ANNALS OF SURGERY
Vol. 227, No. 1, 57–62

APC Genotype, Polyp Number, and Surgical Options in Familial Adenomatous Polyposis

James S. Wu, M.D.,* Philip Paul, Ph.D.,†, Ellen A. McGannon, B.S.W.,* and James M. Church, M.D.*

From the Departments of Colon and Rectal Surgery and Cancer Biology,† The Cleveland Clinic Foundation, Cleveland, Ohio*

Objective

This study was performed to examine the relation between phenotypic expression in patients with familial adenomatous polyposis (FAP) and the site of mutations in the *APC* (adenomatous polyposis coli) gene. The ability of *APC* mutations to predict surgical outcome was also investigated.

Summary Background Data

Germline mutations in the *APC* gene cause FAP and can now be identified by direct mutational analysis. Such an analysis can identify affected persons for close surveillance and spare unaffected persons. Phenotypic expression varies within and among FAP kindreds, but certain mutations have been associated with severe disease. Patients with severe polyposis are frequently offered total proctocolectomy rather than colectomy and ileorectal anastomosis out of concern for increased rectal cancer risk. Mutation analysis may offer a more rational basis for these decisions.

Methods

The postsurgical courses of 58 patients from 19 FAP kindreds with identified *APC* gene mutations were reviewed. *APC* gene mutations were identified by analysis of leukocyte DNA using single-strand conformational analysis and DNA sequencing. FAP severity was defined according to the number of polyps in the colon at the time of resection (<1000, mild; >1000, severe). Operations included subtotal colectomy with ileorectal anastomosis (IRA), total proctocolectomy with ileal pouch/anal anastomosis, total proctocolectomy with end ileostomy, and partial colectomy (PC).

Results

Eight different *APC* mutations were identified. Mutations at codons 1309 and 1328 in exon 15G were associated with a uniformly severe polyposis phenotype. For other mutations, the phenotype was more variable. Patients with *APC* mutations at codons 1309 and 1328 more commonly underwent proctectomy. Among the 43 patients who initially underwent either IRA or PC, the rectum was later removed in 8. Seven of these patients had a mutation at codon 1309 or 1328. With one exception, all patients with mutations outside the 1309 or 1328 site who initially had IRA have retained their rectum.

Conclusions

Our data support an association between severe polyposis phenotype and mutations at *APC* gene codons 1309 and 1328. For patients with these mutations, the prognosis for retaining the rectum is poor.

Familial adenomatous polyposis (FAP) is caused by germline mutation of the *APC* gene that results in multiple adenomatous polyps throughout the colon and rectum. Without prophylactic surgery, virtually all patients with FAP become symptomatic in the third to fourth decade of life and usually develop colorectal cancer by age 50 years.[1] The severity of the FAP phenotype, as defined by the number of adenomas in the large bowel, varies both within a family and from one family to another. Patients with a severe phenotype (>1000 polyps) tend to present earlier, are more often symptomatic, and may be more likely to develop colorectal cancer. Debinski et al.[2] showed that a resected specimen with >1000 polyps has double the chance of containing a cancer compared with one with <1000 polyps.

The *APC* gene was identified in 1991,[3,4] and since then several reports have shown a correlation between the severity of polyposis and the site of mutation.[5–8] Patients with an *APC* mutation at a locus associated with severe disease are likely to have a high risk of developing cancer. Treatment of colorectal disease in FAP is chiefly surgical and is designed to prevent cancer from developing. There are two primary surgical options: subtotal colectomy with ileorectal anastomosis (IRA) or total proctocolectomy with ileal pouch/anal anastomosis (IPAA). IRA is a simpler operation with less disturbance of bowel function, but its role in the management of FAP is controversial because of the risk of cancer in the retained rectum.[9–12] If patients could be triaged preoperatively according to their risk of developing rectal cancer, IRA could be used more appropriately.

We compared *APC* genotype with FAP phenotype to determine whether any specific mutations correlate with severe polyposis. The clinical course of FAP patients with known mutations was also reviewed to determine whether there was any association between the site of mutation and the fate of the rectum after IRA.

METHODS

Patients and DNA Samples

FAP families in the David G. Jagelman Polyposis Registry at the Cleveland Clinic Foundation with successful mutational analysis were included in this study. DNA was obtained from peripheral blood leukocytes as previously described by Miller et al.,[13] and *APC* gene mutations were identified and characterized by single-strand conformational analysis.[14] The polymerase chain reaction was used to amplify genomic DNA[6] using primers to amplify *APC* exons 1 to 14 and overlapping segments of exon 15, as described by Groden et al.[3] Sequencing of polymerase chain reaction products after single-strand conformational analysis was carried out as described by Paul et al.[6]

The number of polyps was determined from surgical pathology reports of excised specimens. Terms such as "innumerable," "diffuse," "too numerous to count," or "carpeted" or an absolute number of polyps >1000 were considered to indicate severe disease. An absolute number of polyps <1000 was defined as mild disease. The type of first operation performed varied depending on surgeon preference and on the year of surgery. In the prepouch era (before 1983), IRA was preferred over total proctocolectomy and ileostomy, except when rectal involvement was severe. In the postpouch era, the choice of operation depended on the number of rectal polyps as well as patient or surgeon preference.

