

Identification and molecular characterization of a novel mutation in *MSH2* gene in a Lynch syndrome family

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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, including *MLH1*, *MSH2*, *MSH6*, *PMS2*, *MLH3* and *MSH3*. The molecular characterization of mutations in these MMR genes facilitates the pre-symptomatic diagnosis of subjects at risk to develop a colon cancer or a cancer LS-related. *Methods:* DHPLC and direct sequencing were performed for the mutation detection analysis. *Results:* In this study, we identified a novel frame shift mutation, the named is c.170delT in *MSH2* gene that determined a premature stop codon and consequently, the formation of a truncated protein (p. Val56Glyfs*7). This is a novel mutation, as it has not been reported before in the international scientific literature. This mutation was found in two subjects (father and son) belonging to a LS family. However, they showed a different phenotype disease. *Conclusion:* In this study, we identified and characterized a novel *MSH2* mutation; moreover, this study reaffirmed the importance of genetic testing in Lynch syndrome.

Key words: Lynch syndrome, HNPCC, *MSH2* gene, frame-shift mutation, novel variant *MSH2* gene

Introduction

Colorectal cancers are frequent causes of death in the world (1). Although most cases are sporadic, up to 5-6% develops in the context of gastrointestinal hereditary syndromes (2). Some syndromes are inherited in mendelian manner as they are due to alteration in a specific gene. These syndromes include familial adenomatous polyposis (FAP), hereditary non-polyposis colorectal cancer (HNPCC), PTEN hamartoma tumor syndrome (PHTS) and Peutz-Jeghers (3-7). With genetic testing for detection the mutation responsible of disease, it is possible to make differential diagnosis among these syndromes and to perform a pre-symptomatic diagnosis identifying the carriers of specific

mutation in family at high colon cancer risk. Thus, DNA testing may change the individual's presumed risk status and affect decision-making by patients and their physicians regarding surveillance and management (8,9).

The HNPCC, also known as Lynch syndrome (LS) is inherited as dominant autosomal. The Lynch Syndrome is characterized by onset of colon cancer on average around 45 years as well as an increased risk of developing extra-colonic tumors such as endometrial cancer, ovarian, stomach, urinary and biliary tract (10,11). Identification of families affected by LS occurs by the Amsterdam Criteria (AC) and Bethesda guidelines (BG) (12, 13). LS is associated with mutations in MisMatch Repair (MMR) genes. Most of

mutations were found in the *MLH1* and *MSH2* genes that account for about 50% and 40% respectively of all mutations reported; only 10% of mutations were identified in the *MSH6* and 5% in *PMS2* (14, 15); a low percentage of mutations were identified in *MLH3* (16) gene and only one variant in *MSH3* gene was associated with LS phenotype (17). Mutations are distributed heterogeneously along each *MMR* gene, denoting the absence of “hot spot” mutations. The most frequently detected pathogenetic variants in *MLH1* and *MSH2* are small insertions/deletions or large genetic rearrangements (large deletions/insertions) that, at protein level, result in premature stop codon formation (18–20). Each mutation in one *MMR* genes is considered pathogenetic if determines the loss or malfunction of *MMR* complex. The loss of function of one *MMR* protein prevents to repair's complex to work properly and this determines a genetic instability known as microsatellite instability (MSI) at somatic level (21). At somatic level the MSI is detectable by immunohistochemistry (IHC) analysis (22).

In this study, we reported a novel frameshift mutations in *MSH2* gene that was identified in two affected subjects belonging to a family with Lynch syndrome.

Materials and methods

Patients

Our probands are a 20-year-old male (n. 1440) and his father with age of 49 years (n. 1439). The first developed a left colon cancer and two dysplastic adenoma at 19 years of age, while his father developed a left colon cancer at 44 years of age. The probands' family history was also positive for colorectal and extra-colonic cancer. A detailed pedigree is shown in Figure 1.

Furthermore, as negative controls we collected 100 healthy samples from Clinical Department of Laboratory Medicine of our Hospital (Federico II of Naples).

