Familial adenomatous polyposis: more evidence for disease diversity and genetic heterogeneity

R J Scott, C Meldrum, R Crooks, A D Spigelman, J Kirk, K Tucker, D Koorey, the Hunter Family Cancer Service

Hunter Area Pathology Service, Locked Bag No 1, Hunter Regional Mail Centre, Newcastle NSW 2310, Australia
R J Scott
C Meldrum
R Crooks

Medical Genetics, Faculty of Medicine and Health Sciences, University of Newcastle, Newcastle NSW 2308, Australia
R J Scott
A D Spigelman

Discipline of Surgical Science, University of Newcastle, Callaghan NSW 2308, Australia
A D Spigelman

Family Cancer Unit, Westmead Hospital, Westmead NSW 2145, Australia
J Kirk

Hereditary Cancer Clinic, Department of Medical Oncology, Prince of Wales Hospital, Randwick NSW 2031, Australia
K Tucker

A W Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Camperdown NSW 2050, Australia
D Koorey

Hunter Genetics, Cnr Turton and Tinonee Roads, Waratah NSW 2298, Australia
The Hunter Cancer Family Service†

†The Hunter Family Cancer Service includes Claire Groombridge, Bronwyn Burgess, Anne Hammond, and Gillian Turner

Correspondence to: Professor R J Scott, Hunter Area Pathology Service, Locked Bag 1, Hunter Regional Mail Centre, Newcastle NSW 2310, Australia.
rscott@doh.health.nsw.gov.au

Accepted for publication 31 October 2000

Abstract

Familial adenomatous polyposis (FAP) is characterised by the presence of profuse colonic carpeting of adenomas throughout the entire colon and rectum. The genetic basis of FAP has been shown to be primarily associated with germline mutations in the APC gene. Notwithstanding, several reports have been published indicating that there is genetic heterogeneity in FAP and that the most likely explanation is the existence of another gene. In this report we further delineate the genotype/phenotype correlation in families that harbour germline mutations in the APC gene and identify some previously unreported changes in the APC gene which predispose to an attenuated disease phenotype. From 53 index patients diagnosed with either FAP or attenuated FAP, 27 harboured changes in the APC gene. The remaining 26 patients were further subgrouped according to their colonic phenotype. There were nine patients with a mixed hyperplastic/adenomatous colonic phenotype and there were 17 patients with an adenomatous colonic phenotype. Evaluation of the disease characteristics of these patients and their families is presented which may aid in the identification of new genes associated with colonic polyposis.

(Gut 2001;48:508-514)

Keywords: familial adenomatous polyposis; genetics; hyperplastic polyposis; attenuated adenomatous polyposis coli; hereditary non-polyposis colorectal cancer

Familial adenomatous polyposis (FAP) is characterised by the development of hundreds to thousands of adenomas throughout the entire colon and rectum, which if not removed will almost certainly result in the development of colorectal cancer.¹ Variance in disease expression has also been described and is typified by fewer colonic and rectal adenomas (usually less than 100), later age of onset, and a reduced likelihood of colorectal cancer development. Variant forms of FAP are known as attenuated adenomatous polyposis (AAPC) or the flat adenoma syndrome.^{2,3} The incidence of FAP in the population is approximately 1:7000 according to the Danish Polyposis Registry and it affects both sexes equally.

The genetic basis of FAP was first localised in 1986 to chromosome 5q and the region localised to 5q21 in 1987.^{4,5} In 1991 a gene for FAP was identified and called the *APC* gene.⁶⁻⁹

APC mRNA is 8531 bp in size and codes for a protein of approximately 310 kDa. The *APC* protein is multifunctional and its primary role appears to be linked to the breakdown of β -catenin via the ubiquitin degradation pathway.¹⁰ Several other proteins not associated with that pathway have been shown to bind to the *APC* protein, suggesting a diversity of function.^{11,12}

Identification of mutations within the *APC* gene has revealed the existence of genotype/phenotype correlations between the site of mutation and disease expression. Mutations restricted to 5' of codon 169, exon 9, and 3' of codon 1403 in exon 15 have been associated with an increased likelihood of a mild colonic phenotype.^{3,13-15} In addition, mutations beyond codon 1403 are associated with a much greater probability of extracolonic disease.¹⁴⁻¹⁷ Germ-line mutations between exon 9 and approximately codon 1400 have been correlated with the presence of congenital hypertrophy of the retinal pigment epithelium (CHRPE). Outside of this region, CHRPE also occurs but there is a tendency for these mutation carriers not to express this sign.¹⁸ Finally, a common variant of the *APC* gene has been identified in the Jewish population, leading to lower penetrance than most *APC* mutations. This variant is an amino acid change at codon 1307 where a lysine substitutes for a isoleucine residue and is associated with an attenuated disease phenotype. This alteration of the *APC* gene is the first genetic change that has been identified which cannot be unequivocally assigned as causative.¹⁹