A chi square test was used to compare categorical data. The null hypotheses were stated in terms of no differences in the proportion of subjects between mutation locus and either polyp severity or the fate of the rectum.

RESULTS

Eight different mutations were detected in 58 FAP patients from 19 kindreds (Table 1). Site of mutation and polyp severity are also shown in Table 1. A comparison of polyp severity with mutation site reveals mild polyp density in all patients with known phenotype in the kindred with a mutation at codon 157 (exon 4), in the single patient with a mutation at codon 1464 (exon 15H), and in the 3 patients from the kindred with a mutation at codon 1528 (exon 15I). Polyp density was variable in kindreds with mutations at codons 540 (exon 12), 1060 (exon 15E), and 1068 (exon 15E). All patients with mutations at codons 1309 (exon 15G) or 1328 (exon 15G) had severe polyposis. Only 1 patient had a colon cancer (mutation in codon 1328).

First Surgery

All 28 patients with mutations at codons 157, 540, 1068, and 1528 underwent subtotal colectomy with

Dedicated to the memory of David G. Jagelman, M.D.

Address reprint requests to James M. Church, M.D., The Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195.

Accepted for publication July 3, 1996.

Table 1. CORRELATION OF POLYP SEVERITY WITH *APC* GENE MUTATION: SEVERITY OF POLYPOSIS (NUMBER OF PATIENTS)

Mutation (codon)	Exon	Severe	Mild	Unknown	Patients	Kindreds
157	4	0	6	1	7	1
540	12	1	1	0	2	1
1060	15E	3	4	1	8	5
1068	15E	5	11	0	16	2
1309	15G	16	0	2	18	7
1328	15G	3	0	0	3	1
1464	15H	0	1	0	1	1
1528	15I	0	3	0	3	1
Total		28	26	4	58	19

IRA. Among patients with a mutation at codon 1060, 6 received an IRA, 1 underwent total proctocolectomy with end ileostomy, and 1 had an IPAA. The single patient with a mutation at codon 1464 underwent total proctocolectomy with IPAA at the patient's request, even though the polyposis phenotype was mild. Proctectomy was carried out as part of the first surgery in 12 of 21 cases with mutations at codons 1309 and 1328. These results are summarized in Table 2. Patients with a severe phenotype tended to present and have surgery at a young age: 50% were 16 years of age or younger at the time of the first operation, whereas only 25% of patients with a mild phenotype were 16 years of age or younger.

Fate of the Rectum

Seven of 9 patients with mutations at codons 1309 and 1328 who had their rectum preserved at the first surgery subsequently had it removed because of increasing number or size of rectal polyps (Table 3). Overall, 19 of 21 patients with mutations at codons 1309 and 1328 lost their rectum. In contrast, only 1 patient with a mutation other than at codon 1309 or 1328 required proctectomy after IRA.

A comparison of the number of rectal polyps appearing after IRA was performed by grouping mutations at codons 1309 and 1328 for comparison against all others. For mutations at codons 1309 and 1328, the mean total number of rectal polyps found per patient at follow-up was 266 (standard deviation, 333) during a mean period of 6.75 years (standard deviation, 5.1). For all other mutations, the mean total number of rectal polyps found per patient at follow-up was 33 during a mean follow-up of 8.5 years. The number of follow-up endoscopic examinations ranged from 1 to 26.

Table 4 shows a comparison of surgery type with polyp severity. The most striking finding is the poor prognosis for retaining the rectum in patients with a severe polyposis phenotype: only 2 patients with 15G mutations have retained their rectum, and at last follow-up both had multiple rectal polyps.

Table 2. CORRELATION BETWEEN MUTATION SITE AND FIRST SURGERY PERFORMED

Mutation (codon)	Ileorectal Anastomosis	Partial Colectomy	Ileopouch Anal Anastomosis	Total Proctocolectomy and End Ileostomy
157	7	0	0	0
540	2	0	0	0
1060	6	0	1	1
1068	16	0	0	0
1309	7	1	5	5
1328	1	0	1	1
1464	0	0	1	0
1528	3	0	0	0
Total	42	1	8	7

Table 3. CORRELATION BETWEEN MUTATION AND FATE OF THE RECTUM

Mutation	Rectum Preserved	Rectum Removed
157	7	0
540	2	0
1060	6	2
1068	15	1
1309	2	16
1328	0	3
1464	0	1*
1528	3	0
Total	35	23

* Patient choice, mild phenotype.

