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SHORT COMMUNICATION

Whole-exome sequencing identified a novel mutation of *MLH1* in an extended family with lynch syndrome

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Abstract Hereditary nonpolyposis colorectal cancer or Lynch syndrome is autosomal dominant cancer predisposition syndrome characterized by early onset of colorectal cancer and neoplasia in other organs. This condition typically caused by germline mutations in the mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*. To date, a considerable number of *MLH1* gene mutations have been found to be associated with Lynch syndrome. We were aimed at identifying a genetic mutation in an extended Iranian family affected by Lynch syndrome-related cancers. Here, we applied whole-exome sequencing to identifying mutation in the proband. Furthermore, we applied Sanger sequencing to validate the candidate variant. We found a heterozygous novel single nucleotide deletion (c.206delG) in the exon two of the *MLH1* gene in the proband. Also, Sanger sequencing analysis showed that this mutation has segregated in

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all affected family members. The mutation (c.206delG:p.R69fs) may create a premature stop codon followed by the formation of a truncated (p.R69fs) Mlh1 protein. Our findings expand the mutational spectra of *MLH1* gene related Lynch syndrome which is vital for screening and genetic diagnosis of the disease.

Introduction

In Iran, gastrointestinal cancers are responsible for 57% of all diagnosed neoplasia.[1] In Iran, the incidence of gastrointestinal cancer significantly increased. Based on the new evidence, the incidence of colorectal cancer (CRC) is 14.6 per 100,000 males and 11.1 per 100,000 females per year in Iran.[2] The most common familial colorectal cancer syndrome is Lynch syndrome (LS).[3] The LS is a genetically heterogeneous autosomal dominant syndrome with an increased risk of CRC.[4] Moreover, individuals with LS have a significant increased risk for extra-colonic neoplasia, including cancers of the endometrial, small bowel, stomach, upper urologic tract, ovary, biliary tract, and brain tumors.[5]

Most often the disease is due to a germline mutation in one of the four MMR genes including *MSH2*, *MLH1*, *MSH6*, and *PMS2* accounting for 40—60%, 40—50%, 10—20%, and 2% of LS cases, respectively.[6,7] The synthesis of shorter, nonfunctional proteins may occur as a result of nonsense, splice-site, or frameshift alterations, all of which are often identified as constitutional defects.[8] High frequency of insertion or deletion type somatic mutations within microsatellite repeats is also the characteristic of MMR gene mutations which led to the development of LS-associated tumors.[8] The diagnosis of LS is relying on clinical, pathological, and genetic findings.[9] Accordingly, detection of LS-induced mutations is important for clinical surveillance in carriers and genetic testing for relatives in high-risk families.[10]

The *MLH1* gene is composed of 19 exons, and encode a 756-amino acid protein named Human mutL homolog 1 (Mlh1), which is involved in the mismatch repair process.[11,12] A large body of evidence suggested this gene was characterized as a locus mostly mutated in LS.[7,13]

In this study, we employed whole-exome sequencing (WES) and Sanger sequencing to investigate genetic mutation caused LS in a family with 17 CRC cases, a breast cancer case, an endometrium cancer case and a case with lung cancer.

Methods and methods

Study participants and clinical evaluation

The family pedigree is depicted as Fig. 1. We obtained blood samples from alive affected individuals and healthy family members. Subjects' medical records were reviewed. LS diagnosis was established according to Amsterdam clinical criteria.[14] The study protocol was approved by the Ethical Committee of the Semnan University of Medical Sciences and the study participants gave informed consent.

Whole-exome sequencing

We solely performed WES for the proband. For DNA extraction from peripheral blood, a standard salting out method was used. The patient DNA was captured using Agilent SureSelect Human All Exome Kit V6 (Agilent Technologies, Santa Clara, CA, USA) and thence sequenced in Illumina HiSeq 4000 machine (Illumina, San Diego, CA, USA) as stated by the manufacturer's protocols. The average read depth was more than 100 × and depth of 20 × or greater was achieved in 98.0% of the targeted genomic sequence.

Mutation filtering and annotation

Sequenced reads were trimmed for adaptor sequences, and masked for low-complexity or low-quality sequencing. Then, we mapped reads to the hg19 whole genome using the mem algorithm from the BWA aligner. In order to variant calling, we used the tools mentioned in the GATK Best Practices pipeline (http://www.broadinstitute.org/gatk/). Finally, for variant annotation, we used the ANNOVAR tool.[15]

Mutation validation and co-segregation analysis

The mutation identified by WES in *MLH1* gene was confirmed in the proband. Moreover, the mutation was segregated with LS in all the family members with LS manifestation and none of the healthy individuals. The primers were designed by Primer 3 — the sequences of the primers will be supplied upon request. To validate the candidate mutation we used the ABI 3130 Genetic Analyzer (ABI, California, USA) machine to determine sequences of the polymerase chain reaction products.

