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Request for opinion on manuscript by Miyoshi, Ando, Nagase, Nishisho, Horii, Miki, Mori, Utsunomiya Baba, Petersen, Hamilton, Kinzler, Vogelstein, Nakamura

Title Germ-Line Mutations of the APC Gene in 53 Familial Adenomatous Polyposis Patients

The *Proceedings of the National Academy of Sciences, U.S.A.*, a multidisciplinary journal, publishes brief reports of original research of exceptional importance and novelty. I am writing to ask your opinion on the following points, together with any other comments you may offer. Please reply to all questions. The Editorial Board considers the first two the most important. Please note that the Editorial Policy states that the referees should remain anonymous.

1. **Is this contribution of sufficient general interest to justify publication in the Proceedings rather than a specialty journal?** ☑ Yes ☐ No ☐ Don't know
2. **Is the overall quality of this paper suitable for this journal?** ☑ Yes ☐ No ☐ Don't know
3. Does the evidence justify the conclusions drawn? ☑ Yes ☐ No ☐ Don't know
4. Is this paper clearly written for a diverse audience of scientists? ☑ Yes ☐ No ☐ Don't know
5. Are the procedures described sufficiently well that the work can be repeated? ☑ Yes ☐ No ☐ Not relevant
6. Comments (use additional pages if necessary; send original and two copies): If the answers to questions 1 and 2 are Yes, please describe here the aspects of this paper that are novel and important:

This manuscript represents a very important and timely study of germline mutations at the APC locus. It is very timely, as the gene has only recently been cloned, and as the authors describe, there had been some reason to think that the phenotype of familial adenomatous polyposis might be attributable to mutations in any one of a family of genes located in a very small region based on the tumor specific mutations at the APC locus, as well as at the MCC locus. This is a thorough study that examines the specifics of the mutations with some consideration of the mechanisms by which such mutations might arise as well as the implications for more broadly based genetic testing. The authors further emphasize the population aspects of the study with respect to the frequency of certain types of mutations found in different ethnic groups.

There are only a few relatively minor comments:

1. In figure 1B, it is very difficult to see the alteration in upper band at arrow in lane 6.
2. In figure 2, the writing in the legend needs to be revised and clarified.
3. It would be helpful to describe with an additional sentence or two the quality control measures that were used in the study, such as the number of times the amplification reactions were repeated, use of sense and antisense strands in RNase protection analysis, sequencing of the opposite strand of genomic DNA once the specific fragment with a suspected mutation has been identified, etc. They reference previous work but a simple statement of the procedures would be worth including.

There are a few minor grammatical or typographical errors:

1. On page 11 in the middle of the page, methylcytosine is misspelled.
2. On page 13 line 14, a word should be deleted, either "are" or "ranged."
3. The last sentence on page 13 and continuing on page 14, needs some revision/clarification:"these two patients 6 and 70 contain? Further in the same sentence on page 14 "two mutations at both alleles" should be either "both" alleles or "two" mutations.
4. Reference 15 (page 20) is misaligned.

7. If the manuscript is revised, I would be prepared to rereview it. ☐

Minor points:

1) The last sentence on page 4 requries commas after Another and gene.

2) The last sentence of Figure 2- The work each should be removed.

3) Since there is no scale for the number of mutations at each po

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5. Are the procedures described sufficiently well that the work can be repeated? ☒ Yes ☐ No ☐ Not relevant
6. Comments (use additional pages if necessary; send original and two copies): If the answers to questions 1 and 2 are Yes, please describe here the aspects of this paper that are novel and important:

This manuscript examines the correlation between mutations in the APC gene and the presence of Familial Adenomatous Polyposis (FAP) in 79 unrelated patients. The authors present data showing that the majority of these patients contain mutations in this gene strongly implicating its involvement in FAP disease. The importance of these findings for diagnosis as well as genetic counseling are emphasized in the Discussion. The clustering of these mutations may also provide insights into the functions of the APC protein.

This is generally a well-written paper presenting a great amount of information. I would suggest several changes before acceptance for publication to clarify some of the interesting points.

