Short Report

Pitfalls in molecular analysis for mismatch repair deficiency in a family with biallelic pms2 germline mutations

Heterozygous germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2 cause Lynch syndrome. Biallelic mutations in the MMR genes are associated with a childhood cancer syndrome [constitutional mismatch repair-deficiency (CMMR-D)]. This is predominantly characterized by hematological malignancies and tumors of the bowel and brain, often associated with signs of neurofibromatosis type 1 (NF1). Diagnostic strategies for selection of patients for MMR gene analysis include analysis of microsatellite instability (MSI) and immunohistochemical (IHC) analysis of MMR proteins in tumor tissue. We report the clinical characterization and molecular analyses of tumor specimens from a family with biallelic PMS2 germline mutations. This illustrates the pitfalls of present molecular screening strategies. Tumor tissues of five family members were analyzed for MSI and IHC. MSI was observed in only one of the analyzed tissues. However, IHC analysis of brain tumor tissue of the index patient and his sister showed absence of PMS2 expression, and germline mutation analyses showed biallelic mutations in PMS2: p.Ser46Ile and p.Pro246fs. The same heterozygous mutations were confirmed in the father and mother, respectively. These data support the conclusion that in case of a clinical phenotype of CMMR-D, it is advisable to routinely combine MSI analysis with IHC analysis for the expression of MMR proteins. With inconclusive or conflicting results, germline mutation analysis of the MMR genes should be considered after thorough counselling of the patients and/or their relatives.

Conflict of interest

Nothing to declare.

Introduction

Heterozygous germline mutations in mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2 cause Lynch syndrome (1–5). Carriers of heterozygous MMR gene mutations are at high risk for developing colorectal carcinomas (CRCs) and extracolonic neoplasias such as endometrial, small bowel, ureter, renal pelvis, stomach, ovarian and brain tumors. In Lynch syndrome carriers, these malignancies usually develop during the fourth and fifth decade of life. Biallelic mutations in MMR genes lead to a childhood cancer syndrome.
Pitfalls in molecular analysis for mismatch repair deficiency

This is predominantly characterized by hematological malignancies, brain tumors and gastrointestinal tumors in early childhood. Carriers of biallelic MMR gene mutations often show signs of neurofibromatosis type 1 (NF1), mainly café au lait (CAL) spots. This childhood cancer syndrome is often referred to as constitutional mismatch repair-deficiency (CMMR-D). To our knowledge, a total of 107 cases of children with CMMR-D have been reported in the literature (6–10).

Diagnostic strategies for fast selection of patients with an MMR gene defect suspected for Lynch syndrome include analysis of MSI and immunohistochemical (IHC) analysis of tumor tissue for expression of MMR proteins (11–13). However, the sensitivity of molecular tests in tumor tissue of patients with CMMR-D is unclear. MSI and absent MMR protein staining have been described in gastrointestinal tumors of patients with CMMR-D (9). In contrast, tumor tissue of most reported CMMR-D patients with brain tumors did not show MSI (8, 14).

Here, we report a family with childhood brain tumors and early-onset colorectal cancer with biallelic germline mutations in the PMS2 gene that underscores pitfalls of the present molecular screening strategy.

Case report

Family data

At age 7, the index patient was diagnosed with an anaplastic glial brain tumor (Figure 1: pedigree, individual IV.2). His older sister (individual IV.1) had died from a primitive neuroectodermal brain tumor (PNET) at 4 years of age. Both children had multiple large CAL spots (Figure 2) and the index patient showed freckling. The younger sister of the index patient (individual IV.3) showed one CAL spot. Both non-consanguineous parents were of Dutch origin and showed no signs of neurofibromatosis type I (NF1). At the time of counseling, the family history of both parents was not suggestive of Lynch syndrome. One maternal uncle had been diagnosed with colorectal cancer (individual II.5) at age 62. The parents of the index patient declined endoscopic screening. However, within 2 years of the diagnosis of the brain tumor of our index patient, the father was diagnosed with colorectal cancer at age 43 (pT4N2M1, Dukes stage D). The paternal mother (individual II.2) was diagnosed with colorectal cancer during the same period of time at age 84. The mother of our index patient then underwent surveillance colonoscopy and one adenoma with low-grade dysplasia was removed.

