Clinical evaluation of additional \textit{MLH1} variants indicated in gray in Figure 4

<table>
<thead>
<tr>
<th>Variant</th>
<th>Evaluation</th>
<th>Evidence</th>
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<tbody>
<tr>
<td>I219V</td>
<td>Not pathogenic</td>
<td>Frequent polymorphism (above 30% in the western population) (1). Homozygous carriers are also frequent. Has previously been shown not to significantly affect expression or mismatch repair capacity of the MLH1 protein (2, 3), as also evident from the current data presented in this work. Case-control analyses suggested that it is non-pathogenic (4, 5) or may at most show a very mild positive association with cancer risk under certain evaluation conditions (1). However, it does not display any traits compatible with Lynch syndrome.</td>
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<tr>
<td>V506A</td>
<td>Reports compatible with a pathogenic effect</td>
<td>The alteration was identified in a kindred with Lynch syndrome (3118) (6) and together with K618A in one Amsterdam-positive patient with CRC before the age of 45 years (patient DF260) (7). Furthermore, it was found in two Bethesda-positive patients (11 and 12) with CRC diagnosis at ages 28 years (male) and 26 years (female), respectively (8).</td>
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<tr>
<td>N551T</td>
<td>Reports strongly suggestive of a pathogenic effect</td>
<td>Found in a Swiss Amsterdam-positive individual (MSI-positive CRC with 46 years) (9). It was also described to cosegregate with cancer in three patients of a family from Turkey (average age of cancer onset 53 years); one child of heterozygous, consanguineous parents from this family died of CRC at the age of 13 years, most likely a case of constitutive MMR deficiency syndrome (10).</td>
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<td>L559R</td>
<td>Reports compatible with a pathogenic effect</td>
<td>Found in two Italian patients with MSI-positive colon cancer patients (age of onset 35 and 63 years) without MLH1 staining (patient 230 (11) and patient 3 (12)). It was furthermore identified in a CRC patient designated “high risk” from Great Britain (13).</td>
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<tr>
<td>P640S</td>
<td>Reports strongly suggestive of a pathogenic effect</td>
<td>Described to co-segregate with disease in three siblings of an Amsterdam-positive Columbian kindred (family UN-4) (14). It was furthermore found in a 34 year old colorectal cancer patient from Korea, who came from a family meeting either the Amsterdam-II-criteria or modified criteria (family SNU-H36) (15).</td>
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<tr>
<td>P648L</td>
<td>Reports compatible with a pathogenic effect</td>
<td>The alteration cosegregated in two CRC siblings from a German, Bethesda-positive family (family 123) with proximal colorectal cancer, ages at diagnosis were 43 and 45 years; one was tested for microsatellite instability and tumor MLH1 staining and was positive and negative, respectively. The father of the siblings had CRC at the age of 42 years (but was not genotyped) (16). Other previous examinations most likely referred to the same family and patients (then as family 5) (17, 18).</td>
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