Genotype-Phenotype Correlations in Attenuated Adenomatous Polyposis Coli

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Summary

Germ-line mutations of the tumor suppressor *APC* are implicated in attenuated adenomatous polyposis coli (AAPC), a variant of familial adenomatous polyposis (FAP). AAPC is recognized by the occurrence of <100 colonic adenomas and a later onset of colorectal cancer (age >40 years). The aim of this study was to assess genotype-phenotype correlations in AAPC families. By protein-truncation test (PTT) assay, the entire coding region of the *APC* gene was screened in affected individuals from 11 AAPC kindreds, and their phenotypic differences were examined. Five novel germ-line *APC* mutations were identified in seven kindreds. Mutations were located in three different regions of the *APC* gene: (1) at the 5′ end spanning exons 4 and 5, (2) within exon 9, and (3) at the 3′ distal end of the gene. Variability in the number of colorectal adenomas was most apparent in individuals with mutations in region 1, and upper-gastrointestinal manifestations were more severe in them. In individuals with mutations in either region 2 or region 3, the average number of adenomas tended to be lower than those in individuals with mutations in region 1, although age at diagnosis was similar. In all AAPC kindreds, a predominance of right-sided colorectal adenomas and rectal polyp sparing was observed. No desmoid tumors were found in these kindreds. Our data suggest that, in AAPC families, the location of the *APC* mutation may partially predict specific phenotypic expression. This should help in the design of tailored clinical-management protocols in this subset of FAP patients.

Introduction

Familial adenomatous polyposis (FAP) is an inherited, autosomal dominant syndrome caused by germ-line mutations of the adenomatous polyposis coli (APC) gene, *APC* (Groden et al. 1991; Kinzler et al. 1991). Clinical diagnosis of FAP is usually based on the presence of >100 colonic adenomas (Bussey 1975), which, if left untreated, progress to colorectal cancer (CRC), typically at age ≤40 years (Gardner 1951). Other features include variable age at onset of polyposis (age 10–40 years) and variable expressivity (Groden et al. 1993; Giardiello et al. 1994). Extracolonic manifestations in FAP include the occurrence of desmoids, congenital hypertrophic retinal pigment–epithelial lesions (CHRPE), upper-gastrointestinal (UGI) polyps, and cancers of the thyroid, brain, and hepatobiliary tract. In previous studies, association of colonic polyposis and CRC with osteomas, fibromas, or epidermoid cysts has been described as a distinct entity, "Gardner syndrome" (Gardner and Richards 1953). That FAP and Gardner syndrome are the same genetic entity became apparent with the discovery of the *APC* gene and the identification of germ-line *APC* mutations in patients with those syndromes.

APC is a large (~300 kD) multifunctional protein and consists of distinct sequence motifs that specify its interactions with diverse protein partners (Polakis 1997). The majority of germ-line *APC* mutations cause truncated products and predominantly occur in the first half of the gene (Nagase et al. 1992a). Some phenotypic variants of FAP have been specifically associated with distinct mutation patterns in the *APC* gene. Thus, the occurrence of CHRPE is correlated with *APC* mutations in the region spanning exons 9–15 (Olschwang et al. 1993b), and the desmoid tumors are correlated with mutations in the region spanning codons 1444–1578 (Caspari et al. 1995). With regard to colonic adenomatous polyposis, a "sparse" phenotype (1,000–2,000 polyps) has been correlated with germ-line *APC* mutations in the region spanning codons 213–1597 (Nagase et al. 1992a); whereas a "profuse" (>5,000 polyps) phenotype has been demonstrated to segregate with *APC* mutations

in the region spanning codons 1250–1464 (Nagase et al. 1992*b;* Caspari et al. 1994; Gayther et al. 1994).

Recognition of such genotype-phenotype associations, along with the identification of distinct structural motifs, has provided important insights into APC's function. For example, *APC* carries a series of β-catenin–binding sites in the region associated with profuse polyposis. Using these sites, APC regulates intracellular turnover of β-catenin (Su et al. 1993), a protein involved in cellular adhesion, communication, and cell signaling pathways that affect apoptosis and cell growth (Hülsken et al. 1994; Morin et al. 1996, 1997; Peifer 1996; Rubinfeld et al. 1996; Korinek et al. 1997). Thus, mutant *APC* transcripts with deletions of the β-catenin–binding region function by mediating an imbalance of cell division over cell death, which likely results in an aggressive polyposis phenotype. Another functional domain, localized to the first 55 residues of APC, is involved in the protein-dimerization process (Joslyn et al. 1993). Mutation analyses have shown this domain to be almost always retained in the truncated mutant *APC* transcripts, which suggests that the dimerization domain is critical to APC's function.