Fig. 1. Pedigree of the reported family. Patient details on malignancy, adenomas and age of diagnosis in years are given. † index patient; □ male; ○ female; ♦ males and females; CRC, colorectal cancer; †† brain cancer; / deceased.
MSI and IHC analyses

Tissues of five family members including CRC tissues from the father and his mother, the colonic adenoma from the mother and brain tumor specimens from the index case and his sister were analyzed for MSI and IHC aberrations (Table 1). MSI analysis was performed on DNA retrieved from paraffin-embedded tumor tissues, using five mononucleotide repeat MSI markers (Promega pentaplex) as previously described (14, 15). As controls, normal leukocyte DNA from the index patient, DNA from paraffin-embedded normal tissue from the father (III.2), DNA from paraffin-embedded normal tissue from the grandmother (II.2), and unrelated normal DNA were used. The MSI marker profiles of all these normal DNA samples (three family members, one unrelated normal DNA) were identical, demonstrating the absence of MSI in normal tissues. IHC analysis was performed for four MMR proteins: MLH1, MSH2, MSH6 and PMS2, according to the standard procedure (15).

The brain tumor of the index patient showed an MSI pattern with additional fragments of increased size of markers NR-21 and BAT-26. Surprisingly, microsatellite stability (MSS) was observed, in the brain tumor of the sister of the index patient (Fig. 3a). IHC analysis of brain tumor tissues from both children showed absence of PMS2 expression in the tumor and normal cells. Tumor specimens from all other family members were MSS and showed normal expression of the MMR proteins in the tumor and normal tissue.

Germline mutation analysis

Mutation analysis of the NF1 gene was performed in the index patient but a mutation could not be identified. Mutation analysis in a blood sample

Table 1. Summary of results of the molecular and IHC analyses of tissues from the studied family

<table>
<thead>
<tr>
<th>Case</th>
<th>Malignancy</th>
<th>Age at diagnosis (years)</th>
<th>Skin lesions</th>
<th>NFI gene mutation</th>
<th>Analysis of MSI</th>
<th>IHC</th>
<th>LOH analysis</th>
<th>PMS2 gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>II.2</td>
<td>Adenocarcinoma of the rectum</td>
<td>84</td>
<td>ND</td>
<td>ND</td>
<td>MSS Normal</td>
<td>ND</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>III.2</td>
<td>Adenocarcinoma of the transverse colon</td>
<td>43</td>
<td>None</td>
<td>ND</td>
<td>MSS Normal</td>
<td>no LOH</td>
<td>Heterozygous Pro246fs</td>
<td></td>
</tr>
<tr>
<td>III.3</td>
<td>One adenoma (low-grade dysplasia)</td>
<td>45</td>
<td>None</td>
<td>ND</td>
<td>MSS Normal</td>
<td>ND</td>
<td>Heterozygous Pro246fs</td>
<td></td>
</tr>
<tr>
<td>IV.1</td>
<td>PNET</td>
<td>4</td>
<td>CAL spots &gt;6, hemangioma leg</td>
<td>ND</td>
<td>MSS PMS2 absent</td>
<td>ND</td>
<td>Compound heterozygous Pro246fs, p.Ser46ile</td>
<td></td>
</tr>
<tr>
<td>IV.2</td>
<td>Anaplastic glioblastoma</td>
<td>7</td>
<td>CAL spots &gt;6, axillary melanotic freckling</td>
<td>None</td>
<td>MSI-H PMS2 absent</td>
<td>ND</td>
<td>Compound heterozygous Pro246fs, p.Ser46ile</td>
<td></td>
</tr>
</tbody>
</table>

CAL, café au lait; IHC, immunohistochemistry of MLH1, MSH2, MSH6 and PMS2; LOH, loss of heterozygosity analysis by sequencing; ND, not determined; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable; PNET, primitive neuroectodermal brain tumor.
of the index patient identified the compound heterozygous mutations, p.Pro246fs and p.Ser46Ile. Both mutations were also found in DNA derived from the brain tissue from his sister. No mutation analysis was performed in the younger sister of the index patient. The heterozygous mutations p.Ser46Ile and p.Pro246fs were confirmed in the father and mother, respectively, indicating the compound heterozygous pattern in the index patient and his sister. The paternal grandmother appeared not to carry the p.Ser46Ile mutation, as present in the father of the index patient.
In both the index patient and his father, no germline mutation was detected in the MLH1, MSH2 and MSH6 gene.

