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Mutational Analysis of Patients with Adenomatous Polyposis: Identical Inactivating Mutations in Unrelated Individuals

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Summary

Samples of constitutional DNA from 60 unrelated patients with adenomatous polyposis coli (APC) were examined for mutations in the *APC* gene. Five inactivating mutations were observed among 12 individuals with APC; all were different from the six inactivating mutations previously reported in this panel of patients. The newly discovered mutations included single-nucleotide substitutions leading to stop codons and small deletions leading to frameshifts. Two of the mutations were observed in multiple APC families and in sporadic cases of APC; allele-specific PCR primers were designed for detecting mutations at these common sites. No missense mutations that segregated with the disease were found.

Introduction

Familial adenomatous polyposis coli (APC) is an autosomal dominant disease characterized by the development, at an early age, of hundreds to thousands of colonic adenomatous polyps. There is an inevitable progression to colon cancer if the colon is not removed. Mutation at the *APC* locus is thought to be a rate-limiting step in the development of colorectal neoplasia, since it is one of the earliest genetic events in this pathway, as evidenced by phenotype.

Recently, the gene mutated in APC patients was isolated by positional cloning (Joslyn et al. 1991; Kinzler et al. 1991*a*). This finding was based on (*a*) the initial observation of an APC patient with a cytogenetic deletion removing part of chromosome 5q (Herrera et al. 1986), (*b*) confirmation of this suggested chromosomal localization by linkage analysis (Bodmer et al. 1987; Leppert et al. 1987), and, finally, (*c*) the observation of two patients with overlapping deletions of 100 kb and 250 kb, respectively (Joslyn et al. 1991). The *APC* gene was identified specifically by the presence of inactivating constitutional mutations in individuals affected with either familial or sporadic APC; mutations in this gene were also found in a number of colorectal tumors and in a cell line grown from a colorectal carcinoma (Groden et al. 1991; Nishisho et al. 1991). The present report describes additional *APC* mutations and polymorphisms found by means of single-strand conformation polymorphism (SSCP) analysis, in genomic DNA from affected individuals.

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Patients, Material, and Methods

APC Patients

Our set of APC patients was collected through referrals from private physicians and hospital clinics specializing in the diagnosis and treatment of APC. All human specimens and histories were obtained with the approval of the Institutional Review Board at the University of Utah Health Sciences Center. Patients were