Attenuated APC (AAPC) is a variant of FAP (Leppert et al. 1990) and also has been described as "hereditary flat adenoma syndrome" (Lynch et al. 1988, 1995). AAPC differs phenotypically from classical FAP, in the number of colonic adenomas (usually <100) that tend to be located proximal to the splenic flexure (van der Luijt et al. 1996; Giardiello et al. 1997; Gardner et al. 1997). These adenomas may present with a flat (rather than polypoid) morphology. Gastric fundic polyps and duodenal adenomas are also seen, and, in contrast to what is seen in FAP, CHRPE lesions and desmoid tumors rarely have been described in AAPC (Lynch et al. 1995; Scott et al. 1995). The average age at onset of CRC is later in AAPC (average age 55 years) than in classical FAP (average age 39 years) (Lynch et al. 1995). Furthermore, some AAPC families may phenotypically overlap with the hereditary nonpolyposis CRC syndrome (HNPCC), in which adenomas also are located on the right side of the colon (Jass et al. 1994). Alternatively, in some small nuclear AAPC families, the lack of accurate documentation regarding the number and/or the location of polyps may lead to a misdiagnosis of sporadic CRC.

In 1992, the attenuated variant was localized to the *APC* gene locus by molecular-genetic linkage analysis (Spirio et al. 1992). These observations were subsequently confirmed by the identification of four distinct germ-line mutations occurring at the 5′ end of the *APC* gene (Spirio et al. 1993). Several reports have described AAPC families with unique *APC* mutations (Cama et al. 1994; Dobbie et al. 1994; Smith-Ravin et al. 1994; Varesco et al. 1994; van der Luijt et al. 1995; Giardiello

et al. 1997); however, only a few studies have investigated genotype-phenotype associations in AAPC (Spirio et al. 1993; Giardiello et al. 1997). In the present study, we used molecular-genetic characterization of *APC* to better classify kindreds that have a familial predisposition to colonic adenomas and CRC and that have phenotypic features not typical of FAP or HNPCC.

Subjects and Methods

Selection of Patients

Eleven unrelated kindreds with hereditary predisposition to colonic adenomas and CRC were identified, from the Familial Gastrointestinal Cancer Registry located at our institution. Most affected individuals had >10 but <100 adenomas, and 9 of 11 families had a history of CRC. Endoscopic, operative, and histopathological data were collected retrospectively. Complete follow-up information was obtained as well. Predictive genetic testing was offered to affected individuals, with pre- and posttest counseling (Soravia et al. 1997*a*). Patient accrual, sample collection, and genetic screening were performed according to guidelines of the Human Ethics Committee of the University of Toronto.

Molecular-Genetic Analysis

The protein-truncation test (PTT) assay was used to screen for *APC* germ-line mutations (Powell et al. 1993; van der Luijt et al. 1994). Blood samples were obtained from affected individuals, and lymphoblastoid Epstein-Barr virus–transformed cell lines were established for some of these patients. RNA and DNA extraction was performed by use of TRIzolJ and DNAzolJ, according to the manufacturer's protocol (Life Technologies). Reverse-transcription PCR was performed by use of standard techniques. In brief, cDNA was generated from total RNA (2–5 μg) by use of random hexamers, 1 × first-strand buffer, 0.5 mM of deoxynucleotide triphosphate, 10 mM DTT, and 200 units of Superscript II reverse transcriptase (Bethesda Research Laboratories). cDNA and genomic DNA were amplified under the following conditions: initial denaturation at 95°C for 2 min; 35–40 cycles, each consisting of denaturation at 95°C for 30 s, annealing at 63°C–65°C for 1 min 30 s, and extension at 70°C for 2 min; and a final extension at 70°C for 5 min. Five-microliter aliquots of PCR products were electrophoresed on a 1.5% agarose gel, in order to confirm amplification.