1) The first several paragraphs of the Discussion repeat much of the Results section. These paragraphs should be shortened perhaps by combination. The authors also mention the coiled-coil motif of the APC protein. They should discuss the effects of the observed mutations on this region of the protein.

2) Tables 2 and 3 could be combined into one Table by using the outline of Table 3 to organize Table 2.

3) The legend to Figure 2 requires clarification. Did the authors use a normal individual or a normal PCR product from an affected individual? The information in Figure 2B and C needs better illustration for the general audience of this journal.

7. If the manuscript is revised, I would be prepared to rereview it. ☒

AUTHOR'S COPY 12/20/91

(Classification; Medical Genetics)

Germ-Line Mutations of the APC Gene in 53 Familial Adenomatous Polyposis Patients

(APC gene / familial adenomatous polyposis / germ-line mutation)

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Abbreviation: FAP, familial adenomatous polyposis.

ABSTRACT

We searched for germ-line mutations of the APC gene in 79 unrelated patients with familial adenomatous polyposis (FAP) using an ribonuclease (RNase) protection analysis coupled with polymerase chain reaction (PCR) amplifications of genomic DNA. Mutations were found in 53 patients (67%); twenty-eight of the mutations were small deletions and two were 1-2 bp insertions; nineteen were point mutations resulting in stop codons and only four were missense point mutations. Thus 92% of the mutations were predicted to result in truncations of the APC protein. Over 2/3 (68%) of the mutations were clustered in the 5' half of the last exon, and nearly two-fifth of the total mutations occurred at one of five positions. This information has significant implications for understanding the role of APC mutation in inherited forms of colorectal neoplasia and for designing effective methods for genetic counseling and pre-symptomatic diagnosis.

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal-dominant inherited disease, affecting 1 in 5,000 and 1 in 17,000 of the American and Japanese populations, respectively (1). FAP is characterized by the development 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. Neoplasia is not limited to the colon and rectum of patients with FAP, as some patients are affected with desmoid tumors, osteomas, fibromas, and a variety of other neoplasms in addition to polyps.

Cytogenetic and linkage studies have localized the gene responsible for FAP to chromosome 5q21 (2-5), a region that is also deleted commonly in sporadic colorectal tumors (6-8). Hence, it was considered likely that germ-line or somatic mutations of chromosome 5q21 gene(s) would lead to adenoma formation in familial and sporadic forms (6).

Recently, several novel chromosome 5q21 genes were identified (9-13). One of them the MCC gene was shown to be somatically mutated in a subset of sporadic colorectal cancers. Another the APC gene was shown to undergo similar somatic mutations and

also to be mutated in the germ-line of patients with FAP. Both APC and MCC were predicted to contain coiled-coil proteins that might interact in vivo with themselves or with other proteins.

In our initial study, we examined three exons of APC in 103 kindreds with FAP, and found germ-line mutations in five of them (11). Groden et al. examined a large portion of the APC gene product but found only four mutations in 61 separate kindreds (12). These studies thus left open the question of whether other genes might be involved in a large number of kindreds.

To answer this question, and to further investigate the nature of inherited APC gene mutations, we have now examined the entire coding region of APC in 79 unrelated kindreds with FAP. We were able to identify presumptive mutations in 53 of these kindreds, suggesting that APC is responsible for the great majority of FAP cases. Remarkably, over 90% of the mutations resulted in truncations of the predicted protein product. These results have significant theoretical and practical implications for understanding etiology and diagnosing disease in susceptible individuals.

MATERIAL AND METHODS

Genomic DNA of FAP Patients

FAP patients were identified on the basis of clinical manifestation. Genomic DNA of 79 unrelated patients including 55 American and 24 Japanese kindreds were prepared from white blood cells as described elsewhere (14).

PCR

The coding region of the APC gene was divided into 31 segments (see text), and each segment was separately amplified using PCR (15). The primer pairs used in this study are listed in Table 1. 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. (16).

