

## Involvement of large rearrangements in *MSH6* and *PMS2* genes in Southern Italian patients with Lynch syndrome

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**Summary.** *Background and aim of the work:* The Lynch Syndrome (LS) is associated with germline mutations in one of the Mismatch Repair (MMR) genes. Most of germline mutations are point variants, followed by large rearrangements that account to 15-55% of all pathogenic mutations. Many study reporting the frequency of large rearrangements in the *MLH1* and *MSH2* genes were performed, while, little is known about the contribution of large rearrangements in other MMR genes, as *PMS2* and *MSH6*. Therefore, in this study we investigated the involvement of large rearrangements in *MSH6* and *PMS2* genes in a well-characterized series of 20 LS southern Italian patients. *Methods:* These large rearrangements are not usually detected by methods of mutation analysis, such as denaturing high-performance liquid chromatography (DHPLC) and direct DNA sequencing, but they are detectable by a known technique as the Multiplex Ligation-Probe Dependent Amplification (MLPA) assay. *Results:* No large rearrangements were identified in *MSH6* gene; instead, a large rearrangement was identified in *PMS2* gene. A large duplication including the exons 3 and 4 of the *PMS2* gene was identified in a patient who developed a rectum carcinoma at 45 years of age, an endometrial carcinoma and a vaginal cancer at the 65 years of age. *Conclusion:* We can affirm that the detection of large rearrangements in the *MSH6* and *PMS2* genes should be included in the routine testing for Lynch syndrome, especially considering the simplicity of the MLPA assay.

**Key words:** Lynch syndrome, HNPCC, *MSH6* gene, *PMS2* gene, MMR genes, large rearrangements, large duplication, genetic testing of Lynch syndrome

### Introduction

The main hereditary gastrointestinal cancer syndromes (1) include the Familial Polyposis Adenomatous (2, 3), PTEN Hamartoma Tumor Syndrome (4), Peutz Jeghers (5) and Lynch Syndrome (6). Mutations in Mismatch Repair (MMR) genes are responsible for the early onset of colorectal cancer in Lynch syndrome (LS) (6). Germline mutations in *MLH1*, *MSH2* and *MSH6* genes account to 70-80% of LS cases, while a minor contribution (about 10-30%) is given by mutations in the *PMS2*, *MLH3* and *MSH3* genes (7-9). The mutations are distributed heterogeneously along

each MMR gene, denoting the absence of “hot spots” mutations. Regarding to nature of germline mutations, most of these are point variants, followed by large rearrangements that account to 15-55% of all pathogenic mutations (10). Such alterations are mainly due to the presence of highly repeated sequences such as Alu sequences, which driver the recombination processes (11). A higher percentage of these rearrangements (deletions or duplications) are present in *MSH2* gene (20%) (12, 13); also in *MLH1*, *MSH6* and *PMS2* genes several large rearrangements were described in international literature (14). Molecular screening in suspected LS families attempted to find relationships

between a particular phenotype and a mutation in one of *MMR* gene (15). Although, the correlation genotype-phenotype for LS was not clarified to date (16), it is possible to affirm that the classic forms of LS, characterized by a early onset age of tumor (about 42 years) high penetrance and high degree of microsatellite instability (MSI) (17) were associated with point mutations in *MSH2* and *MLH1* genes. While, *MSH6* point mutations were reported in the literature as causing an “attenuated” forms of LS, with a later onset of tumor (18). Finally, point mutations in the *PMS2* gene were reported to cause early onset of tumors, that showed microsatellite instability but with different somatic features (19). Instead, the large rearrangements in any *MMR* genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*) cause a similar clinical phenotype of disease, that is corresponding to classic forms of LS (20). These large rearrangements are not usually detected by methods of mutation analysis, such as denaturing high-performance liquid chromatography (DHPLC) and direct DNA sequencing, but they are detectable by a known technique as the Multiplex Ligation-Probe Dependent Amplification (MLPA) (12) assay. So far, many large rearrangements in the *MLH1* and *MSH2* genes were described as responsible of Lynch syndrome phenotype, while, little is known about the identification of large rearrangements in other *MMR* genes, as *PMS2* and *MSH6*. In this study we researched the large rearrangements in *MSH6* and *PMS2* genes in a well-characterized series of 20 LS southern Italian patients already negative for point mutations in the *MLH1*, *MSH2*, *MSH6*, *PMS2* and *MLH3* genes and for large rearrangements in the *MLH1* and *MSH2* genes. Identification of mutation responsible to LS phenotype, it is important in order to not exclude from the prevention and treatment program the subjects at risk of developing an early colon cancer.

