GASTROENTEROLOGY 2001;121:1127–1135

Periampullary Adenomas and Adenocarcinomas in Familial Adenomatous Polyposis: Cumulative Risks and *APC* Gene Mutations

JAN BJÖRK,* HELENA ÅKERBRANT,* LENNART ISELIUS,† ANNIKA BERGMAN,§ YVONNE ENGWALL,§ JAN WAHLSTRÖM,§ TOMMY MARTINSSON,§ MARGARETA NORDLING,§ and ROLF HULTCRANTZ*

*Department of Gastroenterology and Hepatology, and †Department of Surgery, Karolinska Hospital, Karolinska Institute, Stockholm; and §Department of Clinical Genetics, Sahlgrenska University Hospital/Östra, Gothenburg, Sweden

See editorial on page 1246.

Background & Aims: **Patients with familial adenomatous polyposis (FAP) have a high prevalence of duodenal adenomas, and the region of the ampulla of Vater is the predilection site for duodenal adenocarcinomas. This study assessed the risk of stage IV periampullary adenomas according to the Spigelman classification and periampullary adenocarcinomas in Swedish FAP patients screened by esophagogastroduodenoscopy (EGD). The genotype of patients with stage IV periampullary adenomas and periampullary adenocarcinomas was also investigated.** ***Methods:*** **A retrospective study of 180 patients screened by EGD in 1982–1999 was undertaken. Kaplan-Meier analysis was performed to evaluate cumulative risk. Mutation analysis was carried out in patients with periampullary adenocarcinomas diagnosed outside the screening program, in addition to patients in the screening group with stage IV periampullary adenomas and adenocarcinomas.** ***Results:*** **Periampullary adenoma stage IV was diagnosed in 14 patients (7.8%), with a cumulative risk of 20% at age 60 years. Periampullary adenocarcinoma was diagnosed in 5 patients (2.8%), with a cumulative risk of 10% at age 60. Three of the adenocarcinomas occurred in patients with stage IV periampullary adenomas compared with 2 in patients with less severe periampullary adenomatosis at screening (odds ratio, 31; 95% confidence interval, 4.6–215). Fifteen (88%) of the *APC* gene mutations were detected; 12 of these were located downstream from codon 1051 in exon 15.** ***Conclusions:*** **The life time risk of severe periampullary lesions in FAP patients is high, and an association between stage IV periampullary adenomas and a malignant course of the periampullary adenomatosis is strongly suggestive. Mutations downstream from codon 1051 seem to be associated with severe periampullary lesions.**

Familial adenomatous polyposis (FAP) is an inherited autosomal dominant disease. The gene locus is on chromosome 5q21.[1–3] The disease is characterized by the formation of adenomatous polyps in the small and large intestine and is associated with a virtual 100% risk of colorectal cancer. Prophylactic colectomy and proctocolectomy have dramatically reduced the morbidity and mortality in colorectal cancer.[4,5] However, patients remain at risk of developing duodenal cancer which, together with desmoid tumours, currently is the main cause of FAP-related death in adequately treated patients.[6]

The most common endoscopic feature of the duodenum is multiple small sessile polyps in the descending part, but some patients develop larger adenomatous polyps, often in the region of the ampulla of Vater. Moreover, the adenocarcinomas frequently occur in the ampulla region.[7] Because of the risk of life-threatening duodenal cancer, regular endoscopic follow-up is recommended, although the efficacy of screening has not yet been fully elucidated.[6,8,9]

A genotype-phenotype correlation, i.e., that the specific germ-line mutation of the adenomatous polyposis coli (*APC*) gene predicts the clinical course of the disease, has been shown to exist for some features of FAP, such as the attenuated phenotype.[10] However, there has still been no satisfactory investigation of whether there is any correlation between the genotype and stage IV periampullary adenomas and periampullary adenocarcinomas.[11,12]

Abbreviations used in this paper: APC, adenomatous polyposis coli; EGD, esophagogastroduodenoscopy; FAP, familial adenomatous polyposis; HD, heteroduplex; PTT, protein truncation test; RT-PCR, reverse-transcription polymerase chain reaction; SSBP, single stranded binding protein; SSCP, single-stranded conformation polymorphism.

0016-5085/01/$35.00
doi:10.1053/gast.2001.28707

The aims of this study were to investigate the prevalence and cumulative risk of duodenal stage IV periampullary adenomas and periampullary adenocarcinomas in the Swedish FAP population screened by esophagogastroduodenoscopy (EGD) and to identify the germline *APC* gene mutations in the FAP families with a history of severe periampullary lesions.

Patients and Methods

Patients

The Swedish Polyposis Registry includes data on 467 patients with verified FAP from 154 Swedish FAP-families registered since the late 1950s.[5] One hundred ten of the patients were dead before introduction of EGD surveillance, and another 62 were not screened because at the end of the study period they were younger than 30 years old, the recommended starting age for screening in Sweden. Thus, 265 patients remained, and the registry had EGD surveillance information for 180 (68%) of these patients (88 male, 92 female) representing 79 FAP families. All patients were asymptomatic when they entered the screening program, and there was no selection of patients based on family history. The reason for EGD surveillance was the known risk of duodenal adenomas. The study period was from the introduction of screening in 1982 through the end of 1999. A total of 537 examinations were performed. The median number of examinations per patient was 2 (range, 1–24). The mean period during which EGD was performed was 72 (range, 4–210) months. Observation time was from birth through the date of last endoscopy or diagnosis of duodenal adenomas, Spigelman stage IV periampullary adenomas, or periampullary adenocarcinomas. In addition to endoscopy reports, information was obtained from medical records, including surgical and autopsy reports. The examinations were performed at several sites in Sweden, and the results of the endoscopy and histopathologic evaluation were reported to the Swedish Polyposis Registry. Standard forward-viewing endoscopes were used. Polypectomy for severe adenomatosis outside the periampullary region was performed in only 2 patients, and major surgery (pancreaticoduodenectomy or duodenctomy) was not performed in any patients except for those accounted for in Table 1.