RNase Protection Analysis

RNase protection assay was performed by the method of Winter et al.(17) as modified by Kinzler et al.(9). Briefly, PCR products were hybridized to ^{32}P-labeled RNA transcripts corresponding to normal APC sequences, cloned and labelled as described by Nishisho et al.(11). The hybrids were digested with RNase A, which cleaves at mismatches within DNA-RNA hybrids. The size of the digestion products was analyzed by polyacrylamide gel electrophoresis.

Cloning and Sequence Analysis

PCR products showing abnormalities in RNase protection patterns were cloned into a plasmid vector (pBluescript SK Stratagene), as described (11). 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.(18).

RESULTS

We examined the entire coding region of the APC gene in the germ-line of 79 unrelated FAP patients by an RNase protection assay. The coding region of the APC gene is contained within 15 exons (no. 1-15) (9,11), preceded by at least one 5' non-coding exon (author's unpublished data). The coding region was divided into 31 segments. The first 14 segments corresponded to 14 individual exons. The coding region in the last exon (no. 15) is very large (6577 bp), and was divided into 17 overlapping segments each of approximately 400 bp in length. These 31 segments were individually amplified from each of the 79 patients and subjected to RNase protection analyses as described in Material and Methods.

Fig. 1 presents examples of RNase protection analyses, in which variations were observed. PCR

products in which variations were detected were cloned into a plasmid vector and sequenced (examples in Fig. 2). Patient 100 had a C to A transversion at the second nucleotide of codon 932 resulting in a change from serine (TCA) to a stop codon (TAA) (lane 2, Fig. 2 (A)). Patient 16 showed a 2 bp deletion (AG) of codon 1465 (AGT) (lane 2, Fig. 2 (B)) and a T insertion at the second nucleotide of codon 1211 (ATTG) of patient 39 (lane 4, Fig. 2 (C)) beginning at arrows.

The results from the PCR-RNase protection analyses are summarized in Table 2. Presumptive mutations altering the sequence of the predicted protein product were observed in 53 of the 79 patients studied. No patient had more than one of these mutations, and none of these mutations was observed in the germ-line DNA of at least 100 individuals without FAP studied by RNase protection assay or direct sequencing of PCR products.

Nature of Mutations

As summarized in Table 3, 23 of the 53 alterations were point mutations. Nineteen of them generated stop codons. Two of the four amino acid changes were non-conservative (resulting in the substitution of cysteine for serine or arginine), and two were functionally conservative (serine to

threonine or leucine to phenylalanine, respectively). Twenty-one of the 23 mutations resulted in a change from C to some other nucleotides in which C to T was the most common (Table 4). The point mutations were scattered throughout the gene and no "hot-spot" was detected; only the mutations at codon 302 and 625 were observed in more than one kindred (Table 2).

Thirty of the 53 mutations were associated with frameshifts due to deletions (28 cases) or insertions (2 cases) (Table 3). Deletions of 1 to 5 bp were observed, with 5 bp being most common (18 of the 28 deletion mutations involved 5 bp). Interestingly, a 5 bp deletion at codon 1309 was observed in 10 separate kindreds; this was so far the most common genetic alteration observed among the families. All the deletion and insertion mutations altered the reading frame and created stop codons at immediate downstream.

Distribution of Mutations

The distribution of mutations in the APC gene in the 53 FAP patients is shown in Fig. 3. Point mutations were found in exon 5, 6, 8, 9, 12, 13, 14, 15. However, all but one of the 30 cases of deletion or insertion were found in exon 15 (one case was in intron 3). Thirty-six (68%) of the

total mutations (including both point mutations and frameshifts) were clustered within the 5' half of exon 15 (codon 713 to 1597) represented less than one-third of the coding region. Five specific mutations were found in more than one unrelated kindred (Table 2 and Fig. 3).

