RFLP Report

NINE-bp REPEAT POLYMORPHISM IN EXON 1 OF THE *hMSH3* GENE

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Summary We have identified a polymorphic 9-bp repeat sequence in exon 1 of the *hMSH3* gene using polymerase chain reaction (PCR). Five alleles were observed in unrelated Japanese individuals with heterozygosity of 0.57.

Key Words *hMSH3* gene, polymorphism, PCR

Introduction. A length polymorphism due to a variable number of 9 nucleotide tandem repeat was found in exon 1 of the gene for *hMSH3* (previously named MRP1 (Fujii and Shimada, 1989)). The repeating unit starts 151 nucleotides downstream from the first ATG, and the consensus sequence appears to be (GC)CPy-(GC)CAGCG which encodes alanine or proline.

Primers for PCR. A primer set designed from sequences flanking repeat as 1M: 5'-GCAAGCGGTTTGTAGCCGA-3', and 1AS: 5'-CAGTCCCAGACAG-AACCTAC-3' (Chen *et al.*, 1984).

PCR condition. The PCR reaction was performed with 50 ng genomic DNA, 330 pmol of dNTPs, 3.3 pmol of each primer end-labeled with [γ -³²P]ATP, 0.25 U Taq polymerase and 500 pmol of dimethyl sulfoxide (DMSO), in a total volume of 10 μ l. After initial denaturation for 5 min at 94°C, amplification for 30 cycles consisting of 30 sec at 94°C, 30 sec at 55°C, 60 sec at 72°C, and a final extension of 5 min at 72°C.

Polymorphism. We have previously found that the *hMSH3* cDNA clone isolated from HL-60R cells contains 6 copies of the 9 nucleotide repeating unit, while the *hMSH3* genomic clone from HeLa cells contains 3 copies (Fujii and Shimada, 1989). PCR amplification of the corresponding region of the genomic DNA from mononuclear cells from 58 individuals showed five allelic fragments (Fig. 1). Comparison with the sequencing ladder revealed that the length of each fragment was a function of the number of copies of 9 nucleotide repeating unit (Table 1).

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Fig. 1. Polymorphism in exon 1 of the *hMSH3* gene. Some common alleles are shown. Lane M represents the DNA size marker pBR322 digested with *Msp*I. PCR product contained 3, 4, 5, 6, and 7 repeat should be 171, 180, 189, 198, and 207 bp, respectively. Each lane from 1 to 8, the PCR product from the peripheral blood cells of the unrelated individuals. Lane 1; 3 repeat, lane 2; 4 and 6 repeats, lane 3; 5 and 7 repeats, lanes 4, 5; 6 repeats, lanes 6, 7; 6 and 7 repeats, lane 8; 7 repeat.

Table 1. Allelic sizes and frequencies at exon 1 of the *hMSH3* gene in 116 unrelated Japanese individuals.

Repeats	Sizes (bp)	n (total, 116)	Frequencies
3	171	18	0.155
4	180	5	0.043
5	189	1	0.009
6	198	70	0.603
7	207	22	0.190

Allele frequencies. Allele frequencies were estimated from 58 unrelated Japanese individuals and the heterozygosity was found to be 0.57 (Table 1). The heterozygosity we use as a measure of allelic diversity was calculated according to the Chakraborty theory (Chakraborty *et al.*, 1988).

Chromosomal localization and Mendelian inheritance. The *hMSH3* gene is located immediately upstream of the dihydrofolate reductase gene which has been mapped on 5q11-13 (Anagnou *et al.*, 1988). Mendelian inheritance was observed in 11 informative families with a total 44 individuals.

Comments. The *hMSH3* gene and the dihydrofolate reductase gene are organized in a head-to-head configuration separated by an 90 bp sequence (Fujii and Shimada, 1989). The *hMSH3* gene encodes a protein highly homologous to a

bacterial mismatch protein MutS, although the exact function of this gene is not known at this moment (New *et al.*, 1993).

The polymorphic region identified in this study is located downstream from the first ATG in exon 1 of the *hMSH3* gene. Therefore, the protein structure differs depend on the number of repeats, although the reading frame is conserved in all cases. These structural alterations may affect the activity of *hMSH3*.

Recently, another MutS homolog, *hMSH2*, has been shown to play an essential role in human DNA mismatch repair (Fishel *et al.*, 1993; Parsons *et al.*, 1993). Genomic instability due to mutations of the *hMSH2* gene is considered to be involved in the development of hereditary nonpolyposis colorectal cancer (Leach *et al.*, 1993). These results suggest that mutations of the *hMSH3* gene may be associated with some types of cancers or genomic diseases. The polymorphic marker should be useful for genetic analysis of such *hMSH3* related disorders.

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