Molecular analysis of the APC gene in 205 families: extended genotype-phenotype correlations in FAP and evidence for the role of APC amino acid changes in colorectal cancer predisposition

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Abstract
***Background/aims*—The development of colorectal cancer and a variable range of extracolonic manifestations in familial adenomatous polyposis (FAP) is the result of the dominant inheritance of adenomatous polyposis coli (APC) gene mutations. In this study, direct mutation analysis of the APC gene was performed to determine genotype-phenotype correlations for nine extracolonic manifestations and to investigate the incidence of APC mutations in non-FAP colorectal cancer.**
***Methods*—The APC gene was analysed in 190 unrelated FAP and 15 non-FAP colorectal cancer patients using denaturing gradient gel electrophoresis, the protein truncation test, and direct sequencing.**
***Results*—Chain terminating signals were only identified in patients belonging to the FAP group (105 patients). Amino acid changes were identified in four patients, three of whom belonged to the non-FAP group of colorectal cancer patients. Genotype-phenotype correlations identified significant differences in the nature of certain extracolonic manifestations in FAP patients belonging to three mutation subgroups.**
***Conclusions*—Extended genotypephenotype correlations made in this study may have the potential to determine the most appropriate surveillance and prophylactic treatment regimens for those patients with mutations associated with life threatening conditions. This study also provided evidence for the pathological nature of amino acid changes in APC associated with both FAP and non-FAP colorectal cancer patients.**
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Keywords: familial adenomatous polyposis; genotype-phenotype; familial colorectal cancer

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Familial adenomatous polyposis (FAP) is characterised by the development of hundreds to thousands of colorectal adenomatous polyps and a variable range of extracolonic manifestations.[1] It is caused by the dominant inheritance of a constitutional adenomatous polyposis coli (APC) gene mutation which carries with it a virtual 100% risk of developing colorectal cancer.[2 3]

The identification and characterisation of an APC gene mutation in one affected member of an FAP family means that at risk subjects may then be tested directly for the same mutation thereby circumventing many problems associated with linkage analysis.[4] Premature death of an FAP gene carrier may subsequently be prevented by the prophylactic removal of the colorectum before the development of malignancy. The majority of FAP causing APC mutations to date result in the formation of a truncated protein product.[5 6]

The diagnosis of FAP is confirmed upon histological verification of at least 100 adenomatous polyps.[1] However, the West Midlands Polyposis Registry contains a number of less well clinically defined colorectal cancer patients, in which a familial inheritance of colorectal cancer exists in the absence of the usual pathognomonic features of FAP. In addition, the registry also contains a number of sporadic colorectal cancer patients in whom the disease is associated with FAP extracolonic manifestations, in particular congenital hypertrophy of the retinal pigment epithelium (CHRPE). For the purpose of this study, patients in these two groups have been termed non-FAP patients. To date, chain terminating APC mutations have not been reported in non-FAP colorectal cancer cases. However, the role of missense mutations remains to be elucidated.[7]

In this study the whole of the APC coding region has been analysed for constitutional mutations in 190 FAP and 15 non-FAP colorectal cancer patients. The purpose of this analysis was threefold: firstly, to facilitate accurate presymptomatic diagnosis in all FAP families, especially those not amenable to linkage analysis; secondly, to determine the incidence of constitutional APC gene mutations in patients with non-FAP colorectal cancer; and thirdly, to determine whether the heterogeneous phenotype of FAP may in fact be partly explained by the heterogeneous distribution of constitutional APC mutations in the hope that precise genotype-phenotype correlations may aid in the implementation of appropriate treatment and surveillance programmes.

Materials and methods

PATIENT GROUPS

The APC gene was analysed for constitutional mutations in 205 cases, of whom 190 were diagnosed with FAP and 15 were non-FAP patients. Histological verification of at least 100 adenomatous polyps was made for all FAP

patients. The non-FAP colorectal cancer patients did not meet the diagnostic criteria for FAP and were divided into three groups: (1) five patients belonged to families exhibiting a clustering of colorectal cancer (CRC) or polyps associated with congenital hypertrophy of the retinal pigment epithelium (CHRPE); (2) five patients belonged to families exhibiting a clustering of CRC or polyps not associated with CHRPE; (3) five remaining patients were isolated cases of CRC associated with CHRPE. The incidence of the following nine extracolonic manifestations of FAP was ascertained for all patients: desmoid tumours, osteomas, epidermoid cysts, duodenal adenomas, gastric polyps, hepatoblastoma, dental anomalies, periampullary carcinoma, and brain tumours. This information was extracted directly from medical records.

