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BRIEF REPORT — MUTATION REPORT

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# Novel germline mutations of *hMSH2* in a patient with hereditary nonpolyposis colorectal cancer (HNPCC) and in a patient with six primary cancers

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**Abstract** We screened for germline mutations of mismatch repair genes, *hMLH1* and *hMSH2*, in five Japanese families carrying hereditary nonpolyposis colorectal cancer (HNPCC) and in a patient with multiple primary cancers. Screening the entire coding regions of both genes using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis, we found two novel germline mutations in *hMSH2*. One was a 1-bp insertion in exon 12, detected in a patient who had undergone surgery six times for independent tumors (four primary colorectal carcinomas, a small intestinal carcinoma, and an endometrial cancer). The other, in a second patient, was a missense mutation from CTT to TTT at codon 390 in exon 7 that resulted in substitution of phenylalanine for leucine. This conservative alteration was not found in any of 50 normal controls, but we cannot exclude the possibility that it may represent a rare polymorphism rather than a factor in the disease.

**Key words** Hereditary nonpolyposis colorectal cancer (HNPCC) · Mismatch repair gene *hMSH2* · Multiple primary cancers

## Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC), a common autosomal dominant cancer syndrome, is characterized by early onset of cancers in various tissues such as colorectum, uterus, stomach, biliary tract, urinary tract, and ovary (Lynch et al. 1993); many affected individuals are diagnosed with two or more primary cancers. HNPCC results from germline mutations in the human homologues of bacterial and yeast mismatch-repair genes; these include *hMLH1* (Bronner et al. 1994 ; Papadopoulos et al. 1994), *hMSH2* (Fishel et al. 1993; Leach et al. 1993), *hPMS1, hPMS2* (Nicholaides et al. 1994), and *GTBP* (Akiyama et al. 1997; Miyaki et al. 1997). Although the proportion of HNPCC attributable to each of these genes remains unclear, defects of *hMLH1* and *hMSH2* are considered to be the most common causes; each accounts for 30%–40% of HNPCC families (Liu et al. 1994, 1996; Han et al. 1995). In this study, using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis we screened the *hMLH1* and *hMSH2* genes for mutations in five HNPCC families and in a patient with a history of six independent primary cancers.

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## Materials and methods

*Patients.* We obtained blood samples with informed consent from members of five families clinically defined as having HNPCC, and from one patient who had no family history of cancer as far as we could ascertain, but whose personal history included six independent primary cancers (four colorectal, one small intestinal, and one endometrial). Peripheral blood samples were obtained from up to three members of each of the families (including one or more affected individuals) and DNAs were extracted from these samples according to methods described elsewhere (Sato et al. 1990).

*Polymerase chain reaction (PCR).* For SSCP analysis, each exon of the *hMLH1* and *hMSH2* genes, along with flanking intronic sequences, was amplified from genomic DNA. The oligonucleotide primer sequences were described before (Han et al. 1995). Each of the 19 exons of *hMLH1* and the 16 exons of *hMSH2* was amplified in a total volume of 20 μl

containing 50 ng genomic DNA, 2 μl of 10 × PCR buffer, 10 pmoles of each primer, 4.5 μM of each dNTP, and 1 unit of *Taq* DNA polymerase, for 35 cycles at the annealing temperature calculated according to the composition of its primers, on the GeneAmp PCR system 9600 (Perkin Elmer Cetus, Norwalk, CT, USA).

*Single strand conformation polymorphism (SSCP) analysis.* Nonradioisotopic SSCP analysis was carried out as previously described (Oto et al. 1993). Mixtures containing 2 μl of each PCR product and 2 μl of a denaturing stop solution (95% formamide, 10 mM ethylenediaminetetraacetic acid (EDTA), 0.25% bromophenol blue, and 0.25% xylene cyanol) were heated to 80°C for 5 min and quenched on ice; 2 μl of each denatured mixture was loaded on a 5% polyacrylamide gel containing 10% glycerol and electrophoresed for 16 h under running conditions of 400–500 V at 4°C. After electrophoresis, the gels were stained with SYBR Green II (FMC BioProducts, San Jose, CA, USA) and visualized by means of a FMBIO II Multi-View fluorescent image analyzer (Takara, Otsu, Japan).

*DNA Sequencing.* Products that showed abnormal patterns on SSCP analyses were reamplified with *Taq* polymerase, subcloned into pBluescript II SK(-) vector (Stratagene, La Jolla, CA, USA), and sequenced using the T3 and T7 primers. The sequencing analyses for each product were performed using an Applied Biosystems model 377 DNA sequencer and the Perkin Elmer Dye Terminator Cycle Sequencing FS Ready Reaction Kit.

## Results and discussion

We identified possibly disease-causative mutations of the *hMSH2* gene in two of the six individuals screened by PCR-SSCP. One was a 1-bp insertion at codon 596 (AAT to AAAT) in exon 12, detected in the patient whose medical history included six primary cancers; this change led to a frameshift and a premature termination (Fig. 1). This patient, who had no family history of cancer clustering, had undergone surgery six times for removal of independent carcinomas; ascending colon cancer at age 37, transverse colon cancer at age 38, rectal cancer at age 40, ileal cancer at age 47, and sigmoid colon cancer and endometrial cancer at age 50. As no DNA samples were available from her parents, we do not know whether the mutation occurred de novo or was inherited. However, each offspring of the patient is likely to carry a 50% chance of being at high risk of cancer unless the mutation in this proband is chimeric and her germline cells do not contain the mutation.

