?</td>
<td>Yes</td>
<td>NE</td>
<td>–/+</td>
<td>Yes</td>
<td>Yes</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>p.Cys559Tyr</td>
<td>Yes</td>
<td>No</td>
<td>NE</td>
<td>No</td>
<td>No</td>
<td>–/+</td>
<td></td>
</tr>
<tr>
<td>p.Glu619Asp</td>
<td>NE</td>
<td>No</td>
<td>NE</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td></td>
</tr>
<tr>
<td>p.Ala780Gly</td>
<td>NE</td>
<td>No</td>
<td>NE</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td></td>
</tr>
<tr>
<td>p.Lys854Metc</td>
<td>Yes</td>
<td>No</td>
<td>+/+</td>
<td>No</td>
<td>Yes</td>
<td>y, mMSH6</td>
<td></td>
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<tr>
<td>p.Ala942del</td>
<td>Yes</td>
<td>No</td>
<td>–/+</td>
<td>No</td>
<td>Yes</td>
<td>TAQ, mMSH6</td>
<td></td>
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<tr>
<td>p.Tyr969Cys</td>
<td>Yes</td>
<td>No</td>
<td>–/+</td>
<td>No</td>
<td>Yes</td>
<td>y, mMSH6</td>
<td></td>
</tr>
<tr>
<td>p.Phe1104fs</td>
<td>Yes</td>
<td>NE</td>
<td>–/+</td>
<td>Yes</td>
<td>–/+</td>
<td>–/+</td>
<td></td>
</tr>
</tbody>
</table>

NE, not examined; (+), normal protein expression; (−), lack of protein expression; TAQ, Thermophilus aquaticus; mMSH6, MSH6 from mouse; yMSH6, MSH6 from yeast; WT, wild type sequence; MT, mutated sequence.

aObmolova et al. (2000) and BLAST.
bhttp://rulai.cshl.edu/tools/ESE.
cDescribed both as a possible missense mutation and a polymorphism (6, 15).

In the present study we analyzed 11 patients with multiple primary cancers, with the resulting frequency of detected mutations being 18% (2/11). The literature data reported on correlations between multiple primary tumors and hMSH6 abnormalities are not consistent. No mutations were detected in a group of 20 cases with primary CRC and EC without CRC, EC and OC among their relatives (12), but in the group of 78 patients affected by CRC and EC, the frequency of hMSH6 mutations was 7.7%, although the high frequency of changes in hMSH6 was the result of a founder effect (13).

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J Suchy1
G Kurzawski1
K Jakubowska2
ME Rac2
K Safranow2
J Kladny3
I Rzepka-Górskal
M Chosia5
B Czeszyńska6
O Oszurek1
RJ Scott7
J Lubiński1

1International Hereditary Cancer, Cancer-Department of Genetics and Pathology
2Department of Biochemistry and Chemistry
3Department of General and Oncological Surgery
4Department of Surgical Gynecology and Oncology of Adults and Children
5Department of Pathology
6Clinic of Neonatology, Pomeranian Medical University, Szczecin, Poland
7Discipline of Medical Genetics, Faculty of Health, University of Newcastle and the Hunter Medical Research Institute, Newcastle, NSW Australia.

References


Correspondence:
Janina Suchy
Department of Genetics and Pathology
Pomeranian Medical University
ul Polabska 4, Szczecin 70-115
Poland
Tel./fax: 0-48-91-4661533
e-mail: janusch@interia.pl