DENATURING GRADIENT GEL ELECTROPHORESIS

The PCR based technique of denaturing gradient gel electrophoresis (DGGE) was used to analyse directly 33 segments of the APC coding region covering codons 1 to 1499 and 2215 to 2844 as described in detail elsewhere.[8] The sequences of the GC rich clamp and primers used in the analysis have previously been published.[9 10]

PROTEIN TRUNCATION TEST

The assay was used for the analysis of four overlapping exon 15 segments of the APC gene. Four primer pairs amplified the overlapping segments 2, 3, 4, and 5, containing codons 658-1283, 1099-1701, 1547-2339, and 2131-2844, respectively. The nucleotide sequences for each primer pair were as follows. Segment 2: forward, 5'-GAGAACAACTGTC TACAAACT-3' and reverse, 5'-AGCTGA TGACAAAGATGATAATG-3'. Segment 3: forward, 5'-GTTTCTCCATACAGGTC ACG G-3'. Segment 4: forward, 5'-GA AAACCAAGAGAAAGAGGCAG-3' and reverse, 5'-GAGGACTTATTCCATTTCT ACC-3'. Segment 5: forward, 5'-GGTTTATCTAGACAAGCTTCG-3', and reverse, 5'-ATTTTCTTAGTTTCATTCTT CCTC-3'. Each forward primer was T7 modified by the addition of a GGATCCTAATACGACTCACTATAGGGA-GACCACCATG sequence to the 5' end containing a T7 promoter/translation initiation signal. The PTT was performed using an Amersham kit as previously described in detail.[10] Truncated protein products were confirmed by at least two separate analyses. Protein products were resolved by 15% SDS-polyacrylamide gel electrophoresis and bands visualised following autoradiography.

DNA SEQUENCING OF DGGE VARIANT PRODUCTS

Variant DGGE PCR products (either directly excised mutant homoduplex bands or mutant clones obtained using the pT7Blue® T vector and NovaBlue competent cells from Novagen) were amplified using original DGGE PCR primers. Templates were sequenced using a thermal cycle dideoxy DNA sequencing kit (New England Biolabs) using the original non-GC clamped DGGE primer. Each variant PCR product was sequenced on at least two occasions, using a freshly synthesised template each time.

DNA SEQUENCING OF APC CODONS 1680-1880

PCR primers were specifically designed to amplify a segment of the APC gene spanning codons 1680 to 1880 (forward primer sequence is 5'-AACGAGATACCATTCCTAC AGAAG-3' and reverse primer sequence is 5'-CCTGGAAAGGTCAACATCATCATC-3') as this region was not screened by DGGE or PTT for the majority of patients. The resultant PCR product was sequenced as described above.

DIRECT TESTING FOR 5 BP DELETIONS AT CODONS 1061

Newly referred patients were screened directly for the common 5 bp deletion of "ACAAA" starting at codon 1061 following a protocol which facilitated direct detection of the deletion by agarose gel electrophoresis. A pair of primers was designed (forward primer sequence is 5'-GAATGAAAGATGGGCAAG ACC-3' and reverse primer sequence is 5'-GATTCCTTGAT TGTCTTGC-3') to amplify a region spanning the 5 bp deletion. Normal fragments were 77 bp in size and deleted fragments were 72 bp. PCR products were resolved by 3% NuSieve agarose gel electrophoresis.

Results

In this study DGGE, PTT, and sequencing were used to investigate the APC gene for constitutional mutations in 190 FAP and 15 non-FAP patients. A summary of DNA sequence alterations identified in the two patient groups is given in table 1.

CHAIN TERMINATING APC MUTATIONS

Frameshift mutations

Twenty-seven different mutations caused by the deletion or insertion of a small number of nucleotides were identified in 47 unrelated FAP patients (table 2). All mutations were out of frame and therefore resulted in a downstream premature chain terminating signal. The removal of 5 bp was the most frequently found frameshift, with two common deletions of "ACAAA" from codon 1061 and "AAAGA" from codon 1309 identified in 11 and 10 families, respectively.

