The Novel Germline Mutation of \( hMSH2 \) Gene in a Case of a Hereditary Non-polyposis Colorectal Cancer (HNPCC) Patient Who Meets the Revised Amsterdam Criteria

Naohiro Tomita\(^1\), Mutsumi Fukunaga\(^1\), Hiroki Ohzato\(^1\), Shigeyuki Tamura\(^1\), Keishi Sugimoto\(^1\), Tomohiko Aihara\(^1\), Hiroyuki Miki\(^1\), Yuuichi Takatsuka\(^1\), Nariaki Matsuura\(^2\), Takeshi Iwanaga\(^3\), Noriko Fukayama\(^4\) and Kokichi Sugano\(^4\)

\(^1\)Department of Surgery, Kansai Rosai Hospital, Amagasaki, Hyogo, \(^2\)Department of Pathology, School of Allied Health Science, Faculty of Medicine, Osaka University, Suita, Osaka, \(^3\)Aino-Gakuen College, Ibaraki, Osaka and \(^4\)Oncogene Research Unit/Cancer Prevention Unit, Tochigi Cancer Center Research Institute, Utsunomiya, Japan

Received April 18, 2003; accepted August 8, 2003

Hereditary non-polyposis colorectal cancer (HNPCC) is a very important clinical entity in oncology. In order to identify HNPCC, the international diagnostic criteria named ‘Amsterdam criteria’ have been used. In this report, we present a case of an HNPCC patient who met the revised Amsterdam criteria after the sequential history taking in which a novel germline mutation of \( hMSH2 \) gene was detected by genetic testing. The proband was a 69-year-old Japanese female who was admitted to our hospital with a diagnosis of advanced ascending colon cancer. Microsatellite instability (MSI) analysis revealed high MSI in the resected tumor tissue. PCR/direct sequencing analysis of the genomic DNA revealed the TTG(Leu) to TAG(Stop) nonsense mutation at codon 302 in exon 5 of the \( hMSH2 \) gene, which was considered to be a pathogenic mutation. According to the Human Mutation Database and International Collaborative Group on HNPCC (ICG-HNPCC) Database, this type of nonsense mutation is the first report in the \( hMSH2 \) gene.

Key words: hereditary non-polyposis colorectal cancer (HNPCC) – genetic testing – \( hMSH2 \) – microsatellite instability (MSI) – revised Amsterdam criteria

GENETICS SUMMARY
Disorder: Hereditary non-polyposis colorectal cancer
Ethnicity of patient: Japanese
Gene: \( hMSH2 \)
GenBank accession number: U04045
Chromosomal assignment: 2p21
Type of DNA variant: A germline nonsense mutation
Mutation: Base transversion of TTG(Leu) to TAG(Stop) at codon 302 in exon 5 of the \( hMSH2 \) gene
Type of DNA variant: A germline missense mutation (polymorphism)
Mutation: Base transition of ATA(Ile) to GTA(Val) at codon 169 in exon 3 of the \( hMSH2 \) gene
Method of mutation detection: RT-PCR/direct sequencing and PCR/direct sequencing

CASE REPORT AND GENETIC ANALYSIS
Hereditary non-polyposis colorectal cancer (HNPCC) is a very important clinical entity because of its clinical characteristics such as relatively high frequency (1.0–5.0%) among all colorectal cancers and relatively early onset of malignancies in various organs, etc. (1). In order to identify HNPCC, the international diagnostic criteria named ‘Amsterdam minimum criteria’ were proposed in 1991 and have been used thereafter. However, at least three colorectal cancer patients in the pedigree were needed to fulfil these criteria. Moreover, considering the variety of malignancies other than colorectal cancer occur-
ring in HNPCC families, this might miss some proportion of true HNPPC. Therefore, a revised version of these criteria, ‘Amsterdam criteria II’ was proposed in 1999. In this report, we present a case of an HNPCC patient who met the revised Amsterdam criteria after the sequential history taking in which a novel germline mutation of hMSH2 gene was detected by genetic testing.

The proband was a 69-year-old Japanese female who was admitted to Kansai Rosai Hospital with a diagnosis of advanced ascending colon cancer. Right hemicolectomy was carried out in January 2002. A histopathological diagnosis of moderately differentiated adenocarcinoma of clinical stage Duke’s C was made.

The initial history taking was done at the outpatient’s clinic and revealed that the patient had a history of operation for intestinal obstruction and her mother had died with duodenal cancer and a younger sister with an operation history for uterine cancer, although the details of these histories were unknown. These data prompted us to check the microsatellite instability (MSI) status of the tumor tissue to investigate further the possibility of HNPCC. As shown in Fig. 1, MSI analysis revealed that among five microsatellite markers used, four (D2S136, D3S1067, D18S51 and BAT26) were positive, resulting in high MSI in the tumor tissue. This result, taken together with the patient’s history, further suggested that the patient might be HNPCC kindred and made us ask again about the precise history as shown in Fig. 2, indicating that the patient might be HNPCC kindred and made us ask again about the precise history. As the result, two possible mutations were detected by long RT-PCR/direct sequencing analysis and PCR/direct sequencing analysis. One was the ATA(Ile) to GTA(Val) missense mutation at codon 169 in exon 3 of the hMSH2 gene. This mutation had been confirmed to be detected in one out of 100 normal DNAs in Japanese and was considered as the rare polymorphism seen in the Japanese population. The other was the TTG(Leu) to TAG(Stop) nonsense mutation at codon 302 in exon 5 of the hMSH2 gene and was considered to be a significant functional mutation in the disease. Representative profiles of germline DNA analysis are shown in Figures 3 and 4. No mutation was detected in the hMLH1 gene.

