Genetic and clinical characterisation of familial adenomatous polyposis: a population based study

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Background: Familial adenomatous polyposis (FAP) is a rare autosomal dominantly inherited disease predisposing to colon cancer and caused by germline mutations in the APC (adenomatous polyposis coli) gene.

Aims: We conducted a population based study to evaluate the prevalence and clinical implications of APC mutations among Finnish FAP kindreds. A possible founder effect in parallel with previous observations in hereditary non-polyposis colon cancer (HNPPC) was addressed.

Patients: Affected individuals from 65 kindreds were included.

Methods: The APC gene was screened for mutations using the protein truncation test and heteroduplex analysis. Haplotype analysis was performed with four flanking microsatellite markers. Families that failed to show any mutations were scrutinised with Southern blot hybridisation and allelic expression analysis.

Results: Thirty eight different germline mutations in APC were identified in 47 kindreds (72%). The majority of these mutations were novel and unique to each family. Although sharing the classical polyposis phenotype, families without detectable APC mutations differed from mutation positive families in the following respects: firstly, mean age at polyposis diagnosis was higher (38.6 years (48 individuals) v 30.0 years (140 individuals); p=0.001); and secondly, the proportion of kindreds lacking extracolonic disease was higher (6/18 v 5/47; p=0.04).

Conclusions: Our results may pave the way for predictive testing in mutation positive families and should stimulate further molecular studies in mutation negative families. No founder effect was observed, which is in contrast with HNPPC in the same population.

The discovery of germline mutations in the adenomatous polyposis coli (APC) gene has provided the tools to clarify the pathogenesis of familial adenomatous polyposis (FAP). For example, the human genome mutation database (http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html) presently contains information on more than 300 of such mutations. However, the precise function of the 2843 amino acid APC protein in tumour development is not yet fully understood. The protein has many domains that interact with other cellular proteins, including microtubules (microtubule end binding protein and Drosophila discs large homologue), as reviewed by Kinzler and Vogelstein. Most pathogenic mutations (both germline and somatic) cause loss or disruption of the central β-catenin binding region of the APC protein. β-Catenin is involved in cell-cell adhesion via its binding to E-cadherin, and it has a pivotal role in mammalian homologue (Wnt) for Drosophila wingless (Wg) (Wnt/wg) signalling. In normal cells, the cytoplasmic level of β-catenin is maintained low by APC mediated degradation. If the APC protein is not functional, levels of β-catenin rise in the cytoplasm and enter the cell nucleus where they associate with T cell transcription factor (Wnt/wg signal mediator), and this complex enhances transcription of many oncogenes, including c-myc.

The observation of distinct functional domains in vitro has been substantiated by numerous clinical analyses showing correlations between the FAP phenotype and mutations identified in different parts of the APC gene. Retinal lesions (congenital hypertrophy of retinal pigment epithelium) are associated with truncating mutations between codons 463 and 1387. Desmoid tumours and mandibular osteomas (typical of Gardner’s syndrome) accompany mutations between codons 1403 and 1578. Truncating mutations between codons 169 and 1600 are associated with the classical polyposis phenotype. A milder or more variable phenotype called attenuated adenomatous polyposis (AAPC) is characterised by less than 100 polyps and later age at onset. It has been ascribed to mutations in the 5′ part of the gene (before codon 157 in exon 4), in the alternatively spliced part of exon 9, or in the most 3′ part of the gene beyond codon 1595 in exon 15. These seemingly straightforward correlations between genotype and phenotype are complicated by reports describing kindreds with similar mutations but different phenotypes. Furthermore, the tissue specific role of given APC mutations in the development of rare associated malignancies such as hepatoblastomas, brain tumours (Turcot’s syndrome) or thyroid cancers remains to be elucidated. Finally, even with the best available techniques, approximately 20% of clinically typical FAP kindreds fail to show any APC mutations, raising the possibility of additional susceptibility genes for FAP.