DISCUSSION

Most of the mutations described in this study are predicted to have profound effects on the predicted gene products. Forty-nine (92 %) of the mutations led to incomplete products of the APC gene due to translational termination; 19 of the 23 point mutations were nonsense mutations and all 30 frameshift mutations created new stop codons at immediate downstream. Missense mutations were observed in only 4 patients. In two of these four cases, the change to cysteine from arginine or serine is expected to have significant effects on the structure of the predicted protein. The other two changes were relatively conservative, and we cannot be sure that these substitutions represent true mutations rather than rare variations with no functional effect. However, this serine to threonine (patient 3) or leucine to phenylalanine (patient 89) change was the only change detected by

RNase protection analyses of the entire coding region, except one amino acid polymorphism of patient 3 (describe later). As the biochemical and/or physiologic properties of the APC protein are undiscovered, however, these missense mutations might prove to be valuable for assessing function, and to provide clues for localizing the critical effector domains of the very large protein encoded by the APC gene.

91%
~~87%~~ of the 23 point mutations resulted in a substitution for C, most commonly with a T (Table 4). These occurred at 7 CA sites, 5 CG sites and one CT site. Deamination of 5-metylcytosine in the CpG dinucleotide has been implicated as a mechanism for point mutation from C to T (19). But only 5 of our cases involved CpG sites (Table 2). The mechanism for CpA to TpA and CpT to TpT change is not known. However, as the most common DNA polymerase error is thought to be a G mispairing with T (20) with a lack of repair at this mismatch (21), the CpA to TpA and CpT to TpT mutations we observed might have been generated in this manner.

Twenty-eight of the 30 cases with frameshifts were associated with small deletions (Table 2). It is well known that deletions occur at repeated bases, perhaps because of mis-alignment; for

example, we observed a C deletion from CCC (at codon 1427), an A deletion from several A's (codon 142) and an AA from AAA (codon 1250). A model for generating mis-alignment within a stretch of common bases has been proposed (22,23). Some deletions were observed at positions containing several copies of a direct repeat; for example, an AG deletion was detected at the sequence of AAAGAGAGAGAGTG (codon 1465); an AAAGA deletion was observed at ATAAAAGAAAAGATT (codon 1309); an ACAAA deletion from ATAAAACAAAGT (codon 1061) and a TGAAA deletion from TCAAATGAAAAC (codon 1546). All of these deletions might have occurred during DNA replication as a result of slippage of the template strand and subsequent mis-alignment (24). After the synthesis of the first copy of the direct repeat, the template strand could slip and mis-align with the second copy of the repeat, result in deletion of the intervening sequences.

All but one of the frameshift mutations due to insertions or deletions were detected only within exon 15 but point mutations were scattered from exon 5 to exon 15. The most frequent mutation was observed at codon 1309. As this mutation was observed in Caucasian, Black and Japanese populations, a founder effect is excluded.

In the course of RNase protection analyses, we found several polymorphisms within the coding sequences resulted in both with and without amino acid changes. Four major polymorphisms are TAC/TAT (at codon 486), GCA/GCG (at codon 545), ACG/ACA (at codon 1493) and GGA/GGG (at codon 1678). All of these polymorphisms have not changed coding amino acids; tyrosine, alanine, threonine and glycine, respectively. The first polymorphism of the tyrosine coding sequences creates restriction fragment length polymorphism with Rsa I. This polymorphism and the last glycine coding polymorphism are the same reported previously (12). The allelic frequencies of these polymorphisms are ranged from 0.36 to 0.64 (data not shown). Including two amino acid polymorphisms, the other four rare polymorphisms of which allelic frequencies were less than 0.01 were also recognized (data not shown). Two amino acid polymorphisms were ATA (isoleucine)/ GTA (valine) at codon 1304 of patient 6 and TCA (serine) / TTA (leucine) at codon 2578 of patient 3 and 70. Because these two patients 6 and 70 contain other mutations of 5 bp deletion that have profound effect on the predicted gene products at codon 1309 (patient 6) and at 1061 (patient 70) (Table 2), we

think these amino acid changes might not alter the function of the predicted proteins. But, the possibility of two mutations at both alleles is not excluded. The others were TTA/TTG at codon 548 of patient 90 and ATA/ATT at codon 1055 of patient 22 without amino acid changes; leucine and isoleucine, respectively.