The patients with diagnoses of stage IV periampullary adenomas and/or periampullary adenocarcinomas in the screening group were selected for mutation analysis. The germline *APC* gene mutation was able to be detected for all but 1 patient with stage IV periampullary adenomas (not found) and for all but 1 of 4 patients with periampullary adenocarcinomas (refused testing). We also included 5 patients outside the screening program who had verified periampullary adenocar-

Table 1. Details of the FAP Patients With Periampullary Adenomas Spigelman Stage IV and Periampullary Adenocarcinomas

Case/ family	Year of birth (sex)	No. of EGDs	Spigelman stage at screening (age)	Symptom	Spigelman stage at symptom	Minor surgery (age)	Major surgery (age)	Spigelman stage at surgery	Age at death (year of death)
1/18	1934 (F)	6	IV (59)						64 (1998)
2/2	1943 (F)	5	IV (51)						
3/55	1954 (F)	7	IV (37)						
4/20	1944 (F)	8	IV (50)						
5/134	1944 (M)	3	IV (45)						
6/200	1945 (M)	5	IV (49)			Local excision (53)			
7/44	1938 (M)	10	IV (55)			Local excision (49)	PD (56)	IV	
8/69	1925 (M)	4	IV (64)	Cholestasis			PD (64)	IV	74 (1999)
9/66	1960 (F)	13	III (32)				PD (32)	IV	
10/300	1950 (M)	?	III (44)				PD (44)	IV	
11/45	1937 (F)	1	IV (57)				PD (57)	Cancer	
12/207	1941 (F)	8	IV (52)	Pancreatitis	IV	Local excision (53)	PD (57)	Cancer diss dis	57 (1998)
13/221	1950 (F)	24	IV (39)	Cholestasis	IV	Papillotomy (47)	PD (48)	Cancer	
14/19	1951 (M)	4	II (44)	Cholestasis	IV		PD (44)	Cancer + ln	45 (1996)
15/149	1939 (M)	5	Normal (50)	Cholestasis	Cancer	Local excision (47)	PD (50)	Cancer	
16/221	1943 (M)			Cholestasis	III		PD (55)	Cancer + ln	55 (1998)
17/94	1958 (M)			Cholestasis	Normal		PD (39)	Cancer	
18/67	1925 (M)			Cholestasis			PD (51)	Cancer + ln	52 (1977)
19/17	1939 (M)			Duodenal obstruction	Cancer			Cancer diss dis	57 (1996)
20/5	1921 (F)			Cholestasis				Cancer diss dis	58 (1979)

PD, pancreaticoduodenectomy; diss dis, disseminated disease; ln, lymph node metastasis.

cinomas and had been reported to the registry since the first case was diagnosed in 1976. The germline *APC* gene mutation was identified in 3 of these patients (one could not be analyzed because there was nobody alive with FAP in the family, and one mutation was not found). All patients, except 2 siblings who developed periampullary carcinomas, belonged to different families. Mutation analysis data were also available for 25 Swedish FAP families without evidence of stage IV periampullary adenomas or a history of duodenal cancer.

Histopathology

Biopsy specimens were taken from polyps, including those in the region of ampulla of Vater when it appeared abnormal. Histopathologic diagnosis was also obtained from surgical specimens taken from patients undergoing surgery. The Spigelman classification was used to classify the periampullary adenomas (Table 2).[9] Only adenomas fulfilling the criteria of Spigelman stage IV were included: number, 1 (score 1); size, >10 mm (score 3); histology, tubulovillous (score 2) or villous (score 3); dysplasia, high grade (score 3).

Molecular Genetic Analyses

Genomic DNA was isolated from EDTA-treated blood with the Purgene DNA isolation kit (Gentra Systems, Minneapolis, MN) using standard methods. RNA was isolated from peripheral blood lymphocytes. Histopaque (Sigma, St. Louis, MO) was used for purification of lymphocytes, and total RNA was extracted using RNAstat 60 (Tel-Test, Friendswood, TX).

Polymerase chain reaction and reverse-transcription polymerase chain reaction. Complementary DNA (cDNA) was synthesized with 2–3 μg total RNA using random primers and 200 U Superscript II reverse transcriptase according to the manufacturer's protocol (Gibco/BRL, Gaithersburg, MD). The *APC* gene was amplified in a total of 6 fragments. Exons 1–14 were amplified using a nested reverse-transcription polymerase chain reaction (RT-PCR) approach in 2 amplicons. Primers used were described by Prosser et al.[13] Exon 15 was amplified in 4 fragments using genomic DNA as the template. Two primer pairs covering codons 1038–1700 were described by Van der Luijt et al.,[14] and two new primer pairs were designed covering the 3′ part of the *APC* gene:

APC-5* fp 5′-CAG GAA AAT GAC AAT GGG AAT GA-3′

APC-5* rp 5′-TAG GGC TTT TGG AGG CTG GAG TCT-3′

APC-6* fp 5′-TCA CAG GGA GAA CCA AGT AAA CC-3′

APC-6* rp 5′-GTG CCT CCC AAA ATA AGA CCA GTG -3′

Primer pair *APC*-5* included codons 1528–2261, and primer pair *APC*-6* included codons 1994–2907. All forward primers used in these reactions were flanked by a 5′ sequence containing the T7-promotor and a eukaryotic translation initiation sequence (5′-GGATCCTAATACGACTCACTATAG-GAACAGACCACCA-TG-3′). Protein truncation test (PTT) analyses were carried out by addition of 200–400 ng of the PCR product to the TNT Quick Coupled Transcription/Translation System (Promega, Madison, WI), 10 μCi ^{35}S-methionine was incorporated, and the reactions were carried out according to protocol. The synthesized protein products were analyzed using the Protean II electrophoretic separation cell (BioRad Laboratories, Hercules, CA). Homogenous 12.5% Ready-Gels (BioRad) were used as gel matrix, and the separation was performed according to the manufacturer's recommendation. Gels were fixated, dried, and exposed to radiographic film.

