APC gene mutations and extraintestinal phenotype of familial adenomatous polyposis

F M Giardiello, G M Petersen, S Piantadosi, S B Gruber, E I Traboulsi, G J A Offerhaus, K Muro, A J Krush, S V Booker, M C Luce, S J Laken, K W Kinzler, B Vogelstein, S R Hamilton

Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, USA
F M Giardiello
K Muro
A J Krush
S V Booker

Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, USA
E I Traboulsi

Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, USA
S R Hamilton

Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, USA
F M Giardiello
G M Petersen
S Piantadosi
S B Gruber
S J Laken
K W Kinzler
B Vogelstein
S R Hamilton

Department of Epidemiology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, USA
G M Petersen

Departments of Pathology and Clinical Epidemiology, Academic Medical Centre, Amsterdam, Netherlands
G J A Offerhaus

LabCorp, Triangle Park, North Carolina, USA
M C Luce

Correspondence to:
Dr F M Giardiello, Blalock 935, Johns Hopkins Hospital, 600 N Wolfe Street, Baltimore, MD, 21287, USA.

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Abstract

***Background*—Familial adenomatous polyposis (FAP) is caused by germline mutation of the adenomatous polyposis coli (APC) gene on chromosome 5q.**

***Aims*—This study assessed genotype-phenotype correlations for extraintestinal lesions in FAP.**

***Methods*—Mutations of the APC gene were compared with the occurrence of seven extraintestinal manifestations in 475 FAP patients from 51 families. The frequency of manifestations was adjusted for different ages of patients using person years of exposure. In pedigrees without identified APC gene mutation, analysis of linkage to chromosome 5q and/or assessment of neoplasms for replication errors characteristic of mutation in mismatch repair genes were performed.**

***Results*—FAP patients from the 42 families (82%) with identified mutations of the APC gene had more frequent expression of extraintestinal manifestations than affected individuals without identified mutations (risk ratio 1·2–4·0; significant difference for cutaneous cysts). The presence of a cutaneous cyst or extraintestinal cancer significantly increased the likelihood of detection of a mutation in the APC gene (94% and 92% respectively; $p<0·05$). In patients without identified APC gene mutation, linkage to the APC gene was found in one large family (lod=5·1, theta 0·01), and replication error phenotype was absent in all 24 neoplasms from 16 members of these nine pedigrees. Expression of pigmented ocular fundus lesions was strongly associated with mutations in codons 541–1309, but no other extraintestinal manifestations were related to mutation position. Multiplicity of extraintestinal manifestations was high with mutation in codons 1465, 1546, and 2621.**

***Conclusions*—Patients with the colorectal phenotype of FAP but no extraintestinal manifestations may have non-truncating mutations of the APC gene or mutation in a gene other than APC or mismatch repair genes. The site of APC gene mutation is associated with pigmented ocular fundus lesions (codons 542–1309) and predisposition to multiplicity of extraintestinal manifestations (codons 1465, 1546, and 2621).**

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Keywords: familial adenomatous polyposis, APC gene mutation, phenotype, extraintestinal lesions, Gardner syndrome.

Familial adenomatous polyposis (FAP) is an autosomal dominant disease characterised by the development of hundreds of colorectal adenomas in young adults.[1] Germline mutations of the APC (adenomatous polyposis coli) gene located on the long arm of chromosome 5 in band q21[2–5] have been shown to result in the FAP phenotype. The APC gene has 15 exons and encodes a gene product of 2843 amino acids with a molecular weight of about 309 000 Da. Mutations of the APC gene have been documented in more than 170 patients with FAP.[2 4–16] Frameshift and point mutations are the most common types of alterations and are primarily located at the 5' half of the coding region of the APC gene. Most germline mutations in FAP patients result in stop codons and lead to truncation of the APC gene product.[17]

The phenotype of FAP is well known to be variable, especially with respect to various extraintestinal lesions.[18] In fact, Gardner syndrome, a variant of FAP with polyposis and extraintestinal manifestations, was originally described as a distinct entity.[19] Patients with FAP can develop cutaneous cysts, osteomas, pigmented ocular fundus lesions (POFLs) (also known as congenital hypertrophy of the retinal pigment epithelium or "CHRPE"),[20] occult radio-opaque jaw lesions, dental odontomas, and desmoids.[21–24] Extraintestinal cancers reported in association with FAP include tumours of the thyroid gland, adrenal gland, biliary tree, pancreas, and central nervous system.[21–23 25 26]