Several screening strategies have been developed to identify mutations in the *APC* gene. They include single strand conformation polymorphism analysis (SSCP), heteroduplex analysis, denaturing gradient gel electrophoresis (DGGE), the protein truncation test (PTT), and direct sequencing. Despite these various mutation detection strategies, 20-50% of families do not appear to have changes in the *APC* gene.²⁰ It is unlikely that the screening methods employed for *APC* gene mutation detection are incapable of detecting such a high proportion of cases. Linkage analysis in one series clearly identified the presence of genetic

Abbreviations used in this paper: FAP, familial adenomatous polyposis; APC, adenomatous polyposis coli; AAPC, attenuated adenomatous polyposis coli; CHRPE, congenital hypertrophy of the retinal pigment epithelium; SSCP, single strand conformation polymorphism; DGGE, denaturing gradient gel electrophoresis; PTT, protein truncation test; ARMS, amplification refractory mutation system; HNPCC, hereditary non-polyposis colorectal cancer; PCR, polymerase chain reaction.

heterogeneity²¹ which may explain some of these findings. More recently, Heinimann *et al* identified phenotypic differences in FAP patients who did not harbour germline mutations in the *APC* gene. Typically, *APC* mutation negative patients were older than their *APC* mutation positive counterparts and presented with fewer extracolonic symptoms. Colonic disease in the *APC* mutation negative patients tended to be less severe as they usually presented with fewer than 100 colonic adenomas compared with the *APC* mutation positive patients who presented with more than 100 colonic adenomas.²⁰

In this study we have analysed 53 unrelated patients who fulfilled the clinical criteria of FAP or attenuated FAP. Mutation analysis was performed using DGGE for exons 1–14 and the PTT assay for exon 15. All polymorphisms and truncated proteins were subjected to DNA sequencing for confirmation and characterisation. All families that did not harbour any obvious change in the *APC* gene were further studied to determine if they harboured the I1307K *APC* polymorphism. Phenotypic characteristics were analysed in a total of 194 affected individuals and compared according to their *APC* mutation status.

Patients and methods

A group of 53 unrelated patients diagnosed with colonic polyposis at colonoscopy or colectomy were examined for mutations in the *APC* gene. All probands gave informed consent for genetic testing. Of the 53 patients, 12 presented with colonic and extracolonic disease, four were described as having attenuated disease, and there were 14 patients who did not have a family history of disease. The remaining 23 patients only presented with colonic polyposis but did have strong family histories of colorectal cancer consistent with an autosomal dominant inheritance pattern. The diagnostic criteria for FAP were the presence of more than 100 adenomas throughout the colon and rectum. Included in the study were patients who satisfied the diagnosis of attenuated FAP. This included patients with a family history of disease that was associated with multiple colonic adenomas (>20) at a relatively young age.

Genomic DNA was isolated from Na₂EDTA blood according to the method described by Miller and colleagues.²² Briefly, blood samples were mixed with EL buffer (155 mM NH₄Cl, 10 mM KHCO₃, 1 mM Na₂EDTA, pH 7.4) and left on ice for 15 minutes. The lysate was centrifuged, washed twice with EL buffer, and the intact lymphocyte pellet resuspended in NL buffer (2 ml; 100 mM Tris HCl, pH 8.0, 0.1 M NaCl, 2 mM Na₂EDTA, 1% SDS, and 1 mg/ml proteinase K), and incubated overnight at 55°C. The next day, 333 µl/ml of 6 M NaCl was added, mixed, and centrifuged to remove cellular proteins. The supernatant containing the DNA was placed in a fresh tube and the DNA precipitated with ethanol. The resulting DNA pellet was washed with 70% ethanol, dried briefly, and dissolved in TE buffer (10

mM Tris HCl, pH 8.0, 1 mM Na₂EDTA) to a concentration of 500 ng/µl.

DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) AND AMPLIFICATION REFRACTORY MUTATION SYSTEM (ARMS) ANALYSIS

Screening of the *APC* gene was performed in two stages. Exons 1–14 were screened for mutations using DGGE and exon 15 by the PTT assay. The I1307K polymorphism was screened using the ARMS technique.¹⁹ Briefly, for DGGE, amplification of exons 1–14 was performed using primer pairs similar to those previously described⁷ except that the forward primer of each pair included a GC clamp. The polymerase chain reaction (PCR) was used to amplify genomic DNA. Amplification was performed according to the method of Fodde and colleagues.²³ In summary, a 25 µl PCR mixture contained 100 ng of genomic DNA, 0.8 µM of each primer, 200.0 µM of each dNTP, 3 mM MgCl₂, 1.0 U Taq DNA polymerase, and 1× reaction buffer (10 mM/l Tris HCl, pH 7.5, 50 mM/l KCl, 0.2 mg/ml BSA). The reaction parameters were one cycle of 95°C for one minute followed by 35 cycles of 95°C for one minute, 58°C for 1.5 minutes, and 72°C for two minutes. PCR products were subjected to DGGE using gradients ranging from 10% to 80% urea in 10% polyacrylamide (29:1) and visualised by ethidium bromide staining. A permanent record was made by photography. The ARMS technique was similar to that previously described²⁴ without modification.