We detected mutations in 67% of FAP patients using an RNase protection assay. We did not find mutations in all patients because (i) some mismatches are protected from RNase digestion, in fact, the reported sensitivity of detecting mutations with RNase is only 35-50 % (11,25,26). We attribute our higher success rate to the fact that so many of the mutations in the APC gene were small deletions or insertions, which are usually quite susceptible to digestion with RNase A; (ii) the promoter region of the APC gene has not yet examined; (iii) non-examined sequences within introns may have a significant influence to gene expression; (iv) there may be a second FAP gene. We think, however, that the latter possibility is now unlikely.

The results of the studies described above provide significant insights into the nature of the mutations leading to FAP. They suggest that the

carboxy-terminus is required for function, because deletions that remove this end of the protein (including a deletion that removes only the last 200 amino acids of the 2843 residue protein) result in disease. Further studies will be required to determine whether specific mutations are associated with specific phenotypes (such as early age at onset or a high prevalence of extra-colonic neoplasms). Already, however, these studies provide a basis for pre-symptomatic diagnosis. In 53 kindreds reported here, such diagnoses can now be made with virtually 100% accuracy simply by testing for the relevant mutation. In kindreds not yet studied, it would seem advisable to begin screening of mutation at the five positions accounting for nearly 40% of the total alterations detected (Table 2). If these were negative, the next logical step would be to examine the 5' half of exon 15, which contained over two-thirds of the mutations. Only if this failed would an analysis of the remainder of the gene be warranted. Finally, these studies suggest an alternative method of examination for APC mutations. 92% of the total mutations detected are predicted to result in truncated protein products. Thus, detection of these shortened proteins using

antibodies against the APC protein is likely to be a valuable screening method in a high fraction of kindreds.

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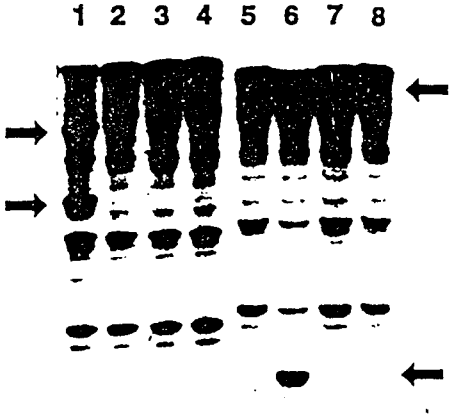
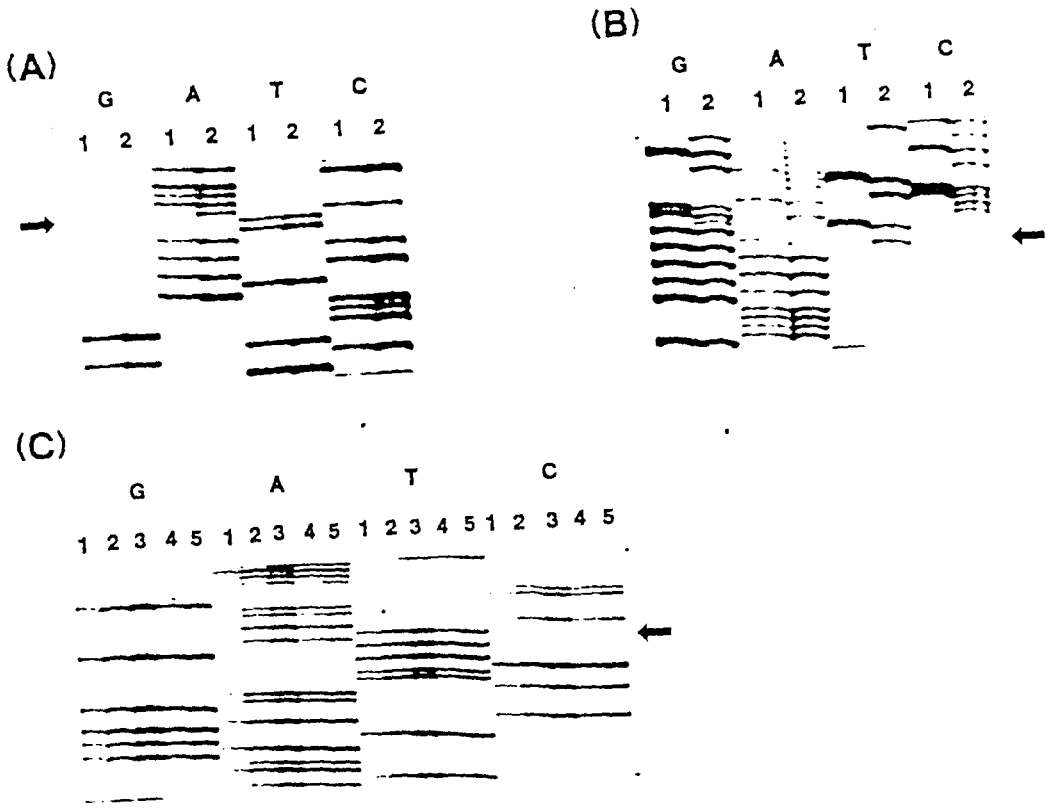
(A)

