CASE REPORT

Identification and verification of a pathogenic MLH1 mutation c.1145dupA in a Lynch syndrome family

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Abstract: Lynch syndrome (LS), an autosomal-dominant disorder with an increased risk of predominantly colorectal and endometrial cancers, is caused by germ-line mutations in mismatch repair genes. The identification of germ-line mutations that predispose to cancer is important to further our understanding of tumorigenesis, guide patient management and inform the best practice for healthcare. A 45-year-old woman with atypical endometrial hyperplasia who suffered colon cancer at the age of 30 years underwent hysterectomy and genetic counseling. Pedigree analysis revealed her family fulfilling the Amsterdam I criteria. Next-generation sequencing was offered to the patient. A mutation in the MLH1 gene, c.1145dupA, was identified and verified by Sanger sequencing. In addition, her nine family members were tested for the mutation. Two were affected (colon cancer at the age of 43 years and 45 years) and one healthy relative carried the same mutation in the MLH1 gene. The mutation resulted in a frame-shift (p.Met383Aspfs*12) located in exon12, as well as a polypeptide truncation of 393 amino acids by the formation of a premature stop codon. An immunohistochemistry analysis of endometrial hyperplasia tissues revealed defects in MLH1 and PMS2 protein expression in the patient. Based on the 2015 American College of Medical Genetics and Genomics (ACMG) guideline, we report this MLH1 c.1145dupA variation to be a pathogenic mutation that contributes to a strongly increased cancer risk in this LS family. Proper screening suggestions were offered to the three affected patients and the healthy carrier. To the best of our knowledge, this germ-line mutation of MLH1 was previously submitted to the Leiden Open Variation Database (LOVD) database, but no comprehensive evidence or supporting observations were reported previously in the literature. The present report found a single nucleotide insertion in exon12 of the MLH1 gene, which can be considered causative of Lynch phenotype. Moreover, identification of individuals at risk for hereditary syndromes is important, as they can benefit from genetic counseling and increased surveillance.

Keywords: Lynch syndrome; MLH1 mutation; atypical endometrial hyperplasia; colon cancer


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Introduction

Lynch syndrome (LS), an inherited cancer predisposition syndrome with a substantially increased risk of colorectal and endometrial cancer, along with increased risk of malignant ovarian, gastric, small bowel, brain, hepatobiliary, pancreatic, kidney, bladder, urothelial, prostate, and breast tumors, is caused by germ-line mutations in mismatch repair (MMR) genes MLH1, MSH2, MSH6, and PMS2[11–11]. The majority of Lynch syndrome cases (69%–90%) arise from germ-line MLH1 and MSH2 mutations; carriers of MSH6 and PMS2 mutations account for the rest[12–15].

DNA mismatch repair proteins are critical for maintaining the fidelity of DNA replication and preserving genome integrity. They are involved in numerous cellular
functions including (1) repairing DNA synthesis errors, (2) repairing double-strand DNA breaks, (3) apoptosis, (4) anti-recombination, and surprisingly, (5) destabilization of DNA. When MMR is lost or defective, there is a decrease in apoptosis, an increase in cell survival, and a potential increase in damage-induced mutagenesis. This can provide a selective growth advantage to the cell, thus causing increased susceptibility to tissue-specific cancers.

MMR proteins function in a heterodimeric manner in which $MLH1$ and $MSH2$ are the pivotal players. $MSH2$ interacts with $MSH6$ (MutSa, predominant form) and $MSH3$ (MutSβ) to form the MutS complex, which recognizes DNA mismatches. $MLH1$ interacts with $PMS2$ (MutLa, predominant isoform), $PMS1$ (MutLβ) and $MLH3$ (MutLγ) to form the MutL complex, which is recruited by MutS and has endonuclease activity to catalyze a nick formation at the mismatch site. Hence, more pathogenic mutations have been observed in the $MLH1$ and $MSH2$ genes that are essential for formation of all the MutS and MutL homologue heterodimers. Lynch syndrome is occasionally associated with defects in other mismatch repair genes. The rarity of defects in these other MMR genes is probably a consequence of their functional redundancy [16,17].