To conclude the locations of the mutations detected by PTT, a combined approach of single-stranded conformation polymorphism (SSCP) and heteroduplex (HD) analyses was used. For PCR, genomic DNA was amplified using primers flanking exons 1–14 and the 5′ half of exon 15 as described by Miyoshi et al.[15] except for nt 4141 to nt 4639 and nt 2580 to nt 3046, where 2 new primer pairs were designed:

15:3*fp 5′-AGG CAA CTA CCA TCC AGC AAC AG-3′
15:3*rp 5′-C ATC CAT ATG ATT TGC ACT ATG TA -3′
15:7**fp 5′-CCA CTC ATG TTT AGC AGA TGT A -3′
15:7 rp 5′-CAT TTG ATT CTT TAG GCT GCT CT -3′

Reactions were carried out in a volume of 25 μL, containing 50–200 ng DNA, 200 μmol/L deoxynucleotide triphosphates, 20–30 pmol of each primer, 1 U Expand High Fidelity PCR System enzyme mix, and 2.5 μL of 10× PCR buffer (Roche Diagnostics, Mannheim, Germany).

Electrophoretic separation. For denaturation, 2 μL of the PCR reaction mixture was mixed with 6 μL of formamide–dye solution (98% formamide, 20 mmol/L EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanole). The samples were heated at 98°C for 5 minutes and then immediately put on ice. HD/SSCP analyses were performed using the Phast system (Amersham Pharmacia Biotech, Uppsala, Sweden) with a subsequent automated silver staining step for visualization of DNA fragments. Native buffer strips equilibrated (overnight) in a 0.2 mol/L Tris-Tricine buffer, pH 8.3, and homogenous 20% polyacrylamide Phast gels were used to perform the electrophoretic separation.

All mutations were verified by DNA sequence determination from both the 5′ and 3′ directions. Cycle sequencing was performed using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit on the ABI 310 automated sequencer (PE Biosystems, Foster City, CA). PCR was carried

Table 2. The Spigelman Classification of Duodenal Adenomas in FAP

Score[a]	1	2	3
n	1–4	5–20	>20
Size (*mm*)	1–4	5–10	>10
Histologic type	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

[a]Spigelman stage I, score 1–4; stage II, score 5–6; stage III, score 7–8; stage IV, score 9–12.

out in the same way as for the SSCP/HD analyses using the same PCR primers. These fragments were used as template in the sequencing reactions. Forward and reverse primers were used as sequencing primers.

Statistical Analyses

Survival analysis was performed using the Kaplan-Meier method. The Fisher exact test was used for categorical data. Results are presented as odds ratios, and the variability of the data is presented as 95% confidence intervals (95% CIs).

Results

Duodenal Adenomas

Duodenal adenomas were found in 134 (74%) of the FAP patients. As shown in Figure 1, the cumulative age-dependent risks of duodenal adenomatosis were 80% (95% CI, 72.9–87.1) and 98% (95% CI, 95–100) at ages 60 and 75 years, respectively.

Stage IV Periampullary Adenomas

As shown in Table 1, stage IV periampullary adenomas were found in 14 patients (7.8%): 11 at screening gastroduodenoscopy (cases 1–8 and 11–13), 1 at gastroduodenoscopy performed because of symptoms (case 14), and 2 at surgery (cases 9 and 10). The reasons for surgery in these 2 patients were histopathology (case 9) and histopathology combined with alkaline phosphatase elevation (case 10). The time course for the development of stage IV periampullary adenomas stage IV from normal duodenum could be evaluated for 7 patients and was 7.1 (range, 5.3–9.8) years. The median age at detection was 50 (range, 32–64) years, and the cumulative age-dependent risk was 20% (95% CI, 9.7–30.3) at age 60 years (Figure 1). The 5 patients who did not undergo surgery had a median follow-up time of 7 (range, 4.5–10.5) years. One patient developed symptoms. Local resection of the ampulla region was performed in 4 patients (cases 6, 7, 12, and 15), and neoplastic recurrence, leading to major surgery, occurred in 2 of the patients (cases 12 and 15; Table 1). One patient died of disseminated rectal cancer (case 1), and 1 died of an infection (case 8).

Periampullary Adenocarcinomas

Periampullary adenocarcinomas were diagnosed in 5 patients in the screening program (2.8%), of whom 3 had a previous diagnosis of stage IV periampullary adenoma at EGD screening (cases 11–13) and 2 had less severe periampullary adenomatosis (cases 14 and 15) at screening. Thus, 3 of 11 patients in the subgroup who already had stage IV periampullary adenomas at screening endoscopy later had adenocarcinomas diagnosed, compared with 2 of 169 patients without stage IV periampullary adenomas (odds ratio, 31; 95% CI, 4.6–215; $P \leq 0.003$). None of the adenocarcinomas were diagnosed during EGD screening. One was diagnosed at EGD performed because of symptoms (case 15), and 4, 3 of which had symptoms, were diagnosed at surgery (cases