The correlation of extraintestinal phenotype with specific APC mutations has not been evaluated in detail. Three studies have suggested that the occurrence of POFLs is restricted to APC mutation in codons 463 to 1444.[27–29] Two investigations also reported that patients with mutations between codons 1445 and 1578 frequently developed desmoid tumours[28 29] and mutations of the APC gene 3' to codon 1403 were associated with a more variable phenotype.[30] Previous studies, however, have not accounted for differences in age of expression and cohort effects. Therefore, we evaluated seven specific extraintestinal manifestations in patients with FAP with and without identified mutation of the APC gene and determined the relation of specific extraintestinal lesions to the location of APC gene mutations with use of person year analysis. In pedigrees without identified APC gene mutation, analysis of linkage to chromosome 5q and/or assessment of neoplasms for replication errors characteristic of mutation in mismatch repair genes were done.

Methods

SUBJECTS

Data from subjects in the Johns Hopkins Polyposis Registry were used. This registry was initially gathered in 1973 from the six state area of the mid Atlantic region and now contains 369 pedigrees with FAP. Comprehensive patient medical and family information was obtained and subsequently computerised. The diagnosis of FAP in family members was verified by clinical and pathological criteria.[1]

GENOTYPE ANALYSIS

Among the 369 families with FAP in the Johns Hopkins Polyposis Registry, an affected member of 51 families was evaluated for mutation of the APC gene after informed consent was obtained. The APC gene was analysed in peripheral blood leucocytes by RNAse protection assay or by in vitro synthesised protein assay, and by cloning and sequencing the entire coding region of the APC gene, as described previously.[6 13 17] All affected members of a family were assumed to have the same mutation as the analysed affected member.

Linkage analysis was performed on one large, informative family in which no APC gene mutation had been detected by the methods described above (none of the other families was sufficiently large for linkage analysis). Linkage was based on 12 members of this 18 member three generation family. Markers which flank the APC gene (D5S112, D5S107, D5S82, D5S346, and D5S529) were utilised.

Ten colorectal cancers, 12 colorectal adenomas, a glioblastoma multiforme, and a breast carcinoma from 16 FAP patients representing all five pedigrees with no identified mutation of the APC gene (including the pedigree in which linkage analysis was performed) were evaluated for replication errors (RER, microsatellite instability), characteristic of mutations in mismatch repair genes. DNA from histopathological sections was used for analysis of simple repeated genomic sequences (microsatellites).[26] Three dinucleotide repeats on chromosome 18q (D18S55, D18S58, and D18S64) and a polyA tract in the type II transforming growth factor β receptor gene were evaluated.[31 32] The minimum criterion for an error in replication for this study was that at least one of the markers tested contained a band in the tumour PCR product that was not found in the corresponding non-neoplastic PCR product.

EXTRAINTESTINAL PHENOTYPE

The records of FAP patients in tested kindreds were evaluated for extraintestinal lesions without knowledge of APC gene mutation status. Cutaneous cysts and osteomas were identified by physical examination. Pigmented ocular fundus lesions (congenital hypertrophy of the retinal pigment epithelium) were determined by indirect ophthalmoscopic examination, and occult radio-opaque jaw lesions and odontomas by evaluation of panoramic radiographs of the maxilla and mandible, as previously reported.[20 24] The presence of desmoids and other neoplasms was substantiated by review of clinical, surgical, and pathological records. The age at examination of each subject for each extraintestinal manifestation was recorded for use in person year analysis.

STATISTICAL METHODS

The primary statistical outcome of this investigation was estimating risk of extraintestinal lesions in patients with and without APC gene mutation. Differences in the prevalence of extraintestinal manifestations between these two groups were analysed by χ^2 test. Statistical significance was considered at p values $<0{\cdot}05$. Because of differences in age and follow up or exposure time between groups, lesion frequencies were expressed as rate per person year of exposure, lambda, with 95% confidence limits for each estimated rate. For example, two lesions in patients examined at ages 50 and 25 would yield a rate of $2/(50+25)=2/75=0{\cdot}0267$ per person year exposure. Statistical significance was considered with non-overlapping 95% confidence intervals.

The influence of the APC gene mutation site on the expression of specific extraintestinal manifestations was analysed by plotting the mutated codon against prevalence of positive patients and rate of each extraintestinal manifestation expressed as events/person year, lambda. Multiple logistic regression to evaluate and adjust for potential confounding due to differing follow up and relative's distance from the proband was done.

