Variable phenotype of familial adenomatous polyposis in pedigrees with 3' mutation in the APC gene

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Abstract

Background—Germline mutation in the adenomatous polyposis coli (APC) gene on chromosome 5 causes familial adenomatous polyposis. "Attenuated" phenotype has been reported with mutation in the 5' end of the gene (5' to codon 158), but genotype-phenotype relations at the 3' end (3' to codon 1596) have not been described fully.

Aims—To describe and compare colorectal and extracolonic phenotypes in a case series of families with mutation in the 3' end of the APC gene.

Methods—Thirty one at risk or affected members from four families with a mutation in the APC gene located at codon 1979 or 2644 were evaluated.

Results—Variable intrapedigree colorectal phenotype was observed: some members at older age had oligopolyposis (fewer than one hundred colorectal adenomas) whereas other members had classic polyposis at young age. Colorectal cancer was diagnosed at older mean age (50 (7) years) in the four families than in classic FAP pedigrees (39 (14) years). Extracolonic lesions characteristic of FAP occurred with 3' APC mutations, but variability in intrapedigree and interpedigree extracolonic phenotype and dissociation of severity of extracolonic manifestations from number of colorectal polyps was noted.

Conclusions—Families with 3' mutations of the APC gene exhibit variable intrapedigree phenotype similar to the heterogeneity noted in families with proximal 5' mutations. Genotyping of FAP and oligopolyposis pedigrees can guide appropriate surveillance of the upper and lower gastrointestinal tract in affected members.

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Keywords: familial adenomatous polyposis; attenuated adenomatous polyposis coli; adenomatous polyposis coli gene mutation; extracolonic lesions

Familial adenomatous polyposis (FAP) is an autosomal dominant disease classically characterised by the development of hundreds of adenomatous colorectal polyps, usually in the teenage years.¹ Most affected individuals who are not treated with colectomy develop colorectal cancer by the sixth decade of life.² Germline mutations in the adenomatous polyposis coli

(APC) gene, located on the long arm of chromosome 5 in band q21, cause FAP.³⁻⁶ The APC gene has 15 exons and encodes a predicted gene product of 2843 amino acids with a molecular weight of about 312 000 daltons. Both frameshift and point mutations of the APC gene occur in FAP and are generally distributed in the 5' half of the coding region.

Genotype-phenotype correlation studies have reported that mutations in the extreme 5' end of the APC gene are associated with a less severe phenotype of FAP,⁷⁻¹⁵ termed attenuated adenomatous polyposis coli (AAPC) by Spirio *et al* in 1992.¹¹ The clinical characteristics of the attenuated variant include fewer than 100 colorectal adenomas (oligopolyposis) at presentation but notable phenotypic variation within pedigrees, and a delayed onset of colorectal cancer which occurs on average 12 years later than in classic FAP.^{10 12 15} Further evaluation revealed that APC mutations 5' to codon 158 (proximal 5' mutations) predict the attenuated phenotype, while mutations 3' to codon 167 are associated with classic FAP.¹⁰

Recently, several pedigrees with mutations in the distal 3' portion of the APC gene have also been reported to have an attenuated phenotype.¹⁶⁻²⁵ These pedigrees have mutations of the APC gene 3' to codon 1596, while mutations of codons 1444 to 1578 have been associated with classic polyposis and multiple extracolonic manifestations.^{26 27} We performed a detailed evaluation of the phenotype of four families with mutations that occurred in codon 1979 or 2644 and compared them to other reports of families with distal 3' mutations. The implications for clinical management of patients in these pedigrees are addressed.

Methods

At the time of this study, The Johns Hopkins Polyposis Registry contained 340 pedigrees with FAP. After informed consent was obtained, at least one member with FAP from 112 available pedigrees was evaluated for mutation of the APC gene. The APC gene was analysed using DNA and/or RNA from peripheral blood leucocytes by *in vitro* synthesised protein (IVSP) assay and/or cloning and sequencing the entire coding region of the gene, as described previously.²⁷⁻²⁹ Only two of the registry families were found to carry a mutation 3' to codon 1596 (families 1 and 4). Two additional families with mutation in this region (families 2 and 3) are included. Family 2 is reported in collaboration with Indiana University Medical