Additional molecular analysis: loss of heterozygosity (LOH)

To assess whether the tumor from the father of the index patient was caused by the PMS2 germline mutation, LOH analysis was performed (Fig. 3b). For that purpose, DNA extracted from normal and tumor tissue of the father was sequenced, according to a previously described method (14). No LOH of the PMS2 locus was found, while the tumor percentage was high enough to detect LOH, which was indicated by the presence of LOH at the P53 and APC loci (Fig. 3c).

Discussion

The above-mentioned family displays a CMMR-D phenotype in the presence of compound heterozygous PMS2 mutations (p.Ser46Ile and p.Pro246fs). MSI was only found in the brain tumor of the PMS2 compound heterozygous index patient. The brain tumor of his compound heterozygous sister, as well as the CRCs of the father and his mother and the colorectal adenoma of the mother, were MSS. IHC analysis showed absence of PMS2 staining in both the brain tumor and normal tissue of the index patient and his sister, but not in the analyzed CRCs of their father and grandmother.

PMS2 is considered a tumor suppressor gene (5). In tumors of carriers of a heterozygous PMS2 mutation, MSI and absence of IHC staining of PMS2 can be expected as a result of the loss of the wild-type allele. In case of a biallelic germline mutation, MSI and especially absence of PMS2 expression can be expected already in normal tissue, as well as in tumor tissue.

Both parents of the index patients were found to carry a heterozygous PMS2 mutation. The p.Pro246fs mutation of the mother (individual III.3) is a previously described pathogenic frameshift mutation (16). The p.Ser46Ile missense germline mutation of the father (individual III.2) has been found in 7 cases in a cohort of 400 selected Dutch patients suspected to have an MMR gene defect. In contrast, this mutation was not detected in 927 controls (unpublished data of the Department of Human and Clinical Genetics, LUMC). Also, the amino acid involved in this mutation, is positioned in a highly conserved small helix domain (codon 35–48) and in addition serine and isoleucine have very different physical and chemical properties. In the literature, there is a clear overrepresentation of p.Ser46Ile in patients with PMS2 negative tumors (17–21). These findings support the pathogenicity of this mutation.

Surprisingly, no MSI and IHC aberrations were found in the CRC of the father. Eight additional CRCs in heterozygous carriers of the p.Ser46Ile mutation have been reported (Table 2). Unfortunately, only data on the MSI status of the tumor tissues of three of these eight patients were available, all displaying MSI. Absence of PMS2 expression was found in all described tumors in contrast to our observations in the CRC of the father. Because additionally no LOH of the PMS2 locus was detected in the tumor of the father, a role of PMS2 in the development of the early-onset CRC of the father cannot be demonstrated at the moment. It is possible that other colorectal cancer susceptibility genes are involved. As the tumor tissue of the father’s CRC was found to be MSS, it is unlikely, however, that this concerns the other MMR genes. Also, germline mutation analyses of

Table 2. Genetic and clinical summary of nine reported cases (including our case) of patients with CRC and the heterozygous PMS2 mutation: (c.137G>T), p.Ser46Ile