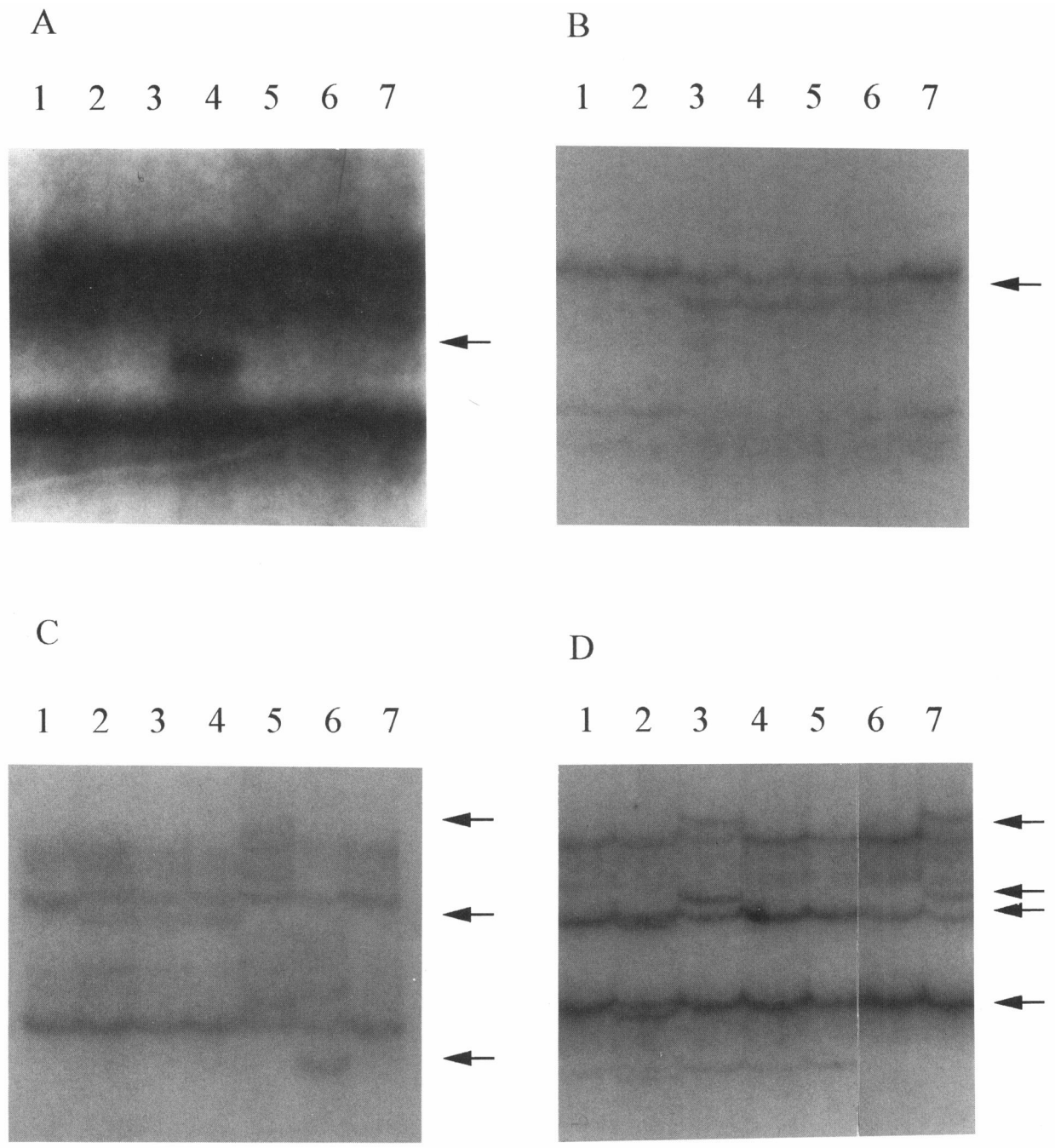

Figure 1 PCR-SSCP conformers in the *APC* gene specific to APC patients. Each panel shows the PCR-SSCP conformers found in APC patients, with a unique pair of primers. Arrows mark the position of the new conformers. The flanking lanes in each panel show the expected pattern of conformers in DNA from other affected individuals and from normal controls. *A*, PCR products of primer pair 15-D, which amplify the *APC* gene from nucleotides 2780–3161. Lane 4, DNA from patient 3794, which exhibits a new conformer. *B*, PCR products of primer pair 15-E, which amplify nucleotides 3082–3511. Lanes 3–6, DNA from patients FP-5-04, FP-8-03, 12046, and 12297, respectively. Each of these samples gives an identical new conformer. *C*, PCR products of primer pair 15-G, which amplify the *APC* gene from nucleotides 3767–4151. Lanes 2–4, Identical novel conformer in DNA from patients 3442, 3493, and 12255, respectively. Lane 5, Second novel conformer pattern, in DNA from patient 3400. Lane 6, Third novel conformer pattern, in DNA from patient 3461. *D*, PCR products using primers 15-U that amplify nucleotides 7694–8095. Lane 2, DNA from patient 3407, which exhibits a novel conformer pattern. Lanes 3 and 5, Identical and novel conformer pattern in DNA from patient 3408 and his nonaffected offspring, respectively.

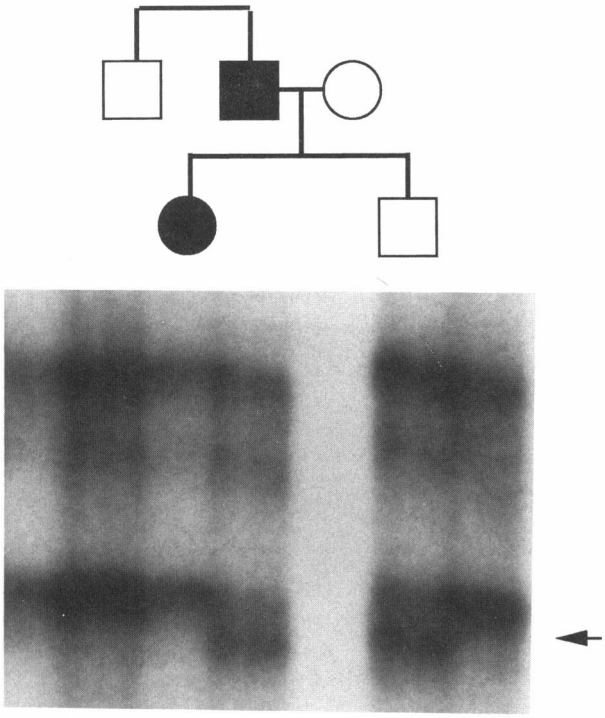

Figure 2 Pedigree and PCR-SSCP analysis of APC patient 3408. *Top,* Pedigree of patient 3408, the affected father, shown above. Individuals with APC are represented by the blackened square and circle. *Bottom,* PCR-SSCP analysis of DNA from individuals in the pedigree above, amplified with APC primer pair 15-U. Each lane is centered below the individual from whom the corresponding DNA was obtained. The unique conformer originally observed in DNA from patient 3408 can be seen only in DNA from his unaffected offspring and is not seen in DNA from his affected offspring.

referred from the Department of Surgery, Division of Gastroenterology, at the University of Utah Medical Center, Salt Lake City; the Johns Hopkins School of Medicine, Baltimore; St. Mark's Hospital, Salt Lake City; Latter-Day Saints Hospital, Salt Lake City; Cleveland Clinic, Cleveland; and the University of Illinois College of Medicine at Peoria Medical Center, Peoria.