A specific protocol was designed to facilitate the detection of the most common codon 1061 5 bp deletion by agarose gel electrophoresis. This procedure therefore provided a quick initial screening test for newly diagnosed FAP patients as well as making predictive testing more convenient in those families already known to have the 1061 deletion. Primers were designed to amplify across the deletion so that normal subjects produced 77 bp PCR fragments and affected subjects produced a 72 bp fragment in addition to the wild type product.

A rather unusual mutational event was identified in patient 44. Sequencing analysis showed that the 2 bp deletion was a summation of two 1 bp deletions (of an "A" and a "T")

Table 1 Summary of APC sequence alterations identified in FAP and non-FAP patients

Exon	*Codon*	*Nature*	*Alteration*	*Consequence*	*Family No*
4	177	Deletion	T	Frameshift (7, TGA)	162
5	Splice site	Base substitution	g to t (−1)	Frameshift (7, TGA)	9
5	Splice site	Base substitution	g to a (−1)	Frameshift (7, TGA)	101, 178, 233
6	232	Base substitution	C to T	Arg to stop (TGA)	42, 122, 149
6	233	Base substitution	C to T	Gln to stop (TAG)	142
7	278	Base substitution	C to T	Gln to stop (TAG)	8
8	283	Base substitution	C to T	Arg to stop (TGA)	17, 38, 120, 121, 155, 156, 199, 201, 220
10	452	Insertion	TG	Frameshift (2, TAA)	60
10	456	Deletion	TTTCA	Frameshift (2, TGA)	69
10	457	Base substitution	C to A	Ser to stop (TAA)	5
11	Splice site	Base substitution	g to a (−1)		187
11	495	Insertion	A	Frameshift (41, TAA)	215
11	486	Base substitution	C to G	Tyr to stop (TAG)	21
11	500	Base substitution	C to T	Arg to stop (TGA)	33
12	527	Deletion	AGCACTTGTG	Frameshift (4, TAA)	56
12	541	Base substitution	C to T	Gln to stop (TAG)	179, 200
13	564	Base substitution	C to T	Arg to stop (TGA)	40
14	585	Deletion	C	Frameshift (5, TGA)	246
14	609	Deletion	G	Frameshift (1, TAG)	57
14	625	Base substitution	C to T	Gln to stop (TAG)	62, 231
14	640	Deletion	G	Frameshift (5, TGA)	137
14	648	Insertion	A	Frameshift (2, TGA)	131
14	Splice site	Base substitution	t to g (+2)		51
15A	657	Deletion	A	Frameshift (13, TAA)	54
15B	764	Base substitution	T to A	Leu to stop (TAA)	27
15B	770	Insertion	A	Frameshift (4, TGA)	99
15C	931	Deletion	TTCA	Frameshift (23, TAG)	63
15C	934	Deletion	TTAC	Frameshift (20, TAG)	97
15C	935	Base substitution	C to A	Tyr to stop (TAA)	22
15C	935	Base substitution	C to G	Tyr to stop (TAG)	150
15C	935	Insertion	A	Frameshift (1, TAA)	10
15D	964	Insertion	A	Frameshift (16, TGA)	163
15D	1023	Insertion	T	Frameshift (5, TAG)	37
15E	1045	Base substitution	C to T	Gln to stop (TAG)	18, 144
15E	1057	Base substitution	A to G	Aspartic acid to glycine	133
15E	1057	Deletion	A	Frameshift (4, TAA)	140
15E	1061	Deletion	ACAAA	Frameshift (2, TGA)	6, 14, 25, 29, 110, 136, 138, 146, 175, 186, 193
15E	1068	Deletion	TCAA	Frameshift (57, TAA)	16, 141
15E	1122	Deletion	AATC	Frameshift (3, TAA)	1
15F	1152	Base substitution	C to T	Gln to stop (TAG)	95
15F	1171	Base substitution	C to T	Arginine to cysteine	102
15F	1182	Deletion	A	Frameshift (83, TAG)	47
15F	1193	Deletion	CA	Frameshift (5, TAA)	164
15F	1198	Base substitution	C to G	Ser to stop (TGA)	96
15F	1198	Insertion	A	Frameshift (5, TAG)	189
15G	1309	Deletion	AAAGA	Frameshift (4, TAG)	2, 3, 32, 43, 106, 116, 135, 200, 208, 225
15H	1395	2 deletions	A and T	Frameshift (2, TGA)	44
15H	1449	Base substitution	A to T	Lys to stop (TAG)	52
15H	1450	Base substitution	C to T	Arg to stop (TGA)	24
15H	1465	Deletion	G	Frameshift (8, TAA)	147
15H	1493	Deletion	A	Frameshift (14, TGA)	92
	1822	Base substitution	A to T	Asparagine to valine	87, 89, 160
15V	2738	Base substitution	T to C	Isoleucine to threonine	1
PTT2	658-1283		Stop		15 families
PTT3	1099-1701		Stop		6 families
PTT4	1545-2338		Stop		2 families