PROTEIN TRUNCATION TEST (PTT)

The PTT was performed essentially as described by Powell and colleagues.²⁵ Exon 15 of the *APC* gene was divided into four overlapping segments containing codons 686–1217, 1099–1693, 1555–2256, and 2131–2843. The primers used for amplification were designed to introduce a T7 promoter sequence for the initiation of transcription by T7 RNA polymerase, as well as a Kozak consensus sequence and ATG codon for initiation of translation. Similar PCR parameters were used as for DGGE analysis. PCR products were used directly as templates in 10 µl coupled transcription-translation assays (Promega, Madison, Wisconsin, USA) using 30 µCi of [³⁵S] methionine (Amersham, Bucks, UK). Samples were incubated for one hour at 30°C, diluted in sample buffer, heated for five minutes at 95°C, and analysed on a 4–20% gradient SDS/polyacrylamide gel (Biorad). Protein was visualised by autoradiography.

DNA SEQUENCING

New DGGE polymorphisms, prematurely truncated proteins identified by the PTT, and the overlap regions of exon 15 were subject to DNA sequencing. Dideoxy sequencing of the PCR products was performed using a BIGDYE dideoxy sequencing ready reaction kit and analysed on an ABI 310 automated sequencer (PE Foster City, California, USA).

STATISTICAL ANALYSIS

Statistical differences between different groups of patients were compared using a Student's *t* test.

Results

All patients entered into this study had a clinical diagnosis of adenomatous (44 probands) or adenomatous and hyperplastic polyposis (nine probands). Of the 53 patients, 27 were found to harbour causative germline mutations in the *APC* gene and 26 patients were not found to harbour any change. The 26 patients that did not have a discernable causative germline *APC* mutation were further analysed for the I1307K mutation: none was found to harbour this change.

APC GENE POSITIVE FAMILIES

The 27 mutations identified were spread across the *APC* gene with the majority clustered more towards its 5' end (see fig 1). All but seven mutations were in exon 15 and four of these were within regions that have previously been associated with an attenuated FAP phenotype. There were three families harbouring a common mutation at codon 1061 and two unrelated families shared an identical base insertion at position 4330, which resulted in a stop codon at amino acid 1454. The common mutation at codon 1309 was not identified in this series. Most mutations were either small insertions or deletions, which gave rise to a truncated protein (see table 1 for a summary of mutations). Of particular note was the finding that four mutations that resulted in a predicted protein truncation were not identified by the PTT but were found on DNA sequencing of the overlap regions of exon 15 (see fig 1). In all patients who had a family history of disease, additional family members were tested for the identified mutation.

Two mutations were unusual. One mutation was a 17 bp duplication of the sequence between nucleotides 5772 and 5790 inserted at position 5790, which resulted in a premature stop codon at amino acid 1969. The predicted protein was identical to the APC protein until codon 1930, thereafter the remaining 39 amino acids coded for a nonsense C terminal peptide. Patients harbouring this mutation tended to

present with relatively sparse colonic polyposis and had a predilection for disease outside of the gastrointestinal tract (osteomas and desmoid disease).

The other interesting mutation was a 14 bp deletion beginning at nucleotide position 1005 through to 1019. This mutation is in exon 9 and is associated with an attenuated form of the disease. Colonic polyposis tended to be right sided with sparse presentation in the descending colon. An interesting feature described in two patients was the presence of mixed hyperplastic adenomatous polyposis. Extracolonic disease was not generally a feature in this family, but several patients presented with small cystic hamartomatous fundic gland polyps. Disease development tended to be at older ages compared with typical FAP families (34.5 (range 21–51) *v* 26.5 (range 11–65)) but this was not statistically significant, which is probably a reflection of the sample population.

A comparison between the mutations occurring within regions of the *APC* gene previously identified as being associated with an attenuated phenotype revealed different disease characteristics compared with those families harbouring mutations associated with a typical FAP phenotype. The average age of diagnosis of the attenuated FAP patients was significantly different from typical FAP patients ($p=0.019$), being 42 years (range 21–73) compared with 26.5 years (range 11–65). In the attenuated FAP families, there were no reports of disease occurring at other sites apart from the gastrointestinal tract whereas 12 FAP families were associated with recognised extra-colonic manifestations.