The in vitro–synthesized PTT assay was performed by use of a commercial kit (TNT7 T7 Quick Coupled Transcription/Translation System; Promega), according to the manufacturer's protocol. In brief, *APC* exon 15 was amplified in four overlapping segments, as described elsewhere (Powell et al. 1993). Exons 1–14 were amplified

Table 1

Characterization of *APC* Mutations

Kindred(s)	Exon	Nucleotide Change	Consequence
1–3	4	Exon 4 deletion	Frameshift deletion (premature truncation) with TGA stop at nucleotide 551 (exon 5)
4	4	C<u>AG</u>→<u>T</u>AG	C→T substitution, Gln→stop, nucleotide 487
5	9	<u>A</u>TGT→TGT	Deletion of A, frameshift; termination at nucleotide 1014
6	9	C<u>G</u>A→T<u>G</u>A	C→T substitution, Arg→stop, nucleotide 994
7	15	TC<u>C</u>G→TCG	Deletion of C, frameshift, termination at nucleotide 6218

in two overlapping segments: 1A (exons 1–9) and 1B (exons 8–14). The 5′ end of each forward primer had a T7 promoter sequence and a translation-initiation site for coupled in vitro transcription and translation. PCR products were separated on 12.5% polyacrylamide gels. Positive PTT results were confirmed by two independent PCR reactions.

For sequence determination, PCR products of the putative positive-PTT samples were electrophoresed on a 1.5% agarose gel for 45–60 min at 100 V and were stained with ethidium bromide. The band of interest was excised with a sterile scalpel blade, and DNA was extracted by use of the QIAquick Gel Extraction Kit (QIA-GEN). The dideoxy-mediated chain-termination method was used for DNA sequencing (Sanger et al. 1977). For this purpose, we used $\alpha[^{33}P]$ dideoxynucleotide and a ThermoSequenase radiolabeled terminator sequencing kit (Amersham Life Science); sequencing was performed according to the manufacturer's protocol. Finally, the gel was autoradiographed by use of BioMax MR film (Kodak) for 24–48 h at room temperature. Additional confirmation was obtained by genomic DNA sequencing. Primers used for PTT-PCR and sequencing reactions are available on request.

Results

In 7 of the 11 kindreds, putative truncating mutations were identified as novel transcripts, by PTT analysis. Sequence characterization revealed five germ-line *APC* mutations in these seven families (table 1). To the best of our knowledge, all five *APC* mutations are novel (Béroud and Soussi 1996; De Vries et al. 1996). In three AAPC kindreds, a truncated-protein product of ~16 kD was identified, in addition to the wild-type protein for

APC segments 1 (codons 1–811; fig. 1*A*, lanes 3 and 4) and 1A (codons 1–379; figs. 1*A*, lanes 1 and 2, and 1*B*, lanes 5–7). Sequence analysis revealed this truncation to be caused by exon 4 deletion, leading to a frameshift mutation and resulting in a stop codon (TGA) at nucleotide 551 (codon 184). In kindred 4, the size of the novel PTT product was ~20 kD (fig. 1*B*, lane 5). DNA sequence analysis revealed a C→T transversion at nucleotide 487 (exon 4, codon 163), resulting in a substitution of glycine by a stop codon (CAG→TAG). In kindreds 5 and 6, the truncated proteins showed a similar pattern (doublet), ~46 kD in size (fig. 2*A*). Subsequent characterization by sequencing identified two distinct *APC* germ-line mutations within exon 9. Specifically, in kindred 5, the mutation occurred at nucleotide 988 (deletion A), resulting in a frameshift leading to a stop codon (TAG) at nucleotide 1014, and, in kindred 6, a C→T transversion at nucleotide 994 resulted in a substitution of arginine by a stop (GCA→TGA) at codon 332. Finally, in kindred 7, the size of the truncated protein was ~48 kD (fig. 2*B*). By sequence analysis, the mutation was identified to be a 1-bp frameshift deletion (C) at *APC* nucleotide 6138 (codon 2047), leading to a stop codon (TGA) at nucleotide 6218.