In a second patient, another aberration was detected: a missense mutation involving a C (CTT) to T (TTT) transition at codon 390 (Leu to Phe) in exon 7 (Fig. 2). The same substitution was found in all of her three children, one of whom was found to have a colorectal cancer at the age of 20; the other two children, in their twenties, have so far had no tumors. Although this amino-acid substitution was a conservative change, it was not found in any of 50 normal controls. Hence, we think it may be responsible for HNPCC in this family, but we are unable to exclude the possibility that it represents a very rare polymorphism. To our knowledge, most mutations discovered so far in *hMSH2* have been deletions/insertions or nonsense alterations resulting in truncations of the predicted protein products; few missense mutations in this gene have been reported. However, most of the mutations described to date were revealed by in vitro transcription/translation reaction (IVTT) assays (Hall et al. 1994), and the link between translation-terminating mutations in *hMSH2* and carcinogenesis was clear (Luce et al. 1996). In the case reported here, the patient's family had a typical HNPCC pedigree. Since the same missense mutation was also detected in her two children, careful clinical surveillance is indicated for them even though neither has yet shown signs of cancer.

HNPCC is caused by mutations in genes encoding proteins that play significant roles in DNA mismatch-repair systems. It should be possible to improve clinical management through accurate presymptomatic diagnosis of carrier status among members of HNPCC families (Froggatt et al. 1995). However, molecular diagnosis remains difficult because (a) most HNPCC kindreds are unsuitable for genetic linkage studies that could indicate which gene needs to be examined; (b) most reported mutations have been unique to single families, so the mutation in each HNPCC family

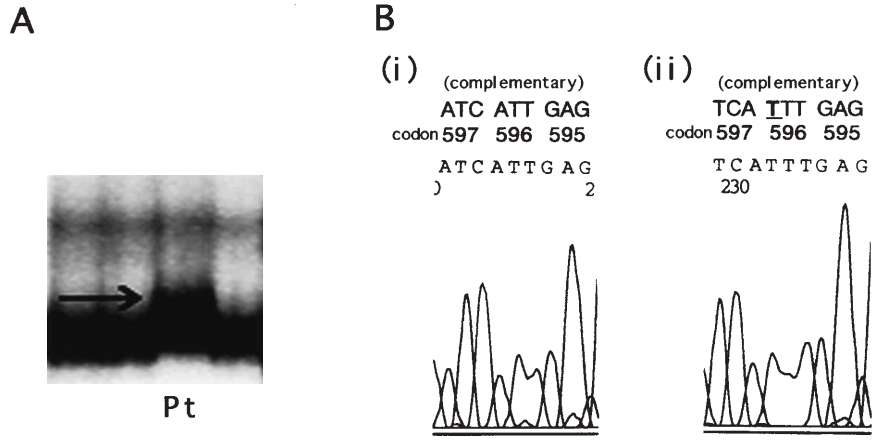


**Fig. 1** **A** Polymerase chain reaction – single strand conformation polymorphism (PCR-SSCP) patterns in exon 12 of the *hMSH2* gene. A mutant band *(arrow)* is present in the patient *(Pt)*. **B** Nucleotide sequences revealing a 1-bp insertion in exon 12 of *hMSH2*. *(i)*, normal allele; *(ii)*, mutant allele of the patient. Both alleles were sequenced from single colonies

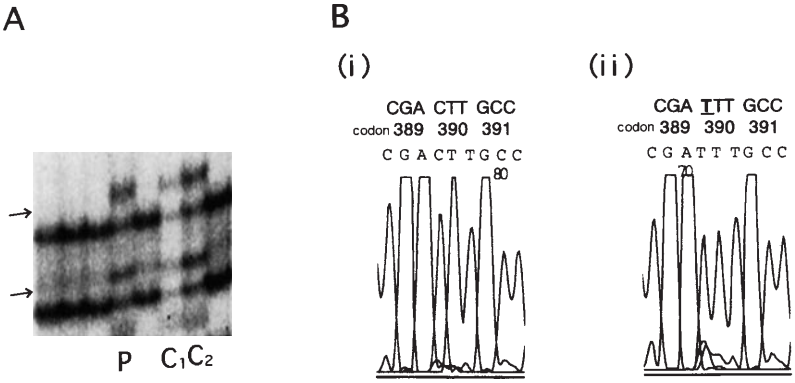


**Fig. 2** **A** PCR-SSCP patterns in exon 7 of the *hMSH2* gene. Mutant bands *(arrows)* are present in the proband *(P)* and her two asymptomatic children (*C1* and *C2*). **B** Nucleotide sequences revealing a C (CTT) to T (TTT) transition at codon 390 (Leu to Phe) in exon 7 of *hMSH2*. *(i)*, normal allele; *(ii)*, mutant allele of the patient. Both alleles were sequenced from single colonies

must be newly defined; (c) of the several techniques used to search for mutations, none has achieved both accuracy and simplicity; and (d) if several genes must be examined before a family's mutation is found, the expense will be prohibitive for most people.

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