APC MUTATION NEGATIVE FAMILIES

To determine if there were specific features of the polyposis families that did not appear to harbour *APC* gene mutations, a comparison between age of disease onset and other clinical features was made. The average age of diagnosis for *APC* mutation positive families was 28.5 years (range 11–73) with an unusual gender difference with females representing 61.1% and males 38.9% of patients. The mutation negative families were diagnosed at an average age of 41.4 years (range 8–81) and there were no sex differences. The difference in age at

Figure 1 Schematic diagram of the *APC* gene indicating functional regions, mutation sites, and overlap regions of analysis. Proteins (including the homodimerisation region) described above the gene bind at their respective positions to *APC*. Vertical lines above the gene represent causative changes. Repeated mutations are indicated by the height of the lines.

Table 1 Clinical characteristics of the 53 index patients tested for APC germline mutations.

Patient No	Age at diagnosis (y)	Colonic polyposis†	Extracolonic disease*	Family history
1	27	C (Ad)	1, 2	Yes
2	30	C (Ad)	0	Yes
3	16	A (Ad)	2, 3, 4	No
4	45	C (Ad)	0	No
5	37	C (Ad)	0	Yes
6	41	B (Ad)	0	Yes
7	35	B (MHAd)	1, 2, 3	Yes
8	27	C (Ad)	1	Yes
9	41	C (Ad)	1, 5	Yes
10	41	C (Ad)	0	No
11	12	B (Ad)	0	Yes
12	45	B (Ad)	1	Yes
13	19	B (Ad)	6	No
14	35	B (Ad)	0	Yes
15	10	A (Ad)	1, 2	Yes
16	34	C (Ad)	1	Yes
17	13	C (Ad)	0	Yes
18	32	C (Ad)	7	Yes
19	11	A (Ad)	0	No
20	15	C (Ad)	5	Yes
21	23	C (Ad)	5, 7, 8	Yes
22	39	A (MHAd)	0	Yes
23	38	A (MHAd)	0	No
24	12	A (Ad)	0	No
25	64	C (Ad)	0	No
26	37	C (Ad)	0	No
27	62	B (Ad)	0	No
28	41	C (Ad)	7	Yes
29	42	C (Ad)	0	Yes
30	20	B (Ad)	0	Yes
31	2	A (too young)	1	Yes
32	41	A (Ad)	0	No
33	52	B (Ad)	0	No
34	38	C (Ad)	7	Yes
35	23	C (Ad)	0	Yes
36	42	B (MHAd)	0	Yes
37	33	B ((Ad)	0	No
38	47	C (Ad)	0	Yes
39	68	B (MHAd)	0	No
40	56	B (MHAd)	0	No
41	72	A (Ad)	0	No
42	14	A (Ad)	0	Yes
43	18	B ((Ad)	0	Yes
44	42	C (Ad)	0	Yes
45	57	B (MHAd)	0	Yes
46	22	C (Ad)	1, 5	Yes
47	55	C (Ad)	0	Yes
48	57	B (MHAd)	0	Yes
49	22	C (Ad)	0	Yes
50	48	C (Ad)	0	No
51	25	C (Ad)	0	Yes
52	37	C (Ad)	0	Yes
53	52	B (MHAd)	0	No

†Colonic adenoma numbers were subdivided into three groups: A, 10<n<50; B, 51<n<100; C, >100.

Ad, adenomatous polyposis; MHAd, mixed hyperplastic/adenomatous polyposis.

*1, desmoid disease; 2, osteomas; 3, epidermoid cysts; 4, supernumerary teeth; 5, duodenal polyposis; 6, angiofibroma; 7, congenital hypertrophy of the retinal pigment epithelium (CHRPE); 8, gastric adenomas.

diagnosis between mutation positive and mutation negative families was found to be statistically significant ($p=0.0004$).

Within the mutation negative families two subgroups could be differentiated: an adenomatous polyposis group (group A) and a mixed adenomatous hyperplastic polyposis group (group B). The average age for group A was 32.7 years (range 8–72) with a sex distribution of 56.3% females and 43.7% males and for group B (excluding the APC mutation positive family reported with a mixed colonic phenotype) 54.4 (range 11–81) and a sex distribution of 40% females and 60% males. This result was found to be highly statistically significant ($p=0.00003$). In fig 2 the age distribution at diagnosis is shown for group A, group B, and

Figure 2 Age of diagnosis of APC mutation positive families compared with mutation negative families combined and when divided into a mixed hyperplastic adenomatous colonic phenotype (group B) and an adenomatous phenotype alone (group A). Numbers on the x axis represent age ranges: 1, 1–9 years; 2, 10–19 years; 3, 20–29 years; 4, 30–39 years; 5, 40–49 years; 6, 50–59 years; and 7, >60 years.

groups A and B compared with APC mutation positive families. The majority of mutation positive cases are diagnosed within the first three decades of life whereas the majority of patients with a mixed adenomatous hyperplastic phenotype are diagnosed in their sixth and seventh decades of life. Group A families tended to be diagnosed at an intermediate age compared with the APC mutation positive group and group B. Other disease characteristics were examined to further refine any similarities and differences between the three groups (APC gene mutation positive families, group A, and group B). The major clinical features observed between all groups are presented in tables 2–4. Desmoid disease was identified in eight mutation positive families, in one mutation negative group A family, and in none of the group B families. Osteomas were identified in four APC mutation positive families and none of the other families. Mixed adenomatous hyperplastic polyposis was observed in eight mutation negative families and in one mutation positive family (as shown in fig 3). All hyperplastic polyps described in this study were identified within the colons of affected patients and displayed typical features with serrated glandular profiles but without nuclear hyperchromasia or atypia. The distribution of these lesions in all cases was pancolonic with no apparent sites of proclivity.