The influence of APC gene mutation site on severity of extraintestinal phenotype was analysed by plotting the mutated codon of the APC gene against the sum of all extraintestinal manifestations expressed as all events/person year, lambda, for all seven analysed extraintestinal lesions. Exploratory multiple regression analysis was done to ascertain whether combinations of extraintestinal manifestations were associated with mutation site.

Results

Among 51 families, germline mutations of the APC gene were found in 42 families (82%) comprising 391 people with FAP. Mutations spanned codons 99 to 2644. Each specific mutation represented a single family except mutations at codon 625 (two families), codon 1061 (three families), and codon 1309 (nine families). No mutation by RNAse protection assay was detected in nine FAP families with 84 affected individuals. In vitro synthesised protein assay performed in six of these pedigrees also revealed no APC gene mutation.

There was an excess of cutaneous cysts, osteomas, and extraintestinal cancers in the group with identified APC gene mutations (p values $<0{\cdot}001$, $0{\cdot}032$, and $0{\cdot}029$, respectively). The rate of all extraintestinal lesions was higher in patients with detectable mutations (risk ratio range of 1·2–4·0, Table I). However, after

TABLE I *Comparison of rates of extraintestinal lesions among FAP patients with and without identified mutations of the APC gene*

Extraintestinal Lesion	*Group*	*No of patients*	*Events/ person year*	*Lambda (95% CI)*	*Risk ratio*
Cysts					
	APC+	132	86/3383	0·025 (0·021–0·031)	3·6*
	APC–	35	7/994	0·007 (0·003–0·014)	
Osteomas					
	APC+	114	43/3135	0·014 (0·010–0·018)	2·8
	APC–	31	4/833	0·004 (0·002–0·013)	
POFLs					
	APC+	54	40/1729	0·023 (0·017–0·031)	1·6
	APC–	8	4/280	0·014 (0·005–0·040)	
Jaw lesions					
	APC+	64	48/1829	0·026 (0·020–0·034)	1·2
	APC–	17	11/495	0·022 (0·12–0·040)	
Odontomas					
	APC+	64	11/1829	0·006 (0·003–0·011)	3·0
	APC–	17	1/495	0·002 (0·0003–0·014)	
Desmoids					
	APC+	330	49/11424	0·004 (0·003–0·006)	4·0
	APC–	81	3/2761	0·001 (0·0003–0·003)	
Extraintestinal Ca					
	APC+	326	34/10948	0·003 (0·002–0·004)	2·0
	APC–	81	4/2742	0·0015 (0·0005–0·004)	

*Non-overlapping confidence intervals.
POFLs=pigmented ocular fundus lesions; Ca=cancer.

accounting for person years of exposure, only the rate of cutaneous cysts distinguished patients with APC gene mutations when confidence limits between the two groups were considered. Adjustment for relative's distance from the proband in multiple logistic regression models found no evidence of significant confounding.

Among the 475 patients studied, the presence of an extraintestinal manifestation increased the likelihood that the person would have an identified APC gene mutation. If an FAP patient was affected by extraintestinal cancer or cutaneous cysts, the conditional probability of detection of an APC gene mutation in the family was 94% and 92%, respectively, compared with 82% of all families in the study ($p<0·05$). The presence of osteomas (91%), POFLs (91%), and desmoids (89%) also indicated a trend towards increased likelihood, but occult radio-opaque jaw lesions (81%) and odontomas (79%) did not.

Linkage analysis was peformed on one large, informative family in which no germline APC gene mutation was identified. Two point lod scores for markers on chromosome 5q21 flanking the APC gene are shown in Table II. Close linkage to this region was detected by the high lod score (5·1) for D5S346 for recombination fraction values (theta) less than 5%.

Replication errors characteristic of tumours with mutations in mismatch repair genes were absent in the DNA from neoplasms of all 16 patients representing the five pedigrees in which no germline mutation of the APC gene

TABLE II *Linkage analysis of chromosome 5q in a family without APC gene mutation*

	Lod scores recombination fraction (theta)					
Marker	*000*	*0·001*	*0·05*	*0·1*	*0·2*	*0·3*
D5S112	0·569	0·543	0·457	0·379	0·272	0·168
D5S107	–	–0·895	–1·105	0·184	0·302	0·210
D5S82	–	0·242	0·646	0·585	0·263	0·060
D5S346	5·196	5·106	4·739	4·261	3·235	2·104
D5S529	–	–0·577	0·607	0·932	0·936	0·632

mutation could be identified, including the family evaluated by linkage analysis.