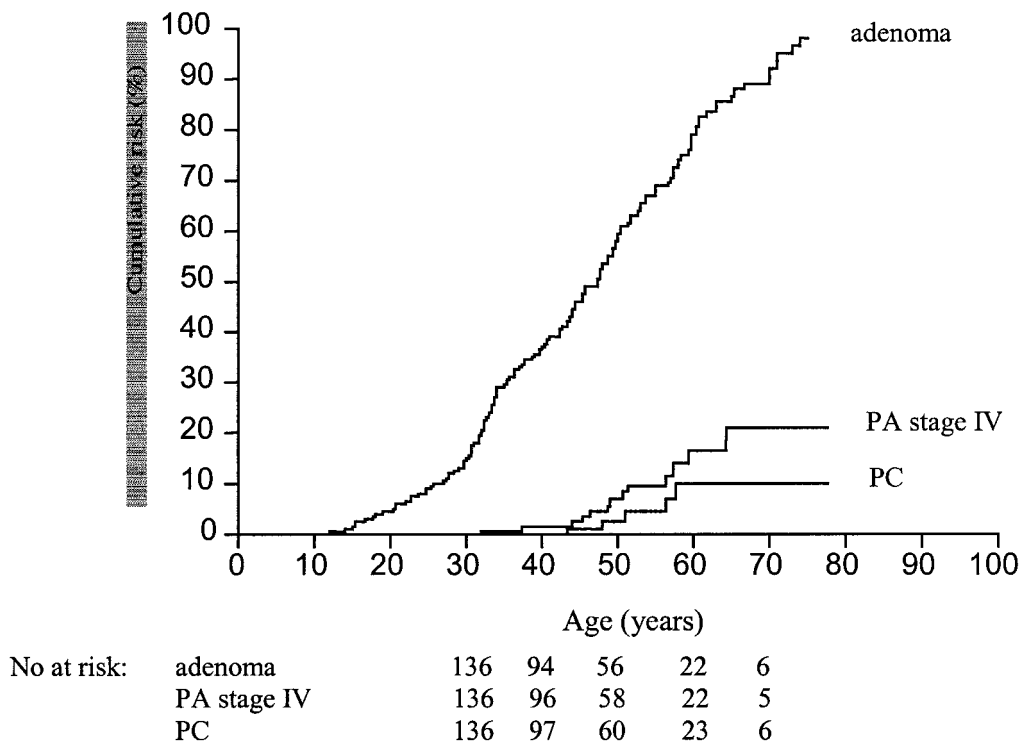

Figure 1. The cumulative risk of developing duodenal adenomas, Spigelman stage IV periampullary adenomas (PA stage IV), and periampullary adenocarcinomas (PC) in 180 Swedish FAP patients screened by EGD from 1982 through the end of 1999.

11–14). Last screening gastroduodenoscopy was performed a median of 16 (range, 4–31) months before detection of cancer, and EGD or duodenoscopy because of symptoms (n = 4) was performed at a median of 2.5 (range, 1–10) months before surgery. The time course for the development of periampullary adenocarcinoma from stage IV periampullary adenomas (n = 4) was 3.6 (range, 0.5–9) years. The median age at diagnosis of cancer was 50 (range, 44–57) years, and the cumulative age-dependent risk of developing cancer was 10% (95% CI, 1.0–19.0) at age 60 years (Figure 1). Two patients died of disseminated periampullary adenocarcinoma (cases 12 and 14), and the median postoperative follow-up time of the 3 patients who were still alive at the end of the study period was 5.1 (range, 1.5–11.1) years. Two patients developed periampullary cancer 3 and 4 years after local resection of the periampullary lesion (cases 12 and 15, respectively).

The 5 patients outside the screening group with periampullary adenocarcinomas (cases 16–20) had a median age at diagnosis of cancer of 55 (range, 39–58) years. All periampullary adenocarcinomas were diagnosed because of symptoms. Two of the cases were diagnosed in the 1970s (cases 18 and 20), i.e., before the introduction of endoscopic screening, but it is more important that the remaining 3 patients, although symptoms led to diagnosis in the 1990s, had not been screened. Four patients died within 1 year after diagnosis, and the postoperative follow-up time of the only patient alive at the end of the study (case 17) was 2 years.

In summary, 9 of the 10 cases of periampullary adenocarcinomas were diagnosed because of symptoms, and 6 of these patients died during follow-up. Adenocarcinomas were not found at other sites of the duodenum, either in the screening group or in other FAP patients reported to the registry.

APC Gene Mutations

Fifteen (88.2%) of 17 mutations were identified in the families accessible for analysis. As shown in Table 3 the germline mutation was found in 9 of the 10 families with stage IV periampullary adenomas that were available for analysis, and all had different mutations. The mutations were located in 3 different regions of the *APC* gene: near the 5′ end at codon 262 and 283 in exons 7 and 8, respectively, in the region from codon 1061 to codon 1170 in exon 15, and at codon 1533. Six of the mutations were frameshift mutations with 1–5 base pair deletions or insertions, and 3 were base substitutions, both causing premature stop codons. Five of these germline mutations have not been described elsewhere (codons 262, 1083, 1120, 1170, and 1533).

The germline *APC* mutation was identified in 6 of 7 families accessible for analysis with a history of periampullary carcinomas. The mutations were located from codon 728 to codon 1556 in exon 15, and 5 of these were located downstream from codon 1051. All of the identified mutations were frameshift mutations with 1–86 base pair deletions or insertions, causing premature stop

Table 3. Germline APC Mutations in Swedish FAP Families With Periampullary Adenomas Spigelman Stage IV and Periampullary Adenocarcinomas