<table>
<thead>
<tr>
<th>Patient</th>
<th>Case</th>
<th>Malignancy</th>
<th>Age at diagnosis of malignancy (years)</th>
<th>Analysis of MSI</th>
<th>IHC</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>CRC: cecum</td>
<td>32</td>
<td>NA</td>
<td>PMS2 absent</td>
<td>Senter et al., 2008 (20)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>CRC: cecum</td>
<td>47</td>
<td>NA</td>
<td>PMS2 absent</td>
<td>Senter et al., 2008 (20)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>CRC: sigmoid</td>
<td>44</td>
<td>NA</td>
<td>PMS2 absent</td>
<td>Senter et al., 2008 (20)</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>CRC: transverse</td>
<td>43</td>
<td>NA</td>
<td>PMS2 absent</td>
<td>Senter et al., 2008 (20)</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>CRC: sigmoid</td>
<td>62</td>
<td>NA</td>
<td>PMS2 absent</td>
<td>Senter et al., 2008 (20)</td>
</tr>
<tr>
<td>6</td>
<td>Patient 1</td>
<td>CRC</td>
<td>31</td>
<td>MSI-H</td>
<td>PMS2 absent</td>
<td>Nakagawa et al., 2004 (19)</td>
</tr>
<tr>
<td>7</td>
<td>66603/current report; III.2</td>
<td>CRC</td>
<td>43</td>
<td>MSS</td>
<td>Normal</td>
<td>Van der Klift et al., 2010 (21)</td>
</tr>
<tr>
<td>8</td>
<td>74028</td>
<td>CRC</td>
<td>70</td>
<td>MSI-H</td>
<td>PMS2 absent</td>
<td>Van der Klift et al., 2010 (21)</td>
</tr>
<tr>
<td>9</td>
<td>74055</td>
<td>CRC</td>
<td>54</td>
<td>MSI-H</td>
<td>PMS2 absent</td>
<td>Van der Klift et al., 2010 (21)</td>
</tr>
</tbody>
</table>

CRC, colorectal carcinoma; IHC, immunohistochemistry; NR, not reported; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable.
Table 3. Results of analysis for MSI and IHC analysis of patients with brain cancer from families with biallelic MMR mutations

<table>
<thead>
<tr>
<th>Family</th>
<th>Case</th>
<th>Gene</th>
<th>Malignancy</th>
<th>Age at diagnosis of malignancy (years)</th>
<th>Signs of NF1</th>
<th>Analysis of MSI</th>
<th>IHC</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patient 1</td>
<td>PMS2</td>
<td>Glioblastoma, colonic adenomas, NHL of the rectum</td>
<td>4</td>
<td>CAL spots</td>
<td>MSI-H</td>
<td>NA</td>
<td>Hamilton et al., 1995 (25)</td>
</tr>
<tr>
<td>2</td>
<td>V.2</td>
<td>MSH2</td>
<td>Glioblastoma</td>
<td>4</td>
<td>NR</td>
<td>MSS</td>
<td>NA</td>
<td>Bougeard et al., 2003 (35)</td>
</tr>
<tr>
<td>3</td>
<td>III.1</td>
<td>PMS2</td>
<td>Giant cell glioblastoma, duodenal cancer, colonic adenomas</td>
<td>17</td>
<td>CAL spots</td>
<td>MSS, glioblastoma, MSI-H, duodenal cancer</td>
<td>PMS2 absent in glioblastoma. MSH6 and PMS2 absent in duodenal tumor tissue</td>
<td>Agostini et al., 2005 (17)</td>
</tr>
<tr>
<td>4</td>
<td>V.4</td>
<td>MSH6</td>
<td>Oligodendroglioma, rectosigmoid cancer</td>
<td>10</td>
<td>CAL spots</td>
<td>MSS</td>
<td>MSH6 absent in CRC, MSH6 present in oligodendroglioma</td>
<td>Menko et al., 2004 (8)</td>
</tr>
<tr>
<td>5</td>
<td>IV.3</td>
<td>MSH6</td>
<td>Glioblastoma multiforme</td>
<td>8</td>
<td>CAL spots, axillary freckling, IgA deficiency</td>
<td>MSI-H</td>
<td>NA</td>
<td>Hegde et al., 2005 (37)</td>
</tr>
<tr>
<td>6</td>
<td>Patient 1</td>
<td>MSH6</td>
<td>Astrocytoma</td>
<td>9</td>
<td>CAL spots, axillary freckling, IgA deficiency</td>
<td>NA</td>
<td>MSH6 absent</td>
<td>Ostergaard et al., 2005 (38)</td>
</tr>
<tr>
<td>7</td>
<td>I</td>
<td>MLH1</td>
<td>Glioblastoma, Wilms tumor</td>
<td>4</td>
<td>CAL spots</td>
<td>MSS</td>
<td>MLH/PMS2 absent in brain tissue</td>
<td>Poley et al., 2007 (14)</td>
</tr>
<tr>
<td>8</td>
<td>I.2</td>
<td>MSH6</td>
<td>Glioblastoma multiforme</td>
<td>9</td>
<td>Hyper and hypopigmentation skin</td>
<td>MSS</td>
<td>MSH6 absent, MSH2 expression reduced</td>
<td>Etzler et al., 2008 (36)</td>
</tr>
</tbody>
</table>