Cell Lines and Tissue Culture

Epstein-Barr virus was used to transform lymphocytes from APC patients and from normal controls. The lymphoblastoid cell lines, cultured with standard conditions, provided DNA for mutational analysis. DNA was prepared according to standard protocols.

Oligonucleotide Primers and PCR Amplification

Oligonucleotide primers were designed to amplify the 15 coding exons of the *APC* gene. Each pair of primers was tailed with UP (M13 universal primer) and RP (M13 reverse primer) sequencing primers. With two exceptions, all of the primer sequences had been published elsewhere (Groden et al. 1991); replacement primers were synthesized for 1UP and 3UP, ATTAACACAATTCTTCTTAAACGTCandGACCCAAGTGGACTTTTCAGG, respectively (5′ to 3′), to allow scanning of both the entire coding region and splice junctions for each of these two exons. Primers were designed from intronic sequences flanking the first 14 exons and from the 5′ intronic sequence of exon 15. Exon 15 primers were placed in coding regions in an overlapping fashion, to cover the 6.5-kb contiguous DNA of the last exon. Two of the four primers amplifying the alternatively spliced exon (exon 9) also were placed in coding regions. This work concludes the survey of the *APC* gene by using SSCP in this patient set.

DNA samples were amplified for SSCP analysis using PCR (5 min at 95°C, 1 time; 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C, for 35 cycles; and 5 min at 72°C, 1 time) and the primers described above. Reaction mixtures contained the following: 200 ng of DNA template, 0.5 μM each primer, 70 μM each deoxynucleoside triphosphate, 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.25 U of *Taq* polymerase, 0.25 mM spermidine, and 0.1 μl of [α-^{32}P]dCTP (3,000 Ci/ml) in a volume of 10 μl.

PCR-SSCP Gel Analysis

To detect conformational changes in DNA, PCR products were electrophoresed through 5% polyacrylamide gels by using the nondenaturing conditions described by Orita et al. (1989*a*, 1989*b*). PCR products were diluted with 50 μl of 0.1% SDS, 10 mM EDTA, 50 μl of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol. They were denatured at 95°C for 3 min and were kept on ice until loaded onto the gels. Each set of PCR reactions was run under two conditions: a gel containing 90 mM Tris-borate (pH 7.5) and 2 mM EDTA at 4°C and a gel containing 90 mM Tris-borate (pH 7.5), 2 mM EDTA, and 10% glycerol at room temperature (maintained by fans). Electrophoresis was carried out at 40 W constant power for both types of gels. Following electrophoresis (run time depended on the size of the DNA samples being analyzed), gels were transferred to Whatman 3MM paper and were dried on vacuum-slab dryers. Autoradiographic exposure of Kodak X-Omat AR film for 1–2 d, without screens, usually was sufficient to render bands detectable.

Table 1

APC Patients in Whom a Mutation Was Observed

Patient	Age at Diagnosis (age at last medical exam)[a]	Mutation	Nucleotide Position	Result	Extracolonic Symptoms[b]
3794	N (N)	TAC→TAA	2805	Stop	N
FP5-04	N (33)	−ACAAA	3183	Frameshift	−
FP8-03	8 (18)	−ACAAA	3183	Frameshift	+
12046	18 (23)	−ACAAA	3183	Frameshift	+
12297	30 (30)	−ACAAA	3183	Frameshift	−
3442	29 (57)	−AAAAG	3926	Frameshift	+
FP7-04	N (N)	−AAAAG	3926	Frameshift	−
12255	23 (28)	−AAAAG	3926	Frameshift	−
3461	N (N)	−AAAAG	3926	Frameshift	N
3493	N (N)	−AAAAG	3926	Frameshift	N
3400	N (N)	CAG→TAG	4015	Stop	N
3407	N (N)	−TTAT	7935	Frameshift	+

[a] N or (N) = patient for whom age(s) could not be verified.

[b] A plus sign (+) denotes that patient is known to have fibromas, desmoids, osteomas, or dental anomalies; a minus sign (−) denotes that patient is known to be free of these characteristics; and "N" denotes that patient could not adequately be assessed.

Sequencing of SSCP Conformers

Individual SSCP bands chosen for sequencing were cut directly from the dried gels. Gels were marked with fluorescent dye to align the film and the gel. These bands were eluted into 100 μl of water and shaken for 30 min at room temperature. A 10-μl aliquot was removed for PCR amplification in a 100-μl reaction volume, using the custom primers from the original amplification. Reaction conditions were as follows: 10 μl of 10 × Perkin Elmer Cetus buffer and 2.0 μl of a 1.25-mM dNTP mixture. Samples were centrifuged, placed on ice, and overlaid with mineral oil. Two units of *Taq* polymerase were added. Reaction tubes were placed in a preheated DNA thermal cycler (95°C) and amplified (1 min at 95°C, 1 min at 60°C, and 1 min at 72°C, for 25 cycles). Samples were purified on a Centricon 100 column (Amicon) and were stored at −20°C until sequencing was carried out on an Applied Biosystems model 373A DNA sequencer (Applied Biosystems, Foster City, CA). Aliquots were sequenced with the dideoxy-termination method using *Taq* polymerase and fluorescently tagged M13 universal and reverse sequencing primers.