Discussion

Using a combined strategy of PTT, DGGE, and direct DNA sequencing of the overlap regions within the interval covered by the PTT, several families that otherwise would have been assigned as APC mutation negative were found to harbour mutations. Furthermore, families in which we did not find an APC mutation were examined for the common Jewish I1307K polymorphism by the ARMS technique.²² Using this approach the likelihood of missing mutations is very low and could not account for the number of families that were screened and found to be APC mutation negative. From our experiences we recommend this screening strategy for the analysis of the APC gene.

The mutation spectrum identified in this series of families is similar to that reported by others (reviewed in Nagase and Nakamura,²⁶ Miyaki and colleagues,²⁷ and Polakis²⁸). Two

Table 2 Mutations identified in the APC gene

Family No	Exon/ intron	Position	Nucleotide change*	Mutation type	Consequence of mutation
1	Ex 3	301	GGGAAGGATC → GGGAATGATC	T substitution	Stop at codon 101
2	Ex 4	487	AACCTTCAGAA → AACCTTAGAA	T substitution	Stop at codon 163
3	Ex 4	451	AAGAAGAAA → AAGAAA	4 bp deletion	Stop at codon 169
4	Ex 9	996	GTCGCGAACT → GTCGTGAAC	C deletion	Stop at codon 453
5	Ex 9	996	TGTCGCGAACT → TGTCGTGAAC	T substitution	Stop at codon 332
6	Ex 9	1005	TTGCTAGCTATGCTAGCTCCAAGAC → TTGCTCCAAGAC	14 bp deletion	Stop at codon 364
7	Ex 11	1495	TAAGACGATAT → TAAGATGATAT	T substitution	Stop at codon 499
8	Ex 13	1619	CTTACAGCAG → CTTAACAGCAG	A insertion	Stop at codon 577
9	Ex 14	1907	GAGGTGGGAT → GAGGGTGGGAT	G insertion	Stop at codon 650
10	Ex 15	2096	GCATTATGGGA → GCATTAAAGGA	A substitution	Stop at codon 699
10	Int 14	—	tatatacaga → tatatataga	T substitution	Unknown significance
11	—	2225	CAATATTATGT → CAATAATGT	TT deletion	Stop at codon 754
12	Ex 15	2537	TCGTTCTGAAA → TCTCGTTTGAA	C deletion	Stop at codon 860
13	Ex 15	2799	AAACACTTACAAT → AAACACAAT	ACTT deletion	Stop at codon 954
14	Ex 15	2803	CACTTACAATTT → CACTATTT	TACA deletion	Stop at codon 954
15	Ex 15	2907	GTAGTAGTGA → GTAGAGTGA	T deletion	Stop at codon 1004
16	Ex 15	3183	AATAAAACAAAGTG → ATAAAGTG	ACAAA deletion	Stop at codon 1063
17	Ex 15	3183	AATAAAACAAAGTG → ATAAAGTG	ACAAA deletion	Stop at codon 1063
18	Ex 15	3183	AATAAAACAAAGTG → ATAAAGTG	ACAAA deletion	Stop at codon 1063
19	Ex 15	3231	CCTGTTTATACTG → CCTGTCTG	TTATA deletion	Stop at codon 1079
20	Ex 15	3459	CAGCATGAAGAAG → CAGCAGAAG	TGAA deletion	Stop at codon 1164
21	Ex 15	3283	TTGGACAGCA → TTGGATAGCA	T substitution	Stop at codon 1125
22	Ex 15	3620	AGTAAACCG → AGTAAACCG	A deletion	Stop at codon 1297
23	Ex 15	4330	TCCTCAAACA → TCCTCAAACA	A insertion	Stop at codon 1454
24	Ex 15	4330	TCCTCAAACA → TCCTCAAACA	A insertion	Stop at codon 1454
25	Ex 15	4424	TGCTGCAGTT → TGCTGAGTT	C deletion	Stop at codon 1506
26	Ex 15	4647	AACCAAGAGA → AACCAAGAGA	A insertion	Stop at codon 1457
27	Ex 15	5773	AACCCATACTTCAGAAACAACCATA → CTTCAGAAACAATCCAC	17 bp duplication	Stop at codon 1965

*Intronic sequences are described in lower case and exonic in upper case.

