Novel variants of unknown significance in the PMS2 gene identified in patients with hereditary colon cancer

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Background: Lynch syndrome is associated with genetic variants in mismatch repair (MMR) genes. Pathogenic variants in the MLH1 and MSH2 genes occur in most families in which the phenotype is highly penetrant. These testing criteria are likely to miss individuals with Lynch syndrome due to the less penetrant MMR genes, such as MSH6, MLH3, MSH3, and PMS2. So far, several mutations in the PMS2 gene have been described as responsible for the clinical manifestation of Lynch syndrome. Recent data have reported that families with atypical Lynch phenotype were found to have primarily monoallelic mutations in the PMS2 gene.

Methods: We analyzed the PMS2 gene to detect mutations in members of 64 Lynch syndrome families by direct sequencing.

Results: We report the identification of several genetic variants in patients with LS, of which three are novel variants. The carriers of these novel variants were also carriers of other variants in PMS2 gene and/or in other MMR genes.

Conclusion: Therefore, we think that these novel PMS2 variants may act in additive manner to manifestation LS phenotype.

Keywords: Lynch syndrome, PMS2 gene, MMR genes, PMS2 variants, synergist effect of MMR variants

Introduction

The primary clinical manifestation of Lynch Syndrome is the development of colon cancer at an average age of 45 years; this syndrome is also characterized by an increased risk of developing extra-colonic tumors such as endometrial, ovarian, stomach, urinary, and biliary tract cancer.1 Families affected by LS were identified using the Amsterdam Criteria (AC) and Bethesda guidelines (BG).2,3 Lynch syndrome (LS) testing criteria were developed based on families with cancer histories across multiple generations. LS is associated with genetic variants in DNA Mismatch Repair (MMR) genes and it is characterized at somatic level by high instability of microsatellite sequences (MSI).4,5 Pathogenic variants in the MLH1 and MSH2 genes occur in most families in which the phenotype is highly penetrant.6,7 Pathogenetic variants in the less penetrant MMR genes, such as MSH6, MLH3, MSH3, and PMS2 were also shown in LS patients.8–10

The PMS2 gene is located on chromosome 7p22 in a region spanning 16 kb and is made up of 15 exons and 862 codons.11 The PMS2 protein acts as...
a heterodimer together with MLH1 protein forming the 
MutLa complex that is associated with the MutSα complex, 
which primarily repairs single-nucleotide mismatches.12 Its function is redundant with the MLH3 gene product. Mice that have the PMS2 gene deleted develop lymphomas and sarcomas but not gastrointestinal 
tumors; instead, mice lacking PMS2 and MLH3 develop a non-distinguishable phenotype from MLH1 knock-out mice.13 Finally, it has also shown that the PMS2 gene is also involved in the apoptotic pathway.14

So far, several mutations in the PMS2 gene have been described as responsible for the clinical manifestation of Lynch syndrome.15–17 The large rearrangements in the 
PMS2 gene are rarely described.18,19 Recent studies have demonstrated a reduced penetrance for monoallelic carriers of PMS2 mutations compared to the other MMR genes.20 Moreover, very often homozygous and/or biallelic point mutations in the PMS2 gene have been reported to be responsible for Lynch phenotypes;21,22 as it has been also 
described that PMS2 mutations together with mutations in 
other MMR genes should also be considered in patients 
suspected to present a Lynch syndrome with an unusual 
early-onset of tumors.23

The aim of this study was to analyze the PMS2 gene to 
detect mutations in members of 64 LS families. We have 
identified 21 variants in the PMS2 gene; of which no 
variant was of certain pathogenetic significance. Most of 
these variants had previously been described in the literature, only three of these are novel, of which two are 
missense variants that could alter the functionality of the protein.

Materials and methods
Patients and isolation of genomic DNA
Sixty-four LS patients were recruited from several hospi-
tals in southern Italy; patients were previously estab-
lished to be negative for pathogenetic mutations in other 
MMR genes including MLH1, MSH2, MSH6, and MLH3 and included large rearrangements in PMS2 gene.19
Twenty-three families with classic LS phenotype were 
selected by AC 21 and 41 families with atypical Lynch 
phenotype were selected by MSI analysis as suggested 
BG1 Sixty samples from healthy patients collected from 
the Clinical Department of Laboratory Medicine of the 
hospital affiliated with Federico II University (Naples, 
Italy) were used as negative controls. The experiments 
were performed on DNA extracted from peripheral blood 
lymphocytes. Total genomic DNA was extracted from 
4 mL peripheral blood lymphocytes using a BACC2 
Nucleon kit (Amersham Pharmacia Biotech, Amersham, 
UK). The Clinical Department of Laboratory Medicine of 
the hospital affiliated to Federico II University (Naples, 
Italy) recruited the subjects after receiving authorization 
from the local ethics committee “Comitato etico per le 
attivitá Biomediche Carlo Romano” of the University of 
Naples, Federico II (protocol no. 120/10). Once the 
authorization was obtained, the study received ethical 
approval, and participants’ informed and written consent 
was obtained. The experiments were performed on DNA 
and on cDNA extracted from peripheral blood 
lymphocytes.