Information concerning exon, codon, nature, nucleotide alteration, and consequence is given for each characterised mutation. Types of termination signal and position of downstream stop codon caused by frameshift mutations are given in parentheses. The number of families with truncated protein products identified within each PTT segment is shown at the bottom of the table.

from codon 1395 in exon 15. The net result of the two mutational events was the production of a "TGA" stop signal in codon 1396.

Base substitutions

Twenty-five different single base substitutions were identified in 40 unrelated FAP families (table 2). The majority of base substitutions exchanged a cytosine for a thymine, occurring on 12/25 (48%) occasions. The most common single base change was a "C" to "T" transition at codon 283, converting an arginine amino acid to a "TGA" stop codon.

In four unrelated patients the "G" base of the virtually invariant "AG" dinucleotide present at the splice acceptor site immediately preceding exon 5 was substituted either for a "T" (patient 9) or an "A" (patients 101, 178, and 233). The use of a downstream cryptic "AG" splice site at positions +15/16 in exon 5 was subsequently confirmed using a reverse transcriptase PCR approach. Aberrant splicing of exon 4 to the new site 17 bp into exon 5 was predicted to create a downstream in frame stop codon. Amplification across exons 4-6 using cDNA templates generated from two of these patients confirmed these predictions (not shown).

AMINO ACID CHANGES

Heterozygous nucleotide changes

Single-base substitutions producing heterozygous amino acid changes were identified in two FAP (1 and 102) and one non-FAP patient (133). The changes identified in patients 1 and 102 were not considered to be responsible for the FAP phenotype since the "C" to "T" transition in codon 1171 predicted to cause an

Table 2 Summary of APC mutations identified in FAP and non-FAP patients

Mutation type			*FAP*	*Non-FAP*
Base substitutions	Chain terminating mutations		35	
	Amino acid changes		3	3
	Splice defects		2	
Frameshifts	Insertions	1 bp	7	
		2 bp	1	
	Deletions	1 bp	9	
		2 bp	2	
		4 bp	5	
		5 bp	22	
		10 bp	1	
PTT truncated proteins			23	
Unknown			13	
Total			123	3

Table summarises chain terminating mutations and amino acid changes identified in FAP and non-FAP patients; 13 patients with an unknown status are yet to be sequenced.

arginine to cysteine substitution in patient 102 was subsequently identified in unaffected relatives and the "T" to "C" change in codon 2738, predicted to convert an isoleucine to a threonine residue in patient 1, was identified in addition to a previously characterised chain terminating mutation at codon 1122 (table 1).

However, in patient 133, the pathological significance of the "A" to "G" base change in codon 1057 predicted to cause an aspartic acid (negatively charged) to glycine (uncharged) change is yet to be determined. No other DNA alteration has to date been identified in the whole APC coding region. This scenario is particularly interesting since the family members of patient 133 exhibit a non-FAP phenotype in which FAP associated extracolonic manifestations are found alongside both colorectal and gastric cancer. The daughter of 133 died of hepatoblastoma aged 2 years and her father has had a long history of rectal bleeding and at least eight adenomatous sigmoid/rectal polyps resected in his sixties. In addition, her paternal father and uncle both died from gastric carcinoma. Despite this strong family history of gastrointestinal disease, the index case herself was reported both adenoma and cancer free at the age of 47 years. However, like her father, she has multiple pigmented cutaneous lesions.

