

Correlation between the Location of Germ-Line Mutations in the *APC* Gene and the Number of Colorectal Polyps in Familial Adenomatous Polyposis Patients¹

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Abstract

Recently we have isolated the adenomatous polyposis coli (*APC*) gene which causes familial adenomatous polyposis (FAP), and its germ-line mutations in a substantial number of FAP patients have been identified. On the basis of this information, we compared the location of germ-line mutations in the *APC* gene in 22 unrelated patients (12 of whom have been reported previously) with the number of colorectal polyps developed in FAP patients; 17 were sparse types and five were profuse types. All but one of the mutations were considered to cause truncation of the gene product by frame-shift due to deletion (14 cases) or nonsense mutation (seven cases). The location of the germ-line mutations seems to correlate with the two clinical types; germ-line mutations in five FAP patients with profuse polyps were observed between codon 1250 and codon 1464, whereas mutations in 17 FAP patients with fewer polyps were observed in the other regions of the *APC* gene. The result suggests that the number of colorectal polyps in FAP patients may be associated with a difference in the stability or biological function of the truncated *APC* protein.

Introduction

FAP³ is an autosomal dominant inherited disease with high penetrance that is characterized by a large number of colorectal adenomatous polyps. One or more of these polyps develop into colorectal carcinoma if left without surgical treatment (1, 2). In addition to colorectal tumors, neoplasms in other organs frequently develop: soft tissue tumor; osteoma; gastric cancer; carcinoma of the gall bladder and bile ducts; and papillary carcinoma of the thyroid (1-4).

Based on the number of adenomatous polyps, FAP patients are clinically divided into two groups, the profuse and sparse types. A profuse type of FAP patient develops more than 5000 adenomatous polyps, and a sparse type of patient develops 1000 to 2000 polyps (1, 2). The number of polyps is usually consistent among the patients in the same pedigree (2). Furthermore, the average age at onset for developing cancer in the profuse type (34.0 yr) is younger than that observed in the sparse type (41.8 yr) (2). This evidence implies the possibility of different types of genetic alteration in the two groups.

To address this question, we have determined germ-line mutations of the *APC* gene (5-8) in FAP patients and compared the mutations in two types of phenotypically different groups.

Materials and Methods

FAP Patients. Twenty-two unrelated Japanese FAP patients, for whom detailed clinical and pathological information was available, were used in this study. The germ-line mutations indicated in 12 cases were reported previously (9). Patients were divided into two phenotypically different groups; five were in a profuse type and 17 were in a sparse type. These criteria were based on the total number of colorectal polyps (more than or less than 5000) or the number of polyps, determined microscopically, in 1 cm² (profuse type, 10 or more; sparse type, less than 10) (1, 2, 10). Genomic DNAs from FAP patients were prepared from WBC as described elsewhere (11).

PCR. The entire coding region of the *APC* gene was divided into 31 segments, and each segment was separately amplified using PCR as reported previously (9, 12). PCR was performed with 38 cycles for 0.5 min at 95°C, 2 min at 51°C, and 2 min at 70°C, as described by Baker *et al.* (13).

RNase Protection Analysis. RNase protection assays were performed for the PCR products by the method of Winter *et al.* (14) as modified by Kinzler *et al.* (15). Briefly, the PCR products were hybridized to ³²P-labeled RNA transcripts corresponding to the normal *APC* sequence, cloned, and labeled as described by Nishisho *et al.* (6). The DNA-RNA hybrids were digested with RNase A, which cleaved RNA at the site containing mismatches. The size of RNA product was analyzed by polyacrylamide gel electrophoresis.

Cloning and Sequence Analysis. PCR products revealing alterations in RNase protection patterns were cloned into a plasmid vector (pBlue-script SK Stratagene), as described (6). DNAs from a pool of at least 50 subclones were used as a template for each DNA sequencing reaction. DNA sequencing was carried out according to the method described by Nigro *et al.* (16).

Results

The examples of DNA sequencing of the *APC* gene in two FAP patients are shown in Fig. 1. A nonsense mutation from CAA (Gln) to TAA (stop codon) (Fig. 1A) and a 5-base pair deletion causing frame-shift mutation (Fig. 1B) are clearly detected. Germ-line mutations of 22 FAP patients, including 12 mutations reported previously (9), are summarized in Table 1. All affected individuals belonging to the same pedigree used in this study were phenotypically similar to their probands, and no significant difference in the number of polyps was observed. The age at diagnosis, location and type of germ-line mutation, and number of polyps are also indicated in Table 1.

Germ-line mutations in all patients except Patient 1157 were observed in the 5' half of the coding region and resulted in truncation of the gene product. Among these, seven were caused by nonsense mutations, and 14 were caused by frame-shift mutations because of one- to eight-base deletions. A missense germ-line mutation (Pro-Leu) at codon 1176 was found in

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³ The abbreviations used are: FAP, familial adenomatous polyposis; PCR, polymerase chain reaction; APC, adenomatous polyposis coli.