Case/family	Exon	Codon	Mutation	Nucleotide change	Effect	Phenotype
1/18	7	262	783-784 ins A[a]	Ins A	Frameshift	PA
2/2	8	283	847 C > T	CGA > TGA	Nonsense	PA
12/207	15	728	2183-2184 ins A[a]	Ins A	Frameshift	PC
20/5	15	1051	3151 del A[a]	Del A	Frameshift	PC
6/200	15	1059	3175 G > T	GGA > TGA	Nonsense	PA
3/55	15	1061	3183-3187 del 5	Del ACAAA	Frameshift	PA
17/94	15	1061	3183-3187 del 5	Del ACAAA	Frameshift	PC
4/20	15	1067	3202-3205 del 4	Del TCAA	Frameshift	PC
9/66	15	1083	3249 del T[a]	Del T	Frameshift	PA
7/44	15	1120	3358 G > T[a]	GGA > TGA	Nonsense	PA
8/69	15	1170	3508-3509 ins G[a]	Ins G	Frameshift	PA
15/149	15	1246	3736-3821 del 86[a]	Del 86 bp	Frameshift	PC
11/45	15	1309	3927-3931 del 5	Del AAAGA	Frameshift	PC
10/300	15	1533	4599 del T[a]	Del T	Frameshift	PA
13,16/221	15	1556	4666-4667 ins A[a]	Ins A	Frameshift	PC
5/134	NF					PA
18/67	Obsolete					PC
19/17	NF					PC
14/19	NT					PC

NF, not found; NT, not tested; PA, periampullary adenoma; PC, periampullary adenocarcinoma.
[a]Germline APC mutations not previously reported.

codons. Four not previously reported mutations were identified (codons 728, 1051, 1246, and 1556).

Figure 2*A* shows the distribution of the germline *APC* mutations that we found in the families with stage IV periampullary adenomas and periampullary adenocarcinomas in relation to reported germline *APC* gene mutations in FAP families worldwide.

The distribution of the Swedish germline *APC* mutations identified so far are illustrated in Figure 2*B*. Eighty percent (12 of 15) of the mutations in the families with a history of stage IV periampullary adenomas or periampullary adenocarcinomas were located downstream from codon 1051 in exon 15, compared with 40% (10 of 25) of the mutations in families without evidence of severe periampullary lesions (odds ratio, 6.0; 95% CI, 1.34–26.8; $P \leq 0.03$). In a comparison of Figure 2*A* and *B,* no obvious difference in the distribution of mutations along the APC gene is observed between Swedish and non-Swedish FAP families. However, 38% of the mutations plotted in Figure 2*B* have been found in families selected for analysis because of severe periampullary lesions.

Discussion

We found that 74% of the FAP patients screened by upper endoscopy had duodenal adenomas, compared with the 33%–92% reported in previous studies of 100 or more patients.[8,16–19] However, the cumulative risk in