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Table 1 Clinical characteristics of individual family members

Subject no	Age (y)	Sex	Gene status	Examination type	Age at examination	Number of colon polyps	Colon cancer (Y/N)	Age at cancer diagnosis	Extracolonic manifestations
Family 1									
I:2	79	F	NK	Colonoscopy	74	>100	N	N/A	NK
II:1	54	F	–	Sigmoidoscopy	54	0	N	N/A	None
II:2	53	M	+	Colonoscopy	48	>100	Y	48	None
II:5	52	F	+	Colonoscopy	46	6	N	N/A	Lipomas, gastric polyps*
II:6	50	F	+	Colonoscopy	44	4	N	N/A	Gastric polyps*
III:1	24	M	NK	Sigmoidoscopy	21	0	N	N/A	NK
III:2	23	F	+	Sigmoidoscopy	22	0	N	N/A	None
III:3	29	M	+	Sigmoidoscopy	29	1	N	N/A	Lipomas
III:4	28	M	+	Sigmoidoscopy	27	0	N	N/A	Lipomas
III:5	25	F	–	Sigmoidoscopy	24	0	N	N/A	None
Family 2									
I:2	N/A	F	NK	NK	NK	NK	Y	46	NK
II:1	60	M	+	Colonoscopy	51	>100	N	N/A	Osteoma, adrenal tumour, gastric and duodenal polyps*
III:1	34	M	+	Colonoscopy	33	6	N	N/A	Sebaceous cysts
III:2	32	F	NK	Colonoscopy	28	>100	N	N/A	Gastric polyps, desmoid tumours, osteomas*
III:3	29	F	NK	Colonoscopy	25	>100	N	N/A	Desmoid tumours, sebaceous cysts
III:4	19	M	+	Colonoscopy	18	0	N	N/A	None
Family 3									
I:1	NK	M	NK	NK	NK	NK	Y	NK	Pancreatic cancer
II:1	51	M	+	Colonoscopy	50	>100	N	N/A	None*
III:1	18	M	NK	None	N/A	N/A	N	N/A	NK
III:2	14	M	NK	None	N/A	N/A	N	N/A	NK
I:1	58	M	NK	None	N/A	NK	N	N/A	Liver or bladder cancer
II:2	68	F	+	Colonoscopy	51	>100	N	N/A	Osteomas, fibrosarcoma, lipoma, desmoids, duodenal polyps*
II:3	67	M	NK	Colectomy	60	>100	Y	60	Osteoma, bladder cancer
II:5	73	M	NK	Colectomy	59	>100	N	N/A	None
II:6	71	M	NK	Sigmoidoscopy	54	0	N	N/A	None
III:1	47	M	NK	None	N/A	N/A	Y	46	Epidermoid cysts, desmoids, cancer-multiple primaries
III:2	45	F	NK	Colonoscopy	36	>100	N	N/A	Desmoid*
III:3	39	M	NK	Sigmoidoscopy	23	0	N	N/A	None
III:4	35	M	NK	Sigmoidoscopy	19	0	N	N/A	None
III:5	41	F	NK	Colonoscopy	40	0	N	N/A	Jaw lesion, hepatoma, desmoid*
III:6	50	F	NK	Colonoscopy	33	0	N	N/A	Jaw lesion

*Upper endoscopy performed.

NK, not known; N/A, not applicable.

Center, Indianapolis, Indiana, and family 3 with the University of Colorado Cancer Center, Denver, Colorado.

The records of the members from these four families with mutation in the 3' region of the APC gene were reviewed in detail. The age at diagnosis of FAP, number of polyps at first examination of the colon, age at diagnosis of colorectal cancer, and extracolonic manifestations were evaluated in the members of each pedigree. The diagnosis of FAP in family members was verified by clinical and pathological criteria. The age of colorectal cancer diagnosis in these 3' pedigrees was compared with that of FAP families from the Johns Hopkins Polyposis Registry with mutations 5' to codon 1596.¹⁵

Results

Families 1–3 had a four base pair deletion (ACAA) at codon 1979, which resulted in a detectable truncated protein by IVSP assay. There was no evidence of a founder effect for these three families. Family 4 had a four base pair deletion (TTAT) at codon 2644 which did not produce a truncated protein by IVSP assay.