(B)




(A)
G A T C
1 2 1 2 1 2 1 2
(B)
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(C)
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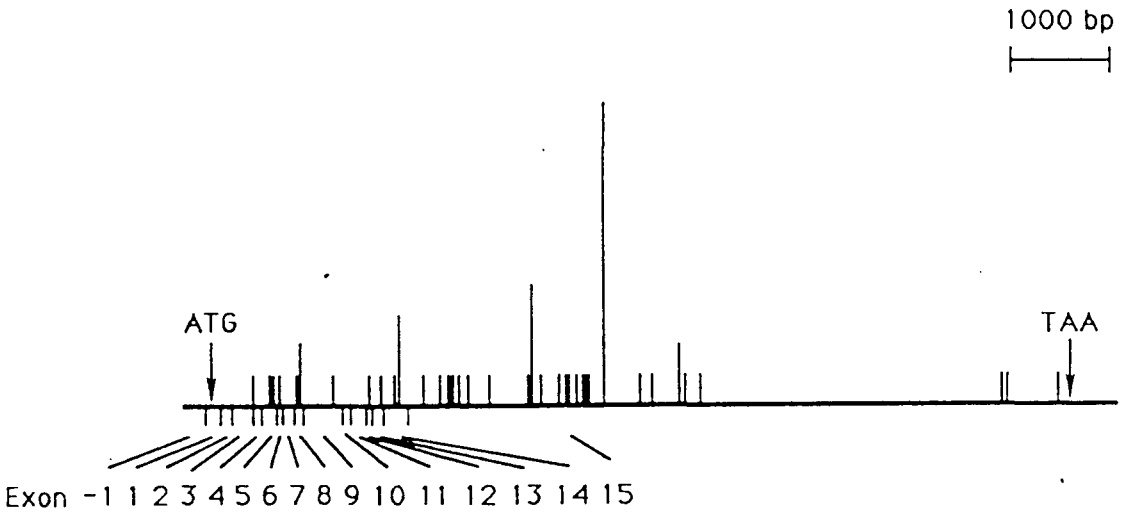
1000 bp
ATG
TAA
Exon -1 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

(Figure legend)

Fig. 1. RNase protection analyses. In (A), lane 1 and 4 show the same variant RNase protection patterns (arrows) in patient 5 and 10, respectively, using Ex 15-4 probe containing codon from 999 to 1139 and 426 bp in length (Table 1) resulted in the same 5 bp deletion. In (B), lane 1 and 6 represented different abnormal patterns (arrows) in patient 15 and 91 with the probes of Ex 15-5 containing codon from 1125 to 1283 and 478 bp in length resulted in different mutations.

Fig. 2. Sequence analyses of normal and variant PCR products. Patient 100 (lane 2 in (A)) shows a C to A change (arrow) at the second nucleotide of codon 932 resulted in a change from serine (T<u>C</u>A) to a stop codon (T<u>A</u>A). Patient 16 (lane 2 in (B)) shows 2 bp (AG) deletion of codon 1465 (<u>AG</u>T) and patient 39 (lane 4 in (C)), a T insertion at the second nucleotide of codon 1211 (AT<u>T</u>G) beginning at arrows. The each reaction samples of different patients are grouped so that mutations could be easily recognized.