Mutation analysis and in silico analysis
The entire coding region of the PMS2 gene was amplified 
in 17 fragments, 1 for each exon and 3 overlapping 
fragments for exon 11, using customized primer sets, 
available upon request. The polymerase chain reaction 
(PCR) products were separated on a 1–2% agarose gel 
to check for unspeci c amplicons. Subsequently, the PCR 
products were sequenced in both the forward and reverse 
directions using an ABI 3100 Genetic Analyzer (Applied 
Biosystems).

The Sorting Intolerant From Tolerant (SIFT) (http:// 
blocks.fhcrc.org/sift/SIFT.html) and Polymorphism 
Phenotyping (PolyPhen) (http://genetics.bwh.harvard. 
edu/pph/) tools were used for functional impact predic-
tion of the novel variants, as described in our previous 
studies.6

Immunohistochemistry (IHC)
IHC was performed on a Benchmark XT automatized 
immunostainer (Ventana Medical Biosystems, Tucson, 
AZ, USA). The antibodies used were anti-MSH6, mouse 
monoclonal clone 44; anti-MSH2, mouse monoclonal 
clone G219-1129; anti-MLH1, mouse monoclonal clone 
M1 (Ventana); and anti-PMS2. The procedure was per-
formed as described previously on sections of colon cancer 
tissues.9

Results
The mutation detection analysis of the PMS2 gene, per-
formed on samples from patients with LS, as described in 
the Materials and Methods section, identified 21 genetic
<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Aminoacid change</th>
<th>Reference</th>
<th>Pathogenicity (<a href="http://www.insight-group.org">www.insight-group.org</a>)</th>
<th>Frequency in hereditary CRC</th>
<th>Frequency in healthy samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5'UTR c.-154g&gt;c</td>
<td></td>
<td>Thompson et al.\textsuperscript{11} 2004</td>
<td>ND</td>
<td>12/64 (19%)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5'UTR c.-195t&gt;c</td>
<td></td>
<td>Thompson et al.\textsuperscript{11} 2004</td>
<td>ND</td>
<td>3/64 (4.7%)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5'UTR c.+72c&gt;t</td>
<td>p.(Ile17Val)</td>
<td>This study Likely Class 1</td>
<td>12/64 (19%)</td>
<td>4/60</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>c.52A&gt;G</td>
<td></td>
<td>Hendrinks et al.\textsuperscript{24} 2006</td>
<td>Class 2</td>
<td>1/64 (1.6%)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>c.59G&gt;A</td>
<td>p.(Arg20Gln)</td>
<td>Nicolaides et al.\textsuperscript{25} 1994</td>
<td>Class 1</td>
<td>6/64 (9.4%)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>c.250+108a&gt;g</td>
<td></td>
<td>Hendrinks et al.\textsuperscript{24} 2006</td>
<td>ND</td>
<td>7/64 (11%)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>c.705+17g</td>
<td></td>
<td>Thompson et al.\textsuperscript{11} 2004</td>
<td>ND</td>
<td>10/64 (1.5%)</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>c.780C&gt;G</td>
<td>p.Ser260=</td>
<td>Viel et al.\textsuperscript{26} 1998 Class 1 Heterozygous 3/64 (4.7%); Homozigous 13/64 (20%)</td>
<td>ND</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>c.1454C&gt;A</td>
<td>p.(Thr485Lys)</td>
<td>Wang et al.\textsuperscript{27} 1999 Class 1</td>
<td>4/64 (6.3%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.1621G&gt;a</td>
<td>p.(Glu540Lys)</td>
<td>Viel et al.\textsuperscript{26} 1998 Class 1</td>
<td>10/64 (1.58%)</td>
<td>ND</td>
<td></td>
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<tr>
<td></td>
<td>c.1789A&gt;T</td>
<td>p.(Thr596Ser)</td>
<td>Viel et al.\textsuperscript{26} 1998 Class 1</td>
<td>1/64 (1.6%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2007−4g&gt;a</td>
<td></td>
<td>Hendrinks et al.\textsuperscript{24} 2006</td>
<td>ND</td>
<td>15/64 (23.4%)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>c.2174+24c&gt;a</td>
<td></td>
<td>Hendrinks et al.\textsuperscript{24} 2006</td>
<td>ND</td>
<td>6/64 (9.4%)</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>c.2248G&gt;A</td>
<td>p.(Gly749Ser)</td>
<td>This study Likely Class 3</td>
<td>1/64 (1.6%)</td>
<td>0/60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2253T&gt;C</td>
<td>p.Phe 751=</td>
<td>Viel et al.\textsuperscript{26} 1998 Class 1</td>
<td>10/64 (1.58%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>c.2324A&gt;G</td>
<td>p.(Asn774Ser)</td>
<td>Viel et al.\textsuperscript{26} 1998 Class 1</td>
<td>14/64 (2.22%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2340C&gt;T</td>
<td>p.Pro780=</td>
<td>Hendrinks et al.\textsuperscript{24} 2006</td>
<td>Class 1</td>
<td>3/64 (4.7%)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>c.2380C&gt;T</td>
<td>p.Pro793Ser</td>
<td>This study Likely Class 3</td>
<td>2/64 (3.2%)</td>
<td>0/60</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>c.2466T&gt;C</td>
<td>p.Leu822=</td>
<td>Viel et al.\textsuperscript{24} 1998 Class 1</td>
<td>10/64 (1.58%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2570G&gt;C</td>
<td>p.(Gly857Ala)</td>
<td>Viel et al.\textsuperscript{24} 1998 Class 1</td>
<td>23/64 (36%)</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** NCBI accession number: NM000535.