Result

Clinical data

We have enrolled an Iranian family with LS from Sari city (Mazandaran province, Iran). The proband (III-10), is a 45-year-old man diagnosed with CRC. His personal medical

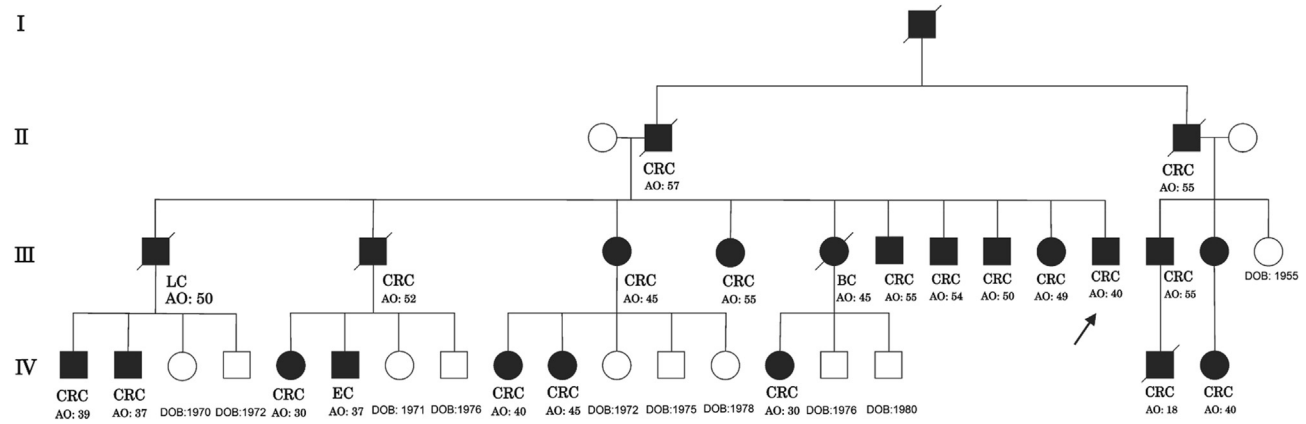

Figure 1 Pedigree of the family. The index case is the first person in the family in which the genetic alteration predisposing to LS was detected—is a 45-year-old male. CRC; colorectal cancer, LC; lung cancer, BC; breast cancer, EC; endometrium cancer, AO; age of onset.

history revealed that he has undergone partial colon resection for a CRC in transverse colon diagnosed at the age of 40 years. Akin to the proband, in most of cases, primary manifestation was CRC. However, family history was also positive three extra-colonic cancers including cancers of the lung (III-1), breast (III-5) and endometrium (IV-6).

Genetic analysis

Genetic sequence analysis detected a novel, heterozygote deletion at c. 206 (NM_000249) in exon 2 of the *MLH1* gene (Fig. 2). The mutation is predicted to be pathogenic by introducing a premature stop codon into the sequence and finally the formation of truncated Mlh1 protein. Further, genetic analysis of members of the family showed segregation of the mutation with disease status (Fig. 3). These observations suggest that NM_000249 (*MLH1*):c.206delG mutation could be the cause of the progress of LS.

Discussion

Lynch syndrome is the most common autosomal dominantly inherited cancer syndrome and affects less up to 5% of all CRC cases.[16] It is associated with highly penetrant germline mutations in MMR genes, which increase the risk of colorectal cancer and also a range of extra-colonic cancers.[16] To identify families with autosomal dominantly inherited CRC without a polyposis phenotype, the Amsterdam criteria were proposed. Nevertheless, these criteria have limited sensibility and specificity since 40% of families with a pathogenic mutation in MMR gene do not justify the Amsterdam criteria and virtually 50% of families who meet the Amsterdam criteria do not have a detectable pathogenic variant in the MMR genes.[17] Nowadays, the most reliable approach to diagnose LS is to detect a variant in the MMR genes in the suspected case.[18] To date, more than 3000 pathogenic mutations identified in the MMR genes and submitted into the ClinVar database, most of them in the *MLH1* and *MSH2* genes.