Tables 2–4 summarize phenotypic and clinical data on the seven kindreds, according to the location of the *APC* mutations. Kindred 2 is a large AAPC family and spans four generations with 27 affected individuals. In this family, a variability was observed with respect to the number of colorectal adenomas (mean 142, range 2–580). A predominance of right-sided colonic polyps with rectal sparing was observed in all AAPC families. In kindreds with 5′-end *APC* mutations (table 2), the mean age at polyposis diagnosis was 42 years (range 39.5–45.2 years), with a mean of 96 polyps (range
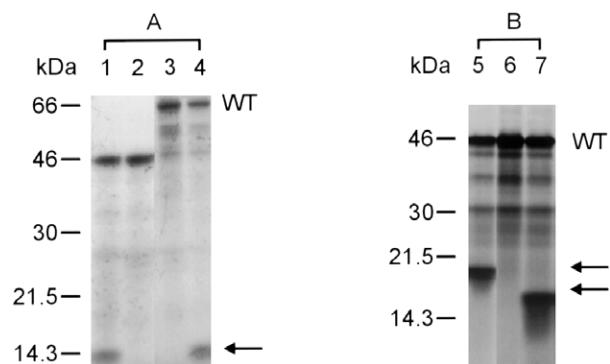

Figure 1　Representative PTT analysis for *APC* segment 1A (codons 1–379) (lanes 1, 2, and 5–7) and *APC* segment 1 (codons 1–811) (lanes 3 and 4). The size of the protein marker is shown. The single arrow in the left-hand panel indicates truncated mutant protein. Lanes 2, 3, and 6, Non-FAP controls. Lane 1, Kindred 1. Lane 4, Kindred 2. Lane 7, Kindred 3. Lane 5, Kindred 4. WT = wild type.
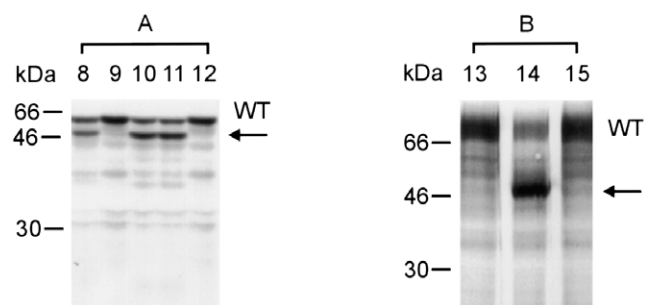
Figure 2 Representative PTT analysis for *APC* segment 1A (codons 1–379) (lanes 8–12) and *APC* segment 4 (codons 1555–2256) (lanes 13–15). The size of the protein marker is shown. The single arrow in the left-hand panel indicates truncated mutant protein. Lanes 9, 12, 13, and 15, Non-FAP controls. Lane 8, Kindred 5. Lanes 10 and 11, Kindred 6. Lane 14, Kindred 7. WT = wild type.

1–580), and the mean age at CRC diagnosis was 58.1 years (range 29–76). In the group with mutations in *APC* exon 9, although both the mean age at polyposis diagnosis (39.7 years [range 12–64]) and the mean age at CRC diagnosis (54.2 years [range 42–67]) were comparable to those in the first group, the average number of polyps (22 [range 1–150]) was less (table 3). In the AAPC kindred with 3′-end *APC* mutations, mean age at polyposis diagnosis was 49 years (range 41–65), and mean number of colonic polyps was 14 (range 1–36); thus far, no CRC has been diagnosed in this family (table 4).

Discussion

In this study, we have shown that AAPC is caused by mutations in three distinct regions of the *APC* gene—that is, at the 5′ end, in the region spanning exons 4 and 5; in exon 9; and at the extreme 3′ end. Phenotypic expression in these three groups of kindreds is variable but is definitely milder than that in classical FAP, indicating modified clinical-management protocols for affected AAPC patients and their at-risk relatives. Our data demonstrate that AAPC families carrying 5′ germline *APC* mutations exhibit greater variability with regard to the frequency and age at onset of polyposis, such that the phenotypes of some affected patients are quite similar to that of classical FAP. Furthermore, duodenal adenomas occur with an increased severity in these families.

We have identified four AAPC kindreds with *APC* mutations located proximal to codon 168. Of these four kindreds, three carry the same *APC* mutation, and the fourth carries a truncating mutation within *APC* exon 4. To date, 13 AAPC families with germ-line *APC* mutations proximal to codon 168 have been described (Spirio et al. 1993; Olschwang et al. 1993*a;* Giardiello et

al. 1997). 5′ *APC* mutations predict the synthesis of proteins shorter than those predicted from the *APC* mutations commonly associated with classical FAP (Horii et al. 1993; Samowitz et al. 1995). Therefore, different properties of the two types of mutant APC proteins may be expected (Fodde and Meera Khan 1995). Short APC peptides may be unable to compete with other peptide complexes, because of a lack of essential sequence motifs required for such protein-protein interaction. Alternatively, such alleles are likely to be unstable and may result in null alleles.