Fig. 3. Distribution of germ-line mutations in the APC gene listed in Table 2 is shown with a bar. The length of a bar indicate the number(s). The positions of translational initiation (ATG) and termination (TAA) codon are described with arrows.

Table 1. Sequences of primers used for PCR of RNase protection analyses

Upstream primers	Exon*	Downstream primers
G31 ATGGAATTCTTCTTAAACTGCTTAAGAG*	(Ex1)	G18 TTTACAAGAGGGAATACTGAAT
G21 CCTGAATTCAAGAAATACAGAATCACGTC*	(Ex2)	G22 ATGAAGCTTGTACTTGGATCTACACACC§
G29 ATGGAATTCCATTAAGAATATTTTAGACTGCT*	(Ex3)	G28 TTAAAGCTTAACAATAAACTGGAGTACACA§
G27 ATGGAATTCCAACTGATGTAAGTATTGCT*	(Ex4)	G30 ATGAAGCTTTAATGGATTACCTAGGTACT§
G17 CAGGAATTCTTTATTGGTTCTTATATGCT*	(Ex5)	G26 CTGAAGCTTCCTAATAGCTCTTCGCTG§
G23 CAAGGATCCTGAGCTTTTAAGTGGTAG‡	(Ex6)	G20 CTGAAGCTTTTCTCAGAATAACTACCTA§
G19 ACTGAATTCCTTGGGCTAAGAAAGCCT*	(Ex7)	G24 ATGAAGCTTCTTAGAACCATCTTGCTTC§
G5 CATGATGTTATCTGTATTTACC	(Ex8)	G4 CTTAGCAAAGTAGTCATGGC
G1 GGATATTAAAGTCGTAATTTTGT	(Ex9)	G2 CATGCACTACGATGTACACT
G13 CATCATTGCTCTTCAAATAACA	(Ex10)	G14 CACCAGTAATTGTCTATGTCA
G9 TAGATGATTGTCTTTTTCCTCT	(Ex11)	G10 TCATACCTGAGCTATCTTAAG
G7 GCTTGGCTTCAAGTTGTCTT	(Ex12)	G8 CAGAGTGAGACCCTGCCT
G11 GCAACTAGTATGATTTTATGTATAAA	(Ex13)	G6 ACATGAAATTCATATTATAGTACT
G15 CAACTCTAATTAGATGACCCA	(Ex14)	G16 GAGAGTATGAATTCTGTACTT
G35 CAATCATATTATGCCTTTTGTC	(Ex15-1)	C22' GATGGCAAGCTTGAGCCAG
E9-1 CGAAGTACAAGGATGCCAAT	(-2)	E9-2 CAGTGGTGGAGATCTGCAA
E9-3 AACTACCATCCAGCAACAGA	(-3)	E9-4 TCTAGTTCTCCATCATTATCAT
C23 TCAATACCCAGCCGACCT	(-4)	E9-6 GGCTTATCATCTTCATAGTCA
E9-5 GTAAGCCAGTCTTTGTGTC	(-5)	E9-8 CAGCTGATGACAAAGATGAT
E9-7 AGACTTATTGTGTAGAAGATAC	(-6)	E9-10 ATGGTTCACTCTGAACGGA
E9-9 TCTGTCAGTTCACTTGATAG	(-7)	C36 CATTTGATTCTTTAGGCTGC
E9-11 ACAGAAAGATGTGGAATTAAG	(-8)	E9-12 TTCTCCAGCAGCTAACTCAT
E9-13 GCTACATCTCTAAGTGATCT	(-9)	E9-14 CTTATCATTGAAGTCCTTGG

