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Insertion/Deletion Polymorphism and Other Restriction Fragment Length Polymorphisms in the *MCC* Gene

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The *MCC* gene is a candidate as a tumor suppressor gene for colorectal neoplasms. Further, *MCC* is tightly linked to the familial adenomatous polyposis (FAP) locus by linkage and physical analysis. Hence, restriction fragment length polymorphisms (RFLPs) of this gene might be very useful for presymptomatic diagnosis of individuals in families segregating mutant alleles of the APC gene. Here we report the identification of five polymorphic systems in *MCC* gene (both cDNA and genomic), one of which is an insertion/deletion polymorphism that is detectable by a polymerase chain reaction method. These five RFLP systems should be useful for linkage studies in FAP and for examining loss of heterozygosity at this locus in colonic polyps and tumors.

Key words: *MCC* gene — RFLP —Colorectal cancer

Cytogenetic and genetic linkage studies have localized the gene responsible for familial adenomatous polyposis (FAP), an autosomal dominant disease, to chromosome 5q21.[1–4] Moreover, deletion mapping in colorectal cancers by the use of restriction fragment length polymorphism (RFLP) markers has indicated that at least one tumor suppressor gene may be present in the same chromosomal region as the putative APC gene.[5,6] These results suggested that the same gene on chromosome 5q21 might be mutated in the germ-line of FAP patients and somatically in sporadic tumors. Furthermore, allelic losses at this locus are associated with early stages of neoplasia in both familial and sporadic colorectal tumors.[7] The search for a tumor suppressor gene in this region identified one candidate gene (*MCC*), which had undergone somatic mutations in some colorectal cancers.[8] Even though it not yet established that *MCC* is responsible for FAP, polymorphisms in this gene might serve as markers not only for detection of loss of heterozygosity in colorectal tumors, but also for presymptomatic diagnosis of individuals at risk in families with FAP. We therefore examined *MCC* for RFLPs and sequenced a region characterized by an insertion/deletion polymorphism in order to amplify the polymorphic site by polymerase chain reaction (PCR).[9]

Genomic DNAs from 120 unrelated individuals including 60 FAP patients were prepared from peripheral white blood cells as described elsewhere.[10]

DNAs were digested with six restriction enzymes: *Msp*I, *Taq*I, *Rsa*I, *Bgl*II, *Pvu*II or *Eco*RI. After electrophoresis on a 0.8% agarose gel, DNA was transferred onto a nylon membrane[11] and hybridized[10] with ^{32}P-labeled DNA fragments: cDNA (69-III) and genomic (L5.71-5) *MCC* clones[8] by the random hexanucleotide-priming method of Feinberg and Vogelstein.[12]

DNA sequencing was done by a modified T7 polymerase method as described by Del Sal *et al.*[13]

PCR was performed by using a GeneAmp™ DNA Amplification Reagent Kit (Cetus, Norwalk, CT) with the oligonucleotides 5′-TCAGGTACGCGGCTCCATTCGGCTTT-3′ and 5′-AAGCTCACTGCATTTCAAAGTGAAGC-3′, for 1 min 95°C, 1 min at 57°C and 5 min at 72°C (25 cycles).[14]

As part of a search for mutations in the *MCC* gene, we have examined DNA from 60 unrelated FAP patients by Southern hybridization experiments. Five RFLP systems were identified with three restriction enzymes and four systems are shown in Fig. 1. Polymorphic bands were detected clearly in DNAs digested with *Eco*RI, *Taq*I or *Msp*I by the 69-III probe. We also found the same frequencies of these polymorphic alleles in 60 unrelated individuals without FAP. The sizes and frequencies of all polymorphic alleles are summarized in Table I. Thirty-nine (65%) of the 60 individuals tested were heterozygous for one or more of the marker systems.

The other system was identified as an insertion/deletion polymorphism involving allele 1 (A1) and allele 2 (A2) detected by the probe L5.71-5, as shown in Fig. 2.