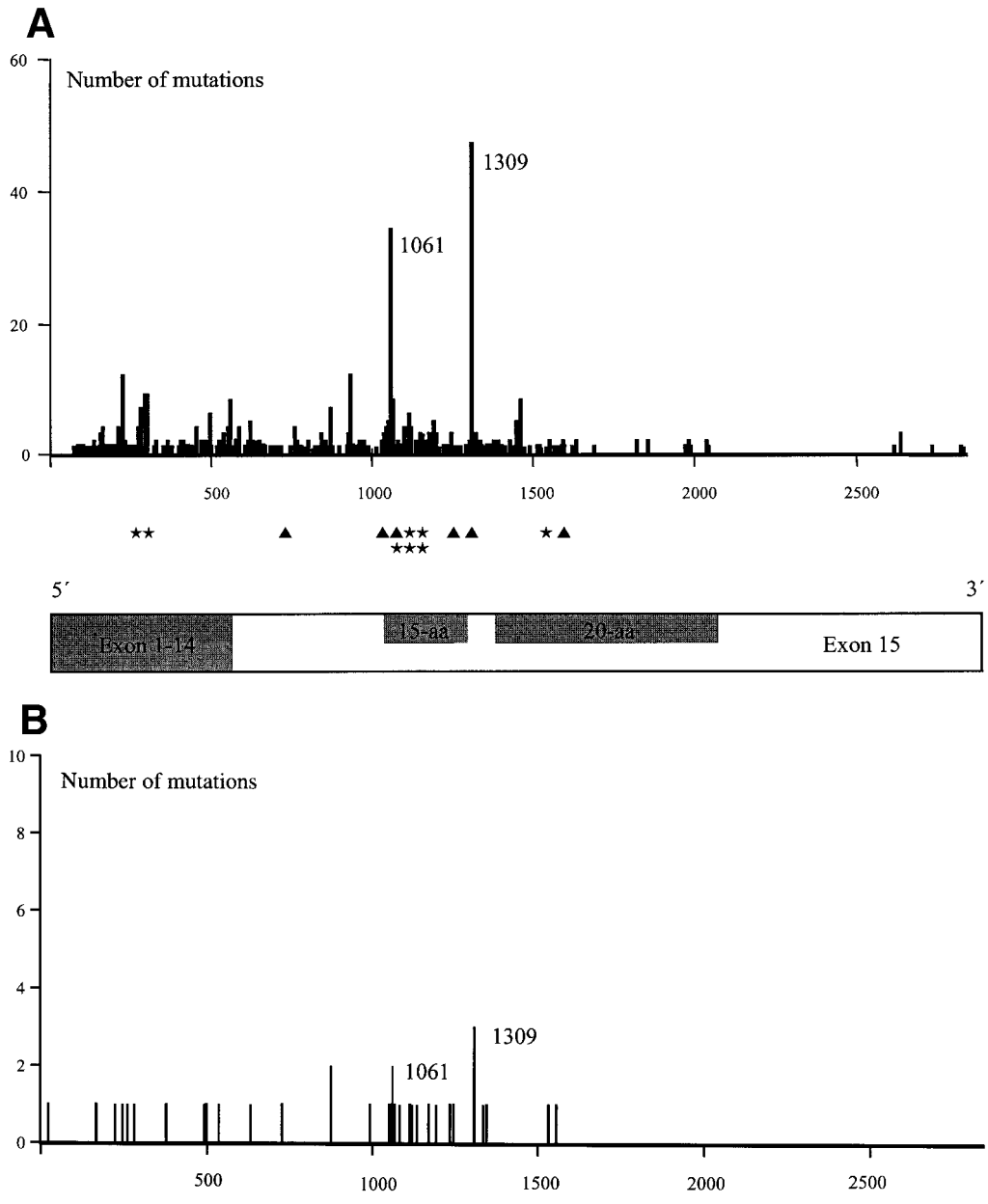

Figure 2. (*A*) The germline *APC* gene mutation spectrum of FAP families derived from the *APC* mutation database (see below) and locations of germline mutations associated with Spigelman stage IV periampullary adenomas (★) and periampullary adenocarcinomas (▲) reported in this study. The regions corresponding to the 15– and 20–amino acid repeats associated with β-catenin interaction are indicated. This image was created in part with the help of data from the APC database (http://perso.curie.fr/Thierry.Soussi/APC.html). (*B*) The germline *APC* gene mutation spectrum of 37 Swedish FAP families.

our material was almost 100% at the age of 75 years, indicating that all FAP patients eventually develop duodenal adenomas. The malignant potential of the most common lesions of the duodenum, the sessile adenomatous polyps predominantly located at the mucosal folds, seems to be low. This is further supported by the absence of duodenal adenocarcinomas outside the periampullary region in our material. The occurrence of dysplasia in the periampullary region has been reported by St. Mark's Hospital and the Cleveland Clinic Foundation to be as high as 74% and 66%, respectively.[8,19] In this study we have focused on advanced periampullary adenomas classified as Spigelman stage IV because there are observations indicating that the adenomas in the periampullary region are more likely to become malignant.[7,20] Stage IV periampullary adenomas were found in 7.8% of the endoscopically screened Swedish FAP patients, and the cumulative risk was as high as 20% at age 60 years. We also found that the cumulative risk of periampullary adenocarcinoma was 10% at age 60 years. This is higher than that of a previous northern European study in which the cumulative risk was estimated to be 3%–4% at age 70 years.[21] However, the absence of age distribution data and data on prophylactic surgery makes it difficult to compare different studies. There were no reports of periampullary adenocarcinoma in the Swedish registry before 1976, and 7 of 9 cases occurred within the last 10-year period of the study, indicating that periampullary adenocarcinoma might become an increasing clinical problem. One possible explanation for the low incidence of periampullary carcinomas before the 1990s is that previous FAP generations died with colorectal cancer at a younger age in which duodenal cancer was unlikely to occur.

Our results also indicate that despite endoscopic follow-up, there is a risk of death caused by periampullary adenocarcinoma. A more detailed analysis also shows that upper endoscopy, including biopsies of the lesion, is of poor diagnostic value in the detection of periampullary adenocarcinoma because only 2 (29%) of 7 patients in the symptomatic phase were diagnosed with cancer even though they were all endoscopically examined close to surgery. In a recent study, the corresponding figure was 32%, supporting the finding that random biopsies from the surface of the lesion are not sufficient for cancer detection.[22] Despite this conclusion, our data indicate that patients with stage IV periampullary adenomas diagnosed by upper endoscopy run a much higher risk of developing periampullary adenocarcinomas than those without evidence of stage IV periampullary adenomas, identification of these lesions seems useful. Therefore, FAP patients should undergo endoscopic surveys at frequent intervals[8] because endoscopy is currently the only screening technique available. Forward-viewing instruments do not always permit optimal visualization of the periampullary region, and side-viewing instruments allow better biopsy precision when the lesion has been recognized. Therefore, the use of side-viewing instruments may improve yields. The benefit of prophylactic surgery is still not clear, but surgical treatment must be considered in severe cases of duodenal adenomatosis.[8,9,21,23,24] In our material, the recurrence rate after local excision of stage IV periampullary adenomas was high, and more extensive surgery was required. When cholestasis or other local symptoms occurred, all but 1 already had cancer and, despite immediate extensive surgery, the prognosis was poor.

We found that most germline *APC* mutations in this selected material of FAP families with severe periampullary lesions were located within relatively restricted regions of the *APC* gene. All but 2 mutations were identified and indicate that chain-terminating mutations might be a prerequisite for severe periampullary lesions. The high sensitivity compared with most other studies is probably an effect of the phenotype selected for this study. Studies on genotype–phenotype correlation have often shown that there is not only a wide genetic heterogeneity but also phenotypic heterogeneity both within a family and between families with mutations in a similar region of the *APC* gene.[25–27] However, the *APC* gene mutations in families with a history of sparse polyposis and late-onset colorectal cancer (attenuated FAP) are located at the proximal 5′ end or in the 3′ end of the *APC* gene.[10,28] It has still not been fully clarified whether the genotype can predict the course of the periampullary adenomatosis. A previous study found no correlation between detectable germline *APC* mutations and the frequency and severity of the periampullary adenomatosis.[11] Another study on genotype–phenotype correlation including 3 cases of periampullary adenocarcinomas showed no correlation, although all periampullary adenocarcinomas were located downstream from exon 10 (codon 457).[12] The clustering of *APC* mutations downstream from codon 1051 in our families with a history of severe periampullary lesions indicates that mutations in this region of exon 15, containing the 2 most common germline deletion hot spots of the *APC* (codon 1061 and 1309), might be associated with an increased risk of stage IV periampullary adenomas and periampullary adenocarcinomas. Further supporting this observation is the more disseminated distribution along the *APC* gene of the mutations observed in Swedish FAP families with-

out evidence of severe periampullary lesions. However, because a minority of Swedish FAP families have been tested, the results so far cannot rule out the possibility that the clustering of the mutations in families with severe duodenal lesions is attributed to a relative homogeneity of the Swedish population.