Two AAPC kindreds carry *APC* mutations in exon 9. This exon is usually alternatively spliced. Germ-line mutations in *APC* exons 6 and 9A, including those causing a splice-site defect, have been reported in AAPC families (Cama et al. 1994; Smith-Ravin et al. 1994; Varesco et al. 1994; van der Luijt et al. 1995). Mutations occurring in alternatively spliced regions of *APC* would support the haploinsufficiency model, in which an unstable truncated mutant protein with a reduced amount of normal APC induces a less severe phenotype (Fodde and Meera Khan 1995; Yang et al. 1997). Furthermore, the splice-site mutations of such *APC* exons are thought to provoke inefficient exon skipping, which may be responsible for the delay in the development of colonic polyposis (Varesco et al. 1994). In the present study, although the mutations in exon 9 were located outside the alternatively spliced region, the phenotypes of these two kindreds are typical of AAPC (table 3). Average age at diagnosis of polyposis is 40 years, and usually there are <100 right-sided colonic adenomas, with rectal-polyp sparing; gastric/duodenal manifestations were not observed in these families.

We found one kindred with a germ-line *APC* mutation at codon 2047, which appears to be one of the most 3′ mutations reported in AAPC. In this kindred, the colonic phenotype is very mild, with <40 polyps, no CRC, and rectal-polyp sparing (table 4). Previous studies have shown that germ-line mutations at the distal 3′ end of *APC* correlate with the AAPC phenotype (Scott et al. 1995; Friedl et al. 1996; van der Luijt et al. 1996; Gardner et al. 1997). Such mutant *APC* transcripts are likely to be proficient in β-catenin binding, thereby mediating signals that are important for cell adhesion and turnover (Friedl et al. 1996). Alternatively, 3′-mutant APC proteins are likely to form stable homodimers and to retain partial function, as in the case of "leaky" mutant alleles (Spirio et al. 1993). Kindreds with desmoid tumors but with almost no colonic manifestations have been reported recently (Eccles et al. 1996; Scott et al. 1996). These families are noteworthy for having *APC* mutations in the 3′ region of the gene. This variant of FAP has been termed "hereditary desmoid disease" and previously had been classified as "familial infiltrative fibromatosis" (MIM 135290). In these kindreds, CRC oc-

Table 2

Clinical Data on Kindreds 1–4

Kindred and Subject (Sex)	Age at Diagnosis (Age at Death) [years]	CRC Status (Location)	No. (Location) of Adenomas	Surgery	Extracolonic Manifestations	Postsurgical Follow-up
1:						
II-1 (F)	59	Absent (…)	Multiple (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	…
III-1 (M)	55	Present (right side [two])	…	Right-side colectomy	Not reported	…
III-3 (F)	64	Absent (…)	Multiple (both sides of colon [approximately equal amounts])	Proctocolectomy with end ileostomy	Not reported	…
III-5 (M)	48 (57)	Absent (…)	Multiple (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	Rectum clear (4 years)
III-7 (F)	43	Absent (…)	150 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Ampullectomy	Rectum clear (20 years)
III-8 (F)	41	Absent (…)	384 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Not reported	…
IV-2 (F)	33	Absent (…)	25 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	…
IV-5 (M)	42	Absent (…)	5 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	…
IV-26 (F)	40	Absent (…)	20 (both sides of colon [more on right side])	None	UGI normal	…
IV-30 (M)	24	Absent (…)	28 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	Rectum clear (13 years)
IV-32 (M)	36	Absent (…)	100 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Not reported	…
V-28 (F)	19	Absent (…)	41 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	UGI normal	Rectum clear (2 years)
2:						
III-2 (F)	57	Absent (…)	<100 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	UGI normal	…
III-6 (M)	59	Present (left side of colon)	Multiple (left side of colon)	Ileo-rectal anastomosis	Not reported	…
III-12 (M)	67 (83)	Present (rectum)	<100 (both sides of colon [approximately equal amounts])	Proctocolectomy with end ileostomy	Not reported	…
III-15 (F)	68 (72)	Present (right side of colon, rectum)	<100 (both sides of colon [approximately equal amounts])	Ileal pouch–anal anastomosis	Not reported	Local cancer recurrence
IV-1 (F)	41	Absent (…)	Multiple (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Not reported	…
IV-2 (M)	39 (42)	Present (right side of colon)	>500 (both sides of colon [approximately equal amounts])	Ileal pouch–anal anastomosis	Duodenal cancer	Liver metastases
IV-3 (F)	38	Absent (…)	Multiple (both sides of colon [more on right side])	Ileo-rectal anastomosis	UGI normal	…
IV-4 (M)	37	Absent (…)	<100 (both sides of colon [more on right side])	Ileo-rectal anastomosis	UGI normal	Rectum clear (12 years)
IV-6 (F)	26	Absent (…)	1	Ileo-rectal anastomosis	UGI normal	Rectum clear (4 years)