CAL, café au lait; IgA, immunoglobulin A; IHC, immunohistochemistry; NA, not available; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable; NHL, non-Hodgkin's lymphoma.
MLH1, MSH2 and MSH6 revealed no aberrations in the father. Nevertheless, other unknown susceptibility genes cannot be excluded. In view of this, first-degree relatives of the father, who test negative for the familial PMS2 mutation, should in our opinion still be offered colorectal surveillance.

The results of MSI and IHC analysis of the tissue of the paternal grandmother are in agreement with analysis of sporadic colorectal cancer. This finding is in concordance with her not being a carrier of the familial PMS2 mutation.

The index patient and his sister inherited both PMS2 germline mutations from their parents, explaining their CMMR-D phenotype. However, MSI was found in only one of the two brain tumors. In gastrointestinal tumors, MSI analysis seems to be a reliable tool to diagnose MMR deficiency. In the literature, results of molecular analyses in 21 patients with gastrointestinal malignancies and biallelic MMR gene mutations have been reported. Nineteen patients were diagnosed with CRC and 2 patients with duodenal cancer. In all tumors, MSI was detected. Additional IHC analysis showed absence of immunostaining of the corresponding affected MMR proteins in 19 of 21 analyzed gastrointestinal tumors (8, 17, 18, 20, 22–34). In addition to the gastrointestinal patients, 43 patients (mean 8 years, range 4–17, 88% male) with biallelic MMR gene mutations and brain cancer have been reported. In 8 of these 43 cases, brain tumor specimens were analyzed for MSI and in 5 of these cases IHC analysis of MMR proteins was performed (Table 3; 8, 14, 17, 25, 35–38). Germline mutation analysis showed one patient with MLH1, one with MSH2, four with MSH6 and two with PMS2 mutations. In six of the analyzed eight cases, no MSI was found in brain tumor tissue. A hypothesis to explain the lack of MSI in brain tumors from germline biallelic PMS2 mutant patients is that in brain tissue a PMS2 deficiency could lead to tumorigenesis through a different mechanism than the MMR pathway (8, 14, 17). Also, the extent and pattern of MSI may differ between CRCs and brain tumors, making the MSI analysis that is routinely used for CRC less reliable for brain tumors (8, 14).

IHC analysis showed the absence of immunostaining of PMS2 in the brain tumor cells as well as in normal cells in the specimens of our index case and his sister. This is in accordance with the absence of expression of the affected MMR protein in all five investigated brain tumors of germline biallelic mutant MMR gene patients described in the literature (8, 14, 17, 35, 36). From the literature and our own data, it can be concluded that MMR and IHC analysis may be more sensitive than MSI analysis to detect MMR deficiency in brain tumors.

The third child in this family (IV.3) is also at risk of being a heterozygous or compound heterozygous carrier of the familial PMS2 mutations. Because single CAL spots are a frequent finding in the general population and this child is 8 years past the age of onset of the brain tumors in her siblings, we estimate her risk for CMMR-D to be lower than the theoretical 25%. However, her risk is not excluded. No guidelines are available yet for the surveillance of children at risk for CMMR-D. In this family, we think regular clinical surveillance by a pediatric oncologist including colonoscopy and possibly brain magnetic resonance imaging (MRI) can be considered. Because of behavioral and psychological problems of the third child, she and her mother declined genetic testing for the PMS2 mutations and surveillance at the moment.

In conclusion, the results of molecular analyses in this family display the diagnostic challenges in PMS2-mutation families. In case of a clinical phenotype of CMMR-D, it is recommended to routinely combine MSI analysis with IHC analysis for the expression of MMR proteins. With inconclusive or conflicting results, mutation analysis of the MMR genes should be considered after thorough counselling of the patients and their relatives.

References

Pitfalls in molecular analysis for mismatch repair deficiency


