# Familial infiltrative fibromatosis (desmoid tumours) (MIM135290) caused by a recurrent 3′ APC gene mutation

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Desmoid tumours are generally very rare but occur about 100 times more frequently in the colorectal cancer predisposition syndrome familial adenomatous polyposis (MIM 175100), being represented in about 10% of patients. In addition to desmoid disease occurring in familial adenomatous polyposis (FAP) there exist familial infiltrative fibromatosis (MIM 135290) kindreds where there is no evidence of FAP. Previously we have described a kindred with familial infiltrative fibromatosis (FIF) in which desmoid tumours were associated with nonpolyposis colorectal cancer. FAP is caused by mutations in the APC gene and various genotype–phenotype relationships have been defined including reports that colorectal polyposis is less severe with mutations 5′ to codon 157 and that the risk of desmoid tumours is high in FAP patients with APC gene mutations between codons 1444 and 1598. There is relatively little information on the phenotype of APC gene mutations 3′ to codon 1598; however, one large family has been reported with a mutation at codon 1987 which presents with a highly variable phenotype which includes desmoid disease. We screened our original FIF kindred and three further families with a similar phenotype for mutations in the APC gene. A 4 bp frameshift deletion in codon 1962 was identified in the original FIF kindred and two further apparently unrelated families. Haplotype analysis suggests a common origin for the APC mutation in all three families. Affected individuals had no evidence of congenital hypertrophy of the retinal pigment epithelium. Colorectal polyposis was variable, and most affected patients had either none or a few late onset polyps. These findings demonstrate (i) that FAP and FIF are allelic, and (ii) that APC gene mutations which truncate the APC protein distal to the beta-catenin binding domain are associated with desmoid tumours, absent CHRPE and variable but attenuated polyposis expression.

## INTRODUCTION

Desmoid tumours (infiltrative fibromatosis) are rare but they occur relatively frequently (between 5 and 10%) in patients with the autosomal dominantly inherited familial cancer syndrome, familial adenomatous polyposis coli (MIM 175100) (1). Familial adenomatous polyposis (FAP) is caused by germline mutations in the APC gene mutation and is characterised by the development of multiple colorectal polyps (>100), duodenal polyps and extra-intestinal manifestations such as congenital hypertrophy of the retinal pigment epithelium (CHRPE), epidermoid cysts, desmoid tumours and osteoma (2–5). In 1992 we described a unique four-generation family with familial infiltrative fibromatosis (FIF) (MIM 135290) (6). Affected individuals developed desmoid tumours or nonpolyposis colorectal cancer, but other features of FAP were not detected, therefore we believed this family to be a variation of the hereditary nonpolyposis colorectal cancer syndrome (HNPCC).

The APC gene is ~8500 bp in size and encodes a protein of 2843 amino acids. Most germline APC mutations are predicted to truncate the APC protein and occur 5′ to codon 1598, so mutations in the 3′ coding region are rare (5,7,8). The colorectal and extra-intestinal manifestations of FAP have been correlated with the position of the APC gene mutation (7,9). Mutations 5′ to codons 157 may cause no or few polyps, referred to as the attenuated FAP phenotype or AAPC (10). CHRPE is usually absent in patients with mutations 5′ of codon 302 and 3′ of codon 1444 (11,12). Truncation of the APC protein between codons 1285 and 1465 has been associated with severe polyposis (5,13). Desmoid tumours are frequent in FAP patients with mutations in codons 1445–1578 (7,8,12,14). Mutations 3′ of codon 1578 are rare in FAP and have not been associated with a distinct phenotype. In a previous study we were unable to identify a 5′ APC gene mutation in familial desmoid kindreds (15) and we hypothesised that familial desmoid tumours with few colorectal polyps might be caused by 3′ APC mutations. We therefore