Oligonucleotide Design for Allele-specific PCR Primers

Specific primers to identify alleles bearing the two common mutations were designed; all primers are listed 5′ to 3′. The first pair, which amplifies the deletion allele at nucleotide position 3183, is L-AGTCTTAAATATTCAGATGAGCAG and R-TGATTGTCTTTGCTCACTTTATTTC. The R primer is specific for the deletion allele; the expected product size is 118 bp. The second pair, which amplifies the deletion at nucleotide position 3926, is L-AAATAGCAGAAATAAAAGATTG and R-ATGAGTGGGGTCTCCTGAAC. The L primer is specific to the deletion allele; the expected product size is 239 bp. PCR conditions are similar to those listed above, with the addition of 5% dimethylsulfoxide to the reaction mixture for the 3926 primer; annealing temperature of both primer pairs is 60°C.

Results

PCR-SSCP Anomalies Detected in DNA from APC Patients

PCR-SSCP analysis of genomic DNA identifies most single- or multiple-base changes in DNA segments up to 400 bases long (Orita et al. 1989*a*, 1989*b*). Base-pair changes are identified by shifts in the pattern of single-stranded DNA conformers on nondenaturing polyacrylamide gels. This technique has been used successfully to identify disease-causing mutations in patients with cystic fibrosis, neurofibromatosis 1, and APC (Cawthon et al. 1990; Dean et al. 1990; Groden et al. 1991). Twenty-three pairs of PCR primers were used to amplify sections of exon 15 in the *APC* gene from the DNAs of 60 unrelated APC patients. New 5′ primers were used to reexamine exons 1 and 3. Twelve normal

DNAs also were examined with each of these primer pairs, as controls to ascertain which changes in conformer patterns were due to common polymorphisms not associated with the disease.

DNAs from 12 APC individuals revealed variant patterns of SSCP conformers, patterns that were not present in DNAs from unaffected individuals (fig. 1). Some of these patterns were identical in DNA from different APC individuals, under both sets of electrophoretic conditions (fig. 1*B* and C).

Common Deletions and Unique Mutations in the APC Gene

The 12 conformers unique to APC patients were sequenced to determine the nucleotide change(s) responsible for their altered mobilities (data not shown). One normal conformer from each of these individuals was sequenced as well.

A novel conformer pattern detected by primer pair 15-D was observed in patient 3794. Sequencing of DNA from this patient revealed, at position 2805, a point mutation that substituted an A for a C, creating a stop codon.

A novel pattern detected by primer pair 15-E revealed an identical deletion in nucleotide sequence in four unrelated patients, representing loss of five bases (ACAAA) from positions 3183–3187 in the *APC* gene. This deletion was confirmed in all four patients by electrophoresing amplified DNA in a denaturing gel with a sequencing ladder to size the two alleles (data not shown).

Six patients exhibited novel conformers with the primer pair 15-G; four of those patterns were identical (three of these are shown in fig. 1C). One control sample showed a novel and unique pattern. Sequence analysis showed that one patient, 3400, carried a C→T substitution at nucleotide position 4015, creating a premature stop codon. Five other patients had the same five-base deletion (AAAAG) at positions 3926–3930. Patient 3461, whose novel conformer pattern differed from that of any other patient, had, in addition to the common deletion, a nucleotide polymorphism (G→C) at position 3949. This silent polymorphism also was observed in the sequence generated from the normal individual with a novel conformer pattern. DNAs with the deletion at 3926–3930 were electrophoresed on denaturing gels accompanied by a sequencing ladder; the deletion of five nucleotides was confirmed in each case (data not shown).

Sequence analysis of the conformers generated with primer pair 15-U showed that, in patient 3407, a four-nucleotide deletion, TTAT, occurred at position 7935–7938. Denaturing-gel electrophoresis with a sequencing ladder confirmed the four-base deletion in this patient (data not shown).

Another APC patient also exhibited a novel conformer pattern in DNA amplified with the 15-U primer pair (fig. 1*D*, lane 3). Sequencing analysis showed a nucleotide transversion from a C to a G at position 7865 (data not shown). This change replaces a serine with a cysteine in the *APC* amino acid sequence. Although this polymorphism has been classified by Miyoshi et al. (1992) as a germ-line mutation in the *APC* gene, PCR-SSCP analysis of this patient's family indicates otherwise. The affected offspring did not inherit this sequence variant, and the unaffected offspring did (fig. 1*D*, lane 5; and fig. 2). The phenotype of each of the 12 APC patients in whom a mutation was newly identified is indicated in table 1.