$MLH1$ was first identified on human chromosome 3p21.3-23 by both Bronner et al. [18] and Papadopoulos et al. [19] groups in 1994. $MLH1$ is one of the most commonly mutated genes in Lynch syndrome, with a heterogeneous mutation spectrum that includes large deletions, non-synonymous and missense mutations, and insertions and deletions that result in frame-shift mutations.

Once the clinical criteria for LS are met, genetic testing of these genes is important in order to identify the mutation carriers that will benefit from specific screening, preventive measures, and therapeutic measures. Additionally, identifying non-carriers among additional family members permits these individuals to be released from intensive surveillance. However, sequence variants of unclear functional and medical consequences are common in genetic test reports, and for many variants, comprehensive data are lacking, especially missense mutations caused by single-base substitutions, whose potential for pathogenicity is much more difficult to predict [20]. These so-called unclassified variants (UVs) cannot be easily classified as either pathogenic or neutral before they have been subjected to a detailed analysis, posing a serious problem to the clinic [21].

The majority of mutations found in LS families have been nonsense/frameshifts predicted to cause protein truncation, which are unambiguously disease-causing [20]. Therefore, a five-tiered scheme for the standardized classification of unique mismatch repair gene variants are lodged on InSiGHT (The International Society for Gastrointestinal Hereditary Tumors) locus-specific database according to the following classes: pathogenic (class 5), probably pathogenic (class 4), no known pathogenicity (class 3), probably no pathogenicity (class 2), and effect unknown (class 1); these classes facilitate consistent management of suspected Lynch syndrome families. Through May 2016, MMR gene variants have been submitted to the InSIGHT colon cancer gene variant databases, including 1,198 unique $MLH1$ gene variants.

In this study, we report a pathogenic $hMLH1$ mutation, c.1145dupA, based on pedigree analysis, genetic testing by both next generation sequencing and Sanger sequencing, and immunohistochemical staining. To the best of our knowledge, this germ-line mutation of $MLH1$ was once submitted to the Leiden Open Variation Database (LOVD) database, but no clinical evidence or observation report has been published in the literature.

Materials and methods

Family data

The proband of the family (individual III:1), a 45-year-old woman, was diagnosed with atypical endometrial hyperplasia and received a hysterectomy. The patient had a history of ascending colon carcinoma with right hemicolectomy and chemotherapy at the age of 30. She had a family history of colon cancer. We collected her family’s (three generations) medical history and did a pedigree analysis. This study was approved by the Ethical Committee of Gynecology and Obstetrics Hospital of Fudan University and the patient provided consent to publication.

Immunohistochemistry (IHC)

Immunohistochemical staining of MMR proteins MLH1, MSH2, MSH6 and PMS2 was carried out for histological sections of the atypical endometrial hyperplasia of the proband (individual III:1).

Testing was performed on a Leica BOND-MAX detection system (Leica Biosystems; Wetzlar, Germany) using the following monoclonal antibodies: MLH1 (DAKO-ES05), PMS2 (DAKO-EP51), MSH2 (DAKO-FE11), and MSH6.
Identification and verification of a pathogenic MLH1 mutation c.1145dupA in a Lynch syndrome family

Nuclear labeling of MLH1, PMS2, MSH2, and MSH6 was considered as positive staining. Total loss of MLH1, PMS2, MSH2, or MSH6 staining in cancer cells was considered deficient, while positivity of at least partial cancer cells was considered intact expression.