Table 3 Disease characteristics of the mixed hyperplastic/adenomatous polyposis group B families

Family No	Age of diagnosis (y) (number of polyps)	Age at cancer diagnosis (y)	No affected relatives (age of crc diagnosis)	Other cancers in relatives (age)
1	56 (>100)	—	None	None reported
2	39 (>30)	52	1 (33)	None reported
3	42 (>25)	—	—	Bladder (72), brain (61), lung (81)
4	57 (>70)	57	—	Melanoma (12)
5	69 (>40)	—	3 (56, 31, ?)	Brain tumour (?)
6	61 (>20)	—	2 (72)	Cervical cancer (40)
7	57 (>10)	—	4 (8, 59, 63, 65)	Stomach (~40)
8	64 (>100)	65	1 (?)	None reported
9	58	—	3 (47, 47, 51)	Breast (56, 39, ?)

?, Unknown; crc, colorectal cancer.

unusual mutations however were identified: a 14 bp deletion and a 17 bp duplication, both of which result in an atypical polyposis phenotype. In addition to the 14 bp deletion, two more mutations were identified in exon 9, increasing the number of families reported with mutations identified within this exon.

Figure 3 Presence of extracolonic disease and hyperplastic polyposis in APC mutation positive families, APC mutation negative families, and families with a mixed hyperplastic/adenomatous polyp colonic phenotype. The occurrence of desmoids in the three groups (APC mutations positive, group A, and group B) is shown in the first three categories, the presence of osteomas is shown in the next three, and mixed adenomatous/hyperplastic polyposis is shown in the last three columns

The 14 bp deletion occurring in exon 9 has not been reported previously and is the largest genetic change that has been described for this exon. This finding adds to the small body of information about mutations in exon 9 that are associated with an attenuated disease phenotype. The differential splicing within exon 9 results in the removal of nucleotides 934–1236 from mRNA which removes 101 amino acids (residues 312–412) from the predicted APC protein.^{6,7} The resultant splice variant can be detected in normal tissue but is less abundant than the full length protein. The mutation in exon 9 described here occurs within the differential splice site and removes codons 335–340 and results in a termination codon at position 364. The presence of a mutation within this differential splice site is likely to reduce the impact of the mutant allele on disease expression and explains the mild phenotype observed in this family. Presumably, differential splicing of this change occurs which renders the mutant product less likely to be involved in disease development.²⁹ In a recent report however it

Table 4 Disease characteristics of the colonic adenomatous APC mutation negative families

Family No	Age at diagnosis (y)	Age at cancer diagnosis (y)	No affected relatives (age at diagnosis)	Other cancers in relatives (age)
1	22	—	6 (21, 26, 28, 52, 40, ?)	None reported
2	47	43, 54	2 (40+*, 40+*)	None reported
3	22	—	6 (22, 25, 61, ?, ?, ?)	None reported
4	33	33	0	None reported
5	41	—	0	None reported
6	52	52	0	None reported
7	23	—	2 (44, 20)	None reported
8	38	47	1 (57)	Melanoma (42)
			0	Unknown cancer
9	41	—	0	None reported
10	24	—	1 (29)	None reported
11	13	14	0	None reported
12	8	—	3 (19, 20, 39)	Desmoid disease
13	62	62	0	None reported
14	53	—	5 (19, 22, 26, 42, ?)	None reported
15	41	41	0	None reported
16	41	—	5 (24, ?, ?, ?, ?)	None reported
17	72	—	2 (63, 78)	Skin cancer (?), laryngeal cancer (?)

? Unknown age.

*Exact age not available.

appears that intrafamilial variation³⁰ is best explained by the presence of modifier genes but in the family reported here, the disease phenotype was remarkably consistent in that disease presentation is very predictable within this family. Notwithstanding, this family is remarkable (described in table 2); hamartomatous polyps were observed in the stomach of some individuals and one patient presented with a single hyperplastic polyp in association with adenomatous polyposis. Furthermore, in affected individuals, colonic disease was restricted to the ascending colon and there was an almost complete absence of adenomatous polyps in the descending colon or rectum. Finally, the disease spectrum observed in this family is reminiscent of hereditary non-polyposis colorectal cancer (HNPCC) illustrating that even though the basis of the two diseases is different, presentation can be remarkably similar. In the other two exon 9 mutation positive families the disease phenotype is consistent with that reported in families harbouring other mutations in this exon, adding further evidence to the notion that these mutations are associated with an attenuated phenotype.

The second mutation of interest resulted in a stop codon at position 1969 making it one of the few 3' mutations identified to date. The genetic change is not a simple one as it involved the duplication of a 17 bp sequence, which results in a nonsense chain of 39 amino acids prior to a termination codon. This change results in an increased propensity to develop extracolonic disease (most notably desmoid disease and osteomas), which appears to be a consistent finding with 3' APC gene mutations. Similar to other 3' APC mutations, the associated colonic phenotype is highly variable; some patients presented with typical symptoms of disease whereas others presented with extremely mild colonic features. The genotype/phenotype correlation in this family is consistent with that observed in other families harbouring 3' APC mutations.^{14 31 32} This family provides further evidence that 3' APC mutations have a distinctive phenotype

whereas mouse models of 3' *Apc* mutations suggest otherwise.³³ A major difference between this set of families and other series that have been reported in the literature was the male to female ratio. Given the size of the population under investigation we believe this to be due to chance and not a real difference between this population and others that have been investigated elsewhere.