Segregation of the Common Mutations in APC Kindreds

DNAs were collected from members of four APC kindreds in which one or the other of the two common mutations had been detected. In one of the Utah kindreds used originally to map the *APC* gene by linkage analysis, an affected male (3304) has four affected offspring (fig. 3C); the mutation in this family was initially identified with DNA from patient 3442. PCR-SSCP analysis of DNAs from affected and nonaffected members of this kindred shows that the novel conformer representing the Δ3926 mutation segregates with the disease in this family. A similar analysis of a second family, FP-7, originally ascertained through detection of the Δ3926 mutation in the affected daughter, revealed the novel conformer pattern only in the two affected individuals (fig. 3*D*). Two kindreds were collected in which the Δ3183 mutation was observed (fig. 3*A* and *B*); again, only DNA from the affected individuals displayed the novel conformer pattern representing the deletion.

Common Deletion Mutations in Sporadic APC

Three kindreds were studied in which only one individual presented with polyposis and in which both parents of each patient were determined by colonoscopy to be normal. Two of these sporadic cases resulted from the Δ3183 deletion, and the third resulted from the Δ3926 mutation. Upon PCR-SSCP analysis (fig. 4), only the patients ascertained with the deletion displayed the novel conformer pattern; the nonaffected parents and siblings did not. These observations confirmed that the novel conformers, known to reflect

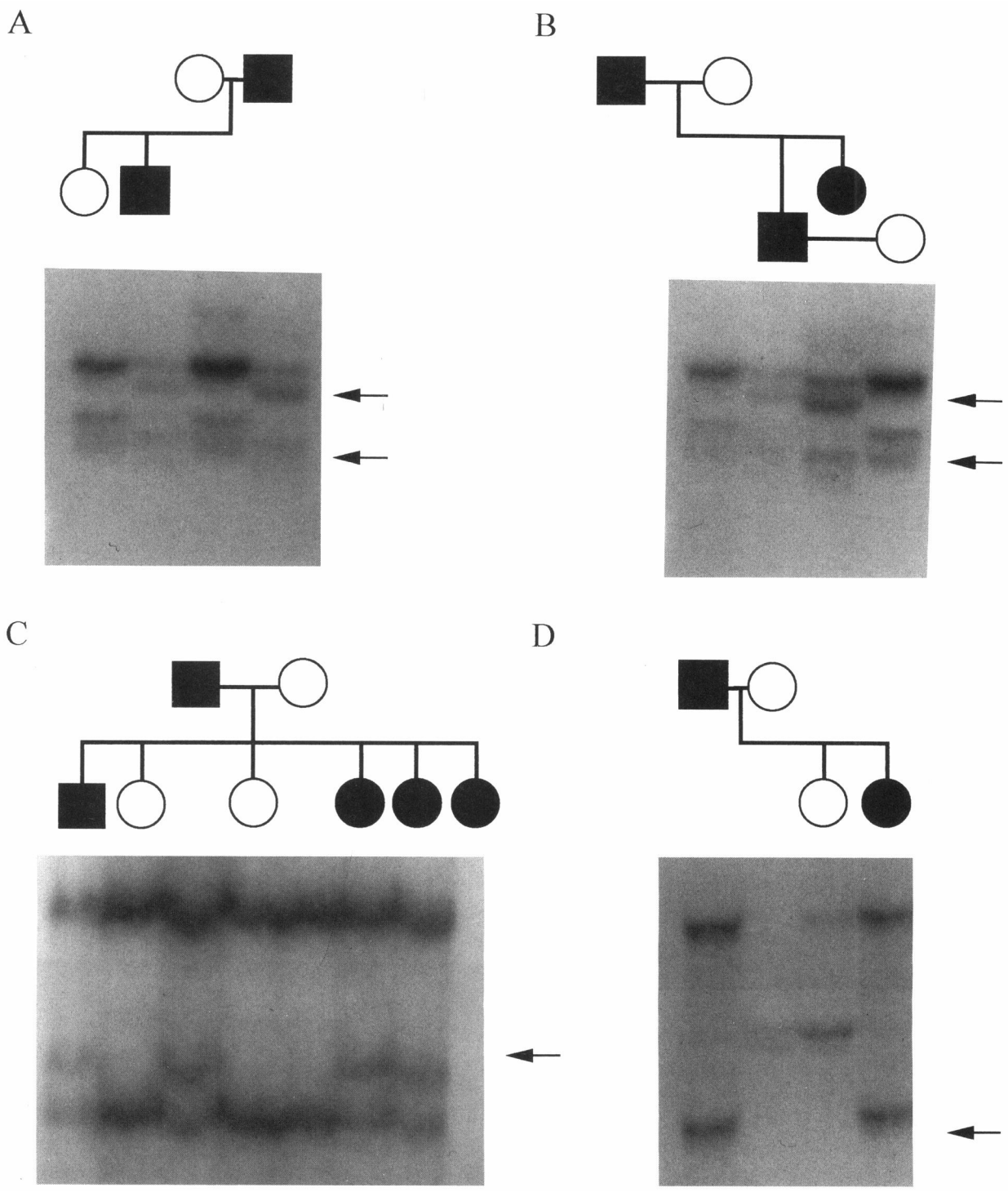

Figure 3 PCR-SSCP gels of four APC kindreds where the common deletions/conformers segregate with the disease. Individuals with APC are represented by the blackened squares and circles. Each lane is centered below the individual from whom the corresponding DNA was obtained. *A*, Pedigree of patient FP-5-04 where the 3183 mutation is found. *B*, Pedigree of patient FP-8-03, who also carries the 3183 mutation. *C*, Pedigree of kindred 1507, including patient 3442, where the 3926 mutation was found. *D*, Pedigree where the 3926 mutation was ascertained in patient FP-7-04.

