SHORT COMMUNICATION

Whole-exome sequencing identified a novel mutation of \textit{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 \textit{MLH1}, \textit{MSH2}, \textit{MSH6}, and \textit{PMS2}. To date, a considerable number of \textit{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 \textit{MLH1} gene in the proband. Also, Sanger sequencing analysis showed that this mutation has segregated in

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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.7 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, non-functional 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

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
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. 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. 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. Nowadays, the most reliable approach to diagnose LS is to detect a variant in the MMR genes in the suspected case. 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. 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-1 individual. Lung cancer is regarded as infrequent cancer in LS. 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. For a better understanding of the
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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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