(*continued*)

Table 2 (continued)

Kindred and Subject (Sex)	Age at Diagnosis (Age at Death) [years]	CRC Status (Location)	No. (Location) of Adenomas	Surgery	Extracolonic Manifestations	Postsurgical Follow-up
IV-8 (M)	27	Absent (…)	Multiple (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	…
IV-19 (M)	49	Absent (…)	<100 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Duodenal adenomas	…
IV-23 (F)	37	Absent (…)	<150 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Not reported	Rectum clear (4 years)
IV-29 (F)	29 (46)	Present (left side of colon)	…	Left-side colectomy	Not reported	…
		Present (right side of colon)	2 (right side of colon)	Right-side colectomy	Not reported	Retroperitoneal metastases
IV-30 (M)	43	Present (rectum)	Multiple (both sides of colon [approximately equal amounts])	Abdominal perineal resection	Ampullectomy	Right-adrenal tumor
IV-37 (F)	46	Absent (…)	69 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	…
IV-43 (F)	46 (49)	Present (right side of colon)	Multiple (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	Liver metastases
IV-45 (M)	50 (53)	Present (left side of colon)	Multiple (both sides of colon [approximately equal amounts])	…	UGI normal	Liver metastases
IV-47 (M)	44	Present (right side of colon)	<100 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Not reported	…
IV-51 (M)	38	Present (left side of colon)	<100 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Dysplastic adenomas of the stomach: partial gastrectomy	…
V-7 (M)	20	Absent (…)	7	None	Not reported	…
V-21 (F)	37	Absent (…)	<100 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	…
V-25 (M)	29	Absent (…)	<200 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	UGI normal	Rectum clear (2 years)
V-32 (F)	23	Absent (…)	580 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	…
V-34 (F)	38	Absent (…)	300 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Not reported	…
V-37 (F)	33	Absent (…)	200 (both sides of colon [more on right side])	Ileo-rectal anastomosis	UGI normal	…
V-40 (F)	28	Absent (…)	<100 (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	UGI normal	Rectum clear (1 year)
V-59 (M)	19	Absent (…)	6 (both sides of colon [approximately equal amounts])	None	UGI normal	…
3:						
I-1 (F)	76 (77)	Present (left side of colon)	Not reported	Left-side colectomy	Not reported	…
II-1 (M)	66 (72)	Present (left side of colon)	Not reported	Left-side colectomy	Not reported	Multiple myeloma

Table 2 (continued)

Kindred and Subject (Sex)	Age at Diagnosis (Age at Death) [years]	CRC Status (Location)	No. (Location) of Adenomas	Surgery	Extracolonic Manifestations	Postsurgical Follow-up
3:						
II-2 (F)	71 (71)	Present (right side of colon)	Not reported	Right-side colectomy	Not reported	Liver metastases
II-3 (F)	64	Present (left side of colon)	Not reported	Left-side colectomy	Not reported	…
III-4 (F)	52	Absent (…)	100 (both sides of colon [more on right side])	Ileo-rectal anastomosis	UGI normal	…
III-9 (F)	51/52	Absent (…)	200 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	…
III-12 (M)	33	Absent (…)	3, right side of colon	Right-side colectomy	Not reported	…
4:						
I-1 (M)	(60)	Present (not reported)	Not reported	Colostomy	Not reported	…
II-1 (M)	35 (69)	Present (left side of colon)	Not reported	Ileal pouch–anal anastomosis	UGI normal	…
II-3 (F)	66	Absent (…)	50 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Duodenal polypectomy	…
II-7 (M)	58	Absent (…)	100 (both sides of colon [approximately equal amounts])	Left-side colectomy	Not reported	6 Polyps, right side of colon
III-2 (M)	37	Absent (…)	40 (both sides of colon [more on right side])	Right-side colectomy	Not reported	Normal colonoscopy
III-10 (M)	30	Absent (…)	1, excised (right side of colon)	…	Not reported	Normal colonoscopy