An association between site of APC gene mutation and extraintestinal manifestation (evaluated by both frequency of positivity and person year analysis) was found only for POFLs (Fig 1). Eye lesions were noted with low frequency of positivity for patients with mutations that occurred 5' to codon 541. All patients with mutations in codons 542–1309 were positive for POFLs and then the frequency of eye lesions decreased 3' to codon 1309. Variance of extraintestinal manifestations within pedigrees did not correlate with the relation to the proband.

The influence of site of APC gene mutation on multiplicity of extraintestinal manifestations is shown in Figure 2. The rate of events/person year (lambda) appeared relatively consistent throughout the gene except for a high rate in patients with mutations in codons 1465, 1546, and 2621 and a dearth of multiplicity of manifestations with mutation in codon 436. Multiple regression analysis of these relations did not reveal significant associations between mutation site and particular combinations of extraintestinal manifestations.

Discussion

This study identified a low frequency of extraintestinal manifestations in patients from FAP families not found to have APC gene mutation. A potential reason for this phenomenon is that some of these individuals have APC mutations which are not detected by current methods and which, at the same time, do not predispose to extraintestinal manifestations. This includes APC mutations in splice sites or intronic regions, mutations that affect the protein product qualitatively and not quantitatively, and mutations that produce subtle modifications in the protein. Alternatively, mutation of another gene could be the

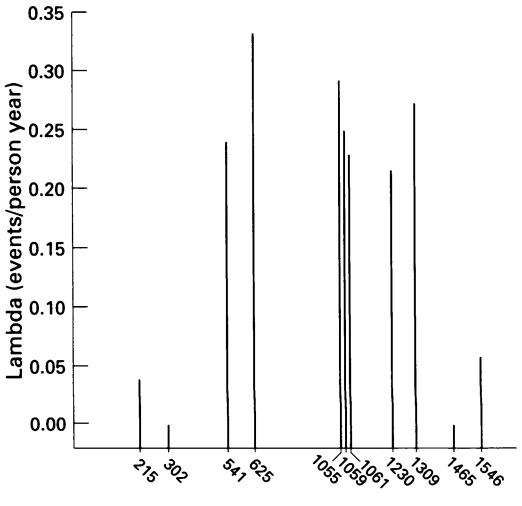

Figure 1: Association between rate (events/person year, lambda) of pigmented ocular fundus lesions (POFLs) and site of codon mutation of the APC gene. The finding of POFLs is strongly associated with mutations in codons 541 to 1309, but this extraintestinal manifestation is uncommon with mutations in more 5' and 3' sites of the gene.

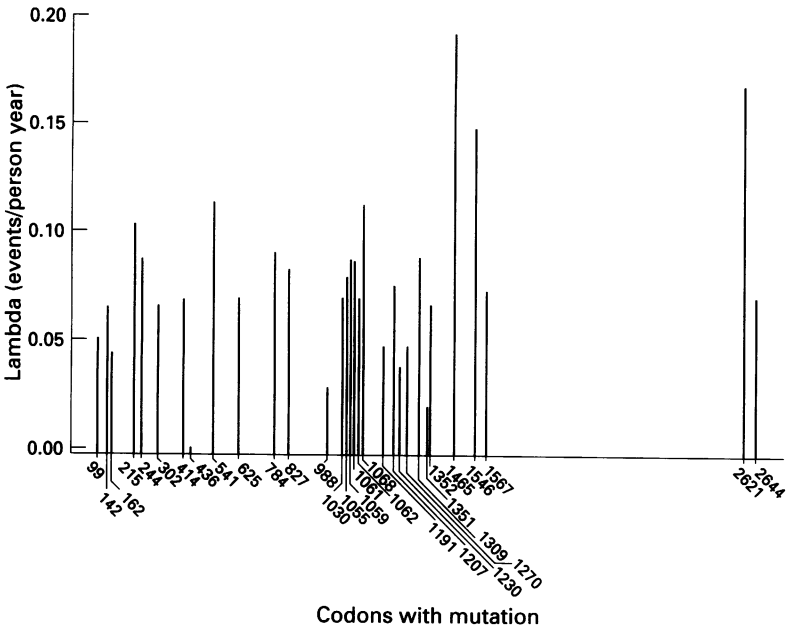

Figure 2: Association between rate (events/person year, lambda) of multiplicity of extraintestinal lesions and site of codon mutation of the APC gene. Mutation in codons 1465, 1546, and 2621 is associated with a high rate of multiplicity of extraintestinal lesions but the rate is low in patients with mutation at codon 436.