This study took advantage of a population based registry with the following aims: (1) to determine the frequency and type of predisposing APC mutations in Finnish FAP kindreds, (2) to determine if there is evidence for a founder effect analogous to another form of dominantly inherited colon cancer, hereditary non-polyposis colon cancer (HNPPC), in the same population, and (3) to analyse genotype-phenotype correlations in these families. Apart from immediate diagnostic implications, our results have further defined the genetic epidemiology of FAP and paved the way for future studies on

Abbreviations: AAPC, attenuated adenomatous polyposis coli; APC, adenomatous polyposis coli gene; FAP, familial adenomatous polyposis; HNPPC, hereditary non-polyposis colorectal cancer; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; PTT, protein truncation test; RT, reverse transcription; Wnt/wg, mammalian homologue (Wnt) for Drosophila wingless (Wg).
possible modifier genes and additional susceptibility genes in this disorder.

MATERIALS AND METHODS
Families, samples, and clinical data
Since 1981, data on 89 FAP kindreds have been reported to the Finnish Familial Polyposis Registry. Twenty-six five kindreds were included in this study. Detecting more than 100 colonic polyps either endoscopically or during operation in at least one affected family member was used as an inclusion criterion. After informed consent, blood samples were obtained from 1–3 affected individuals per family and used for DNA and RNA extraction. Data on age at diagnosis, number of polyps, duodenal adenoma status, occurrence of colonic cancer or other associated malignancies, as well as histologically benign associated extraintestinal features (desmoids, osteomas, and epidermoid cysts) were retrieved from hospital records. The study design was approved by the ethics committee of the Department of Medical Genetics, University of Helsinki.

Mutation analysis of the APC gene
Protein truncation test
Exons 1–14 were amplified from cDNA by reverse transcription-polymerase chain reaction (RT-PCR) in two segments. Segment 1A (exons 1–9) was amplified with primers 1AF 5′-CAT CAG CTT CAT ATG ATC T-3′ and 1AR 5′-AGA GTC TTT GTC ATT GCA T-3′, and segment 1B (exons 8–14) with primers 1BF 5′-GCA TCT TCT GGT AAT GGT C-3′ and 1BR 5′-CAT AAT CAC CAT AGA GAC T-3′. Exon 15 was amplified from genomic DNA in four segments, as described by Powell and colleagues.26 The protein truncation test (PTT) was performed using the TNT coupled reticulocyte lyase system (Promega Corporation, Madison, USA) according to the manufacturer’s instructions. Translation product (44 µg) was run in Novex (Novex (USA), San Diego, USA) precast 4–20% TG gels. The sizes of the observed aberrant protein bands were estimated against size markers, and the respective DNA regions were sequenced. Mutations identified from cDNA (segments 1A and 1B) were further verified in genomic DNA.

Heteroduplex analysis
As mutated RNA coding for truncated protein may be unstable in vitro, heteroduplex analysis was used as an additional screening method for exons 1–14. Single exons were amplified using published primers.27 Amplification products were treated for three minutes at 95°C and allowed to cool for 20–30 minutes until they reached a temperature of 37°C for heteroduplex formation. The samples were loaded onto a 0.5% MDE gel (AT Biochem, Malvern, USA), prepared according to the manufacturer’s instructions, and run with 12 mA and 9 W for 11–15 hours at room temperature. The gels were silver stained. PCR products showing aberrant heteroduplex patterns were sequenced.

Allele specific oligonucleotide hybridisation analysis
The frequency of a previously reported missense mutation (E1317Q) was determined in 25 healthy control individuals (50 chromosomes). For PCR, primers for the fragment 15G were used. For hybridisation, we used primers 5′-CTA GGT CAG CTT CAT ATG ATC T-3′ for the normal allele and 5′-CTA GGT CAG CTT CAT ATG ATC T-3′ for the mutated allele.

Haplotype analysis
Family members were genotyped with four dinucleotide repeat markers (D5S82, D5S134, D5S346, and D5S318) spanning an area of 7 cM around the APC gene. PCR with 32P labelled dCTP incorporated was performed and alleles were size separated by 6% polyacrylamide gel electrophoresis. Putative disease haplotypes in each kindred were constructed based on the assumption of allele sharing in affected individuals.

