Case Report

A novel frameshift mutation in the *MLH1* gene in a patient with Lynch syndrome

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Abstract

A novel mutation in the *MLH1* gene likely to be pathogenic for Lynch syndrome was discovered in a proband with a family history of colon cancer. Immunohistochemistry showed negative expression of *PMS2* and *MLH1* in the resected tumor sample. The mutation lies at the highly conserved C-terminus of the *MLH1* protein, the region through which it dimerizes with *PMS2* to carry out its mismatch repair function.

Key Words: Hereditary colon cancer, Lynch syndrome, *MLH1* variant, MMR genes, MutLα

Introduction

Approximately 2%-5% of all colorectal cancers arise from a defined inherited cancer syndrome.[1] Of these, the Lynch syndrome is caused by germline mutations in DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*. Lynch syndrome predisposes to extracolonic malignancies involving the endometrium, stomach, ovaries, small bowel, hepatobiliary tree, urological system, and brain.[2] Immunohistochemistry (IHC) staining provides a rapid, cost-effective way for assessing the protein expression of the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes. Using a lack of staining of any of the MMR proteins, the germline testing can be targeted to one particular gene.[3] Some authors advocate the routine screening of colorectal cancer specimens for microsatellite instability (MSI) testing through PCR and for MMR expression through IHC staining.[4,5]

MLH1 heterodimerizes with PMS2 to form MutLα, which binds to MutSα (MSH2-MSH6) or MutSβ (MSH2-MSH3), both of which are ATPases which play a critical role in mismatch recognition and initiation of repair.[6]

Case Report

A 50-year-old woman initially evaluated for an enlarged gland in the neck underwent a computed tomography scan of the chest and abdomen, during which a circumferentially thickened enhancing wall of the hepatic flexure of colon was detected. Colonic biopsy from hepatic flexure showed a well-differentiated adenocarcinoma with histological features suggestive of mild to moderate microsatellite instability. The proband had a strong family history of cancer at ages ranging from 20 to 52 years, most of which were carcinoma of the colon [Figure 1]. A section of the resected tumor was sent for testing of the MMR genes *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* expression through immunohistochemistry (IHC) staining. The expression of both *MLH1* and *PMS2* proteins was found to be negative in the tumor sample [Figure 2]. Real-time Polymerase Chain Reaction (PCR) testing of the *RAS* genes was positive for *KRAS* (A146X), while no mutations were detected in *BRAF* and *NRAS*.

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Website: www.indianjcancer.com

DOI: 10.4103/ijc.IJC_349_18

In total, 10%-15% of tumors exhibiting MSI and negative expression of MMR genes are sporadic. A negative expression of *MLH1* observed in IHC is also attributed to a somatic hypermethylation of its promoter. Mutations in the *BRAF* exon 15, especially the V600E mutation, are strongly associated with a sporadic origin.[7] The proband tested negative for the V600E mutation, indicating a germline origin of the cancer. Although the negative predictive value of a *BRAF* mutation for promoter methylation of *MLH1* is poor,[8] the patient met the revised Bethesda guidelines as well as the Amsterdam criteria for screening for lynch syndrome, which combined with a negative *BRAF* mutation test was considered sufficient to recommend germline mutational analysis.

A germline mutation panel for genes associated with commonly inherited cancers was carried out using next-generation sequencing using a standard v2 kit on Illumina MiSeq, with expected data output of 4-5 GB. Besides the MMR genes, the panel included *APC*, *BMPR1A*, *CDH1*, *CHEK2*, *MUTYH*, *PTEN*, *SMAD4*, *STK11*, *TP53*, *BRCA1*, *BRCA2*, *MEN1*, *NF2*, *RB1*, *RET*, *SDHAF2*, *SDHB*, *SDHC*, *SDHC*, *TSC1*, *TSC2*, *VHL*, and *WT1*. Trimmed FASTQ files were generated using MiSeq Reporter from Illumina and aligned against the whole genome build hg19.