Several families were identified that, using our detection strategy, did not harbour germline mutations in the APC gene but did present with a colonic phenotype which was consistent with a diagnosis of either FAP or attenuated FAP. Clinical features of these families were compared with those found in families where an APC germline mutation was identified. From the families that did not harbour detectable germline APC mutations two groups could be clearly identified. Group A consisted of patients who presented with only colonic adenomatous polyposis (albeit towards the lower range, 20 to several hundreds of adenomas). This group also tended to be older at diagnosis of colorectal cancer compared with group B and the mutation positive families and it affected slightly more males than females. Group B consisted of affected individuals harbouring a mixed hyperplastic/adenomatous polyposis phenotype who were older at diagnosis compared with the APC mutation positive group but not group A. Age at diagnosis is expected to be older in groups A and B by virtue of the fact that families clearly fulfilling the diagnostic criteria for FAP were more likely to be entered into a colonic screening programme at an earlier age compared with those in groups A and B. Notwithstanding, the average age at diagnosis of FAP occurs in the fifth decade of life, whereas the average age of diagnosis was in the sixth decade of life for group B, indicative of a real difference between the two groups. Group A tended to present at an age which was similar to that of FAP reported in the literature.³⁴

Little is known of the aetiology of hyperplastic polyps or their relationship to adenomatous polyps. Recent reports have suggested that they

may represent a distinct entity that is associated with an increased risk of colorectal cancer which is greater than the population risk but less than that observed for predispositions³⁵ such as FAP or HNPCC. The numbers of polyps in this group were lower than group A (range 20–70). Unfortunately, it was not possible to obtain the ratio of hyperplastic to adenomatous polyps. Similarly, age at diagnosis was older than the mutation positive group but was also younger than the average age in group A. This suggests that there are at least two groups of polyposis patients not linked to the *APC* gene.

The diagnosis of mixed adenomatous/hyperplastic polyposis in one *APC* gene positive family may be related to the family specific mutation in exon 14 of the *APC* gene. Furthermore, it cannot be ruled out that the hyperplastic polyp is a somatic variant occurring on a genetic background of colorectal cancer risk. In contrast, the nine families that comprised group B where no *APC* mutations were identified presented with only a colonic phenotype of hyperplastic/adenomatous polyposis. Additional evidence to suggest that the two entities are distinct was the presence of extracolonic disease in the adenomatous group (desmoid disease) whereas only colonic disease was observed in the mixed hyperplastic/adenomatous polyposis group.

In summary, the mutation detection strategy used in this report will minimise the number of false negative families screened using the protein truncation test assay. Accurate description of polyposis families that do not harbour *APC* germline mutations will aid in classifying unique features that could help in the identification of additional genes related to at least one other genetic entity associated with colonic polyposis.

This work was supported in part by the Hunter Area Pathology Research Grant and the Hunter Area Health Service. We would like to thank all clinicians and patients who provided us with information so that this study could be performed.

- 1 Bufill JA. Colorectal cancer genetics. Closing the gap between genotype and phenotype. *Cancer* 1995;**76**:2389–92.
- 2 Spirio L, Kutchera W, Groden J, et al. Alleles of the *APC* gene: an attenuated form of familial polyposis. *Cell* 1993;**75**:952–7.
- 3 Lynch HT, Smyrk TC, Lanspa SJ, et al. Upper gastrointestinal manifestations in families with hereditary flat adenoma syndrome. *Cancer* 1993;**71**:2709–14.
- 4 Herrera L, Kakati S, Gibas L, et al. Gardner syndrome in a man with an interstitial deletion of 5q. *Am J Med* 1986;**25**:473–6.
- 5 Bodmer WF, Bailey CJ, Bodmer J, et al. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987;**328**:614–16.
- 6 Joslyn G, Carlson M, Thliveris A, et al. Identification of deletion mutations and three new genes at the familial polyposis locus. *Cell* 1991;**66**:601–13.
- 7 Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991;**66**:589–600.