RESULTS
Identification and characterisation of APC mutations
In total, 38 different predisposing mutations were detected that occurred in 47 of 65 families screened (72%) (fig 1, table 1). Only 14 of the 38 mutations are presently included in the human genome mutation database. Sixteen mutations were single nucleotide substitutions causing an immediate stop of translation. Twenty mutations, including 15 1–4 bp deletions and five 1–4 bp insertions, caused frameshift and truncation mutations. Furthermore, two kindreds harboured different single nucleotide changes at the splice donor site of exon 4 that led to skipping of exon 4 from the transcript (verified by RT-PCR and sequencing) and a stop after the first six codons in exon 5.

A DNA sample from five families with no APC mutation was subjected to direct sequencing of the first half of exon 15 to determine whether any truncating mutations in this highly mutable region might have been missed by PTT. No such mutations were found. However, one kindred (F3) showed a missense mutation GAA to CAA change causing a glutamic acid to glutamine change in codon 1317 (E1317Q). The E1317Q variant has previously been reported to associate both with colon cancer and multiple adenomas28–30 but the mechanism of its pathogenicity is not known. Furthermore, it seems to be absent in normal control populations,31–34 including ours. Unfortunately, we were unable to test the segregation

Figure 1 Distribution of the adenomatous polyposis coli (APC) mutations found in 47 kindreds. The exonic structure of the APC gene is given. Each kindred is indicated by an identification number and one of four symbols (see box) that specify the type of mutation (see table 1). The occurrence of colorectal cancer and desmoids, clinically the most problematic manifestations of familial adenomatous polyposis, is shown below the gene diagram.
of this variant with disease phenotype as a blood sample from only one affected member was available. Among six polyposis patients from F3, four were diagnosed with colon cancer at 41, 44, 52, and 57 years of age. Because the E1317Q variant is primarily associated with an AAPC-like phenotype, whereas our pedigree showed the classical polyposis phenotype with about 1000 polyps, we concluded that the E1317Q change was unlikely to be the primary underlying defect and this kindred was included in the “mutation unknown” group in subsequent analyses.

Additional studies in families that screened negative
For those 18 kindreds in whom PIT or heteroduplex analysis could not reveal a truncating APC alteration, three additional possibilities were considered. Firstly, Southern analysis screening for large genomic rearrangements in the area was performed with four restriction enzymes (EcoRI, BamHI, HindIII, and KpnI). PCR amplified cDNA fragments 1A and 1B (see materials and methods) as well as the most 5’ and 3’ fragments of exon 15 were used as probes. Following hybridisation with fragment 1A, an extra fragment of 6 kb was detected in BamHI digested DNA in kindreds F9 and F31. However, the same restriction fragment was detected in four individuals among 20 anonymous controls, suggesting that it was a common polymorphism rather than a genomic rearrangement. Secondly, evidence of monoallelic expression possibly due to an unstable gene product or mutational event in the promoter region was sought by advantage of a semi-quantitative sequencing based method, analogous to that applied to detect monoallelic expression in HNPCC.24 Individuals from 11 of 15 tested kindreds were heterozygous for at least one of three single nucleotide coding polymorphisms (C/T at codon 486, A/G at codon 1494, and A/G at codon 1679) but no cases of monoallelic APC messenger RNA expression were detected. Thirdly, the 11307K change originally reported as a risk factor for colon cancer among Ashkenazi Jews was screened by heteroduplex analysis, as described previously.27 No positive cases of the 11307K variant were found, which is compatible with previous population studies among non-Jews.28

Lack of a founder effect
As shown in table 1, the majority of all mutations were unique, while six (of 38, 16%) were shared between 2–4 families. To study if the latter mutations had common versus independent origins, the respective kindreds were genotyped with microsatellite markers flanking the APC gene (table 2). No common haplotypes could be constructed except for three kindreds (F55, F65, F95) sharing a 5p deletion at codon 1059. Our haplotypes could be constructed except for three kindreds (F15, F17, F18). No positive cases of the I1307K variant were found, which is compatible with previous population studies among non-Jews.28