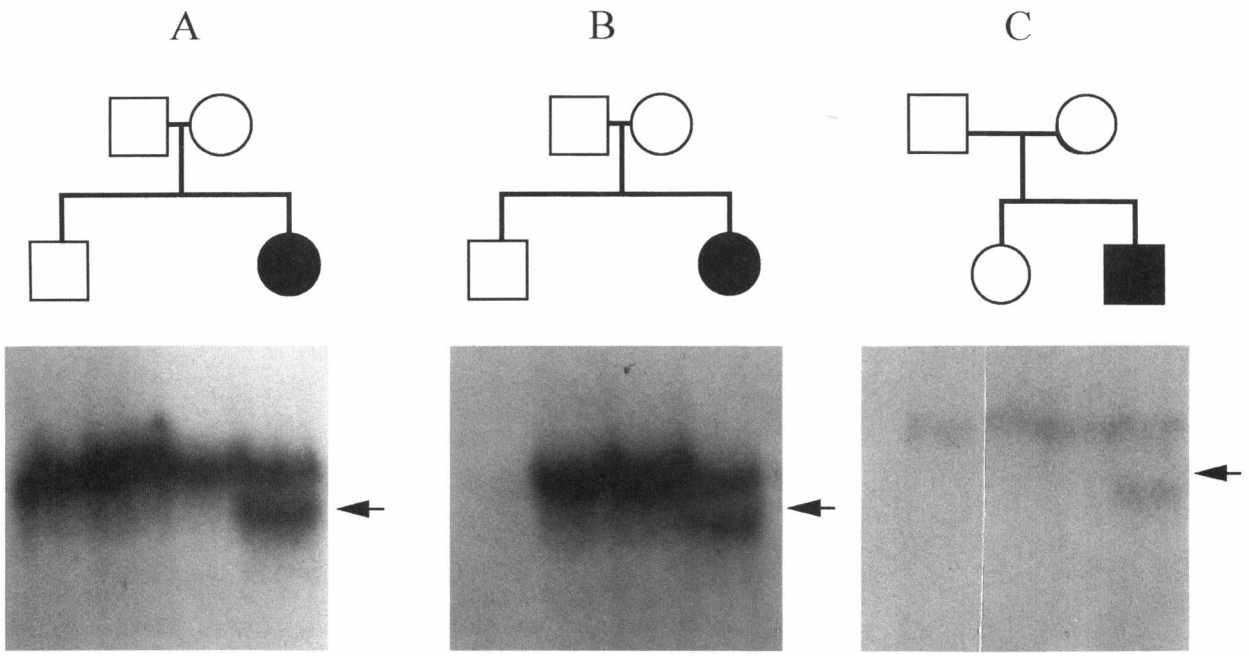

Figure 4 PCR-SSCP gels of three kindreds where the common deletions/conformers appear in sporadic cases of APC. Affected individuals are represented by blackened circles and squares. The novel conformers are identified by arrows. Each lane is centered below the individual from whom the corresponding DNA was obtained. All three sporadic cases show emergence of a novel conformer representing one of the two common deletions. *A*, Amplified DNA from the family of patient 12046. *B*, Amplified DNA from the family of patient 12297. Each of these new patterns represents the deletion occurring at position 3183. *C*, Amplified DNA from the family of patient 12255, where only DNA from 12255 exhibits the new conformer representing the deletion at position 3926.

5-bp mutations in *APC*, appeared spontaneously with APC in these kindreds. Paternity and maternity testing with highly polymorphic genetic markers yielded at least a 99.97% likelihood that all three affected individuals are biologically related to their respective parents (data not shown).

PCR-based Identification of the Common Deletion Mutations

PCR primers to amplify specifically the altered allele were designed for the Δ3183 and Δ3926 mutations. This will allow the identification of these two deletions by an allele-specific PCR reaction, rather than by SSCP. Figure 5 shows ethidium bromide–stained gels containing DNA-amplification products from patients harboring these two deletions. No product at the expected size of these deletion products is visible in the template from normal DNAs. Control primers (15-E) that flank the region of the deletion when used with the 3183 deletion primer amplify DNA from either a deleted or a normal allele. Their inclusion in the PCR reaction results in a larger product in all lanes containing DNA, whether from patients with *APC* deletions or from unaffected individuals. Therefore, they function as a control for the reaction.