Homozygous nucleotide changes

The same homozygous single base substitution was identified in one FAP (89) and two non-FAP patients (87 and 160). In all three cases, the base substitution involved a double "A" to "T" transversion at codon 1822, converting an asparagine (hydrophilic, amide side chain) to a valine (hydrophobic, aliphatic side chain) amino acid.

For patient 89, a histopathologically verified diagnosis of FAP was made upon the death of her sister from colorectal cancer aged 30 years and the FAP phenotype was shown to be associated with the manifestation of congenital hypertrophy of the retinal pigment epithelium (CHRPE). Interestingly, non-penetrance is a possibility in this family as CHRPE lesions were subsequently detected in her mother who is alive and cancer free at the age of 74 years. In addition, her daughter also has CHRPE lesions but does not have polyps aged 25 years.

Non-FAP patient 87 was an isolated case of colorectal cancer associated with CHRPE. Non-FAP patient 160 presented with an hepatic metastasis at the age of 60 and was subsequently shown to have between 30-40 adenomatous polyps in the descending and transverse colon. Rectal sparing prevented definitive diagnosis of FAP. The role of the double amino acid changes in these patients is unclear, but to date no other constitutional change has been identified in these three patients in exons 1-14 and the first 800 bp of exon 15 screened by DGGE or in segments 2, 3, 4, and 5 of exon 15 analysed by the protein truncation test.

The codon 1822 double base change was analysed in the spouses of 45 FAP patients to determine its prevalence in a normal sample population In contrast to the FAP and non-FAP clinical groups, a homozygous change was not identified in any of the normal subjects.

GENOTYPE-PHENOTYPE CORRELATIONS IN FAP

The characterisation of APC gene mutations in a large number of clinically well defined FAP patients facilitated an investigation into genotype-phenotype correlations. A previous study identified a direct correlation between the position of APC gene mutation and the incidence of CHRPE lesions.[11] In the current study, genotype-phenotype correlations were assessed for a further nine extracolonic manifestations (desmoid tumours, osteomas, epidermoid cysts, duodenal adenomas, gastric polyps, hepatoblastoma, dental anomalies, periampullary carcinoma, and brain tumours) by comparing the incidence of these ECMs between three mutation groups (termed C1, C2, and C3) defined by the previous CHRPE phenotype-genotype correlation.[11] Therefore, mutation group C1 patients had mutations between codons 177 and 452, mutation group C2 patients had mutations between codons 457 and 1309, and mutation group C3 patients had mutations between codons 1395 and 1493.

The frequency of each ECM in each mutation subgroup was expressed as the proportion of subjects with a positive expression from the total number of patients for whom clinical data were available (including affected family members), rather than looking just at the ECM expression frequency in index cases alone (table 3). This was considered the most appropriate approach for dealing with

Table 3 Summary of the incidence of extracolonic manifestations in FAP families

Extracolonic manifestation	*Group C1*		*Group C2*		*Group C3*	
	Negative	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*
Desmoids	9	1	22	3	0	11
Osteomas	7	0	16	13	0	6
Epidermoid cysts	7	3	11	11	0	7
Duodenal adenomas	7	4	23	2	0	1
Gastric adenomas	7	3	18	6	1	1
Dental anomalies	6	1	6	2	1	0
Hepatoblastoma	9	0	5	3	1	0
Periampullary cancer	7	0	10	2	0	1
Brain tumour	8	0	9	1	1	0

The table shows the incidence of each extracolonic manifestation (ECM) in three CHRPE subgroups, C1 (codons 177-452), C2 (codons 457-1309), and C3 (codons 1395-1493). For each mutation subgroup, the number of patients found to be negative and positive for each of the nine ECMs is given in the appropriate columns.

intrafamilial differences in ECM expression. Differences between ECM expression frequency between mutation subgroups were assessed using the χ^2 statistical test taking a significance level of $p<0.05$ to be significant.

Generally, few group C1 patients presented with ECMs, found in only 12/79 (15%) patients from 54 unrelated families. Of particular note, osteomas, hepatoblastomas, and tumours of the periampullary region and brain were not seen in any group C1 patient.

