A Novel Germline Mutation of hMLH1 in a Patient with Hereditary Non-polyposis Colorectal Cancer

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DNA mismatch repair genes, hMLH1 and hMSH2, assigned on chromosome 3p21–23 and 2p21–22 are involved in hereditary non-polyposis colorectal cancer (HNPCC). The heterozygous carrier of the mutated allele results in a mutator phenotype and accelerating tumorigenesis, which especially causes carcinomas in the gastrointestinal and genitourinary tracts. We screened germline mutations of mismatch repair genes hMLH1 and hMSH2 in a patient with multiple primary neoplasms (multiple stomach cancers, colon cancer and brain tumor) in a cancer clustered HNPCC family. Screening by long RT-PCR from the RNA extracted from puromycin-treated heparinized blood showed skipping of the exon 2 in hMLH1. The analysis of the genomic DNA showed a GT deletion in the splice-donor site of the exon 2, which is compatible with the splicing variant detected by long RT-PCR analysis. This is a novel germline mutation that has not been reported previously.

Key words: hMLH1 – hereditary non-polyposis colorectal cancer – Turcot’s syndrome

Disorder: Hereditary non-polyposis colorectal cancer (HNPCC)
Ethnicity of patient: Japanese
Gene: hMLH1
GenBank accession number: U59882
Chromosomal assignment: 3p21–23
Type of DNA variant: A germline deletion mutation
Mutation: GT deletion, donor site of exon2, hMLH1
Allelic frequency: Unknown
Methods of mutation detection: PCR/direct sequencing
Databases searched: ICG-HNPCC
(http://www.nfdht.nl/database/mdbchoice.htm)
Human Gene Mutation Database
(http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html)

CASE REPORT AND GENETIC ANALYSIS

Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominantly inherited disorder which predisposes to
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most common cause; each accounts for 30–40% of germline mutations so far reported in HNPCC families (7,8).

Here we report a case of HNPCC with multiple primary cancers carrying germinal mutation in hMLH1 in HNPCC.

The proband was a 35-year-old Japanese female referred to a clinic with a diagnosis of gastric and colon carcinomas. There was no previous history of malignant disorders, except for a duodenal ulcer requiring medication.

She developed a brain tumor at the age of 40 years, verified histologically as an astrocytoma, which was recently related to HNPCC in the context of Turcot’s syndrome (9). This cancer-prone family is shown in Fig. 1. Her mother died of lung carcinoma at the age of 30 years and three of her siblings developed carcinomas of the colon and rectum and one sister developed carcinomas of thyroid and breast, although none of them was histologically verified (Fig. 1). We applied long RT-PCR to amplify whole coding exons from the RNA extracted after puromycin treatment of heparinized blood and subjected the PCR product to direct sequencing (Fig. 2). The mutation detected by long RT-PCR/direct sequencing was confirmed by PCR/direct sequencing of the corresponding regions of genomic DNA (10,11) (Fig. 3).

In RT-PCR analysis of the exon boundary between exons 1 and 2 in hMLH1, a splicing isomer exists in the RNA sample from a normal donor (11), which was 5 bp distant from the 5′ end of exon 2, probably because the AG sequence at nucleotide positions 120 and 121 was regarded as the cryptic splicing acceptor site in the puromycin-treated sample and this made it difficult to detect an abnormally spliced allele (Fig. 2, top and bottom). The signal from the mutated allele was enhanced and easily detectable in the sample treated with puromycin (Fig. 2, middle). We then analyzed the exon–intron boundary of exon 2 in hMLH1 from genomic DNA by PCR/SSCP analysis (data not shown), which showed a band with an abnormal mobility shift (data not shown). Mutated bands were excised from the gel and subjected to direct sequencing, which eventually revealed a GT deletion in the splicing donor site of the exon 2 in hMLH1 (Fig. 3, bottom). As a consequence, skipping of exon 2 results in a termination codon at nt position 115. hMLH1 protein is truncated at nt position 114, yielding a peptide comprising 38 amino acids.

This is a novel germline mutation that has not been reported previously. Other studies reported that the frequencies of

Figure 1. Pedigree showing HNPCC family. Arrow indicates proband 40-year-old female who is a heterozygous carrier of the hMLH1 germline mutation. Filled symbols indicate persons with cancer diagnosis.

Figure 2. Long RT-PCR/direct sequencing. Top, sequencing profile of the sample without puromycin treatment; middle, sample with puromycin treatment; bottom, normal control (wild type).
germline mutations in hMLH1 in complete HNPCC families were 60%. Mutation screening based on long RT-PCR is a high-throughput approach, which permits direct sequencing of the entire coding regions of these two genes from a small amount of the template obtained in a single PCR in a few days.

We believe that the detection of the causative mutations will be of value for the confirmation of diagnoses and for presymptomatic testing of at-risk family members who would like to know their carrier status in HNPCC. The genomic sequencing approach is relatively simple and requires only minute amounts of DNA from one affected family member. Even if it proves to detect mutations in no more than half of the cases, it might be considered as an initial strategy to identify the causative mutations in HNPCC families.

METHODS FOR MUTATION DETECTION

PCR/direct sequencing was performed with the following conditions and parameters.

PCR primer, forward: 5’GATGATTGAGAACTGTTTAG3’.
PCR primer, reverse: 5’ATCAGCTACTGTCTCTT3’.
Size of PCR product: 326 bp.

Thermal cycle profile:
Initial denaturation: 94°C, 1 min.
40 cycles of 94°C, 15 s/65°C, 4 min.
Final extension: 65°C, 10 min.
Sequencing primer: the same as PCR primers.

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References