Discussion

A novel germline variant NC_000003.11: g. 37092135_37092136 del GA (p. Arg755Val fs) was detected in exon 19 of the MMR gene *MLH1* in this patient. She was heterozygous for this frameshift variant, which is predicted to elongate the open reading frame of the protein [Figure 3]. Because the variant lies in the vicinity of pathogenic variants associated with Lynch syndrome, it has been labeled as "variant of unknown significance with probable damaging effects." One known deletion of reported pathogenicity NC_000003.11: g.37092135_37092136 del G (pArg755Glyfs*28) elongates the C-terminus of *MLH1* by 28 amino acids.[9] The *MLH1* database[10] reports

How to cite this article: Pandey AS, Shrestha SK. A novel frameshift mutation in the *MLH1* gene in a patient with Lynch syndrome. Indian J Cancer 2018;55:410-2.

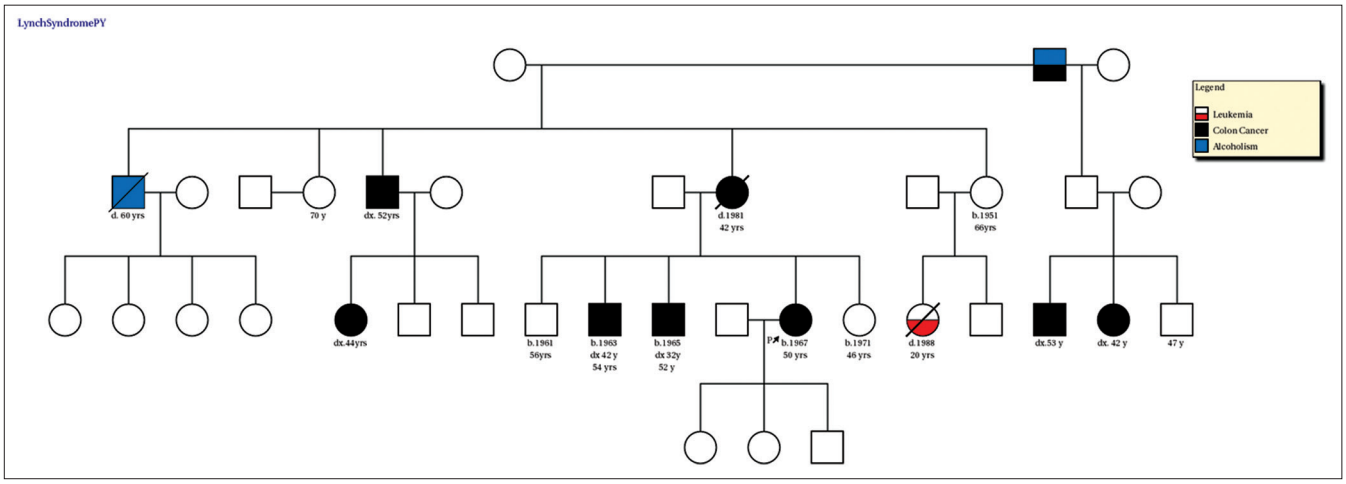

Figure 1: Pedigree chart of patient's family showing strong family history of colon cancer (black) and one first cousin with leukemia (red)

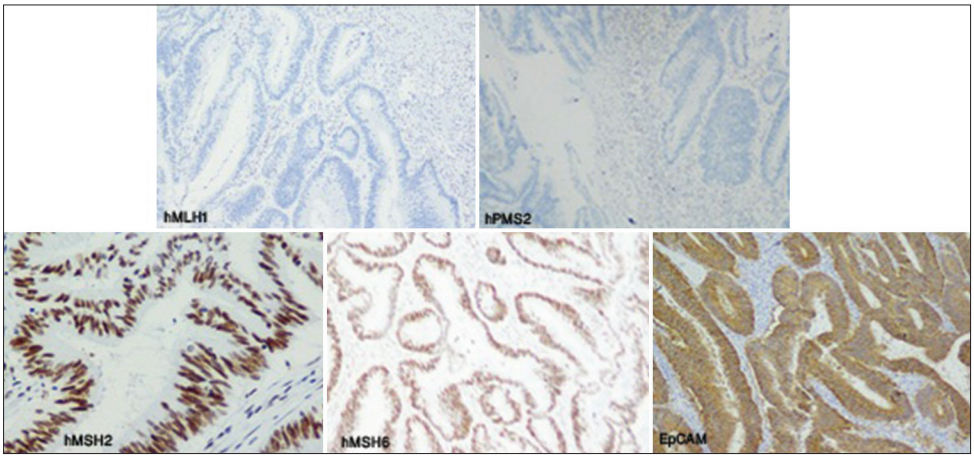

Figure 2: Immunohistochemistry testing for the MMR genes showing negative expression (top) for *MLH1* and *PMS2*. *MSH2*, *MSH6* and *EpCAM* protein expression was normal (below)