**Abbreviations:** ND, not done; CRC, colon rectal cancer.
variants (Table 1). These variants were shown in both families’ groups (AC and BG respecting), casually. No variant of certain pathogenetic significance was identified in this study. Most variants had previously been described in the literature and reported in the MMR variants database of Insight Group (https://insight-database.org/variants/), while three were novel variants, of which two were missense variants. The two novel missense variants were analyzed using a bioinformatic tool that predicted that both these variants were likely pathogenic. These missense variants were identified in three unrelated LS families (Table 2). The genetic variant, c.2248G>A, located in exon 13 of the gene, was identified in a subject who developed right-side colon carcinoma at the age of 34 years. This variant was also identified in the father of this proband, who also developed colon cancer at 40 and in a paternal uncle who developed colon cancer at age 45. Thus, the mutation segregates with the disease in this family. Moreover, this variant was not found in the 60 healthy subjects used as negative controls. In silico analysis indicated that protein function was likely altered as a result of the mutation. The IHC analysis did not provide results due to paraffin-embedded tumoral tissue of poor quality. The father and son of this family were also carriers of unclassified variants in MSH2 genes, a missense variant in exon 3, the c.573C>T (p.Pro125Ser), already reported in “Insight Variants Database” (Table 2). The second novel PMS2 missense variant, the c.2380C>T in exon 14, was identified in two unrelated LS patients, and it was also not found in the 60 healthy subjects used as negative controls. The in silico analysis revealed that this variation has also a high probability of altered protein function; the IHC analysis showed normal expression of the PMS2 protein at the somatic level, (Table 2).

Discussion
For many years, the PMS2 gene has been considered a gene candidate for the development of cancer in Lynch Syndrome. However, to date, its role in the development of cancer in Lynch syndrome is still not well understood. It has been reported that monoallelic mutations in the PMS2 gene are responsible for the phenotype found in families that do not fully comply with the Amsterdam criteria or who develop non-LS-related tumors.20 This can be explained considering that mice lacking PMS2 develop lymphomas and sarcomas but not gastrointestinal tumors.12