DNA extraction and next-generation sequencing

Genomic DNA was extracted from peripheral blood samples of the family members using a ZR Genomic DNA™-Tissue MiniPrep (Zymo Research; Irvine, CA, USA) kit according to the manufacturer’s instructions. DNA from the proband (III:1) was subjected to next-generation sequencing-based genetic testing using a gene panel for inherited tumor susceptibility that included MLH1, PMS2, MSH2, MSH6, MSH3, and PMS1 to detect potential mutations. The detection panel is based on custom-designed Nimblegen probes (Roche; Basel, Switzerland), and sequencing was performed on the Illumina platform (HiSeq 3000 Analyzers; Illumina Inc.; San Diego, CA, USA). Raw data went through a standard informatics pipeline for quality control, read alignment, and variant calling. Tertiary analysis was performed with an in-house software to annotate the variants from the vcf file and integrate the information from multiple databases. The final variants were manually analyzed and evaluated for potential clinical significance. The assessment and classification of variants were based on the 2015 American College of Medical Genetics and Genomics (ACMG) standards and guidelines for the interpretation of sequence variants[22].

Sanger PCR sequencing analysis

The identified mutation site was validated in the proband and the other family members with Sanger sequencing (Applied Biosystems 3730XL; Thermo Fisher Scientific; Waltham, MA, USA). DNA samples were amplified using KAPA Taq Extra kit according to the manufacturer’s instructions. The DNA concentration was measured by spectrophotometry, and the quality was evaluated in 1.2% agarose gel electrophoresis before Sanger sequencing.

Sanger PCR sequencing analysis was performed for the validation of germ-line deletion mutation of the MLH1 gene under specific conditions and parameters. The primers used for the amplification of c.1145dupA of the MLH1 gene were forward primer 5’-AGACTTTGCTACCAGGACTT-3’ and reverse primer 5’-GTCAAGCATCTCCTCATCT-3’. The thermal cycle profiles were an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 58 °C for 15 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 3 min.

Case presentation

Pedigree analysis

The patient had colon cancer at the age of 30 and a precancerous disease (atypical hyperplasia) at the age of 45. She received hysterectomy and bilateral salpingectomy at the Obstetrics and Gynecology Hospital of Fudan University. Her mother (II:2) suffered from colon cancer at the age of 45 but received a hysterectomy because of hysteromyoma. Additionally, her maternal aunt (II:4) was diagnosed with colorectal cancer (CRC) at the age of 43. Moreover, her maternal grandfather (I:2), who had a history of several years of hematochezia, had died of hepatapostema at the age of 50 years (no histology nor pathology report were available). Her brother (III:2) was diagnosed with a rectal polyp at the age of 34. The other family members had not been reported for confirmed or suspected related malignant diseases. All family members are alive except her maternal grandparents. The family met the Amsterdam criteria I for LS[23]; three patients had early-onset colon cancer before the age of 50 years. The family pedigree is shown in Figure 1.

IHC analysis of MMR proteins and detection of mutation sites

After counseling of the proband and obtaining informed consent, testing for LS was performed. First, the atypical hyperplasia endometrial tissue was subjected to MMR protein immunochemistry staining. Results revealed defects in MLH1 and PMS2 protein expression, but no loss of MSH2 or MSH6 protein expression, in the proband (III:1) (Figure 2). Thus, MLH1 mutation was suspected. As PMS2 can be degraded by MLH1 deficiency, PMS2 mutation was less likely.

Then, the peripheral blood of the proband was subjected to genetic analysis using next-generation sequencing. The gene panel we chose covered all the coding regions and the exon/intron boundaries of the MMR genes. After sequencing and analysis, a single nucleotide insertion (c.1145dupA) was detected at the third position of codon 382 in exon12 of MLH1 (NM_000249.3).
frame-shifting variation changed the coding frame, such that methionine 383 was the first affected amino acid replaced by an aspartate; the new reading frame remained open for another 10 amino acids, followed by a UGA stop codon, which led to the premature truncation of the MLH1 protein product (c.1145dupA (p.Met383Aspfs*12)). No other probable causative germ-line variant was identified in the MMR genes in the proband. The proband (III:1) was thus identified as a carrier of a germ-line mutation in MLH1 (Figure 3).