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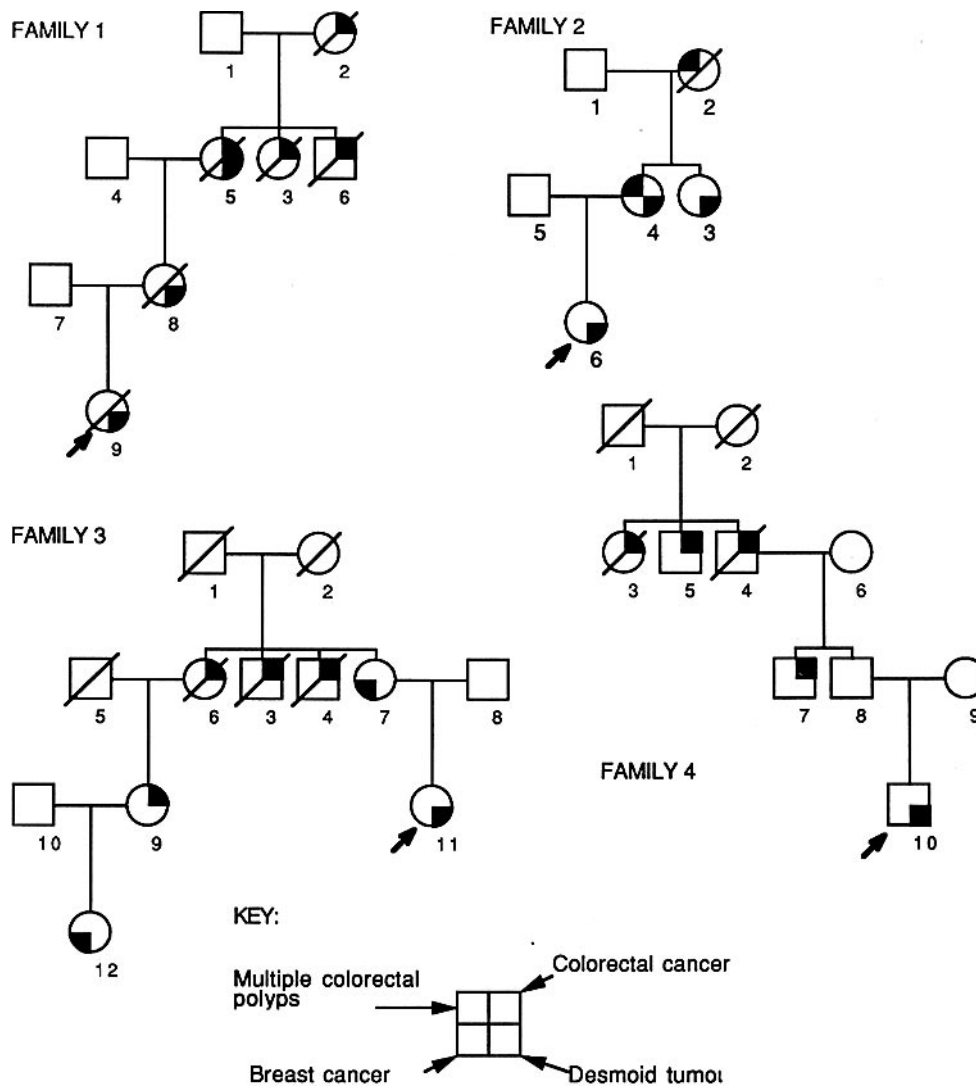
**Figure 1.** Pedigrees of the four families. Further details of the relevant patients are given in the text. DNA was analysed from all patients indicated with an *.