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The sizes of *Msp*I fragments were 2.7 kb (A1), 1.9 kb (A1) and 4.0 kb (A2). Moreover, this probe also detected polymorphic 2.8-kb (A1), 2.2-kb (A2) *Taq*I fragments and 3.0-kb (A1), 2.4-kb (A2) *Eco*RI fragments. The frequencies of alleles 1 and 2 were 0.96 and 0.04, respectively, among 60 unrelated individuals. In this FAP family, the disease cosegregated with allele 2. Results of restriction-enzyme mapping indicated that allele 2 reflected a nearly 600-bp deletion, in which a recognition site of *Msp*I region was included. This deletion was localized in an intron of the *MCC* gene (data not shown).

To examine the deleted sequence, the 2.8-kb (A1) and 2.2-kb (A2) *Taq*I fragments were subcloned and sequenced. Comparing the sequence of these two alleles, we found that on allele 2, a 540-bp segment was deleted from

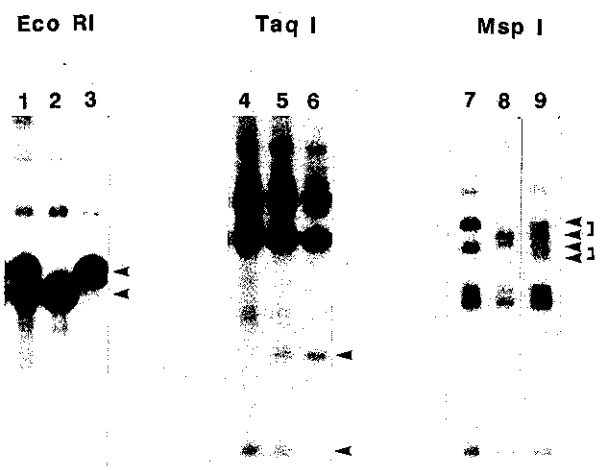

Fig. 1. Autoradiograms of DNA cleaved with restriction enzymes *Eco*RI, *Taq*I or *Msp*I, from unrelated individuals hybridized with the 69-III probe. Arrowheads indicate polymorphic bands. The sizes of polymorphic *Eco*RI fragments were 5.0 kb and 4.5 kb. In addition 1.5-kb, 1.0-kb *Taq*I fragments and 5.4-kb, 5.2-kb, 5.0-kb and 4.8-kb *Msp*I fragments are also polymorphic.

Table I. Single-site RFLPs Detected by the 69-III Probe

Enzyme	Allele size (kb)	Frequency[a]
*Eco*RI	5.0	0.83
	4.5	0.17
*Taq*I	1.5	0.45
	1.0	0.55
*Msp*I	5.4	0.34
	5.2	0.66
	5.0	0.92
	4.8	0.08

a) Estimated among 60 unrelated individuals.