ECMs were only slightly more common in group C2 patients, found in 43/163 (26%) patients from 133 families. All nine ECMs were identified on at least one occasion. Of note, the only positive findings of hepatoblastoma (in three cases) and brain tumour (in one case) were found in patients in this group. In contrast to group C1 patients, osteomas as well as epidermoid cysts were commonly identified with frequencies of 45% and 50% respectively. Similarly to C1 patients, desmoid tumours occurred at a low frequency, identified in only 3/25 (12%) patients.

ECMs were common in group C3 patients, found in 27/31 patients (87%) from 21 unrelated families. Statistically significant differences in the frequencies of desmoid tumours, osteomas, and epidermoid cysts to the levels of $p<0.001$, $p<0.01$, and $p<0.05$, respectively, were found between the patients in groups C1 and C3. Statistically significant differences in the frequencies of desmoid tumours and osteomas to the levels of $p<0.001$ and $p<0.05$, respectively, were found between the patients in groups C2 and C3.

Discussion

This study set out to investigate the nature and distribution of constitutional APC gene mutations in a large panel of patients, with the aim of (1) providing accurate predictive testing in FAP families; (2) investigating the incidence of constitutional APC gene mutations in non-FAP colorectal carcinogenesis; and (3) determining the effect of APC gene mutation position on clinical phenotype. The study used a combination of DGGE, PTT, and direct sequencing to screen the entire APC coding region. Chain terminating mutations (CTMs) predicted to result in the formation of a premature stop codon and therefore a truncated APC protein product were identified in 105 FAP families. One-third of the CTMs were identified in exons 1-14 with the remaining 70 identified in exon 15, comparing closely to the mutation distribution identified in previous studies.[5 6 9 12 13]

Chain terminating mutations were identified in all exons except 1, 2, 3, and the first half of exon 9. The absence of mutations from the first two exons concurs with other studies.[5 8 9 12] In addition, constitutional mutations have rarely been reported in the optional portion of exon 9, a region making up 15% of the coding region for exons 1-14. It has been suggested that mutations in these regions may not elicit a typical FAP phenotype and thereby remain undetected.[14]

In the clinical setting, the identification of a constitutional APC gene mutation in an index case enables at risk family members to be given either a negligible or a 100% estimate of risk for the inheritance of FAP following respectively either a negative or positive mutation result. This level of confidence has important clinical implications for at risk patients, whether mutation analysis shows a positive or negative result. The West Midlands Polyposis register recently reported the removal of 49 at risk family members from screening regimens following a negative mutation result. These patients have therefore been relieved of up to 30 years of endoscopic surveillance, which carries its own morbidity.

Constitutional APC mutations were not identified in all FAP families. There are a number of possible explanations for this. The methods used in the present study would fail to detect directly either whole gene deletions or submicroscopic deletions at the APC locus as identified in other studies.[15] Also, as mutation analysis was restricted to the coding sequence of the APC gene, mutations present in non-coding regions affecting APC gene expression would also be missed, potentially accounting for up to 5% of FAP families.[16] Another region not examined in the present study (and therefore a possible source of missed mutations) was the 54 bp region designated exon 10A or X.[17 18] However, a recent study failed to detect constitutional mutations in exon 10A in 38 FAP patients.[6] Even using a combination of the PTT and allele expression assays, Powell *et al*[16] failed to identify the causative APC gene mutation in eight out of 62 (13%) FAP patients. To date, constitutional mutations responsible for the FAP phenotype have been found only in the APC gene. However, previous linkage analysis studies[19] and the apparent recessive inheritance of the FAP phenotype in one West Midlands family (pedigree not shown) suggests that other genes may occasionally give rise to an identical phenotype to the one produced by mutations in APC. Potential candidates may lie in the WNT-1 signal transduction pathway.[20]

CTMs were not identified in any patient belonging to the non-FAP clinical groups. However in two patients, a double nucleotide substitution was identified at codon 1822 (that is, present on both alleles), a change not seen in a sample of 45 normal subjects. Interestingly, an identical change was identified in an FAP family with a less penetrant form of the disease. Of course, the possibility of a whole gene deletion in these patients would need to be investigated to confirm that the nucleotide change had occurred on both APC alleles. However, this likelihood is small as none of the patients with the apparent double base change exhibited a typical FAP phenotype, as would be expected in patients with whole APC gene deletions.[21]