The region of the APC protein corresponding to the region where most mutations are clustered contains 3 15–amino acid repeats that associate with the multifunctional protein known as β-catenin. Impaired binding causes accumulation of β-catenin in the cytoplasm that could cause dysregulation of several vital cell functions such as transcription, cell cycle regulation, migration, differentiation, and apoptosis.[26] A previous study described a family with a mutation at codon 1520 and severe duodenal adenomatosis,[27] and our only family with 2 members with periampullary carcinomas had the APC mutation at codon 1556, corresponding to a region of the APC protein with 20–amino acid repeats that interact with β-catenin and mediate its down-regulation,[28] providing further evidence of a possible correlation between a severe course of the periampullary adenomatosis and mutations in the region of the *APC* gene encoding for β-catenin–regulating properties.

The detection rate of mutation analyses, covering the entire coding region, in identification of the mutations in different FAP populations is not entirely satisfactory, varying between 44% and 72% in recent studies.[32–35] In this study, we have used both DNA- and mRNA-based analyses, a method that has been shown to be a prerequisite for high-quality mutation analyses of the *APC* gene.[13–15,36]

In conclusion, periampullary adenocarcinomas are an increasing clinical problem in the FAP population in countries where the screening and treatment of colorectal cancer is efficient. Endoscopic screening, including extensive biopsies of the periampullary region for histopathologic evaluation, is essential, and stage IV periampullary adenomas must be taken seriously because our results further support the accepted hypothesis of adenoma–carcinoma progression. The region downstream from codon 1051 in exon 15 of the *APC* gene seems to be associated with severe duodenal lesions. Thus, genetic screening might be used to predict the outcome of the duodenal adenomatosis in FAP families and to provide guidance in terms of surveillance and treatment.

References

1. Bodmer WF, Balley CJ, Bodmer J, Bussey HJR, Ellis A, Gorman P, et al. Localization of the gene for familial adenomatous polyposis on chromosome 5. Nature 1987;328:614–616.
2. Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spiro L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes J, Warrington J, McPherson J, Wasmuth J, LePaslier D, Abderrahim H, Cohen D, Leppert M, White R. Identification and characterization of the adenomatous polyposis coli gene. Cell 1987;66:589–600.
3. Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, et al. Identification of a FAP locus gene from chromosome 5q21. Science 1991;253:661–665.
4. Bülow S, Bülow C, Faurschou Nielsen T, Karlsen L, Moesgaard F. Centralized registration, prophylactic examination, and treatment results in improved prognosis in familial adenomatous polyposis. Results from the Danish polyposis register. Scand J Gastroenterol 1995;30:989–993.
5. Björk J, Åkerbrant H, Iselius L, Alm T, Hultcrantz R. Epidemiology of familial adenomatous polyposis in Sweden: changes over time and differences in phenotype between males and females. Scand J Gastroenterol 1999:34;1230–1235.
6. Griffioen G, Bus PJ, Vasen HF, Verspaget HW, Lamers CB. Extracolonic manifestations of familial adenomatous polyposis: desmoid tumours, and upper gastrointestinal adenomas and carcinomas. Scand J Gastroenterol 1998;225;85–91.
7. Jagelman DG, Decosse JJ, Bussey HJR, and The Leeds Castle polyposis group. Upper gastrointestinal cancer in familial adenomatous polyposis. Lancet 1988;1:1149–1150.
8. Spigelman AD, Williams CB, Talbot IC, Domizio P, Phillips RKS. Upper gastrointestinal cancer in patients with familial adenomatous polyposis. Lancet 1989;1:783–785.
9. Offerhaus GJA, Giardiello FM, Krush AJ, Booker SV, Tersmette AC, Kelley NC, Hamilton SR. The risk of upper gastrointestinal cancer in familial adenomatous polyposis. Gastroenterology 1992;102: 1980–1982.
10. Giardiello FM, Brensinger J, Luce M, Petersen GM, Cayouette M, Krush A, Bacon J, Booker SV, Bufill J, Hamilton SL. Phenotypic expression of disease in families that have mutations in the 5′ region of the adenomatous polyposis coli gene. Ann Intern Med 1997;126:514–519.
11. Sanabria JR, Croxford R, Berk TC, Cohen Z, Bapat BV, Gallinger S. Familial segregation in the occurrence and severity of periampullary neoplasms in familial adenomatous polyposis. Am J Surg 1996;171:136–140.
12. Wallis Y, Morton D, McKeown C, MacDonald F. Molecular analysis of the APC gene in 205 families: extended genotype-phenotype correlations in FAP and evidence for a role of APC amino acid changes in colorectal cancer predisposition. J Med Genet 1999; 36:14–20.
13. Prosser J, Condie A, Wright M, Horn JM, Fantes JA, Wyllie AH, Dunlop MG. APC mutation analysis by chemical cleavage of mismatch and a protein truncation assay in familial adenomatous polyposis. Br J Cancer 1994;70:841–846.
14. Van der Luijt R, Khan M, Vasen H, Van Leeuwen C, Tops C, Roest P, Den Dunnen J, Fodde R. Rapid detection of translation-terminating mutations at the adenomatous polyposis coli gene by direct protein truncation test. Genomics 1994;20:1–4.
15. Miyoshi Y, Ando H, Nagase H, Nishisho I, Horii A, Miki Y, Mori T, Utsunomiya, Baba S, Petersen G, Hamilton SR, Kinzler KW, Vogelstein B, Nakamura Y. Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. Proc Natl Acad Sci U S A 1992;89:4452–4456.
16. Bülow S, Alm T, Fausa O, Hultcrantz R, Jarvinen H, Vasen H. Duodenal adenomatosis in familial adenomatous polyposis. DAF Project Group. Int J Colorect Dis 1995;10:43–46.
17. Sarre RG, Frost AG, Jagelman DG, Petras RE, Sivak MV, McGannon E. Gastric and duodenal polyps in familial adenomatous polyposis: a prospective study of the nature and prevalence of upper gastrointestinal polyps. Gut 1987;28:306–314.
18. Church JM, McGannon E, Hull-Boiner S, Sivak MV, Van Stolk R, Jagelman DG, Fazio VW, Oakley JR, Lavery IC, Milsom MD. Gas-