Statistical Analysis

A calculated chi square showed a significant association between mutation-codon and polyp severity ($p < 0.001$). The predictive value of mutation-codon on polyp severity is expressed in terms of the estimated probability that subjects with mutations at codons 1309 and 1328 will develop severe polyps. In this case, the estimated probability was computed at 100%. We concluded that mutations at codons 1309 and 1328 are associated with severe disease.

When the same statistical procedure was performed to compare mutation-codon and the fate of the rectum, the calculated chi square was also significant ($p < 0.001$). We concluded that mutation-codon and the fate of the rectum were associated. The estimated probability that subjects with mutations at codons 1309 and 1328 would have their rectum removed is 90.5%.

DISCUSSION

When a patient is diagnosed with FAP, one of the most important decisions to be made is the choice of prophylactic surgery. Of the three options (colectomy with IRA, proctocolectomy with IPAA, and proctocolectomy with end ileostomy), IRA is the simplest and least complicated, with least disturbance of bowel function. IPAA, which is more complex and more likely to affect the patient's lifestyle, is generally preferred for patients at high risk for rectal cancer; some authorities recommend it for all FAP patients. Factors that help define rectal cancer risk after IRA include colon cancer at diagnosis and >1000 polyps in the colon at the time of colectomy.[2,15,16] Patients with these factors should have an IPAA as their initial procedure. We have shown in this study that the most frequent *APC* mutation is consistently associated with severe polyposis (defined as >1000 colon polyps) and a high risk for rectal cancer, as reflected by proctectomy rate.

Several studies have attempted to correlate genotype with phenotype in patients with FAP. Mutations have been reported throughout the 15 exons of the APC gene, with the exception of exons 1 and 2. Mutations occurring in the 5′ end of the gene (particularly exons 3 and 4) are associated with an attenuated form of FAP, with fewer adenomatous polyps and delayed cancer onset.[17] One family reported here was found to have a mutation at codon 157 (exon 4) and a uniformly mild phenotype. Several members of this pedigree were not diagnosed until their mid-40s, and no colon cancers have occurred, suggesting a reduced risk for progression compared with typical FAP.

Five papers have reported an association between a severe polyposis phenotype and germline mutations occurring in a specific region of exon 15. In 1992, Nagase et al.[5] reported 5 FAP patients with profuse polyps (>5000) who had germline mutations between codons 1250 and 1464. Patients with mutations before and after this region had a milder phenotype. In 1993, Paul et al.[6] described 5 unrelated FAP patients, each with a deletion mutation at codon 1309 and >1000 polyps at colectomy. Gayther et al.[7] reported that a severe polyposis phenotype was associated with regionally clustered *APC* mutations at codons 1309, 1323, 1368, and 1464. Giardiello et al.[18] showed both inter- and intrafamilial variations of polyp density in patients with mutations at codon 1309. Al-

Table 4. COMPARISON OF SURGERY TYPE WITH POLYP SEVERITY

	Initial Operation		Final Status	
	Ileorectal Anastomosis/ Partial Colectomy	Total Proctocolectomy/ Ileopouch Anal Anastomosis	Ileorectal Anastomosis/ Partial Colectomy	Total Proctocolectomy/ Ileopouch Anal Anastomosis
Severe	16	12	9	19
Mild	25	1	25	1
Unknown	2	2	1	3

though variation in polyp size and density was significant, even patients with the lowest polyp density would likely have met the criteria for severe disease used in other studies. Nugent and Phillips[16] reported severe polyposis associated with mutations at codons 1309, 1323, and 1407 in exon 15, but also with a mutation at codon 233 in exon 6. In the families reported here, mutations at codons 1309 and 1328 were consistently associated with severe polyposis, but there were also persons with mutations outside this region who had >1000 polyps. Furthermore, the patients with distal mutations at codons 1464 and 1528 had mild disease, consistent with the findings reported by Nagase et al.[5] but in contrast to those reported by Gayther et al.,[7] indicating a variable phenotype with mutations distal as well as proximal to the severe polyposis region.

The large exon 15 of *APC* contains 75% of the coding region of the *APC* gene, including three β-catenin binding sites and seven 20-amino-acid repeat domains. The latter domain has been shown by Munemitsu et al.[19] to downregulate β-catenin, a possible functional role for *APC*. All the mutations in the "severe" region of exon 15G would result in truncations of the *APC* protein at, or within a few residues of, the mutated codon and would truncate *APC* between the first and second of the seven 20 repeat elements. Munemitsu et al.[19] have shown that transfection of other portions of *APC* protein, including three separate β-catenin binding sites 5′ of the repeats, can bind β-catenin but not regulate it. Mutations 3′ of 1309 to 1328 would truncate *APC* protein between the second and third repeat (1464) or the third and fourth repeat (1528). The retention of two or three of these repeats may be sufficient for a threshold level of activity that results in reduced disease severity.