The significance, if any, of the 1822 polymorphism is unknown. However, as it is located in the middle of the β-catenin down regulation domain, it may result in disruption

of the putative cell signalling function of the APC protein.[22]

A third non-FAP patient (133) was found to possess an amino acid substitution at codon 1057. The pathological significance of this subtle alteration cannot, however, be dismissed without further investigation. It is possible that the non-conservative amino acid change may itself elicit altered functional properties within a frequently mutated portion of the APC protein. In addition, recent findings suggest that subtle changes in cancer predisposition genes may be responsible for an increased cancer risk by creating mutational hotspots.[23] It would be of interest therefore to investigate somatic APC allelic loss and mutation in the tumours of patients from this family.

The precise localisation of FAP causing mutations facilitated correlations to be made between APC gene mutation position and particular disease phenotypes. In the extreme situation, the existence of 100% reliable genotype-phenotype correlations would be particularly advantageous, since the ability to predict accurately the clinical course of disease progression would facilitate the implementation of patient specific surveillance and treatment protocols. In FAP, successful prophylactic colectomy is reducing the number of deaths from metastatic colorectal cancer and therefore the ability to predict whether an affected subject is at high risk of developing potentially life threatening extracolonic disease is becoming increasingly more important.[24]

We and others have previously reported a CHRPE phenotype-genotype correlation.[11 25] In the present study the effect of constitutional APC mutations on the expression of nine other ECMs associated with FAP was investigated by comparing the incidence of each one in patients belonging to the mutation subgroups previously defined by the CHRPE genotype-phenotype correlation. In contrast to CHRPE, the expression of the nine other ECMs could not be categorically predicted by the APC gene mutation position, suggesting these ECMs were not determined by the aberrant product of the truncated APC protein alone. Nonetheless, the position of the APC gene mutation appeared to have a particular influence on the expression of osteomas, desmoids, and epidermoid cysts. A significantly greater proportion of C3 (mutations between codons 1395 and 1493) patients exhibited a positive expression status for these ECMs compared to C1 (mutations between codons 177 and 452) patients (who never expressed osteomas). In addition, a significantly greater proportion of C3 patients, compared to C2 (mutations between codons 457 and 1309) patients, presented with symptomatic desmoid tumours and osteomas. Patients belonging to the C3 mutation group also exhibited the highest frequency of periampullary cancer and gastric and duodenal adenomas. Also, even though the incidence of hepatoblastoma was too low to allow statistical analysis, it was of interest that hepatoblastoma associated APC mutations clustered within the C2 mutation region. These observations support another recent study[26] and, because this cancer is potentially curable if confined to the liver, may be an indicator for liver imaging in these selected families.

The congenital nature of CHRPE lesions suggests that a single chain terminating mutational event at the APC locus (producing an appropriately sized truncated protein) is sufficient for the development of these lesions. In contrast, it would appear that other stochastic, genetic, or environmental factors are essential for the development of other ECMs, thereby reducing the direct influence of APC gene mutation site. Even though in this study statistically significant differences were only seen for osteomas, desmoids, and epidermoid cysts, the existence of phenotype-genotype correlations for other ECMs of FAP cannot be excluded. The later age of diagnosis of manifestations, such as gastric and duodenal adenomas (frequently only recognised following prophylactic colectomy to reduce the risk of colorectal cancer), may be masking a true correlation with APC gene mutation position. This possibility was alluded to in a recent study in which the only patients with constitutional APC mutations beyond codon 1403 not to possess ECMs were all below the age of 25 years.[27] It would appear that the full extent of phenotype-genotype correlations between APC mutation position and all FAP associated ECMs will only be fully realised by prospective clinical observation of larger numbers of molecularly characterised FAP patients.

This study of a large number of clinically well defined FAP families has confirmed and extended previous correlations between APC mutation position and extracolonic manifestations associated with this disease. In addition, it has also provided evidence for the pathological nature of amino acid changes in APC and further investigations are warranted.

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