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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 involvment 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 µl/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

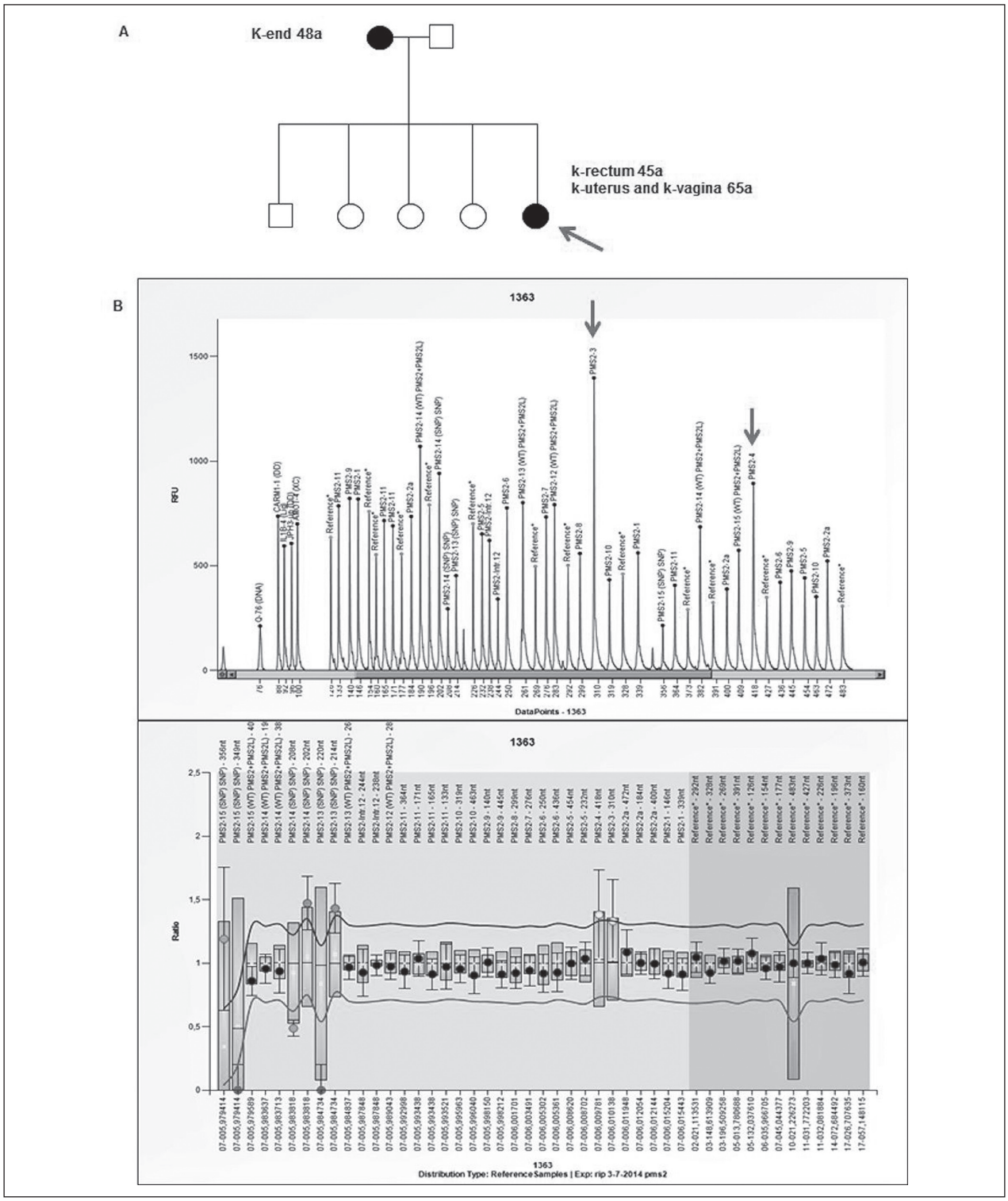

Figure 1. A. Family pedigree of our patient 1363 with a large duplication in PMS2 gene. Symbols and abbreviations used are denoted as fellow: arrows, index case; black symbol, colorectal cancer or tumors associate with LS; Co, colon cancer; End, endometrial cancer; Vag, vaginal cancer. Number next to diagnosis denote age at onset. and **B.** Electropherogram and graphical analysis showing the large duplication including 3 and 4 exons of *PMS2* gene.

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).