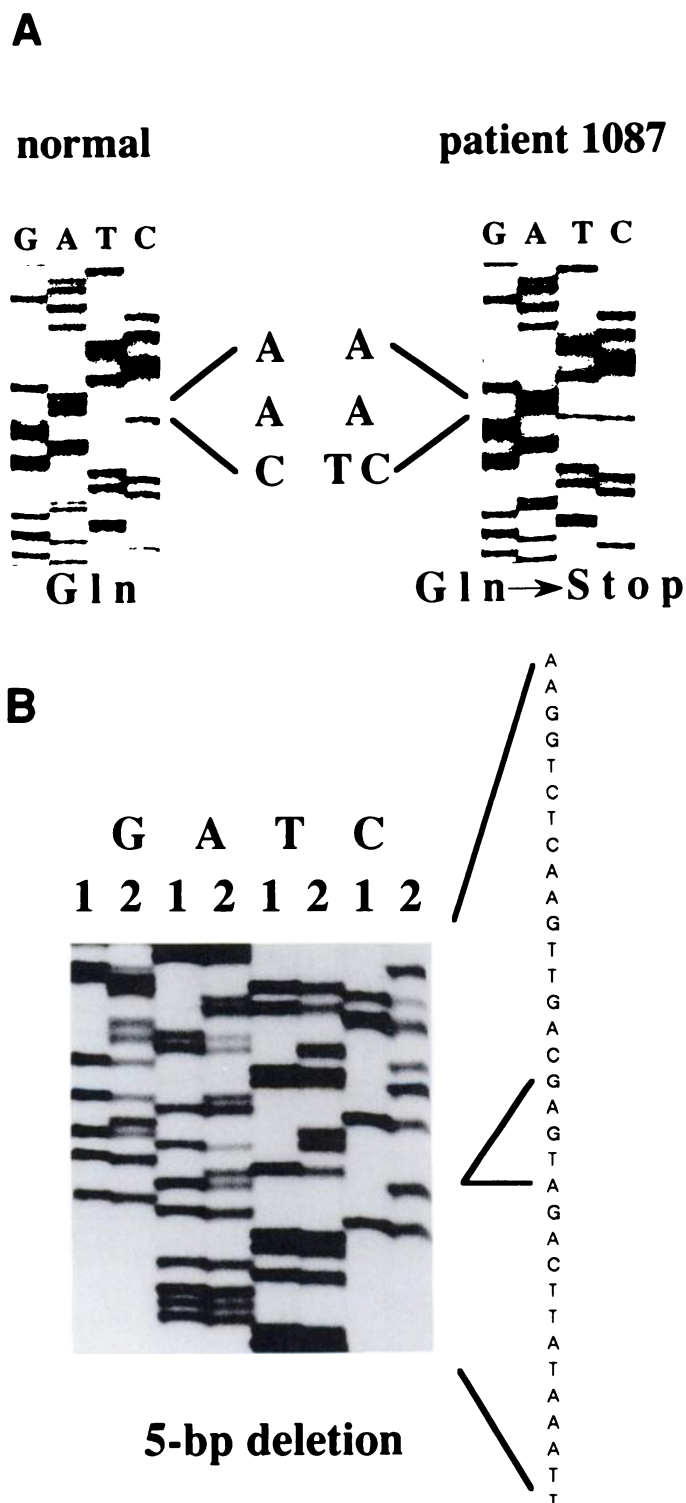


Fig. 1. Sequence analysis of the *APC* gene in FAP patients. PCR products were cloned into a Bluescript vector, and a minimum of 50 clones were pooled for a sequencing template. In *A*, DNA sequences between nucleotides 3119 and 3156 of the *APC* gene in a normal individual and in Patient 1087 show an alteration from CAA (*Gln*) to TAA (*Stop*) at codon 1041. *B*, DNA sequences between nucleotides 3104 and 3136 of the *APC* gene in a normal individual (*Lane 1*) and Patient 1095 (*Lane 2*). Each nucleotide of normal and patient DNAs was electrophoresed side by side. Extra bands corresponding to the mutated allele are observed in *Lane 2*.

Patient 1157, which was the only change detected by RNase protection analysis of the entire coding region.

In the profuse type of FAP patients, the APC protein was truncated in a central region between codon 1255 and codon

1467. FAP patients whose *APC* gene product was truncated outside this region belonged to the sparse type. Fig. 2 illustrates the distribution of germ-line mutations in the two types. In the sparse type of patients, mutations were observed throughout the *APC* gene, except in a central region in which mutations in the profuse type of patients were found exclusively. Furthermore, it is notable that all three patients who had a 5-base pair deletion at codon 1309 developed more than 5000 adenomatous polyps and that both patients for whom we detected a 2-base pair deletion at codon 1465 belonged to the sparse type.