troduodenal polyps in patients with familial adenomatous polyposis. Dis Colon Rectum 1992;35:1170–1173.
19. Burke CA, Beck GJ, Church JM, van Stolk RU. The natural history of untreated duodenal and ampullary adenomas in patients with familial adenomatous polyposis followed in an endoscopic surveillance program. Gastrointest Endosc 1999;49:358–364.
20. Nakatsubo N, Kashiwagai H, Okumura M, Kamoshida T, Takahashi A, Spigelman AD. Malignant change in a duodenal adenoma in familial adenomatous polyposis. Report of a case. Am J Gastroenterol 1998;93:1566–1568.
21. Vasen HFA, Bülow S, Myrhøj T, Mathus-Vliegen L, Griffioen G, Buskens E, Taal BG, Nagengast F, Slors JFM, de Ruier P. Decision analysis in the management of duodenal adenomatosis in familial adenomatous polyposis. Gut 1997;40:716–719.
22. Cahen DL, Fockens P, De Wit LT, Offerhaus GJA, Obertop H, Gouma DJ. Local resection or pancreaticoduodenectomy for villous adenoma of the ampulla of Vater diagnosed before operation. Br J Surg 1997;84:948–951.
23. Penna C, Bataille N, Balladur P, Tiret E, Parc R. Surgical treatment of severe duodenal polyposis in familiar adenomatous polyposis. Br J Surg 1998;85:665–668.
24. Causeret S, Francois Y, Griot JB, Flourie B, Gilly FN, Vignal J. Prophylactic pancreaticoduodenectomy for premalignant duodenal polyposis in familial adenmatous polyposis. Int J Colorectal Dis 1998:13;39–42.
25. Brensinger JD, Laken SJ, Luce MC, Powell SM, Vance GH, Ahnen DJ, Petersen GM, Hamilton SR, Giardiello FM. Variable phenotype of familial adenomatous polyposis in pedigrees with 3′ mutation in the APC gene. Gut 1998;43:548–552.
26. Paul P, Letteboer T, Gelbert L, Groden J, White R, Coppers MJ. Identical APC exon 15 mutations result in a variable phenotype in familial adenomatous polyposis. Hum Mol Genet 1993;2:925–931.
27. Giardiello FM, Krush AJ, Petersen GM, Booker SV, Kerr M, Li Tong L, Hamilton SR. Phenotypic variability of familial adenomatous polyposis in 11 unrelated families with identical APC gene mutations. Gastroenterology 1994;106:1542–1547.
28. Gardner M, Kool D, Edkins E, Walpole I, Macrae F, Nasioulas S, Scott J. The clinical correlation of a 3′ truncating mutation (codon 1982-1983) in the adenomatous polyposis coli gene. Gastroenterology 1997;113:326–332.
29. Heppner Goss KJ, Groden J. Biology of the adenomatous polyposis coli tumor suppressor. J Clin Oncol 2000;18:1967–1979.
30. Leggett BA, Young JP, Biden K, Buttenshaw RL, Knight N, Cowen AE. Severe upper gastrointestinal polyposis associated with sparse colonic polyposis in a familial adenomatous polyposis family with an APC mutation at codon 1520. Gut 1997;41:518–521.
31. Rubinfeld B, Souza B, Albert I, et al. The APC protein and E-cadherin form similar but independent complexes with alpha-catenin, beta-catenin and plakoglobin. J Biol Chem 1995;270:5549–5555.
32. Giarola M, Stagi L, Presciuttini S, Mondini P, Radice M, Sala P, Pierotti M, Bertario L, Radice P. Screening for mutations of the APC gene in 66 Italian familial adenomatous polyposis patients: evidence for phenotypic differences in cases with and without identified mutation. Hum Mutat 1999;13:116–123.
33. Armstrong J, Davies R, Guy S, Frayling I, Evans G. APC mutations in familial adenomatous polyposis families in the northwest of England. Hum Mutat 1997;10:376–380.
34. Van der Luijt R, Khan R, Vasen H, Tops C, van Leeuwen-Cornelisse I, Wijnen J, van der Klift H, Plug R, Griffioen G, Fodede R. Molecular analysis of the APC gene in 105 dutch kindreds with familial adenomatous polyposis: 67 germline mutations identified by DGGE, PTT, and southern analysis. Hum Mutat 1997;9:7–16.
35. Heinimann K, Müllhaupt B, Weber W, Attenhofer M, Scott R, Fried M, Martinoli S, Müller HJ, Dobbie Z. Phenotypic differences in familial adenomatous polyposis based on APC gene mutation status. Gut 1998;43:675–679.
36. Nordling M, Engwall Y, Wahlström J, Wiklund L, Eriksson M, Gustavsson B, Fasth S, Larsson P-A, Martinsson T. Novel mutations in the APC gene and clinical features in Swedish patients with polyposis coli. Antica Res 1997;17:4275–4280.

Received November 6, 2000. Accepted July 10, 2001.

Address requests for reprints to: Jan Björk, M.D., Ph.D., The Swedish Polyposis Registry, Department of Gastroenterology and Hepatology, Karolinska Hospital, S-171 76 Stockholm, Sweden. e-mail: jan.bjork@ks.se; fax: (46) 8-51775768.

Supported by grants from the Cancer Society in Stockholm and the Karolinska Institute and the King Gustav V Jubilee Clinic Cancer Research Foundation.