Table 3

Clinical Data on Kindreds 5 and 6

Kindred and Subjects (Sex)	Age at Diagnosis (Age at Death) [years]	CRC Status (Location)	No. (Location) of Adenomas	Surgery	Extracolonic Manifestations	Postsurgical Follow-up
5:						
III-2 (M)	60	Present (left side of colon [two])	Not reported	Ileo-rectal anastomosis	Not reported	...
	64 (64)	Present (rectum)	Not reported	Proctocolectomy with end ileostomy	Not reported	Liver metastases
III-3 (M)	50	Present (left side of colon)	Not reported	Not reported	Not reported	...
III-4 (M)	55 (71)	Absent (...)	Multiple (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	UGI normal	Lung cancer
III-5 (M)	43	Present (right side of colon)	...	Right-side colectomy	Not reported	...
	58	Absent (...)	21 (left side of colon)	Left-side colectomy	UGI normal	...
III-6 (M)	53	Absent (...)	8 (both sides of colon [more on left side])	Ileo-rectal anastomosis	UGI normal	Rectum clear (26 years)
III-9 (F)	44	Absent (...)	Multiple (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	UGI normal	Rectum clear (24 years)
IV-2 (M)	39	Absent (...)	Multiple (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Not reported	Rectum clear (23 years)
IV-9 (M)	41 (47)	Absent (...)	2, right side of colon	Ileo-rectal anastomosis	Not reported	Esophageal cancer
IV-10 (F)	36	Absent (...)	150 (both sides of colon [more on right side])	Ileo-rectal anastomosis	UGI normal	Rectum clear (13 years)
IV-11 (F)	50	Absent (...)	...	...	...	...
IV-12 (M)	37	Absent (...)	2 (both sides of colon [approximately equal amounts])	...	Not reported	...
IV-17 (F)	27	Absent (...)	20 (both sides of colon [more on right side])	Ileo-rectal anastomosis	UGI normal	Rectum clear (23 years)
IV-18 (M)	16	Absent (...)	Multiple (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	UGI normal	Rectum clear (24 years)
IV-20 (M)	41	Absent (...)	2 (left side of colon)	...	Not reported	...
V-1 (M)	36	Absent (...)	28 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	Rectum clear (1 year)
V-7 (M)	35	Absent (...)	Multiple (both sides of colon [approximately equal amounts])	Ileo-rectal anastomosis	Not reported	Rectum clear (4 years)
V-10 (F)	12	Absent (...)	1 (left side of colon)	...	Not reported	...
6:						
II-1 (F)	67	Present (left side of colon)	None	Left-side colectomy	Not reported	...
III-1 (F)	42	Present (left side of colon)	27 (both sides of colon [more on right side])	Left-side colectomy	UGI normal	Cervical metastases
III-2 (F)	43	Absent (...)	43, right side of colon	Ileo-rectal anastomosis	Not reported	Rectum clear (1 year)
III-4 (F)	35	Absent (...)	20	Ileo-rectal anastomosis	Not reported	...
IV-1 (M)	25	Absent (...)	10 (both sides of colon [more on right side])	...	Not reported	...
IV-2 (F)	30	Absent (...)	2 (both sides of colon [approximately equal amounts])	...	Not reported	...