E9-15	CTCAGACAACAAAGATTCAAA	(-10)	C52	GAGAAAAGCAAACTGGAGTA
E9-17	AGTCATCCAAAGACATACCA	(-11)	E9-22	CTGAATCAGGGGATAGACC
C35	GATATACAGAGACCAGATTCA	(-12)	C38	ACAGGACTTGTACTTGAGGA
C37	CGAGGCAGGACAATGATTC	(-13)	C40	GACTCACTTCTTGGAATACTA
C39	CAGATGAGCCAACAGAACC	(-14)	C42	GCTGGTCTAGATGATGGAG
E9-19	TGGAGGAATCTGCTTCATTT	(-15)	E9-16	TCCTTTTGCGGATACTTGG
E9-21	TGAACTCTATTTCAGGAACC	(-16)	E9-18	GTACGTTCCACTATAGAACT
E9-23	GTCCCTGTATCAGAGACT	(-17)	E9-20	TGTCTATATAGCAGTTGTAATT

* Exon 15 is divided into 17 overlapping segments each of approximately 400 bp in length. Primers are described from the 5' to 3' direction and some of them are created Eco RI (+), Bam HI (‡) or Hind III (§) site within the primers.

Table 2. Germ-line mutations in the APC gene of 79 unrelated FAP patients

Patient	Codon	Nucleotide change*	Amino acid change
13	142	aatag/GTC -> atag/GTC	a deletion
102	213	CGA -> TGA	Arg -> Stop
11	215	CAG -> TAG	Gln -> Stop
33	232	CGA -> TGA	Arg -> Stop
93*	280	TCA -> TGA	Ser -> Stop
24*,34*	302	CGA -> TGA	Arg -> Stop
21*	414	CGC -> TGC	Arg -> Cys
7	541	CAG -> TAG	Gln -> Stop
90	577	TTA -> TAA	Leu -> Stop
86	622	TAC -> TAA	Thr -> Stop
8,38,66	625	CAG -> TAG	Gln -> Stop
60*	713	TCA -> TGA	Ser -> Stop
3	784	TCT -> ACT	Ser -> Thr
49	794	AGTC -> ATC	G deletion
84	806	CATGA -> CGA	AT deletion
80	827	AAT -> AAATT	AT insertion
124	857	GGAATTGG -> GGG	GAATT deletion
100	932	TCA -> TAA	Ser -> Stop
62	1055	ATAATAGA -> AGA /	TAATA deletion
5,10,70,103	1061	AAACAAAG -> AAG	ACAAA deletion

104	1102	TAC -> TAG	Tyr -> Stop
91	1156	GAAGAGA -> GGA	AAGA deletion
43	1175	CAG -> TAG	Gln -> Stop
15	1191	CAGA -> CAA	G deletion
39	1211	ATG -> ATTG	T insertion
1	1230	CAG -> TAG	Gln -> Stop
47	1249	TGC -> TGA	Cys -> Stop
51	1250	AAAGT -> AGT	AA deletion
6,17,20,22,25, 29,46,57,59,61	1309	GAAAAGAT -> GAT	AAAGA deletion
120	1427	CCTG -> CTG	C deletion
16	1465	GAGTG -> GTG	AG deletion
28,78	1546	AATGAAAA -> AAA	TGAAA deletion
79	1567	TCA -> TGA	Ser -> Stop
85	1597	ACTG -> ACG	T deletion
19	2621	TCT -> TGT	Ser -> Cys
18	2644	ATTTATC -> ATC	TTAT deletion
89	2839	CTT -> TTT	Leu -> Phe

* Lower and upper case letters indicate intron and exon, respectively

+ Reported previously (11).

Table 3. Frequency of germ-line mutations in the APC gene

Point mutations			23
	Nonsense mutation		19
	Missense mutation		4
Frameshift mutations			30
	Deletion	(1–5 bp)	28
	Insertion	(1–2 bp)	2
Total			53

Table 4. Summary of point mutations in the APC gene

From/To*	C	T	G	A	Total
C	-	13	5	3	21
T	0	-	0	2	2
G	0	0	-	0	0
A	0	0	0	-	0
Total	0	13	5	5	23

* Listed in coding strand.