Discussion

Inactivating mutations in our panel of 60 unrelated APC patients now have been identified throughout the *APC* gene, from nucleotide 730 to nucleotide 7935. Eleven different inactivating mutations have been found among a total of 18 individuals; six of these mutations were described elsewhere, of which two—a deletion occurring just distal to exon 1 and another occurring just distal to exon 9—were instrumental in identifying the gene (Groden et al. 1991; Joslyn et al. 1991).

Two small deletions account for almost 15% of the disease-causing mutations. Other sets of APC patients have shown a similar representation of these two mutations (Miyoshi et al. 1992; Varesco et al. 1993). Moreover, each has been observed in sporadic polyposis as well as in familial cases. In the general population, a rare single-nucleotide polymorphism (G→C) is found at position 3949, just 3′ to nucleotide 3926. As this polymor-

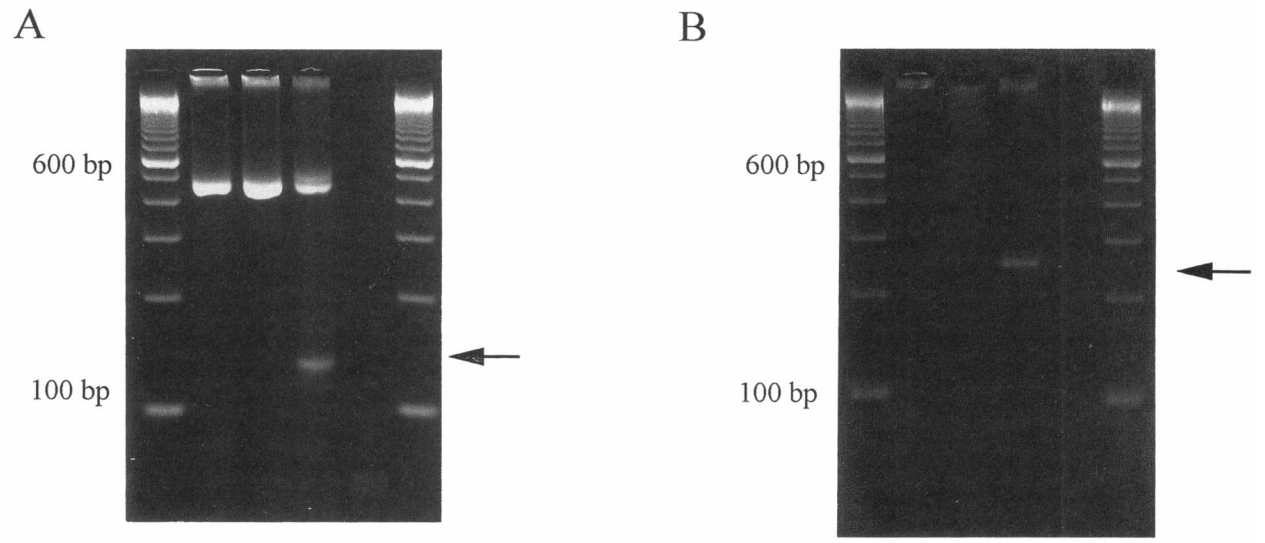

Figure 5 PCR with allele-specific primers for the two common deletions at positions 3183 and 3926. Both panels show ethidium bromide–stained 4% agarose gels containing PCR products from normal individuals and from patients carrying one of the two common deletions. Marker lanes contain a 100-bp ladder. *A*, PCR products amplified with a primer specific to the deletion at 3183. Lanes 1 and 6, Marker DNA. Lanes 2 and 3, DNA from normal individuals. Lane 4, DNA from patient 12046, with a deletion-specific product at 118 bp (product of the 15-E primer pair and the 3183-R primer). Lane 5, Control reaction with no template. *B*, PCR products amplified with a primer specific to the deletion at 3926. Lanes 1 and 6, Marker DNA. Lanes 2 and 3, DNA from normal individuals. Lane 4, DNA from patient 3442, with a deletion-specific product at 239 bp. Lane 5, Control reaction with no template.

phism also is seen in patient 3461, who carries Δ3926, the deletion may have occurred independently in this individual. These observations imply that mutational hot spots are present within the *APC* gene, although the majority of disease-causing *APC* mutations identified to date are unique.

The Δ3926 mutation occurs within a tandem repeat of five nucleotides, AAAAGAAAAG; deletion of one of the two pentamers leads to a frameshift and a downstream stop. This short, direct repeat may be a target for deletion by polymerase slippage during DNA replication or perhaps for unequal sister-chromatid exchange during meiosis or mitosis. Both deletions reported elsewhere by Groden et al. (1991) also occurred within the context of short repeated sequences (TTT and AGAG). Although the Δ3183 mutation, which commonly deletes five nucleotides (ACAAA), does not occur within a direct repeat, it does occur in a region with very similar repetitive sequences.