### Table 1 APC mutations found in Finnish familial adenomatous polyposis kindreds

<table>
<thead>
<tr>
<th>Family ID</th>
<th>Mutation description</th>
<th>Mutation type</th>
<th>Codon number</th>
<th>Coding change</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 (4)</td>
<td>AAGAGAGAAGTAGA*</td>
<td>Splicing</td>
<td>Ex 7</td>
<td>Gln- → stop</td>
</tr>
<tr>
<td>F2 (1)</td>
<td>TACGTG- → TAGA*</td>
<td>Deletion</td>
<td>240-240</td>
<td>Frameshift</td>
</tr>
<tr>
<td>F3 (1)</td>
<td>AAGAGAGAAGTAGA*</td>
<td>Splicing</td>
<td>Ex 7</td>
<td>Gln- → stop</td>
</tr>
<tr>
<td>F4 (4)</td>
<td>TACGTG- → TAGA*</td>
<td>Deletion</td>
<td>240-240</td>
<td>Frameshift</td>
</tr>
<tr>
<td>F5 (5)</td>
<td>TACGTG- → TAGA*</td>
<td>Deletion</td>
<td>240-240</td>
<td>Frameshift</td>
</tr>
<tr>
<td>F6 (6)</td>
<td>TACGTG- → TAGA*</td>
<td>Deletion</td>
<td>240-240</td>
<td>Frameshift</td>
</tr>
<tr>
<td>F7 (7)</td>
<td>TACGTG- → TAGA*</td>
<td>Deletion</td>
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<td>Frameshift</td>
</tr>
<tr>
<td>F8 (8)</td>
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<tr>
<td>F9 (9)</td>
<td>TACGTG- → TAGA*</td>
<td>Deletion</td>
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<td>Frameshift</td>
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<td>F10 (10)</td>
<td>TACGTG- → TAGA*</td>
<td>Deletion</td>
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<td>Frameshift</td>
</tr>
<tr>
<td>F11 (11)</td>
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<td>Deletion</td>
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<td>Frameshift</td>
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</table>

The number of individuals with clinical data available is shown in parentheses after the family ID number. *Mutations not previously included in the human genome mutation database (January 2001).
hypothesis mentioned above. Finally, in 15 kindreds (F28, F32, F34, F35, F44, F51, F66, F68, F82, F88, F91, F92, F94, F96, and F97) there was no family history of polyposis. The value of 23% (15/65) for putative “new mutation” families is consistent with the idea that the APC gene has a high de novo mutational tendency.

Phenotype-genotype correlations

Accurate clinical data were obtained from 188 polyposis patients from 65 kindreds (52% male, 48% female). A total of 140 individuals belonged to kindreds with identifiable mutations and the remaining 48 to the “mutation unknown” group. The number of affected individuals per kindred varied between 1 and 9 in both groups (mean 3.0 in “mutation known” and 2.6 in “mutation unknown” groups).

The polyp count was estimated from the patient records at the time of prophylactic colectomy. Although all families met our inclusion criterion of over 100 polyps in at least one member, four families were suggestive of the attenuated phenotype—that is, polyp number was consistently low (50–200 polyps) in each family. In families F13 and F53, mean age at diagnosis was 44.1 years and the mutation was located in a previously delineated “AAPC region” (in the 5’ part of the gene, fig 1) while no APC mutation was detected in the remaining two (F74 and F83, with mean age at diagnosis 31.4 years).

Mean age at polyposis diagnosis was 32.3 (SD 14.6) years in the whole series. A clear difference in age at diagnosis was observed between the “mutation known” and “mutation unknown” groups, irrespective of the method of calculation (table 3). A greater abundance of apparent de novo kindreds with only one affected individual, usually diagnosed in the symptomatic phase of the disease, in the “mutation unknown” group may constitute a possible bias. However, this did not seem to be the case as the proportion of de novo kindreds was similar in both groups (10/47 or 21% in “mutation known” v 5/18 or 28% in the “mutation unknown” group; p=0.4 by Fisher’s exact test).