The pedigrees are shown in fig 1 and summarised in tables 1 and 2. In the three families which had multiple affected members (families 1, 2, and 4), heterogeneity in the colorectal phenotype within each family was noted (table 1). Gene test results were available for multiple members in families 1 and 2. There were seven gene positive people ranging in age from 22 to 46, each with fewer than seven adenomas on colonic examination, representing oligopolyposis. By contrast, there were five people with polyposis (greater than 100 polyps) diagnosed between 25 and 74 years of age in these same pedigrees. For example, in family 2, subjects III:2 and III:3 (fig 1) had profuse polyposis necessitating colectomy at ages 25 and 28.

Extracolonic manifestations characteristic of FAP (table 1) also showed intrafamilial variation and did not correlate with phenotypic expression of colorectal polyposis. Individual II-6 in family 1 had only four colorectal adenomas at age 44, but she had multiple fundic gland retention polyps of the stomach. Similarly, subjects II:5, III:3, and III:4 were all affected with skin lesions, despite the presence of very few if any colorectal polyps. In family 2,

Table 2 Molecular genetic test results and phenotype of families reported in literature

Mutation and consequence (if reported)	Number of families	Truncated protein	Number of colon polyps	Extracolonic manifestations	Reference
1597delT stop + 53aa	1	Not tested	"Sparse" (<5000)	Unknown	16, 17
1597delC stop + 53aa	2	Not tested	30–200	Gastric cancer, supernumerary teeth	18
1860delTTCT stop + 58aa	1	Not detected	<10–>100	Unknown	19
1924insAA stop + 45aa	1	Detected	0–50	Familial desmoids, gastric polyps, osteomas	20
1962delCAGA	3	Detected	0–200	Desmoids, gastric polyps	21
1982–1983delTAAA stop + 63aa	1	Detected	few–300	Cysts, osteomas, gastric polyps, desmoids, atypical CHRPE	22
1987delA stop + 61aa	1	Detected*	few–1000s	Desmoids, gastric polyps, osteoma, gastric cancer, CHRPE	23
1987delA stop + 61aa	3	Detected*	<100–>1000	Osteoma, lipoma, fibroma, desmoids, small intestinal polyps, gastric polyps	24
2645delTTAT stop + 14aa	1	Not tested	Unknown	Extracolonic "symptoms"	25

*Western blot analysis did not support the existence of a stable truncated APC protein in lymphoblastoid cell lines from individuals carrying the mutation.
+aa, amino acid; CHRPE, congenital hypertrophy of the retinal pigment epithelium.

individual III:1 had sebaceous cysts but only three colorectal adenomas at age 33. The great variety of extracolonic manifestations contrasted with the lack of typical colonic polyposis. Also, despite identical mutations of codon 1979 in families 1–3, interfamilial variability in the severity of extracolonic manifestations was evident (tables 1 and 2).

Four cases of colorectal cancer with known age of cancer diagnosis were noted at 46, 46, 48, and 60 years of age in families 1, 2, and 4. The average age at diagnosis of colorectal cancer (50 (SD 7) years) contrasted with the younger age of colorectal cancer diagnosis (39

(14) years) in classic FAP families in the Johns Hopkins Polyposis Registry.^{15–29} Two patients in whom colorectal polyp number was known had polyposis (more than 100 adenomas) at the time of cancer diagnosis (polyp number in patient I:2 in family 2 and patient III:1 in family 4 could not be quantitated). None of the patients with fewer than 100 adenomas developed colorectal cancer.

Discussion

We found that mutation in the 3' end of the APC gene was associated with intrapedigree variability of both colonic and extracolonic

Figure 1 Pedigrees of families 1–4 with 3' mutation of the APC gene.

phenotype, as evidenced by families 1, 2, and 4 which had multiple affected members available for study. Intrapedigree variability of colorectal phenotype has been noted by other investigators in subjects presumed to be genotypically affected, as reviewed in table 1. Similar variability is also seen in the colorectal phenotype of all pedigrees described to date with mutations 5' to codon 158.¹⁵ Analogous to the boundary for phenotypic manifestations of mutations at the 5' end of the APC gene, a boundary at the 3' end of the gene at codon 1596 separates families with variable intrapedigree phenotype from classic FAP phenotype.