Table 4

Clinical Data on Kindred 7

Subject (Sex)	Age at Diagnosis (years)	CRC Status (Location)	No. (Location) of Adenomas	Surgery	Extracolonic Manifestations	Postsurgical Follow-up
III-7 (F)	65	Absent (...)	36 (both sides of colon [more on right side])	Ileo-rectal anastomosis	UGI adenomas	Rectum clear (1 year)
IV-9 (M)	47	Absent (...)	1 (left side of colon)	...	Not reported	...
IV-12 (F)	43	Absent (...)	2, excised (right side of colon)	...	UGI adenomas	...
IV-13 (M)	41	Absent (...)	20 (both sides of colon [more on right side])	Ileo-rectal anastomosis	Not reported	...

casionally occurs without obvious polyposis. This subset of kindreds could therefore also be considered an AAPC variant with predominance of desmoid tumors. Indeed, in classical FAP, families with desmoid tumors and without CHRPE have been reported to have germ-line mutations in the region of *APC* codons 1445–1578 (Hodgson et al. 1994; Caspari et al. 1995). In our AAPC kindreds, desmoid tumors have not been identified.

A germ-line *APC* mutation was not identified in 4 of 11 kindreds. There are a number of possible explanations: the PTT assay may be unable to detect all mutations in these genes; the mutations may be located in a gene region (e.g., intronic or promoter regions) that was not investigated, or a mutation in a gene(s) other than *APC* may be the underlying defect in these families.

Two phenotypic features of our AAPC families are worth emphasizing: (1) we observed rectal-polyp sparing in patients who underwent total colectomy and ileorectal anastomosis, after a mean follow-up period of 11.7 years (range 1–26 years); and (2) we also observed an increased trend toward development of gastric and duodenal adenomas, with the occurrence of duodenal cancer in one case, as reported elsewhere (Soravia et al. 1997*b*; Zwick et al. 1997). Clinical review of AAPC families with 5′-end *APC* mutations (table 2) demonstrates that, although the number of colorectal adenomas usually is <100, in some cases variability is observed with regard to the number of colonic polyps (i.e., there are >100), with a predominance of polyps located on the right side, as has been described elsewhere (Burt et al. 1995). In families described here, the average age at CRC diagnosis is ∼58 years, whereas the average age at polyposis diagnosis is 42 years; these observations are in agreement with other reports (Dobbie et al. 1994; Lynch et al. 1995; Giardiello et al. 1997).

Differences in the *APC* mutation sites alone cannot completely account for intra- and interfamilial variation in the polyposis phenotypes in these families. Another likely explanation for phenotypic variability is the presence of a modifier gene(s) that may influence the phenotypic expression of the *APC* gene. Such a gene is probably comparatively polymorphic, since phenotypic variability occurs both within and between families. For example, a modifier gene located on chromosome 1p35-36 may influence the severity of duodenal polyposis in FAP (Tomlinson et al. 1996; Dobbie et al. 1997). It is also likely that environmental factors, such as diet, play an important role in AAPC, as has been demonstrated in the mouse models of FAP (Hioki et al. 1997).

The use of molecular-genetic testing has been suggested as an aid in decision making with respect to the type of surgical procedure (total colectomy vs. restorative proctocolectomy) based on the risk of rectal cancer in FAP (Vasen et al. 1996). This suggestion has been challenged by others (Gareth et al. 1997), because few relevant clinical data had been given to support it. The lifetime risk for development of CRC has not yet been estimated in AAPC. Therefore, clinical decision making with respect to the type of surgical procedure should rely on a combination of factors, including age at AAPC diagnosis, number of adenomas (>20), location of polyps (right-side colon), frequency of polyp recurrence, and polyp morphology (confluent vs. scattered polyps). A patient's compliance with the clinical-surveillance regimen should also be taken into account. Finally, this information needs to be correlated with the molecular diagnosis of AAPC in affected and/or presymptomatic individuals of such kindreds.

In view of our experience, the following information seems relevant for the clinical management of AAPC kindreds: (1) predictive genetic testing can be offered to presymptomatic relatives in AAPC families with a known germ-line mutation; (2) colonoscopy, as opposed to sigmoidoscopy, should be advised for endoscopic sur-

veillance, because of the right-side location of colorectal adenomas; (3) UGI endoscopic surveillance is warranted in an attempt to detect premalignant gastric or duodenal tumors; and (4) individuals affected with AAPC may require total colectomy with ileo-rectal anastomosis only when prophylactic colectomy is advised. Finally, the rare occurrence of rectal polyps in AAPC is noteworthy; however, further clinical studies with longer follow-up are needed in order to prove that the risk of rectal cancer is lower in AAPC than in classical FAP (Nugent and Phillips 1992).

Acknowledgments

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for hereditary desmoid disease, or familial infiltrative fibromatosis [MIM 135290])

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