The occurrence and clinical outcome of duodenal adenomas among Finnish FAP patients have been evaluated, and these data were now analysed against the mutation findings. No specific region of APC mutations was associated with duodenal adenomas and these lesions were common regardless of the mutation status of the families (table 3). Desmoid tumours were associated with mutations downstream of codon 1407, in agreement with a previous study. Specifically, nine of 12 individuals with mutations downstream of codon 1059 is indicated in bold.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutated codon</th>
<th>D5S82</th>
<th>D5S134</th>
<th>D5S346</th>
<th>D5S318</th>
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<tr>
<td>F17</td>
<td>216</td>
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<td>5/7</td>
<td>7/7</td>
<td>2/3</td>
</tr>
<tr>
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<td>216</td>
<td>1</td>
<td>7</td>
<td>9</td>
<td>4</td>
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<tr>
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<td>283</td>
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<td>7/7</td>
<td>2/9</td>
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<td>F51</td>
<td>283</td>
<td>5/9</td>
<td>7/7</td>
<td>10/10</td>
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<td>F28</td>
<td>876</td>
<td>5/8</td>
<td>7/7</td>
<td>2/8</td>
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</tr>
<tr>
<td>F32</td>
<td>876</td>
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<td>7/7</td>
<td>5/7</td>
<td>5/6</td>
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<tr>
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<td>4</td>
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<td>3</td>
<td>2/6</td>
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<td>2/5</td>
<td>7</td>
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</table>

The order of markers is D5S82-5 cM-D5S134-1 cM-D5S346 (APC)-1 cM-D5S318. The number of individuals genotyped is shown in parentheses after the family ID. For each marker, an allele shared by all affected members of a given family is shown. If only one member was studied, the individual alleles are shown separately (for example, F17, all markers). The same applies to families where affected members were heterozygous and shared both alleles (for example, F65, marker D5S82). The putative common disease haplotype in three kindreds harbouring a 4 bp deletion in codon 1059 is indicated in bold.

<table>
<thead>
<tr>
<th>Mutation known</th>
<th>Mutation unknown</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=188)</td>
<td>30.0 (13.6)</td>
<td>38.6 (15.4)</td>
</tr>
<tr>
<td>Probands (n=65)</td>
<td>35.6 (14.1)</td>
<td>44.2 (15.2)</td>
</tr>
<tr>
<td>Other than probands (n=123)</td>
<td>27.2 (12.5)</td>
<td>35.3 (14.9)</td>
</tr>
<tr>
<td>Duodenal adenomas (n=104)</td>
<td>63/82 (76.8%)</td>
<td>15/22 (68.2%)</td>
</tr>
<tr>
<td>Colon cancer (n=188)</td>
<td>30/140 (21.4%)</td>
<td>15/48 (31.3%)</td>
</tr>
<tr>
<td>Desmoids (n=188)</td>
<td>21/140 (15.0%)</td>
<td>5/48 (10.4%)</td>
</tr>
</tbody>
</table>

n refers to the number of family members affected with polyposis from whom accurate data were available. Statistical significance was evaluated by the Student’s t test (two sided) or χ² test.
1407 (75%) had desmoid tumours compared with eight of 124 (6.5%) among patients with mutations upstream of the same codon (p<10^-4; see fig 1). The “mutation known” and “mutation unknown” groups did not differ significantly relative to the frequency of desmoids (table 3).

In two kindreds (F7 and F86), early childhood hepatoblastoma was the first clinical manifestation of FAP, and in F86 it occurred simultaneously in identical twins. Both twins later developed polyposis of the colon at ages five and 12 years, probably enhanced by the immunosuppressive treatment given because of liver transplantation for malignancies. The mutations in these two kindreds were located at codons 1061 and 1172 (table 1), in agreement with other series that have included FAP patients with hepatoblastoma. The occurrence of osteomas and epidermoid cysts was not systematically recorded and thus was not analysed further.

The proportion of families lacking both duodenal adenomas and extraintestinal manifestations of FAP was higher in the “mutation unknown” group compared with families with detectable APC mutations (6/18, 33% v 5/47, 11%; p=0.04 by Fisher’s exact test). This difference could be influenced by the slightly lower average number of affected individuals per family in the former group (see above). Alternatively, together with the difference observed in mean age of onset, this raises the possibility of a different basis of tumour susceptibility in these two groups.