References

1. De Rosa M, Pace U, Rega D, et al. Genetics, diagnosis and management of colorectal cancer (Review). Oncol Rep 2015; 34(3): 1087-96.
2. Dodaro C, Grifasi C, Florio J, et al. The role of mutation analysis of the APC gene in the management of FAP patients. A controversial issue. Ann Ital Chir 2016; 87: 321-5.
3. De Rosa M, Galatola M, Borriello S, et al. Implication of adenomatous polyposis coli and MUTYH mutations in familial colorectal polyposis. Dis Colon Rectum 2009; 52(2): 268-74.
4. Paparo L, Rossi GB, Delrio P, et al. Differential expression of PTEN gene correlates with phenotypic heterogeneity in three cases of patients showing clinical manifestations of PTEN hamartoma tumour syndrome. Hered Cancer Clin Pract 2013; 25; 11(1): 8.
5. Meserve EE, Nucci MR. Peutz-Jeghers Syndrome: Pathobiology, Pathologic Manifestations, and Suggestions for Recommending Genetic Testing in Pathology Reports. Surg Pathol Clin 2016; 9(2): 243-68.
6. Liccardo R, De Rosa M, Izzo P, Duraturo F. Novel implications in molecular diagnosis of lynch syndrome Gastroenterol Res Pract 2017; 2017: 2595098.
7. Peltomäki P. Update on Lynch syndrome genomics. Fam Cancer 2016; 15(3): 385-93.
8. Duraturo F, Liccardo R, Izzo P. Coexistence of MLH3 germline variants in colon cancer patients belonging to families with Lynch syndrome-associated brain tumors. J Neurooncol 2016; 129(3): 577-8.
9. Duraturo F, Liccardo R, Cavallo A, et al. Association of low-risk MSH3 and MSH2 variant alleles with Lynch syndrome: probability of synergistic effects. Int J Cancer 2011; 129(7): 1643-50.
10. InSiGHT (The International Society for Gastrointestinal Hereditary Tumours Incorporated) 1985. http://www.insight-group.org/
11. Sen SK, Han K, Wang J, et al. Human genomic deletions mediated by recombination between Alu elements. Am J Hum Genet 2006; 79(1): 41-53.
12. Duraturo F, Cavallo A, Liccardo R, et al. Contribution of large genomic rearrangements in Italian Lynch syndrome patients: characterization of a novel alu-mediated deletion. Biomed Res Int 2013; 2013: 219897.
13. Liccardo R, De Rosa M, Rossi GB, et al. Characterization of novel, large duplications in the MSH2 gene of three unrelated Lynch syndrome patients. Cancer Genetics 2018, 221: 19-24
14. van der Klift H, Wijnen J, Wagner A, et al. Molecular characterization of the spectrum of genomic deletions in the mismatch repair genes MSH2, MLH1, MSH6, and PMS2 responsible for hereditary nonpolyposis colorectal cancer (HNPCC). Genes Chromosomes Cancer 2005; 44(2): 123-38.
15. Lynch HT, Lanspa S, Shaw T, et al. Phenotypic and genotypic heterogeneity of Lynch syndrome: a complex diagnostic challenge. Fam Cancer 2017 Oct 25.
16. Liccardo R, De Rosa M, Duraturo F. Same MSH2 Gene Mutation But Variable Phenotypes in 2 Families With Lynch Syndrome: Two Case Reports and Review of Genotype-Phenotype Correlation. Clin Med Insights Case Rep 2018 Jan 23; 11: 1179547617753943.
17. Yamamoto H, Imai K. Microsatellite instability: an update. Arch Toxicol 2015; 89(6): 899-921.
18. Liccardo R, De Rosa M, Rossi GB, et al. Incomplete Seg-

regation of MSH6 Frameshift Variants with Phenotype of Lynch Syndrome. Int J Mol Sci 2017; 18(5).
19. Li L, Hamel N, Baker K, et al. A homozygous PMS2 founder mutation with an attenuated constitutional mismatch repair deficiency phenotype. J Med Genet 2015; 52(5): 348-52.
20. Smith MJ, Urquhart JE, Harkness EF, et al. The Contribution of Whole Gene Deletions and Large Rearrangements to the Mutation Spectrum in Inherited Tumor Predisposing Syndromes. Hum Mutat 2016; 37(3): 250-6.
21. Park JG1, Vasen HF, Park KJ, et al. Suspected hereditary nonpolyposis colorectal cancer: International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC) criteria and results of genetic diagnosis. Dis Colon Rectum 1999; 42(6): 710-5.
22. Boland CR1, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998 15; 58(22): 5248-57.
23. Duraturo F, Liccardo R, Cavallo A, et al. Multivariate analysis as a method for evaluating the pathogenicity of novel genetic MLH1 variants in patients with colorectal cancer and microsatellite instability. Int J Mol Med 2015; 36(2): 511-7.
24. Brea-Fernández AJ, Cameselle-Teijeiro JM, Alenda C, et al. High incidence of large deletions in the PMS2 gene in Spanish Lynch syndrome families. Clin Genet 2014; 85(6): 583-8. doi: 10.1111/cge.12232. Epub 2013 Jul 28.
25. De Rosa M, Fasano C, Panariello L, et al. Evidence for a recessive inheritance of Turcot's syndrome caused by compound heterozygous mutations within the PMS2 gene. Oncogene 2000; 19(13): 1719-23.
26. Wimmer, Wernstedt A. PMS2 gene mutational analysis: direct cDNA sequencing to circumvent pseudogene interference. Methods Mol Biol 2014; 1167: 289-302.
27. Wernstedt A, Vatorta E, Armelao F, et al. Improved multiple ligation-dependent probe amplification analysis identifies a deleterious PMS2 allele generated by recombination with crossover between PMS2 and PMS2CL. Genes Chromosomes Cancer 2012; 51(9): 819-31.
28. De Rosa M, Rega D, Costabile V, et al. The biological complexity of colorectal cancer: insights into biomarkers for early detection and personalized care. Therap Adv Gastroenterol 2016; 9(6): 861-86.

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