The evolution of colorectal cancer seemed to parallel expression of colorectal polyposis in our study. The two individuals with colorectal cancer and information about the number of polyps both had classic polyposis. An older mean age at diagnosis of colorectal cancer (50 (7) years) was noted in these four patients in families 1, 2, and 4 compared with classic FAP pedigrees.²⁹ This is consistent with the delayed development of colorectal cancer observed in AAPC families with proximal 5' mutations.¹⁵ Nevertheless, the cumulative risk of colorectal cancer seems to reach 100% in attenuated adenomatous polyposis caused by 5' mutation of the APC gene,¹⁵ and 3' families may follow a similar course.¹⁹

Extracolonic manifestations characteristic of FAP also showed intrafamilial variation and did not correlate with phenotypic expression of colorectal polyposis. The great variety of extracolonic manifestations in family members contrasted with the frequent occurrence of oligopolyposis. This phenomenon has also been described by others.²⁰⁻²¹ Specifically, Eccles *et al.*²⁰ reported a family (with a 2 bp insertion at codon 1924 of the APC gene) with hereditary desmoid disease and the presence of other extracolonic manifestations including osteomas, fibromas, and epidermoid cysts, but only one member with colorectal polyps.

Although the four families included in our series were the only ones known to us with mutations 3' to codon 1596, each pedigree included at least one member with classic polyposis (more than 100 polyps) who led to APC gene testing of the family. Our study results are, therefore, biased by selection on the basis of classic disease phenotype. Individuals with oligopolyposis in the absence of a family history of classic disease could also have mutation in the 3' or 5' region of the APC gene but would not have been tested.

Recognition that an attenuated colorectal phenotype can occur in some patients with germline mutations of the APC gene makes routine genotyping of families with multiple adenomas important for clinical management. Colonoscopic rather than sigmoidoscopic screening of at risk members to evaluate the entire colon is appropriate when mutations involve the proximal or distal end of the APC gene because of the occurrence of oligopolyposis.³⁰ Also, gene positive members of families with variable intrapedigree phenotype should consider surveillance with oesophagoduodenoscopy even before the devel-

opment of colorectal polyposis, as several study subjects had upper gastrointestinal polyposis rather than colorectal polyposis. Finally, because of the possibility of a 3' or 5' APC mutation, this study argues that APC gene testing should be considered for patients with small numbers of adenomas (10 or more) if they present at young age (less than 40 years), with extracolonic lesions characteristic of FAP, or with a family history of adenomas and/or cancer.