**DISCUSSION**

In HNPCC, a dominantly inherited form of colon cancer susceptibility without notable polyposis, two ancestral founding mutations in the DNA mismatch repair gene MutL homologue 1 are prevalent in the Finnish population, together accounting for up to 68% of all Finnish HNPCC families with germline mutations. Even in the APC gene, at least two founder mutations have been reported in the literature: one affecting nine kindreds from Switzerland and another affecting five kindreds from Newfoundland. It was therefore of interest to determine whether founding mutations of the APC gene might prevail among FAP families from the Finnish population. Our study showed that this was not the case. Haplotype analysis among Italian Italian FAP kindreds recently arrived at a similar conclusion. In our study, the proportion of FAP families estimated to be due to new mutations was approximately 23%, and a similar value (20–25%) was previously found in a Danish population. For comparison, we are aware of only one reported de novo mutation causing HNPCC. While negative selection against germline mutations in the APC gene compared with those in the DNA mismatch repair genes is expected to be comparable (neither essentially reduce the reproductive fitness of mutation carriers), the mutational tendency of these two classes of genes seems very different. However, a bias in clinical practice may also contribute to this difference: in the absence of any family history, colon cancer patients without polyposis may not be referred as often for detailed molecular studies while polyposis accompanying malignancy is diagnostic of FAP even in de novo cases.

The phenotype-genotype correlations we observed were generally in agreement with previous reports. The two kindreds (F7, F86) presenting with hepatoblastoma in our study illustrate well the notion that the germline APC mutation alone explains only part of the clinical features. The molecular pathogenesis of this rare embryological tumour is still poorly understood. The Wnt/Wg signalling pathway is believed to play a significant role in FAP, although other genetic mechanisms are also involved. The fact that both kindreds originated from the same geographical area of Finland (data not shown) and that identical twins from kindred F86 both developed hepatoblastoma in early infancy suggests a common genetic defect causing susceptibility to hepatoblastoma in conjunction with APC mutation. It is noteworthy that an APC mutation identical to that in F7 was detected in another family (F61), yet without hepatoblastoma, further emphasising the fact that the germline APC mutation alone is insufficient in determining hepatoblastoma development.

Despite a comprehensive mutation detection strategy that consisted of PTT and heteroduplex analysis, supplemented with Southern blotting and allelic expression analysis, no APC mutation was identified in 18/65 families (28%). Mutations most difficult to detect include intronic alterations and changes in the regulatory regions that result in a null expression, as well as large rearrangements. Laken and colleagues demonstrated using the monoallelic mutation analysis method that 7/9 kindreds (78%) that did not have any truncating APC mutations showed non-existent (six cases) or reduced (one case) expression of the disease associated allele, suggesting the presence of hidden APC mutations. The two remaining families had full length APC proteins with a wild-type sequence and normal expression which led the authors to conclude that there may be at least one additional gene apart from APC giving rise to FAP. While our sequencing method could not detect minor imbalances in allelic expression, both alleles were clearly expressed in all of our 11 informative kindreds that failed to show any APC mutations. Given that the mutation negative families also displayed some clinical differences relative to the mutation positive families (this study and Wallis and colleagues), it is conceivable that genes other than APC may be associated with predisposition to FAP in the former families. As somatic inactivating mutations that alter phosphorylation sites in exon 3 of β-catenin have been reported in tumours with a normal APC gene, we tested this region according to the method of Kitaeva and colleagues. No germline mutations were found, suggesting that β-catenin may not play a significant role in FAP predisposition, an observation that was recently reported in two additional series. Other genes involved in the Wnt/Wg pathway remain to be investigated.

In summary, our results are important for two reasons. Clinically, knowledge of predisposing APC mutations provides the basis for genetic counselling and predictive genetic testing among family members at risk and enables the exclusion of non-carrier members from tedious endoscopy surveillance. Scientifically, families with accurate clinical data available and the FAP diagnosis verified by mutation analysis, as well as those with no identifiable APC mutations, provide the basis for a systematic search for genetic modifiers and additional susceptibility genes, respectively, efforts that need to be carried out in collaborative settings.

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