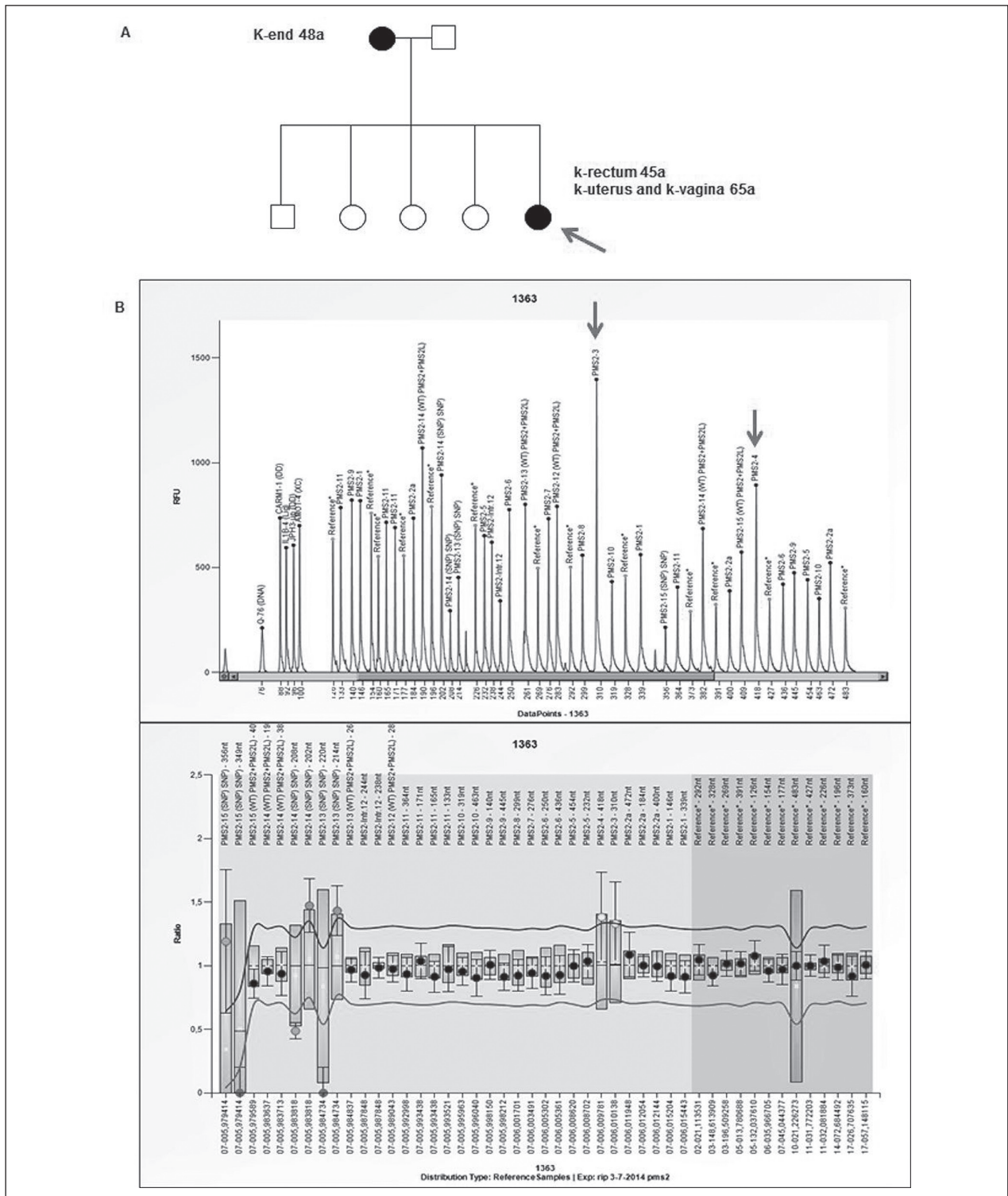
### Case report

In this study, the DNA of 20 selected subjects were analyzed by MLPA analysis to detection of large rearrangements in two *MMR* genes, *MSH6* and *PMS2* genes. These twenty subjects of Italian origin, 12 selected by the diagnostic criteria of Amsterdam (21)