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- 1 Bussey HJR. *Familial polyposis coli. Family studies, histopathology, differential diagnosis, and results of treatment.* Baltimore, Maryland: Johns Hopkins University Press, 1975.
- 2 Petersen GM, Slack J, Nakamura Y. Screening guidelines and premorbid diagnosis of familial adenomatous polyposis using linkage. *Gastroenterology* 1990;100:1658-64.
- 3 Nishisho I, Nakamura Y, Miyoshi Y, *et al.* Mutations of chromosome 5q21 gene in FAP and colorectal cancer patients. *Science* 1991;253:665-9.
- 4 Kinzler KW, Nilbert MC, Su LK, *et al.* Identification of FAP locus genes from chromosome 5q21. *Science* 1991;253:661-5.
- 5 Groden J, Thliveris A, Samowitz W, *et al.* Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991;66:589-600.
- 6 Joslyn G, Carlson M, Thliveris A, *et al.* Identification of deletion mutations and three new genes at the familial polyposis locus. *Cell* 1991;66:600-13.
- 7 Lynch HT, Smyrk T, Lanspa SJ, *et al.* Flat adenomas in a colon cancer-prone kindred. *J Natl Cancer Inst* 1988;80:278-82.
- 8 Lynch HT, Smyrk T, Lanspa SJ, *et al.* Phenotypic variation in colorectal adenoma/cancer expression in two families—hereditary flat adenoma syndrome. *Cancer* 1990;66:909-15.
- 9 Leppert M, Burt R, Hughes JP, *et al.* Genetic analysis of an inherited predisposition to colon cancer in a family with a variable number of adenomatous polyps. *N Engl J Med* 1990;322:904-8.
- 10 Spirio L, Olschwang S, Groden J, *et al.* Alleles of the APC gene: an attenuated form of familial polyposis. *Cell* 1993;75:951-7.
- 11 Spirio L, Otterud B, Stauffer D, *et al.* Linkage of a variant or attenuated form of adenomatous polyposis coli to the adenomatous polyposis coli (APC) locus. *Am J Hum Genet* 1992;51:92-100.
- 12 Lynch HT, Smyrk T, McGinn T, *et al.* Attenuated familial adenomatous polyposis (AFAP): a phenotypically and genotypically distinctive variant of FAP. *Cancer* 1995;76:2427-33.
- 13 Olschwang S, Laurent-Puig P, Groden J, *et al.* Germline mutations in the first fourteen exons of the APC gene. *Am J Hum Genet* 1993;52:273-9.
- 14 Foode R, van der Luijt R, Wijnen J, *et al.* Eight novel inactivating germ line mutations at the APC gene identified by denaturing gradient gel electrophoresis. *Genomics* 1992;13:1162-8.
- 15 Giardiello FM, Brensinger JD, Luce MC, *et al.* Phenotypic expression in adenomatous polyposis families with mutation in the 5' region of the adenomatous polyposis coli gene. *Ann Intern Med* 1997;126:514-19.
- 16 Miyoshi Y, Ando H, Nagase H, *et al.* Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. *Proc Natl Acad Sci USA* 1992;89:4452-6.
- 17 Nagase H, Miyoshi Y, Horri A, *et al.* Correlation between the location of germ-line mutations in the APC gene and the number of colorectal polyps in familial adenomatous polyposis patients. *Cancer Res* 1992;52:4055-7.
- 18 Friedl W, Meuschel S, Caspari R, *et al.* Attenuated familial adenomatous polyposis due to a mutation in the 3' part of the APC gene. A clue for understanding the function of the APC protein. *Hum Genet* 1996;97:579-84.
- 19 van der Luijt RB, Khan PM, Vasen HFA, *et al.* Germline mutations in the 3' part of the APC exon 15 do not result in truncated proteins and are associated with attenuated adenomatous polyposis coli. *Hum Genet* 1996;98:727-34.
- 20 Eccles DM, van der Luijt R, 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.
- 21 Scott RJ, Frogatt NJ, Trembath RC, *et al.* Familial infiltrative fibromatosis (desmoid tumors) (MIM 135290) caused by a recurrent 3' APC gene mutation. *Hum Mol Genet* 1996;5:1921-4.
- 22 Gardner RJM, Kool D, Edkins E, *et al.* The clinical correlates of a 3' truncating mutation (codons 1982-1983)

- in the adenomatous polyposis coli gene. *Gastroenterology* 1997;113:326–31.
- 23 Scott RJ, van der Luijt R, Spycher M, et al. Novel germline APC gene mutation in a large familial adenomatous polyposis kindred displaying variable phenotypes. *Gut* 1995;36:731–6.
 - 24 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.
 - 25 Groden J, Gelbert L, Thliveris A, et al. Mutational analysis of patients with adenomatous polyposis: identical inactivating mutations in unrelated individuals. *J Hum Genet* 1993;52:263–72.
 - 26 Caspari R, Olschwang S, Friedl W, et al. Familial adenomatous polyposis: desmoid tumors and lack of ophthalmic lesions (CHRPE) associated with APC mutations beyond codon 1444. *Hum Mol Genet* 1995;4:337–40.
 - 27 Miyoshi Y, Ando H, Nagase H, et al. Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. *Proc Natl Acad Sci USA* 1992;89:4452–6.
 - 28 Nagase H, Miyoshi Y, Horii A, et al. Germ-line mutations of the APC gene in patients with familial adenomatous polyposis: screening of 150 unrelated patients. *Am J Hum Genet* 1992;51:A39.
 - 29 Powell SM, Petersen GM, Krush AJ, et al. Molecular diagnosis of familial adenomatous polyposis. *N Engl J Med* 1993;329:1982–7.
 - 30 Burt RW, Kuwada SK, Kerber R, et al. The predominance of proximal colonic neoplasms in attenuated adenomatous polyposis coli [abstract]. *Gastroenterology* 1995;108:A453.