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Mutation Report

GERMLINE MUTATIONS OF THE APC GENE IN TWO JAPANESE ADENOMATOUS POLYPOSIS PATIENTS

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Summary Germline mutations of the adenomatous polyposis coli (*APC*) gene have been reported in patients with familial adenomatous polyposis (FAP) and are believed to be an early event in colorectal carcinoma. We report the results of screening for germline mutations of the *APC* gene in 4 cases of 2 kindreds using non-radioactive PCR-SSCP (polymerase chain reaction-single strand conformation polymorphism) analysis. The mutation in kindred 1 was a 4 bp deletion at codon 849 in exon 15, resulting in a frameshift leading to truncation of the *APC* gene product. In kindred 2, a transversion of C to G at codon 2038 was observed, resulting in an amino acid change from leucine to valine. In this case, it is possible to screen presymptomatic diagnosis easily and quickly by digestion with restriction enzyme *Eco*NI.

Key Words *APC*, FAP, non-radioactive PCR-SSCP

Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease. Patients with FAP are characterized by the presence of hundreds to thousands of adenomatous polyps in the colon and rectum, one or more of which can progress to cancer if left without surgical treatment.

The adenomatous polyposis coli (*APC*) gene was identified on chromosome 5q21 in 1991 by positional cloning (Gelbert *et al.*, 1991; Joslyn *et al.*, 1991; Kinzler *et al.*, 1991; Nishisho *et al.*, 1991). Germline mutations of the *APC* gene have been reported in patients with FAP and believed to be an early event in colorectal carcinogenesis (Joslyn *et al.*, 1991; Kinzler *et al.*, 1991; Miyoshi *et al.*,

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1992a; Nagase *et al.*, 1992a, b; Nagase and Nakamura, 1993; Nishisho *et al.*, 1991). These results suggest that inactivation of the *APC* gene plays a significant role in FAP. Because FAP patients have a very high risk of developing colorectal cancer, identification of the individual risk in family members is important to prevent cancer death.

We report the results of screening for the germline mutations of the *APC* gene in 4 cases of 2 kindreds using non-radioactive PCR-SSCP (polymerase chain reaction-single strand conformation polymorphism) analysis.

Materials and Methods

Genomic DNAs were prepared from leukocytes of 4 patients in 2 Japanese familial adenomatous polyposis kindreds (Fig. 1). These patients were diagnosed and operated in Shinshu University hospital. Case 1 had 200 polyps in total colon and 2 of them were well differentiated adenocarcinoma. Case 2 had 700 polyps, case 3 had 250 polyps and case 4 had 300 polyps in total colon, respectively.

To screen for germline mutations in the *APC* gene, 17 segments of exon 15 were amplified by PCR and examined by non-radioactive SSCP (PhastSystem; Pharmacia). The primer pairs used in this study were described by Miyoshi and colleagues (Miyoshi *et al.*, 1992a). In a non-radioactive SSCP, DNA bands on the gel were visualized by silver staining (New PhastGel DNA Silver Staining Kit; Pharmacia). PCR products showing aberrant migration patterns on the SSCP gels were cloned into a plasmid vector. At least 10 subclones were pooled and DNAs were extracted for sequencing templates with the RPM kit (BIO). DNA sequences of subcloned PCR products were determined by the dideoxynucleotide termination method (373A DNA Sequencer; ABI).

PCR products spanning codons 1935 to 2096 in exon 15, exhibiting an aberrant migration pattern by SSCP in kindred 2 and of total 82 normal alleles