Sample from our patient was collected after being granted authorisation from our local Ethic Committee “Comitato etico per le attività Biomediche - Carlo Romano” of the University of Naples Federico II (proto-

col number 120/10). Once the authorisation has been obtained the study has received ethical approval, and participant' informed and written consent has been obtained.

Mutation analysis: Isolation of genomic DNA, amplification, dHPLC and sequencing

The genomic DNA was extracted from peripheral blood lymphocytes. Total genomic DNA was extracted from 4 mL peripheral blood lymphocytes using a BACC2 Nucleon Kit (Amersham Life Science).

All *MLH1* and *MSH2* exons were amplified, including intron-exon boundaries, on DNA extracted from blood lymphocytes of our patient, using customized primer sets (available on request). Prior to dHPLC analysis, the PCR products were run on an 1–2% agarose gel to check for unspecific amplicons. A Transgenomic Wave DNA Fragment Analysis System (3500 HT) was used to perform dHPLC analysis (Transgenomic Inc., Omaha, Nebraska, USA) using personal methods, available on request; subsequently, genomic DNA was re-amplified and sequenced in both the forward and reverse directions using an ABI 3100 Genetic Analyser (Applied Biosystems, Foster City, Ca., USA).

Results

In this study, we have analyzed the DNA of our patients, n.1439 and n.1440. All *MLH1*, *MSH2*, exons were analysed by DHPLC; subsequently, we only sequenced exonic fragments that showed an abnormal chromatogram. In this manner, we identified a novel mutation in the *MSH2* gene that determined a nucleotide deletion (T) in position c.170. This mutation that is named c.170delT, determined a frameshift with premature stop codon and consequently, the formation of a truncated protein (p. Val56Glyfs*7). This mutation has not been reported before in the international database of INSIGHT-Group (20) and it was not detected in 100 healthy controls analyzed. Therefore, this mutation is considered as pathogenetic. No large rearrangements in *MLH1* and *MSH2* genes were identified in our patients.