In this study, all variants identified in the PMS2 gene are unclassified, including also the novel variants. Bioinformatics studies have shown that two novel missense variants may be detrimental to the functionality of the protein. In particular, c.2248G>A, identified in exon 13 of the PMS2 gene, was found to segregate with the disease in the AC family and all family members’ carrier of this variant developed colon cancer. Our previous investigations showed that the index case of this family and his father were also carriers of a missense variant in the MSH2 gene, already described in literature as benign variant. Unfortunately, it was not possible to analyze the other affected family members because didn’t available. The MSI analysis performed on tumoral colon tissue of the index case showed a high MSI status (data not shown). Instead, the IHC investigations did not provide reliable results due to poor quality of the paraffin-embedded tumor tissue from the index case; therefore, we have not been able to escape a deficiency of MMR proteins at the somatic level associated with other causes, such as hypermethylation of the MLH1 promoter.1 Thus, we speculated that likely these two variants identified in this family may act as low-risk alleles that together determine the deficiency of MMR system, as shown by high MSI status on tumoral DNA of index case. Also the other missense variant identified in this study, the c.2380C>T, in exon 14 of the PMS2 gene was found in two unrelated patients that both showed high MSI status on tumoral colon tissues (data not shown) but a poor one significant family history of LS-related cancers. However, the IHC analysis performed on colon cancer tissue of both index cases (07/6 and 00/12) showed normal expression of MMR proteins, MLH1, MSH2, MSH6, and PMS2 (Table 2). This last data does not contrast with the result of the MSI rather it reinforces our hypothesis, first formulated; indeed, a missense mutation is predicted to alter the functionality of the protein but not its production. Moreover, also these two patients were carriers of other variants in MMR gene. In particular, the carrier 07/6 showed in addition to novel missense variant also a high numbers of other variants in PMS2 gene (Figure 1) and a variant in MLH3 gene (Table 2). Also, the carrier 00/12 showed two variants in PMS2 (included the novel variant) and one variant in MLH3 gene. Previously, literature data showed that the monoallelic mutations in the PMS2 gene are likely compatible with a more attenuated Lynch phenotype or a low-penetrance20 and, thus in the case of a classical phenotype,
<table>
<thead>
<tr>
<th>ID</th>
<th>MMR genes variants</th>
<th>Microsatellite instability</th>
<th>Immunohistochemistry</th>
<th>Clinical phenotype</th>
<th>Family history</th>
</tr>
</thead>
</table>
| 05/7 | MSH2: ex3c.573C>T (p. Pro125Ser)  
PMS2: ex7c.780C>G+/+ [p. Ser260=]  
ex13c.2248G>A (p. Gly749Ser)  
ex13c.2253T>C [p.Phe 751=}  
ex14c.2324A>G (p. Asn774Ser)  
PMS2: ex13c.2253T>C [p.Phe 751=}  
ex14c.2324A>G (p. Asn774Ser)  
MLH1: ND  
PMS2: ND | MSI-H | MSH2: +/+  
MSH6: ND  
MLH1: ND  
PMS2: ND | Right-colon cancer at age 44. | Father affected by colon cancer at age 36 and 49 years, PMS2 and MSH2 MUT+; paternal uncle died of colon cancer at age 60, PMS2 MUT+; paternal uncle died of lung cancer at age 60 years and his son affected by three right colon adenoma at age 42. |
| 00/12 | MLH3: ex1-13c.2896T>C (p.Ser965Pro)  
PMS2: ex14c.2324A>G (p. Asn774Ser)  
ex14c.2380C>T (p. Pro793Ser)  
MLH1: ND  
PMS2: +/+  
MLH1: +/+  
PMS2: +/+ | MSI-H | MSH2: +/+  
MSH6: +/+  
MLH1: +/+  
PMS2: +/+ | Right-colon cancer at age 64. | Father affected by colon cancer at age 63; paternal aunt died of breast cancer at age 40; sister died of lung cancer at age 63. |
| 07/6 | MLH1: IVS1+9C>G+/+;  
MLH3: ex1-13c.2896T>C (p.Ser965Pro)  
PMS2: ex2c.59G>A (p. Arg20Gln)  
ex6c.705+17a>g,  
ex7c.780C>G +/+[p. Ser260=]  
ex12c.2007–7c>t,  
ex12c.2007–4g>a,  
ex13c.2253T>C [p.Phe 751=}  
ex14c.2380C>T (p. Pro793Ser)  
MSH6: +/+  
MLH1: +/+  
PMS2: +/+ | Right-colon cancer at age 59. | Parents who died in old age, not affected by neoplastic disease; two maternal aunts died of colon cancer at age 55 and 80, respectively. |

Note: *Novel variant.  
Abbreviations: ID, identification number patient; ND, not done; +/+, homozygous variant; Mut+, carrier mutation; MSI-H, high microsatellite instability; IHC, immunohistochemistry.
a second mutation may also be present in the same PMS2 gene or in another MMR gene. Even if, all variants identified in this study seem unlikely to have pathogenic effects on protein function it cannot be ruled out that such variants represent low-penetration alleles rather than benign variants or polymorphisms. Therefore, it is possible that the synergist effect of these low-risk alleles is causing disease manifestation in our patients.

Figure 1 DNA sequencing electropherograms of the PMS2 exon 2 (A), exon 6 (B), exon 7 (C), exon 12 (D), exon 13 (E), exon 14 (F), and exon 15 (G) for the 07/6 patient sample. Arrows indicate the homozygous variant in the exon 7 (C), and the heterozygous variants in the exon 2 (A), the exon 6 (B), the exon 12 (D), the exon 13 (E), the exon 14 (F), and the exon 15 (G). Each variant is shown in the forward (left) and reverse (right) DNA sequencing analysis.
Disclosure
The authors report no conflicts of interest in this work.

References

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