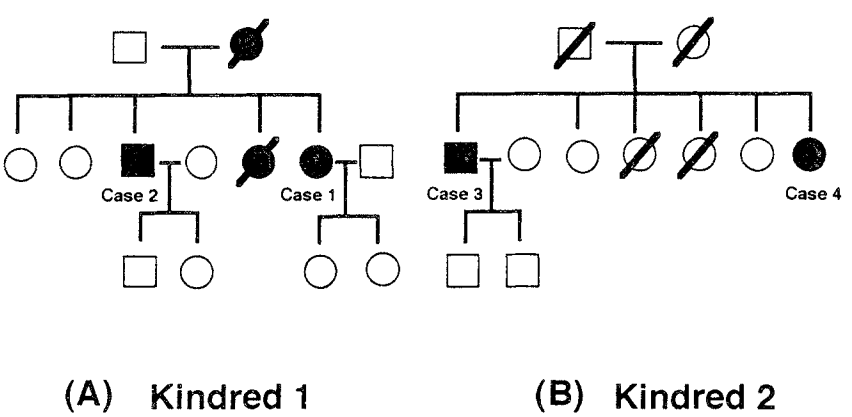

Fig. 1. Pedigrees of two Japanese familial adenomatous polyposis kindreds.

were separated by agarose gel electrophoresis coupled with digestion by restriction enzyme *Eco*NI.

Results

We examined exon 15 (codon 635 to 2843) of the *APC* gene in the genomic DNA of 3 patients with FAP by the non-radioactive PCR-SSCP method to screen variant sequences.

Seventeen different segments of exon 15 were amplified and observed for aberrant migration pattern by SSCP (Fig. 2). PCR products showing aberrant migration patterns on the SSCP gels were cloned into a plasmid vector. DNA sequences of subcloned PCR products were then determined. A mutation in kindred 1 was a 4 bp deletion at codon 849 in exon 15 (Fig. 3A), resulting in a frameshift leading to truncation of the *APC* gene product 30 bp downstream (Table 1). Kindred 2 appeared to carry a C to G missense mutation at codon 2038 (Fig. 3B), resulting in an amino acid change from leucine to valine (Table 1). This missense mutation of codon 2038 disrupted an *Eco*NI restriction site. A 486 bp PCR fragment derived from a normal allele was digested into 312 bp and 174 bp fragments. In contrast, a PCR product from a mutated allele remained at 486 bp (Fig. 4). We examined total 82 normal alleles digested into 312 bp and 174 bp fragments. Therefore, this transition of C to G at codon 2038 is a mutation, not a polymorphism.

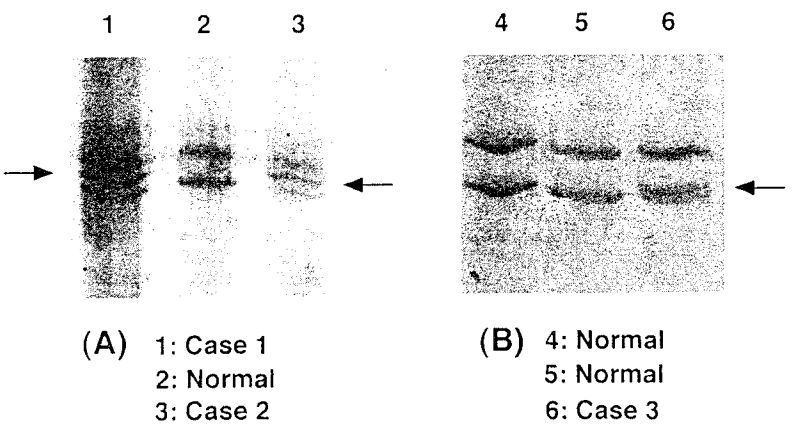

Fig. 2. SSCP analysis. (A) The results of the electrophoresis of the SSCP in kindred 1. PCR products of codon 735 to 883 were amplified and observed aberrant migration pattern on SSCP in 2 patients of kindred 1. A band showing aberrant mobility (lanes 1 and 3) was marked with an arrow. (B) The results of the electrophoresis of the SSCP in kindred 2. PCR products showing aberrant migration pattern were observed at codon 1935 to 2096 by SSCP analysis in kindred 2. A band showing aberrant mobility (lane 6) was marked with an arrow.