In addition, we further analyzed the carrier status of this mutation in other members of this family by Sanger sequencing. This frame-shift mutation was also verified in two family members who had colon cancer (II:2 and II:4);
Identification and verification of a pathogenic MLH1 mutation c.1145dupA in a Lynch syndrome family

it was also found in a healthy relative (III:3) but not in other family members. This result demonstrated that this variation co-segregates with Lynch syndrome and further supports it as pathogenic. The one healthy cousin (III:3) who unfortunately carries this mutation is in her thirties and might be suitable for proper genetic counseling.

This variant had been previously submitted to the LOVD database by Dr. Beate Betz. It is Class 5 (pathogenic) by InSiGHT criteria [20]. However, no comprehensive evidence or supporting observations were provided. Hence, based on our results, we re-evaluate and classify this mutation as pathogenic according to the 2015 ACMG guideline.

Discussion

The family fulfilled the Amsterdam criteria I for LS. LS was suspected because the pedigree (Figure 3) revealed the occurrence of early-onset CRC in three first-degree relatives. Moreover, precancerous endometrial hyperplasia of a second Lynch syndrome-associated cancer occurred in a patient (III:1) after a prolonged period of over 15 years.

The MLH1 mutation c.1145dupA (MLH1_00928) found in the proband caused a polypeptide chain truncation that led to the production of a protein with 393 amino acids instead of 756 amino acids encoded by the wild-type gene. Obviously, this frame shift mutation can be easily classified as pathogenic, leading to nearly half of the region of the expression domain of MLH1 to be missing.

Additionally, MutLa (MLH1, PMS2 heterodimer) is required for mismatch repair; mutations in the protein interaction domain of MLH1 result in reduced affinity with partner protein PMS2, and the MutLa complex is impaired [24]. The interaction of the two MutLa proteins MLH1 and PMS2 is mediated by the carboxy terminal domains of these proteins [25–27]. Previous analyses have shown that the interaction region of MLH1 with PMS2 corresponds to the C-terminal amino acids 492–756 [27]. Therefore, the mutation causing the loss of the protein interaction domain of MLH1 probably resulted in the degradation of the PMS2 protein, which is in concordance with the negative MLH1 and PMS2 protein expression by the IHC test.
this mutation’s pathogenicity and its defective protein function, \textit{MLH1} c.1145dupA likely has functional consequences, and the verification of the mutation in the two CRC relatives supports its contribution to a strongly increased cancer risk in this LS family.

This mutation site was found in a CRC patient that was previously submitted to the LOVD database by Dr. Beate Betz, and it was classified as pathogenic. This is in line with our observation that the mutation does seem to lead to a classical Lynch syndrome phenotype.

Nevertheless, genetic testing and counseling for this family are beneficial not only for the mutation carriers, but also for the non-carriers. The affected carriers need intensive surveillance for early detection of LS-associated second tumors (such as endometrial cancer, ovarian cancer, \textit{etc.}). The healthy carrier needs annual endometrial biopsy and colonoscopy to exclude endometrial cancer and CRC, \textit{etc}. For non-carriers, the results can reassure their nervousness.

**Conclusion**

The \textit{MLH1} mutation c.1145dupA causes a polypeptide chain truncation, which leads to hereditary CRC phenotypes. We can conclude from our results and previous evidence that this mutation should be regarded as a pathogenic mutation with functional effect on the MLH1 protein. Identification of individuals at risk for Lynch syndrome is important, as these individuals and their relatives can benefit from genetic counseling and increased surveillance, which can result in early cancer detection and decreased disease-specific mortality.

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**Author contributions**

Huang F was responsible for diagnosis and treatment of the patient. Jiang W was involved in the management of the psychological aspects. Tao X performed the IHC analysis while Chen J performed genetic testing. Feng W was also responsible for diagnosis and treatment of the patient along with genetic counseling. All authors contributed towards writing, reviewing and final approval of the manuscript.

**Conflict of interest**

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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