In this study, we report the findings of the detection mutation analysis of an extended Iranian family with LS. The mutation detected, the NM_000249 (*MLH1*):c.206delG is a novel frameshift mutation that results in the formation of a pre-mature stop codon and therefore of a truncated protein. A total of 17 individuals belonging to the LS family was diagnosed to be a carrier of the mutation in the *MLH1* gene. Considering the family's medical records, the proband reported showing loss of *MLH1* expression on tissue detected by immunohistochemistry analysis. Therefore, the NM_000249 (*MLH1*):c.206delG mutation is surely responsible for LS-phenotype in the proband and likely in remaining affected subjects of family. Unfortunately, there was no report on microsatellite instability (MSI) status in tumoral tissues. This precluded us to investigate the correlation of the mutation with MSI.

We noticed a significant decreased in the age of onset for LS in the youngest generation members (IV). In the family member II-1, the primary manifestation of LS was reported at the age of 57 years. In contrast, the patient IV-17 was diagnosed to have LS at the age of 18 year and died due to CRC at the age of 20 year. This may imply that the novel mutation was associated with generational anticipation in this family. Up to now, a very limited genotype—phenotype correlation was proposed for LS families.[19] So we could only speculate that modifier genes may also explain at least part of the observed anticipation.

Additionally, the family history was also positive for extra-colonic cancers. Particularly, the case of extra-colonic cancers was lung cancer in the III-1individual. Lung cancer is regarded as infrequent cancer in LS.[20,21] Unfortunately, no DNA sample was available for this individual and hence it was not possible to perform genetic testing.

In conclusion, our study emphasizes the phenotypic heterogeneity of LS and it expands the spectrum of *MLH1* mutations. Identification of LS-causing MMR gene mutations may be beneficial for surveillance and management in at-risk relatives.[18] For a better understanding of the