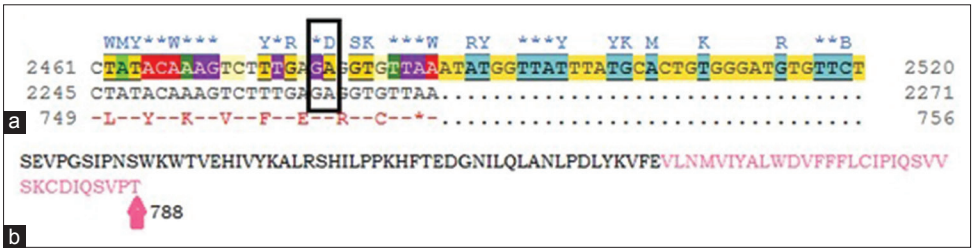

Figure 3: (a) Position of the GA (2262, 2263) that was deleted on the gene, complementary DNA (cDNA), and the protein. (b) The extension of the C-terminal domain as a result of the frameshift is shown in pink

6979 variants, 984 of which are deletions, of which 33 are in exon 19. Roughly half of all Hereditary Non-Polyposis Colon Cancer (HNPCC) mutations are known to lead to MutLα alterations.[11]

A negative expression of *MLH1* in IHC indicates the presence of a mutation in the *MLH1* gene. However, negative expression of *PMS2* can occur due to the absence of *MLH1*. This is because MLH1 binds to PMS2 to form a catalytically functional and correctly localized heterodimer called MutLα.[12,13] Constitutive dimerization of MLH1 with PMS2 occurs via their C-terminal domains (CTDs).[14,15]

The MLH1-PMS2 dimerization interface is located in the CTD, mutations (e.g., p. Gln542Leu, pLeu749Pro, and pTyr750X) in which cause decreased co-expression of PMS2 due to its decreased stability in the absence of interaction with MLH1.[16] The CTD of MLH1 is highly conserved in eukaryotes, in particular the last four invariant residues FERC, which in MLH1 from *Homo sapiens*, constitute residues 753-756. These residues have been shown to form one of the patches on the protein surface that is involved in forming a dimer with PMS1 in *Saccharomyces cerevisiae*.[17] The MLH1-PMS1 heterodimer of *S. cerevisiae* is the MutL homolog of MLH1-PMS2 in humans. In addition, PMS1 shares high sequence identity with human PMS2 (hPMS2), particularly the conserved DQHA (X2) E(X4) E motif of the CTD, which is essential for the endonuclease activity of the MutL complex.[18,19] The C-terminal cysteine residue of

MLH1 is also involved in forming the PMS1 endonuclease site.[15] In the presented case, the FERC terminus is lost with the replacement of Arg755 and Cys756 by a valine and leucine respectively, followed by an extension of 34 amino acids [Figure 3]. The 34 amino acid structure is predicted to form a helix-helix-coil secondary structure using Advanced Protein Secondary Structure Prediction Server.[20] This gives a strong indication that the CTD of MLH1 in this case is unable to associate with PMS2 to form a functional heterodimer to carry out its activity of repairing any mismatches in the DNA.

The patient was counseled about the implications of a yet un-established variant for colon cancer and advised to have other non-affected siblings and children tested for the same. The family was screened for the presence of *Helicobacter pylori* as per the National Comprehensive Cancer Network (NCCN) guidelines for Lynch syndrome, and was found to be negative. The family has decided to postpone the genetic testing for the mutation in unaffected members to near future.

Conclusion

The absence of both *MLH1* and *PMS2* expressions indicates a lack of association of MLH1 and PMS2 to form a functional MutLα endonuclease to carry out the MMR activity. The NC_000003.11: g.37092135_37092136 del GA mutation discovered in this case would result in an extension of the C-terminus of the MLH1 protein, interfering with the formation of the MLH1-PMS2 heterodimer, which could be causative for colon cancer due to faulty MMR.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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