The absolute number of polyps developing in FAP is apparently under the control of multiple determinants, including *APC* mutation, other genetic modifying loci, and environmental factors. We have shown that a region of exon 15 is invariably associated with severe polyposis and includes the most frequent *APC* mutation at codon 1309. The data presented here further define this region and the clinical consequences of these mutations, including a tendency for earlier surgery and a high probability that proctectomy will be needed. Patients with severe polyposis are likely to have a total proctocolectomy initially or to need a completion proctectomy after initial IRA. However, none of the patients with severe polyposis or mutations at the "severe" loci developed cancer, showing the effectiveness of clinical management in compliant patients. Because of the close association between exon 15G mutations and severe polyposis, this clinical pattern can be extrapolated to patients on the basis of genetic analysis.

Therefore, when an FAP family has a known mutation, we suggest that the implications of the site of the mutation be considered when making decisions about screening and surgery. For example, endoscopic screening usually begins at 10 to 15 years of age. In a family with a 15G mutation, where a severe phenotype seems probable, the earlier age should be chosen. Furthermore, mutational analysis may be a more accurate way of selecting patients for IRA or IPAA. We suggest that patients with mutations at these locations be strongly considered for total proctocolectomy and IPAA at the time of initial surgery. If families with these high-risk mutations include patients with an existing IRA, surveillance must be especially thorough. Finally, the association of the common codon 1309 mutation with a severe phenotype can also be used to assist in mutational analysis. In a family with consistently severe disease, the search for the *APC* mutation should begin in exon 15G.

Acknowledgments

The authors thank Ms. Lin T. Williams and Ms. Lisa Rybicki for their statistical analyses.

References

1. Bussey HJR. Familial Polyposis Coli. Baltimore: The Johns Hopkins University Press; 1975.
2. Debinski HS, Love S, Spigelman AD, Phillips RKS. Colorectal polyp counts and cancer risk in familial adenomatous polyposis. Gastroenterology 1996;110:1028–1030.
3. Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell 1991;66:589–600.
4. Kinzler K, Nilbert M, Su LK, et al. Identification of FAP locus genes from chromosome 5q21. Science 1991;253:661–665.
5. Nagase H, Miyoshi Y, Horii 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;52:4055–4057.
6. Paul P, Letteboer L, Gelbert L, et al. Identical APC exon 15 mutations result in a variable phenotype in familial adenomatous polyposis. Hum Molec Genet 1993;2:925–931.
7. Gayther SA, Wells D, SenGupta SB, et al. Regionally clustered APC mutations are associated with a severe phenotype and occur at a high frequency in new mutation cases of adenomatous polyposis coli. Hum Molec Gen 1994;3:53–56.
8. Nugent KP, Phillips RKS, Hodgson SV, et al. Phenotypic expression in familial adenomatous polyposis: partial prediction by mutation analysis. Gut 1994;35:1622–1623.
9. DeCosse JJ, Bulow, Neale K, et al., the Leeds Castle Polyposis Group. Rectal cancer risk in patients treated for familial adenomatous polyposis. Br J Surg 1992;79:1372–1375.
10. Moertel CC, Hill JR, Adson MA. Management of multiple polyposis of the large bowel. Cancer 1971;28:160–164.
11. Sarre RG, Jagelman DG, Beck GJ, et al. Colectomy with ileorectal anastomosis for familial adenomatous polyposis: the risk of rectal cancer. Surgery 1987;101:20–26.

12. Iwama T, Michima Y. Factors affecting the risk of rectal cancer following rectum-preserving surgery in patients with familial adenomatous polyposis. Dis Colon Rectum 1994;37:1024–1026.
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 1988;16:1215.
14. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Nat Acad Sci USA 1989;86:2766–2770.
15. Moertel CG, Hill JR, Adson MA. Surgical management of multiple polyposis. Arch Surg 1970;100:421–525.
16. Nugent KP, Phillips RKS. Rectal cancer risk in older patients with familial adenomatous polyposis and an ileorectal anastomosis: a cause for concern. Br J Surg 1992;79:1204–1206.
17. Spiro L, Olschwang S, Groden J, et al. Alleles of the APC gene: an attenuated form of familial polyposis. Cell 1993;75:951–957.
18. Giardiello FM, Krush AJ, Petersen GM, et al. Phenotypic variability of familial adenomatous polyposis in 11 unrelated families with identical APC gene mutation. Gastroenterology 1994;106:1542–1547.
19. Munemitsu S, Albert I, Souza B, Rubinfel DB, Polakis P. Regulation of intracellular β-catenin levels by the adenomatous polyposis coli (APC) tumor-suppression protein. Proc Nalt Acad Sci USA 1995;92:3046–3050.