The disease-causing mutations have yet to be identified in 40 members of our panel of 60 unrelated APC patients. One reason may be that the method of PCR-SSCP is not sensitive enough to detect all sequence changes in coding regions and splice junctions; alternatively, the specific regions of the gene that contain mutations may not have been examined as yet. Alternative splicing of exons has been observed at the 5′ portion of the *APC* gene, and screening of newly identified exons is in progress (A. Thliveris, unpublished data). The 3′ untranslated region of *APC* has not yet been examined.

No mutation has been found 5′ of exon 7 among the patients in our panel. Although this observation could be insignificant in view of the fact that many of the disease-causing mutations have not been identified, it may reflect some functional significance of a truncated APC protein. Other mutational studies have shown a similar lack of mutations 5′ of exon 3 in the *APC* gene (Fodde et al. 1992; Miyoshi et al. 1992; Olschwang et al. 1993; Varesco et al. 1993). The most 5′ mutation observed in the *APC* gene so far was found in a kindred where the polyposis phenotype is attenuated (Spirio et al., submitted). This mutation, a four-nucleotide deletion leading to a frameshift, disrupts the APC protein between the first and second of the heptad repeats. The first heptad functions potentially as a homodimerization domain (Joslyn et al., submitted). Perhaps mutations immediately flanking the first dimerization domain do not yield a full polyposis phenotype, since they would not have a dominant negative function. Cytogenetic observations of deletion of the entire band of 5q21, where there could be no dominant negative func-

tion, might argue against this hypothesis (Herrera et al. 1986; Rivera et al. 1990).

All of the patient-specific *APC* mutations found thus far in this panel are expected to be inactivating. Although this observation does not rule out the presence of missense mutations in APC patients whose specific mutation has not yet been identified, it implies that the disease requires a loss of one normal *APC* allele, reduction of the normal *APC* product, or interference by an abnormal gene product.

Extracolonic manifestations are observed in some individuals affected with APC. These include osteomas, most commonly of the head and jaw; cutaneous soft-tissue tumors, particularly epidermoid cysts; dental abnormalities; supernumerary teeth; fibrous desmoids; congenital hypertrophy of the retinal epithelium; and a predisposition to neoplasias of the brain, thyroid, and gastrointestinal tract (Burt 1991). These extracolonic manifestations of APC vary greatly in their severity among affected kindreds and even among affected members within a kindred, although generally the severity of extracolonic manifestations is similar within a family (R. Burt, unpublished observations). Their clinical appearance shows more of a continuum from weak to strong, rather than an all-or-none phenomenon.

The data reported here, like those published previously (Groden et al. 1991; Nishisho et al. 1991), reveal no correlation between the mutation found in an individual and the expressivity of the polyposis phenotype. This is an intriguing observation because, in Duchenne and Becker muscular dystrophies, as well as with the pancreatic disease associated with cystic fibrosis, the severity of the clinical course reflects the position and/or type of mutation found in the disease gene (Kerem et al. 1989; Dean et al. 1990; Beggs et al. 1991). However, since many of the genetic anomalies in these other syndromes are missense mutations that seem to alter the function of the protein product, perhaps some mutant *APC* alleles do produce a functional, albeit truncated, protein that has a nonspecific effect in noncolonic cells. If so, one might expect individuals with the same mutation to have an identical phenotype, but, as table 1 shows, they usually do not. Therefore, other hypotheses must be considered.

The Min mouse strain originally was investigated as a mouse model of polyposis, on the basis of phenotype (Moser et al. 1990). Recently, mutational analysis has confirmed the model by showing that a point mutation introduces a stop codon into the coding sequence of the mouse homologue of *APC*, or *mAPC* (Su et al. 1992). Further analysis has identified in the Min mouse at least one unlinked-modifier locus that changes the expressivity but not the penetrance of the *Min* mutation (Moser et al. 1992). The existence of an unlinked modifier in the mouse has important implications for discussion of phenotypic variance in the human, as it suggests that such modifiers may be present in the human genome also.

The cosegregation of extracolonic manifestations with APC within certain kindreds suggests that a linked-modifier locus may exist as well. One candidate that comes to mind is *MCC*. This gene lies within 30–65 kb of *APC* on chromosome 5q, and it has accumulated mutations in a small number of colorectal tumors examined (Joslyn et al. 1991; Kinzler et al. 1991*b*). The predicted product of *MCC* contains heptad repeats, a structural feature thought to participate in formation of dimers; the predicted product of *APC* also contains heptad repeats (Bourne 1991*a*, 1991*b*; Groden et al. 1991). Mutational analysis of *MCC* in APC patients has not revealed any germ-line mutations (J. Groden, unpublished observations), but modifiers other than *MCC* might reside close to the *APC* locus.

Since mutations have been observed only in a subset of the APC patients and since these mutations have, for the most part, been unique, family studies using linkage analysis still offer the best option for the clinician. The use of a highly informative dinucleotide repeat marker (D5S346) within 30–70 kb of *APC* will facilitate this diagnostic approach (Spirio et al. 1991). However, the PCR primers included here, which detect the two more common mutant alleles, can be used for an initial effort to define the mutation segregating in any one family.

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