Discussion

Utsunomiya (2) reported that the number of adenomatous polyps was relatively consistent among affected family members and that 52 (98.1%) of 53 affected relatives had a similar number of polyps to that observed in their probands. This result suggested that the number of polyps may be in accord with the specific germ-line mutations which are carried by FAP families. Our finding presented here supports a possible correlation between the location of the germ-line mutations in FAP patients and the number of adenomatous polyps in the colon and rectum.

All but one mutation caused truncation of the gene product. Why does the size of truncated gene product cause the pheno-

Table 1 Number of polyps and *APC* mutation

Patient	Age (yr)	Codon	Change	Type
102 ^a	40	213	Arg→stop	Sparse
1142	30	553	Trp→stop	Sparse
90 ^a	33	577	Leu→stop	Sparse
86 ^a	20	622	Thr→stop	Sparse
1099	28	665	Frame-shift	Sparse
84 ^a	37	806	Frame-shift	Sparse
124 ^a	50	857	Frame-shift	Sparse
1095	22	1033	Frame-shift	Sparse
1087	43	1041	Gln→stop	Sparse
103 ^a	44	1061	Frame-shift	Sparse
104 ^a	56	1102	Thr→stop	Sparse
91 ^a	35	1156	Frame-shift	Sparse
1157	39	1176	Pro→Leu	Sparse
47 ^a	22	1249	Cys→stop	Sparse
51 ^a	17	1250	Frame-shift	Profuse
46 ^a	29	1309	Frame-shift	Profuse
1091	20	1309	Frame-shift	Profuse
1163	22	1309	Frame-shift	Profuse
1146	40	1330	Frame-shift	Profuse
1141	53	1465	Frame-shift	Sparse
1143	20	1465	Frame-shift	Sparse
85 ^a	59	1597	Frame-shift	Sparse

^a Reported previously (9).

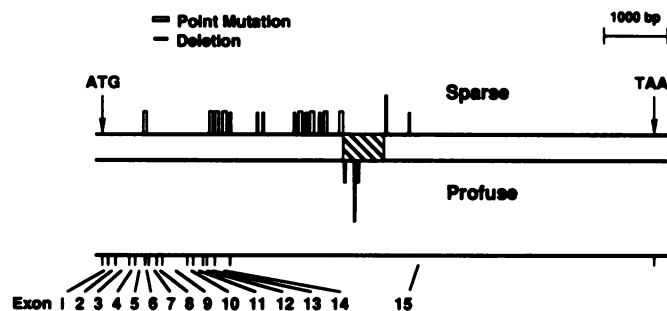


Fig. 2. The distribution of germ-line mutations in the *APC* gene listed in Table 1 is shown with vertical bars. The length of a bar indicates the number(s) of patients carrying a mutation at the position. The sparse type of patients is displayed above and profuse types below the box. The hatched box indicates the region where the germ-line mutations in the profuse type were detected. Positions of the translational initiation (*ATG*) and termination (*TAA*) codons are indicated with arrows.

typic differences? We suspect three possibilities: (a) APC products truncated around codon 1300 have almost no biological activity to suppress adenoma formation, although shorter or longer products have some suppressor activities; (b) APC products truncated around codon 1300 are very unstable compared with shorter or longer products that may still have some suppressor functions; and (c) the APC product interacts with itself or other proteins. APC products truncated around codon 1300 make a stable complex which has no or little suppressor activity. Analysis of the APC sequence has indicated the presence of two regions that have coiled-coil structure, one in the amino-terminal domain and the other in the central portion of APC (5), that are considered to be a structurally important domain for oligomerization with APC itself or other proteins. This evidence might suggest the third possibility, but we need further investigations to address this question.

The number of polyps is expected to increase when patients become older, and this point has been also taken into consideration in our analysis. Maeda *et al.* (10) investigated the number of polyps in 58 affected family members that carry the profuse type of FAP by double-contrast barium enema examination and found that seven members who were 15 yr or younger had already developed more than 5000 adenomatous polyps. As shown in Table 1, FAP patients examined in this study were older than 20 yr except for Patient 51 who had already developed more than 5000 polyps by the age of 17. Although this patient had a 2-base pair deletion at codon 1250, resulting in the creation of a new stop codon 15 base pairs downstream, Patient 47, whose APC product was truncated at codon 1248, had only 425 polyps when she underwent total colectomy at 22 yr of age. This result might define the 5' border between the profuse and sparse types. However, the possibility that Patient 47 might have developed more than 5000 polyps cannot be excluded.

Although we cannot exclude the possibility that these different phenotypes may be caused by a linked modifier gene, our findings suggest a possible correlation between the number of colorectal polyps in FAP patients and the location of germ-line mutations of the APC gene. Furthermore, we need to examine a large number of patients to address the question of whether or not the "profuse" and "sparse" regions are contiguous.

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