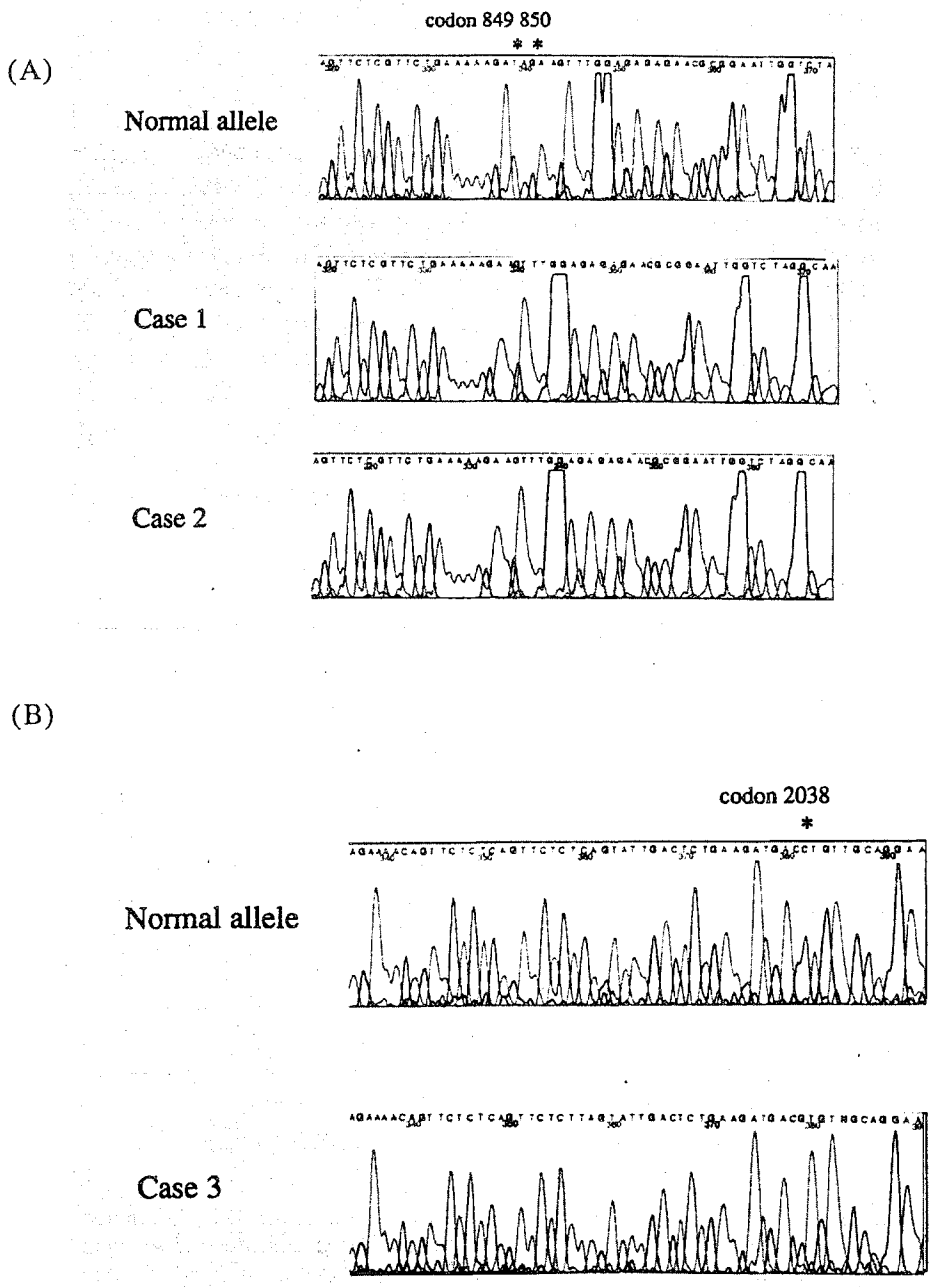

Fig. 3. Sequence analysis. (A) The results of the sequence analysis in kindred 1. 4 bp deletion at codon 849 was determined. (B) The results of the sequence analysis in kindred 2. C to G mutation at codon 2038 was determined.