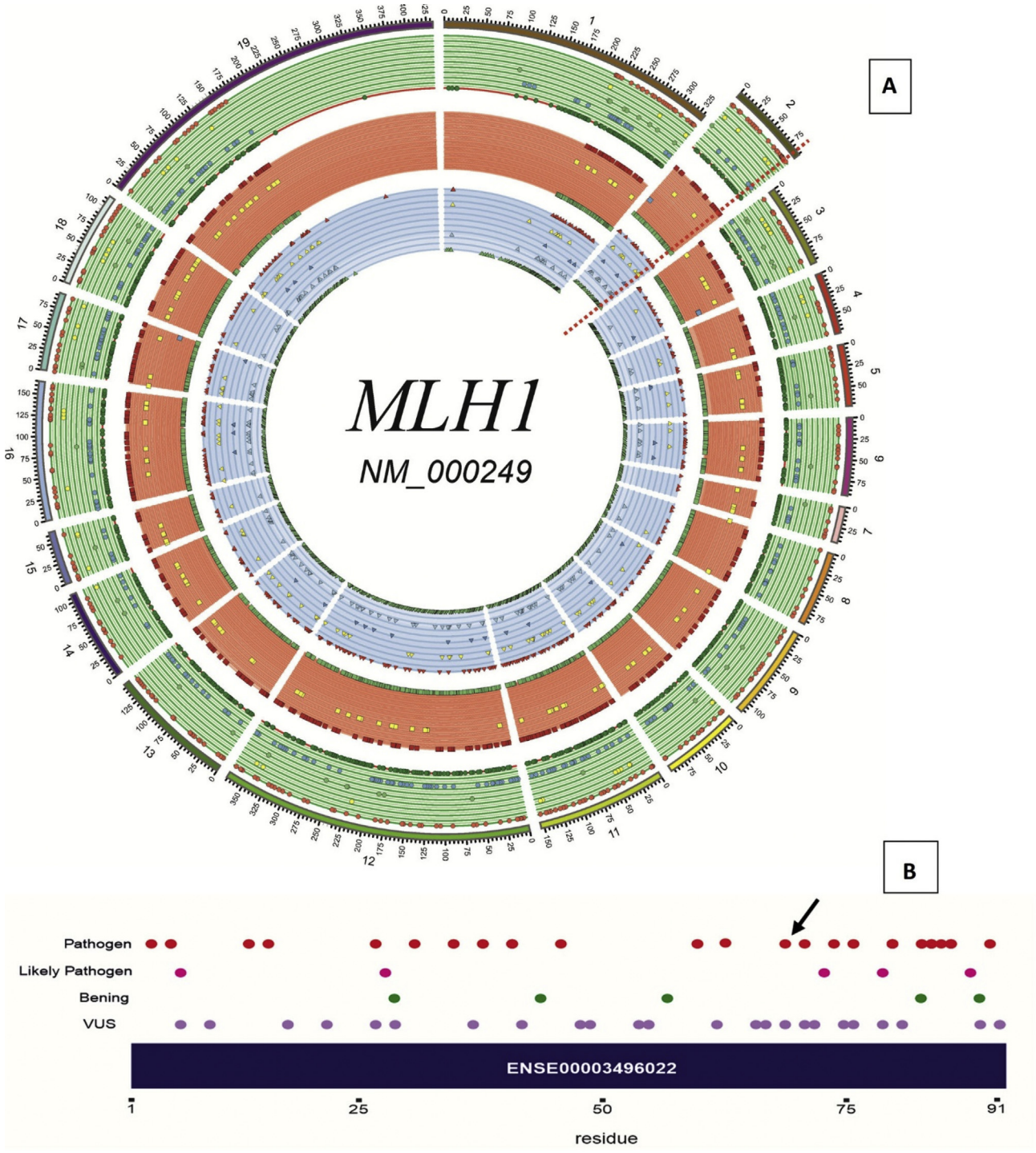

Figure 2 Circular visualization of mutational spectrum in the MLH1 exons. (A) A dashed red line indicates the position of NM_000249 (MLH1):c.206delG. The green blocks are reserved to illustrate clinical significance of MLH1 mutations. In these blocks, red circles represent "Pathogenic " variants, yellow circles represent "likely pathogen" variants, light green circles represent variants with "Conflicting interpretations of pathogenicity", blue circles represent "Benign" and "Likely benign" variants, and dark green circles indicate "uncertain significance" variants. The red blocks are used to illustrate molecular consequences of MLH1 mutations. In these blocks, dark red rectangles represent "Frameshift" variant, yellow rectangles represent "Nonsense" variant, light blue rectangles represent "Splice site" variants and green rectangles represent "Missense" variants. The blue blokes (most inner) are showing type of MLH1 mutations. In these blocks, dark red rectangles represent "Deletion" variants, yellow rectangles represent "Insertion" variants, dark blue rectangles represents "Indel" variants, light blue rectangles represent "Duplication" variants, and rectangles indicate "Single nucleotide" variants.(B) mutation landscape of MLH1 exon 2.

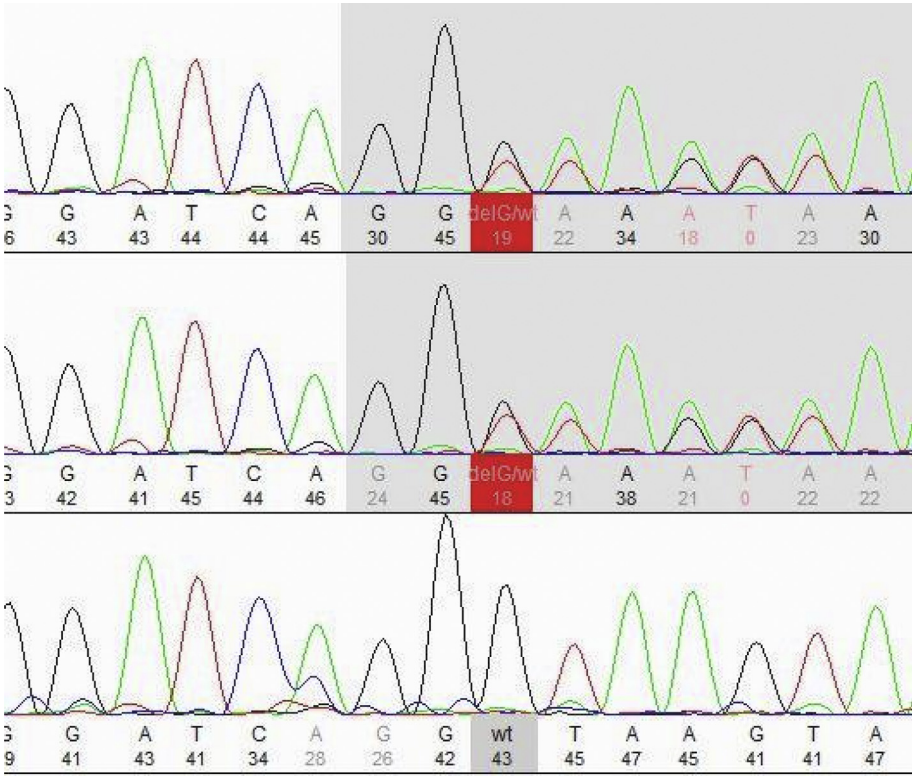

Figure 3 Sanger DNA sequencing chromatogram demonstrates NM_000249 (MLH1):c.206delG segregation with the disease status in the family. Upper, middle and lower panels are chromatograms of an affected mother (III-3), her affected daughter (IV-10), and her healthy daughter (IV-11). IV-3, IV-4 and IV-13 were sequenced but no mutations were found in the samples.

molecular genetics causes of the disease in patients with LS, the WES appeared as a promising approach.

Conflict of interest

The authors declared there is no conflict of interest.

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