and 8 by the Bethesda guidelines (according to MSI high status) (22, 23) were recruited from several health centers in Southern Italy. Furthermore, as negative controls we collected 7 healthy samples from Clinical Department of Laboratory Medicine of our Hospital (Federico II of Naples). All patients received genetic counseling and gave their written informed consent to participate in this study. The detection of large genomic rearrangements in *MSH6* and *PMS2* in our selected patients was performed on genomic DNA using the SALSA MLPA P008-B1 *PMS2* kit -Lot B1-0112 and P072-C1 *MSH6* kit (MRC-Holland, Netherlands) according to the manufacturer’s instructions. No large rearrangements were identified in *MSH6* gene; instead, a large rearrangements was identified in *PMS2* gene. A large duplication including the exons 3 and 4 of the *PMS2* gene was identified in a subject (our number 1363) who developed a rectum carcinoma at 45 years of age, an endometrial carcinoma and a vaginal cancer at the 65 years of age. Figure 1A. For all patients, MLPA results were confirmed in three independent experiments. For the subject with our number 1363 and 7 negative references, we performed other two MLPA experiments using a 4 fold reduced amount of Ligase65 enzyme (0.25  $\mu$ l/reaction), as suggested data sheet of P008-B1 *PMS2* kit, Fig. 1B.

### Discussions

Twenty subjects belonging to families with clinical diagnosis of LS were selected for this study. Of these twenty families, twelve meet the criteria of Amsterdam and eight showing an atypical phenotype were selected by MSI status on DNA extracted from tumoral tissue (data not shown). We performed the detection of large rearrangements in *MSH6* and *PMS2* genes in these LS families already negative for point mutations in *MLH1*, *MSH2*, *MSH6*, *PMS2* and *MLH3* genes and for large rearrangements in *MLH1* and *MSH2*. Therefore, in order to not exclude from the prevention and treatment program these subjects at risk of developing an early colon cancer we extended the research of mutation to analysis of large rearrangements in *MSH6* and *PMS2* genes that not are usually analyzed in genetic testing for LS. However, large rearrangements in these



genes were reported in literature (14, 24). In this study, no large rearrangements were identified in *MSH6* gene among our LS subjects. Instead, we identified a likely duplication including the exons 3 and 4 of *PMS2* gene. This duplication was identified in our LS patient (n. 1363) that developed a rectal carcinoma at the age of 45 and later a uterine and vaginal carcinoma at the age of 65, Fig. 1A. Literature data indicate that monoallelic mutations in *PMS2* gene are responsible of LS phenotype characterized by the presence of multiple tumors (25). The low penetrance could be to explain by redundant function of *PMS2* protein in the MMR complex. This could explain the absence of a significant family history for the subject 1363. Unfortunately, due to limited availability of subjects 1363 and to difficulty of analyzing the *PMS2* gene (26) we were not able to performed other experiments to confirm the MLPA result. However, as suggested data sheet of SALSA MLPA *PMS2* kit P008-B1 to confirm the obtained result we repeated the MLPA experiment using a 4 fold reduced amount of Ligase65 enzyme, to exclude that this duplication of 3 and 4 exons of *PMS2* could be an artifact of MLPA reaction (Fig. 1B). This condition could to occur due to difficulty of analyzing the *PMS2* gene also by MLPA reaction for the presence of numerous pseudogenes (27). In conclusion, we believe that are needed further molecular analysis to confirm the duplication identified in *PMS2* gene. However, we can affirm that the detection of large rearrangements in the *MSH6* and *PMS2* genes should be included in the routine testing for Lynch syndrome, especially considering the simplicity of the MLPA assay. Finally, this study reaffirms the importance to identify pathogenic mutations in LS families to facilitate pre-symptomatic diagnosis and to improve therapeutic pathway in order to promote a personalized medicine (28).

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