The family in this report had been considered to be an HNPCC family who met the revised Amsterdam criteria after the sequential history taking and the genetic testing confirmed the diagnosis. A search of the Human Mutation Database and International Collaborative Group on HNPCC (ICG-HNPCC) Database indicated that this TTG(Leu) to TAG(Stop) nonsense mutation at codon 302 in exon 5 of the hMSH2 gene has not been reported previously and this is considered to be the first case. The mutation detected in the proband was most likely to be derived from her mother, who had died with duodenal cancer at the age of 98 years. If so, it is considered that this mutation was not able to cause any other malignancies in her mother for as long as 98 years. This is intriguing as the same mutation might have caused the advanced colon cancer in the proband at the age of 36 years. In order to confirm that the mutation detected in this report was truly disease-causing mutation, further genetic analysis of the family members, especially the proband’s sister with a history of endometrial cancer, was needed. Also, career diagnosis in this family might be clinically useful and beneficial for each family member. However, the proband did not want the result disclosed to herself, and therefore further genetic analysis has not been undertaken so far.

**METHODS FOR MUTATION DETECTION**

Fluorescence-based PCR was performed with the following conditions and parameters for identification of MSI and LOH on four dinucleotide markers, as described previously (2,3):

**PCR primers for chromosome 2p (D2S136 locus):**

Forward: 5'-AGCTTTGAGACCTTTGTGTC-3'
Reverse: 5'-ATTCAGAAGAACAGTGATGGT-3'
Size of PCR product: 95 bp

**PCR primers for chromosome 3p (D3S1067 locus):**

Forward: 5'-TCATCTATCTCCCACCTGTTGGAG-3'
Reverse: 5'-GAGCATACTGTGTTAAGATGGTT-3'
Size of PCR product: 95 bp

**PCR primers for chromosome 17p (TP53 locus):**

Forward: 5'-CAGCCACTCTCCTTGCCCCATTC-3'
Reverse: 5'-AGGGATACTATTCAGCCCGAG-3'
Size of PCR product: 118 bp

**PCR primers for chromosome 18q (D18S51 locus):**

Forward: 5'-CCGACTACGACCAACAACAC-3'
Reverse: 5'-TACTGACGTGACACTTCACT-3'
Size of PCR product: 278 bp

Reverse primers were labeled with 6-FAM (2p), TET (3p), HEX (17p) or TAMRA (18q) and non-labeled forward primers for the above four regions were synthesized.
Figure 1. PCR profiles showing the DNA replication error at the D2S136 locus in chromosome 2p (upper left), the D3S1067 locus in chromosome 3p (upper right), the TP53 locus in chromosome 17p (middle left), the D18S51 in chromosome 18q (middle right) and the BAT26 locus in chromosome 2p (bottom).

Figure 3. Long RT-PCR analysis of the \textit{hMSH2} gene covering exon 5 suggesting the existence of the mutation [T to A transversion resulting in TTG(Leu) to TAG(Stop) at codon 302 in exon 5 of the \textit{hMSH2} gene].

Figure 4. PCR/direct sequencing analysis of the genomic DNA confirmed one-base substitution of TTG(Leu) to TAG(Stop) at codon 302 in exon 5 of the \textit{hMSH2} gene.
**THERMAL CYCLE PROFILE:**

Initial denaturation: 94°C, 3 min  
Amplification: 35 cycles of 94°C, 45 s/58°C, 1 min/72°C, 1.5 min  
Final extension: 72°C, 5 min  

Fluorescence-based PCR was performed with the following conditions and parameters for identification of MSI on mononucleotide marker BAT26:  
PCR primers for chromosome 2p (BAT26):  
Forward: 5'-CTACTTTTGACTTCAGCC-3'  
Reverse: 5'-ACCAATCAACATTTTAACC-3'  
Size of PCR product: 117 bp  
Forward primer was labelled with HEX (2p) and non-labelled reverse primer was synthesized. The thermal cycle profile was the same as that for the four dinucleotide markers except that the denaturation temperature was 95°C.  
PCR products were then denatured for 5 min at 95°C in formamide dye and electrophoresed in 6% acrylamide gel (5.7% acrylamide, 0.3% N,N'-methylenebisacrylamide) containing 6 M urea using an ABI PRISM 377 DNA sequencer (Applied Biosystems, Perkin-Elmer).  

Long RT-PCR was performed with the following conditions and parameters for identification of the hMSH2 gene containing exon 3 and exon 5:  
Forward: 5'-GGCGGGGAAAACAGCTTAGTGGGTGTG-3'  
Reverse: 5'-CCCATGGGGACTGACACTATGTG-3'  

**Acknowledgments**  
This work was supported in part by a Grant-in-Aid for Medical Research from Kansai Rosai Hospital and a Grant-in-Aid for Cancer Research and by the 2nd Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare, Japan.

**References**  