explanation in these families. Justification for this latter possibility is provided by the circumstances of Turcot syndrome: molecular investigation revealed that some patients with the colorectal phenotype of polyposis and brain tumour were explained by mutations in one of the DNA mismatch repair genes known to cause hereditary non-polyposis colorectal cancer, not by APC gene mutation.[26] In our study, replication errors were not found in the DNA of tumours from at least one member of all families without identified APC gene mutation. This finding argues against mutation in DNA mismatch repair genes as being responsible for the phenotype of these pedigrees. Additional studies of individuals who appear to have FAP but no identifiable APC gene mutations will, therefore, be of interest.

Some investigators have suggested genotype-phenotype associations with extraintestinal manifestations[27–30] while others have not.[15 16 33 34] Olschwang *et al* reported that POFLs in FAP patients did not occur with mutations in codons 136–302 but did occur with mutations in codons 463–1387.[27] Caspari *et al* and Davies *et al* extended these observations by noting an absence of POFLs in patients with mutations in codons 1445–1578.[28 29] Our evaluation, both by frequency and person year analysis, supports the findings that mutations in the 5' and 3' ends of the APC gene do not predispose to POFLs. However, this association was not absolute, since we did find patients with POFLs who had mutations at codons 215 and 1546.

Caspari *et al*[28] also described a high frequency of desmoid tumours (33/36; 92%) in individuals with mutations of codons 1445–1578. Davies *et al* made a similar observation.[29] Our study identified 12 patients with mutations in this region and five (42%) did have desmoids. In addition, desmoids/person year appeared highest in this region of the gene. However, desmoids did occur in patients with mutations throughout the gene, and 95% confidence limits for lambda overlapped with other areas. Only one of 11 patients with mutation 3' to codon 1578 had desmoid tumors.

Nugent *et al* reported that both desmoid disease and extraintesinal cancer were more common in patients with mutation at codon 1309 compared with individuals where knowledge of specific mutation was not available and to people with mutation at seven other sites.[35] In our analysis, all extraintestinal manifestations were more frequently found in patients with mutation of the APC gene compared with those without mutation identified. Also, rates of desmoids and extraintestinal cancers in patients with mutations eleswhere in the APC gene were higher than for 1309 mutation.

We found no clear association between site of APC gene mutation and extraintestinal manifestations other than POFLs. However, analysis of the multiplicity of extraintestinal lesions revealed the highest rate with mutations at codons 1465, 1546, and 2621. This is consistent with another report.[30]

The molecular basis for differences in FAP extraintestinal phenotype resulting from specific sites of APC mutation is not known. Some investigators speculate that tumour suppressor activity of the APC gene protein varies with regard to target tissue and length of transcript.[36] Alternatively, stability and biological activity of APC protein could differ with mutation site. On the other hand, although phenotypic differences seem to be associated with site of mutation, heterogeneity exists within and among families with the same APC gene mutation.[18] This heterogeneity probably occurs through environmental influences and modifying gene(s), as suggested by the MIN mouse model of FAP which has germline mutation in the mouse homologue of APC.[37–40] Understanding the function of the APC gene in various tissues may eventually explain the clinical observations.

Our findings concerning the relation of APC gene mutations and extraintestinal manifestations have implications for patient management. Gene testing is supplanting serial endoscopic procedures for identifying at risk individuals who have inherited a mutated APC gene and will develop FAP.[41] The available method detects APC gene mutation in approximately 80% of FAP pedigrees[17] (82% in this study). Our findings show that the presence of extraintestinal cancer or cutaneous cysts in an FAP pedigree significantly increases the pretest probability that an APC gene mutation will be identified in affected members. Moreover, the presence of POFLs can guide genetic analysis to reduce costs. The expense of APC gene testing, primarily because of the cost of laboratory reagents, can be a barrier to use by patient and physician. In this regard, finding POFLs in the pedigree allows direction of initial molecular analysis to specific segments of the APC gene (codons 463–1387), thereby reducing initial cost which would result if the entire gene was evaluated.

The results of genetic tests can help guide surveillance. For example, families with mutations in codons 1465–1546 deserve clinical attention directed at their proclivity for multiple extraintestinal manifestations. It is clear, however, that extraintestinal lesions can occur with a wide spectrum of APC gene mutations. Lastly, because nearly one fifth of FAP families have no mutation identified with current methods, studies directed at finding mechanisms of germline APC inactivation and at other potential genes remain important.

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