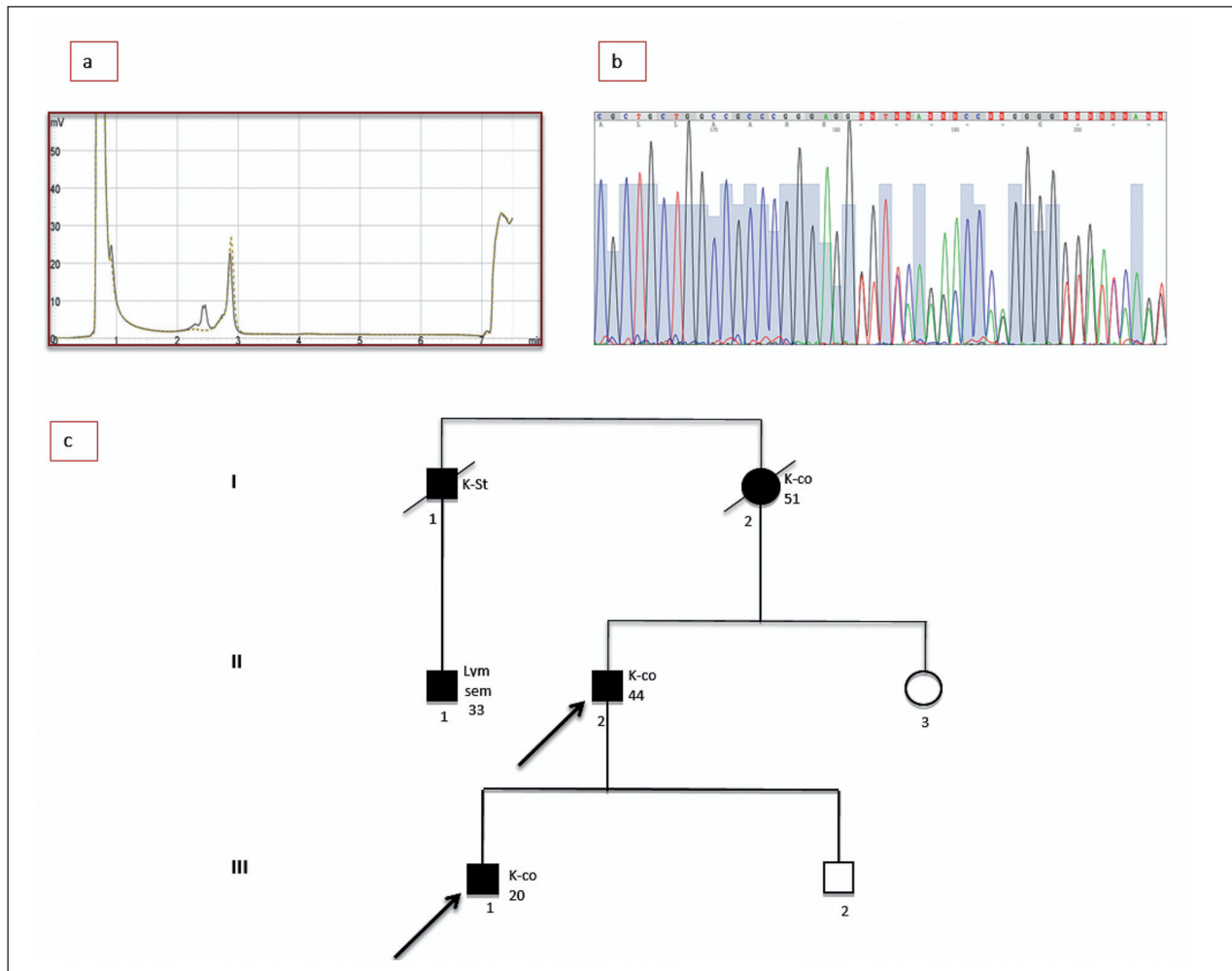


Figure 1. A. and B. Chromatogram and electropherogram showing the novel mutation identified in exon 1 of *MSH2* gene, c.170delT; C. Family pedigrees of our patients with a novel *MSH2* gene mutation. Symbols and abbreviations used are denoted as follow: arrows, index case; black symbol, colorectal cancer or tumors associate with LS; Co, colon cancer; St, stomach cancer; Lym, lymphoma; Sem, seminoma. Number next to diagnosis denote age at onset

Discussions

In this study, we report the results of the detection mutation analysis of a family with Lynch Syndrome. The mutation identified, the c.170delT is a novel frameshift variant that causes the formation of a premature stop codon and hence of a truncated protein. Two subjects belonging to LS family were to carrier of this novel mutation in the *MSH2* gene. Both subjects showed a MSI-H status on DNA extracted from tumoral tissue embedded paraffin and loss of expression of *MSH2* on tissue detected by immunohistochemistry

analysis (data not shown). Therefore, the *MSH2* mutation c.170delT is, surely responsible of LS-phenotype in these patients and likely, in remaining affected subjects of family. These patients (father and son) were both affected by left colon cancer; however, the father, a 49-year-old patient developed an adenocarcinoma in left colon diagnosed at the age of 44 years and, the son developed an adenocarcinoma in left colon diagnosed at the age of 20 years. Hence, this novel mutation was associated with a phenomenon of generational anticipation in this family (23). So far, no clear correlation genotype-phenotype were described for LS families

(24), therefore, we could only speculate that modifier genes could be responsible of this generational anticipation (25). Moreover, this family's history was also positive for colorectal and extra-colonic cancer, Fig. 1. In particular, the cases of extracolonic cancers were a lymphoma and a seminoma onset simultaneously, in a young relative of our probands (II-1, Fig. 1) and these two infrequent cancers in LS (11). Unfortunately, we were not able to performed genetic testing for this subject due to his limited availability.

In conclusion, this study remarks the phenotypic heterogeneity of LS and it enlarges the spectrum of *MSH2* mutations. Moreover, this study reaffirms the importance to identify pathogenic mutations in LS families to facilitate pre-symptomatic diagnosis order to personalize the program of endoscopic surveillance for mutation carrier subjects, in particular in LS families whose the phenomenon of generational anticipation is present.

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