Table 1. Germline mutations in the *APC* gene.

Family	Codon	Nucleotide change	Amino acid change
1	849	GATAGAA→GAA	Codon 860 Stop
2	2038	CTG→GTG	Leu→Val

(A) Restriction site of *Eco*NI

5′-CCT NN* NNN AGG-3′

3′-GGA NNN* NN TCC-5′

Normal allele

5′-GAC CTG TTG CAG GAA-3′

Case 3

5′-GAC GTG TTG CAG GAA-3′

(B)

M: Size marker

1: Case 3

2: Case 4

3-12: Normal control

Fragment size Normal allele: 312 bp, 174bp

mutation allele: Not digested

Fig. 4. (A) Restriction site of *Eco*NI. (B) The missense mutation at first nucleotide of codon 2038 in the kindred 2 disrupts an *Eco*NI restriction site. A 486 bp PCR fragment derived from a normal allele was digested into 312 bp and 174 bp fragments (lanes 3–12). The PCR products from a mutated allele remained at 486 bp (lanes 1 and 2). Size marker: *Hin*fI digested size marker of pUC18 with the partial catalase gene.

Discussion

Germline mutations of the *APC* gene have been reported in patients with FAP and are believed to be early events in colorectal carcinogenesis (Joslyn *et al.*, 1991; Kinzler *et al.*, 1991; Miyoshi *et al.*, 1992a; Nagase *et al.*, 1992a, b; Nagase and Nakamura, 1993; Nishisho *et al.*, 1991). Somatic mutations of the *APC* gene have been reported in sporadic colorectal cancer (Miki *et al.*, 1992; Miyoshi *et al.*, 1992a; Nagase and Nakamura, 1993; Nishisho *et al.*, 1991) and other digestive tumors (Horii *et al.*, 1992a, b; Nakatsuru *et al.*, 1992). These results suggest that inactivation of the *APC* gene plays a significant role in FAP carcinogenesis.

Germline mutations of the *APC* gene have been reported in 174 FAP patients and somatic mutations in 103 colorectal tumors (Nagase and Nakamura, 1993). It was reported that most known mutations are located within the 5′ half of the coding region (codon 713 to 1597), although somatic mutations in colorectal tumors tend to cluster in the MCR (mutation cluster region: codon 1286 to 1513) (Nagase and Nakamura, 1993). Of 174 germline mutations, 106 have been frameshift mutations caused by the insertion and deletion of nucleotides, all creating new stop codons downstream. The remaining 68 had point mutations resulting in nonsense and missense mutations (Nagase and Nakamura, 1993). The two mutations we detected in this study were not observed in these 174 germline mutations.

The exonic location of germline mutations correlated with the number of colorectal polyps had been reported (Nagase *et al.*, 1992b; Nagase and Nakamura, 1993). Germline mutations between codons 1285 and 1465 seem to be associated with a profuse phenotype in which more than 5,000 colorectal polyps develop. The mutations outside of codons 1285 to 1465 were observed in FAP patients having fewer polyps. In our cases, it was suggested that the two mutations outside 1285 to 1465 caused fewer polyps.

It is important to provide presymptomatic diagnosis easily and quickly. Detection of 12 germline mutations in the *APC* gene has been reported by PCR and the germline mutations of nearly 40% of FAP kindreds have been identified (Ando *et al.*, 1993). In our two cases, the mutations could not be determined by this method. However, in kindred 2, it is possible to screen presymptomatic diagnosis easily and quickly with digestion by restriction enzyme *Eco*NI without DNA sequencing.

After germline mutations are detected in FAP patients, we contribute to produce the rapid and easy screening procedure and screen the mutation of the *APC* gene in additional family members. When family members are determined to contain an *APC* mutation, they should be followed up carefully and surgical treatment to prevent the development of colorectal cancer would be advised.

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