- 8 Nishisho I, Nakamura Y, Miyoshi Y, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 1991;**253**:665–9.
- 9 Kinzler KW, Nilbert MC, Su LK, et al. Identification of FAP locus genes from chromosome 5q21. *Science* 1991;**253**:661–5.
- 10 Rubinfeld B, Albert I, Porfiri E, et al. Binding of GSK3 β to the APC- β -catenin complex and regulation of complex assembly. *Science* 1996;**272**:1731–3.
- 11 Su L-K, Burrell M, Hill DE, et al. APC binds to the novel protein EB1. *Cancer Res* 1995;**55**:2972–7.
- 12 Matsumine A, Ogai A, Senda T, et al. Binding of APC to the human homolog of the Drosophila discs large tumor suppressor protein. *Science* 1996;**272**:1020–3.
- 13 Spirio L, Olschwang S, Groden J, et al. Alleles of the *APC* gene: an attenuated form of familial polyposis. *Cell* 1993;**75**:951–7.
- 14 Dobbie Z, Spycher M, Huerlimann R, et al. Mutational analysis of the first 14 exons of the adenomatous polyposis coli (*APC*) gene. *Eur J Cancer* 1994;**30A**:1709–13.
- 15 Scott RJ, van der Luijt R, Spycher M, et al. Novel germline mutation in a large familial adenomatous polyposis kindred displaying variable phenotypes. *Gut* 1995;**36**:731–6.
- 16 van der Luijt RB, Meera Khan P, Vasen HFA, et al. Germline mutations in the 3' part of *APC* exon 15 do not result in truncated proteins and are associated with attenuated familial adenomatous polyposis. *Hum Genet* 1996;**98**:727–34.
- 17 Dobbie Z, Spycher M, Mary JL, et al. Correlation between the development of extracolonic manifestations in FAP patients and mutations beyond codon 1403 in the *APC* gene. *J Med Genet* 1996;**33**:274–80.
- 18 Caspari R, Olschwang S, Friedl W, et al. Familial adenomatous polyposis: desmoid tumours and lack of ophthalmic lesions (CHRPE) associated with *APC* mutations beyond codon 1444. *Hum Mol Genet* 1995;**4**:337–40.
- 19 Laken SJ, Petersen GM, Gruber SB, et al. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in *APC*. *Nat Genet* 1997;**17**:79–83.
- 20 Heinemann K, Muellhaupt B, Weber W, et al. Phenotypic differences in familial adenomatous polyposis based on *APC* gene mutation status. *Gut* 1998;**39**:675–9.
- 21 Tops CMJ, Van der Klift HM, Van Der Luijt RB, et al. Non-allelic heterogeneity of familial adenomatous polyposis. *Am J Med Genet* 1993;**47**:563–7.
- 22 Miller SA, Dykes DD, Polesky HF. A salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;**16**:739.
- 23 Fodde R, Lisekoot M. Mutation detection by denaturing gradient gel electrophoresis (DGGE). *Hum Mutat* 1994;**3**:83–94.
- 24 Frayling IM, Beck NE, Ilyas M, et al. The *APC* variants I1307K and E1317Q are associated with colorectal tumors but not always with a family history. *Proc Natl Acad Sci* 1998;**95**:10722–7.
- 25 Powell SM, Petersen GM, Krush AJ, et al. Molecular diagnosis of familial adenomatous polyposis. *N Engl J Med* 1992;**329**:1982–7.
- 26 Nagase H, Nakamura Y. Mutations of the *APC* (adenomatous polyposis coli) gene. *Hum Mutat* 1993;**2**:425–34.
- 27 Miyaki M, Tanaka K, Kikuchi-Yanoshita R, et al. Familial polyposis: recent advances. *Crit Rev Oncol Hematol* 1995;**19**:1–31.
- 28 Polakis P. Mutations in the *APC* gene and their implications for protein structure and function. *Curr Opin Genet Dev* 1995;**5**:66–71.
- 29 Soravia C, Berk T, Madlensky L, et al. Genotype-phenotype correlations in attenuated adenomatous polyposis coli. *Am J Hum Genet* 1998;**62**:1290–331.
- 30 Nasioulas S, Jones IT, St. John DJB, et al. Profuse familial adenomatous polyposis with an adenomatous polyposis coli exon 3 mutation. *Fam Cancer* (in press).
- 31 Scott RJ, Froggatt NJ, Trembath RC, et al. Familial infiltrative fibromatosis (desmoid tumours) (MIM135290) caused by a recurrent 3' *APC* gene mutation. *Hum Mol Genet* 1996;**5**:1921–4.
- 32 Eccles DM, Van der Luijt RB, Breukel C, et al. Hereditary desmoid disease due to a frameshift mutation at codon 1924 of the *APC* gene. *Am J Hum Genet* 1996;**59**:1193–201.
- 33 Smits R, Kielman MF, Breukel C, et al. Apc 1638T: a mouse model delineating critical domains of the adenomatous polyposis coli protein involved in tumorigenesis and development. *Genes Dev* 1999;**13**:1309–21.
- 34 Houlston R, Slack J, Murday V. Risk estimates for screening adenomatous polyposis coli. *Lancet* 1990;**335**:484.
- 35 Place RJ, Simmang CL. Hyperplastic-adenomatous polyposis syndrome. *J Am Coll Surg* 1999;**188**:503–7.