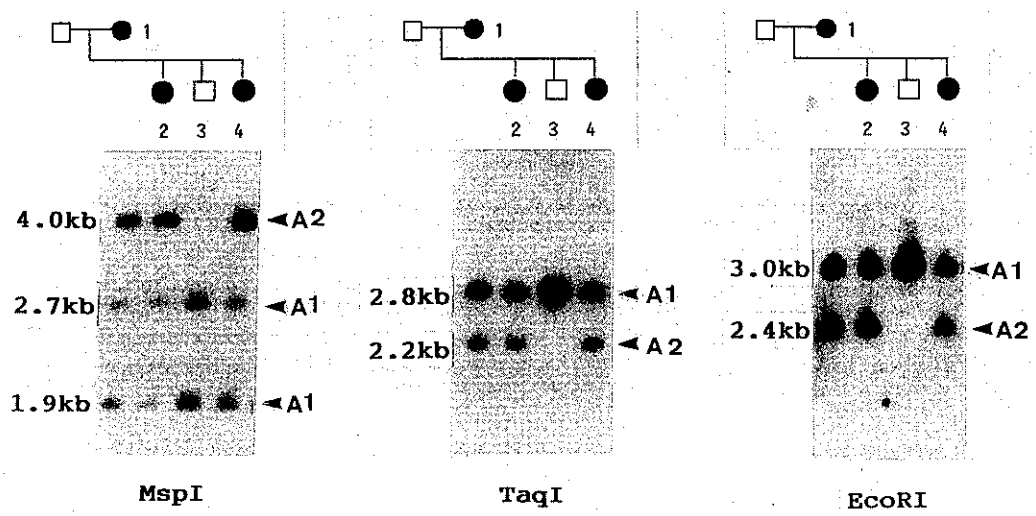

Fig. 2. Detection of polymorphic bands in a FAP pedigree. The blackened symbols indicate affected members. Bottom, hybridization pattern of probe L5.71-5 with genomic DNAs of FAP patients (lanes 1, 2 and 4) and a healthy sibling (lane 3) digested with three restriction enzymes (*Msp*I, *Taq*I and *Eco*RI). An insertion/deletion polymorphism (allele 1 (A1) and allele 2 (A2) were recognized. The disease cosegregates with allele 2. The polymorphic fragments of *Msp*I were 2.7 kb (A1), 1.9 kb (A1) and 4.0 kb (A2). The 2.8-kb (A1), 2.2-kb (A2) *Taq*I fragments and 3.0-kb (A1), 2.4-kb (A2) *Eco*RI fragments are also polymorphic.

