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RFLP Report

AN *Eco*RI RFLP IN HUMAN *APC* GENE

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Summary A novel *Eco*RI-restriction fragment length polymorphism in the genomic sequence of the human adenomatous polyposis coli (*APC*) gene is described. This polymorphism in the *APC* gene would be very useful for examination of segregation of the mutated *APC* gene in the kindreds of familial adenomatous polyposis, and also for studying the loss of heterozygosity at the *APC* region in certain tumors.

Key Words restriction fragment length polymorphism (RFLP), adenomatous polyposis coli (*APC*) gene

Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominant genetic disorder characterized by the development of hundreds to thousands of colorectal adenomas. The disorder is caused by inherited germline mutation in a putative tumor suppressor gene, *APC* (Kinzler *et al.*, 1991; Nishisho *et al.*, 1991). Even in sporadic colorectal cancer, the *APC* gene is also inactivated biallelically by two somatic mutations. Polymorphisms in the *APC* gene are very useful for examination of segregation of the mutated *APC* gene in the FAP kindreds, and also for studying the loss of heterozygosity at the *APC* region in certain tumors. Several polymorphisms in the *APC* gene were reported previously (Nagase *et al.*, 1992). Here we report a novel *Eco*RI-restriction fragment length polymorphism (RFLP) in the *APC* gene detected by Southern hybridization.

Materials and Methods

SW41B is a 2.2 kb cDNA clone including the 5′ portion of the *APC* gene. The *APC* gene had been isolated from 5q21 by positional cloning (Kinzler *et al.*, 1991; Nishisho *et al.*, 1991). The 0.8 kb *Eco*RI subfragment of SW41B was used for Southern hybridization. Southern hybridization was carried out as previously

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described (Kurahashi *et al.*, 1995). A total of 55 unrelated healthy Japanese individuals was examined for RFLP.

Results and Discussion

Representative autoradiogram of Southern hybridization is shown in Fig. 1. An RFLP characterized by the appearance of 5.0 and 3.0 kb bands was detected in *Eco*RI digested DNA samples. The allelic frequencies were estimated from a total of 55 unrelated Japanese subjects as listed in Table 1.

Mendelian co-dominant segregation was observed in two informative pedigrees (data not shown). As other restriction enzymes, including *Msp*I, *Taq*I, *Pst*I, *Bgl*II, *Pvu*II, and *Rsa*I, did not detect polymorphic bands, this polymorphism is considered as a site polymorphism.

The entire coding region of the *APC* gene is approximately 8.5 kb, which is too long to identify a specific mutation in a given FAP kindreds. This RFLP in

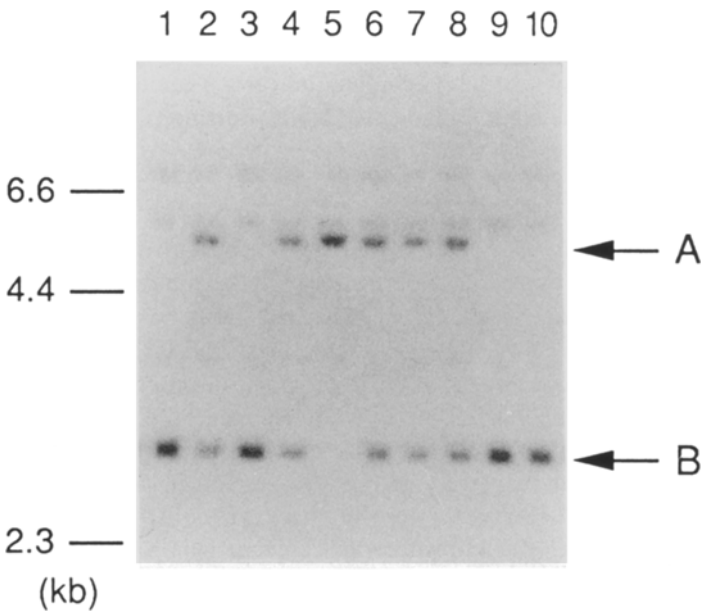

Fig. 1. Representative autoradiogram of Southern hybridization. Lanes 1–10: unrelated healthy individuals. Arrows indicate polymorphic bands (A, B). Size markers are on the left.

Table 1. Allele frequency of *Eco*RI RFLP in *APC* gene.

Allele	Frequency	Band size (kb)	%Heterozygosity
A	0.32	5.0	44%
B	0.68	3.0	

the *APC* gene would be very useful for examination of segregation of the mutated *APC* gene in the FAP kindreds. Furthermore, some FAP patients often suffer from extracolorectal tumors, such as infantile hepatoblastoma. It is still unknown whether these tumors are also implicated with genetic alterations of the *APC* gene. This RFLP would also be very useful for studying the loss of heterozygosity at the *APC* region in these tumors as well as colorectal tumors.

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