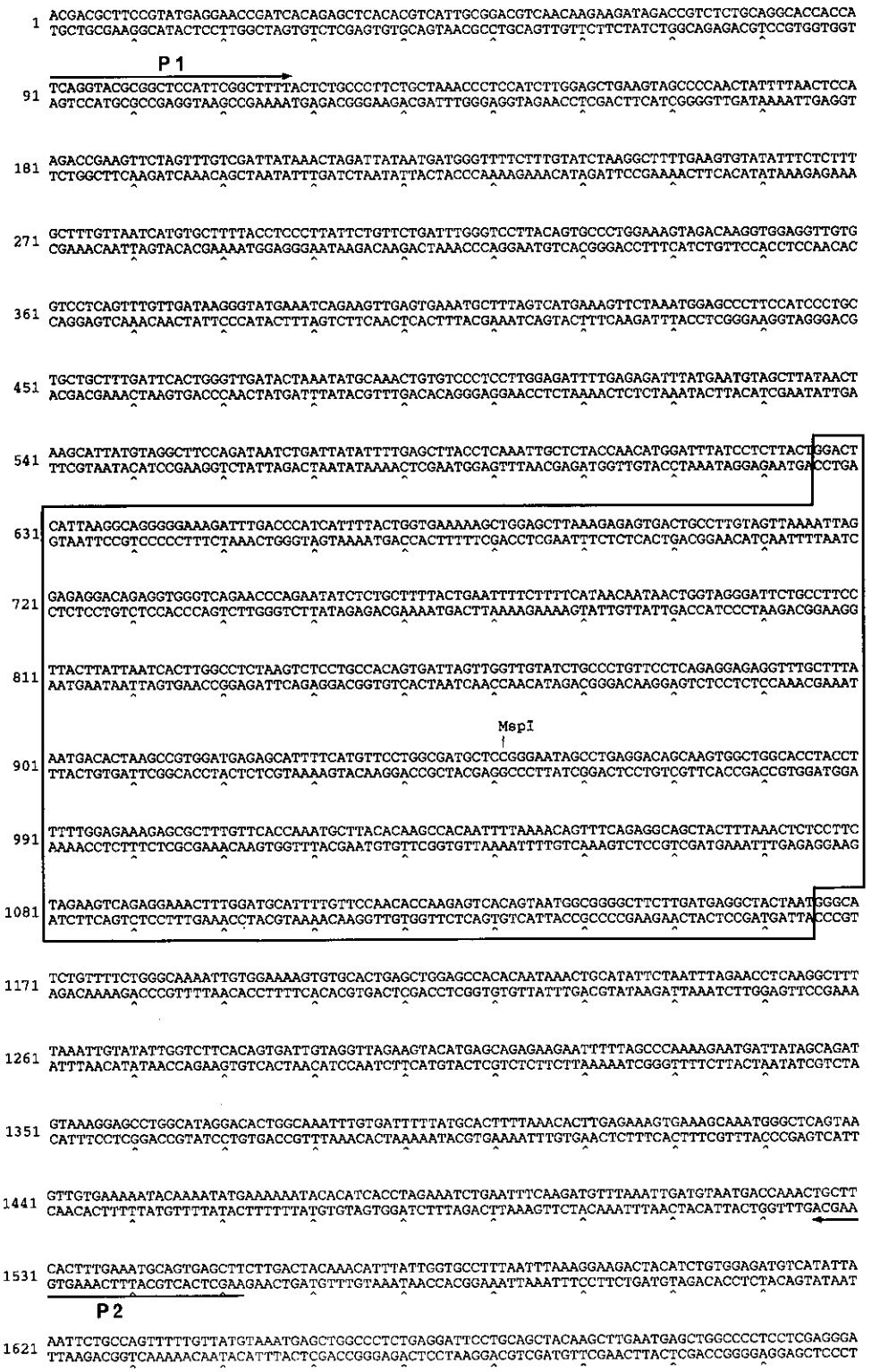

Fig. 3. Sequence of allele 1 at the locus defined by probe L5.71-5 (an intron sequence of the *MCC* gene). From an individual whose DNA showed insertion/deletion polymorphism with three enzymes, the boxed 540-bp region including the *Msp*I site (626–1165) is deleted on allele 2. The primers used for PCR are marked by arrows (P1 and P2).

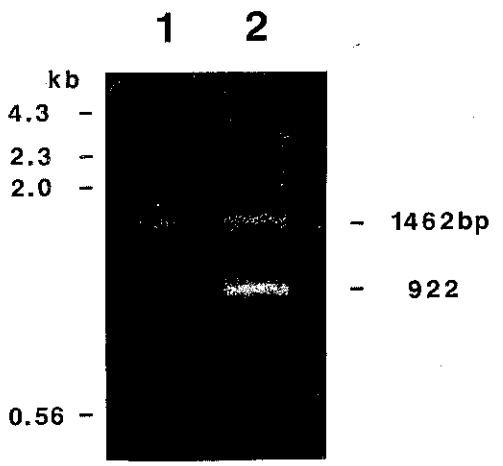

Fig. 4. The PCR products from individuals who showed insertion/deletion polymorphism of L5.71-5 were separated by electrophoresis through an agarose gel. Lane 1 was a homozygous (A1/A1) and lane 2 was a heterozygous (A1/A2) individual. On lane 2, we can identify 1462-bp and 922-bp PCR products.

allele 1 of the intron of the *MCC* gene. The DNA sequence of allele 1 is shown in Fig. 3; the boxed 540-bp segment was deleted on allele 2.

Knowledge of the sequence allowed us to apply the PCR method to detection of the insertion/deletion polymorphism in genomic DNA at this locus. Oligonucleotides for PCR primers were derived from sequences nearly 500-bp upstream and downstream of the deleted region shown in Fig. 3. PCR was performed with members of the FAP family who showed the insertion/deletion polymorphism. Two bands at 1462 bp and 922 bp are easily detected by ethidium bromide staining (Fig. 4). Lane 1 reveals a homozygous (A1/A1) and lane 2 shows a heterozygous (A1/A2) individual.

FAP is the most common hereditary disease that is predisposing to cancer. The ability to diagnose, at a presymptomatic stage, those members of a FAP family who carry the disease allele would be highly desirable for clinical management. The *MCC* gene is located only 150 kb apart from the APC gene[15,16] and the estimated frequency of recombination between these two loci is below 1%. Furthermore, one of the systems (L5.71-5) showed tight linkage to FAP (maximum lod score of 11.9 at zero recombination; Y. Nakamura, unpublished data). Hence, the five RFLP systems reported here should provide very reliable information for presymptomatic diagnosis in members of families who carry FAP.

Somatic deletion of alleles from the long arm of chromosome 5, detected as loss of heterozygosity (LOH) with RFLP markers, has been reported in sporadic colorectal cancers. Moreover, frequent LOH for FAP-linked markers on chromosome 5q has also been found in renal cell carcinomas (RCCs)[17] and hepatocellular carcinomas (HCC).[18] A tumor suppressor gene common to many types of cancers, such as the p53, might exist in the APC region. The markers reported here should be useful in studies to examine that possibility.

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