screened our original FIF kindred and three further families with desmoid tumours without classical FAP for germline APC gene mutations. Intriguingly, an identical mutation at codon 1962 was found in three of the four apparently unrelated kindreds.
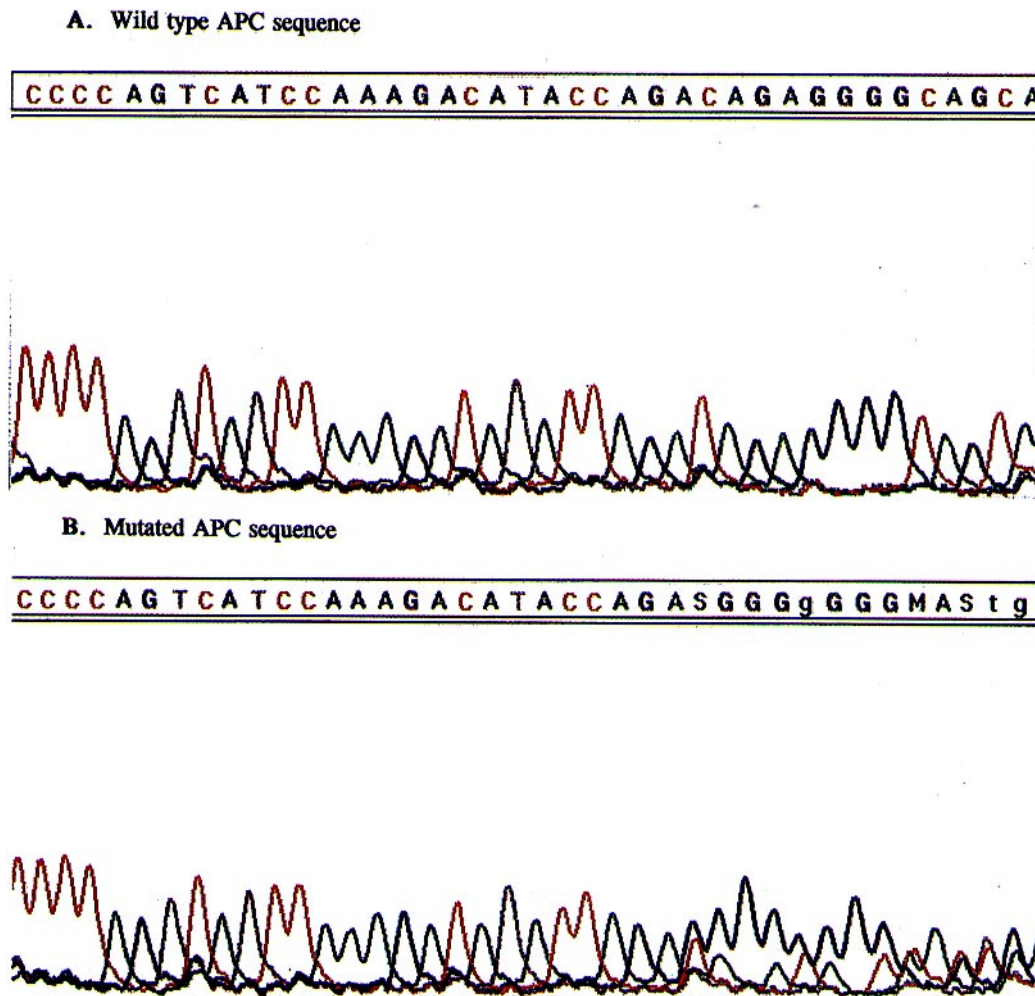
## RESULTS

### Clinical phenotype

Pedigree and clinical details are summarised in Figure 1. All four families were thought to be unrelated, although families 1–3 were from eastern England. Families 1, 2 and 3 contained two or more patients with desmoid tumours and family 4 a single case (see Fig. 1). Family 1 was described previously and displayed a four-generation history of desmoid tumours and nonpolyposis colorectal cancer with no other evidence of intestinal (duodenal polyps) or extra-intestinal features (osteomas, CHRPE) of FAP. In family 2 the proband had multiple thoracic wall desmoid tumours but no colorectal polyps at age 30 years. Her mother had an intra-abdominal desmoid and a carcinoma of the colon and 30 colorectal polyps at age 31 years. A maternal aunt had an intra-abdominal desmoid age 60 years and the maternal grand-

mother had a colectomy for 200 colonic polyps at age 66 years. In family 3, two individuals had had intra-abdominal desmoids without features of FAP, but multiple intervening relatives had a history of colorectal cancer and one relative had a history of multiple gastric polyposis. In family 4 the proband had an intra-abdominal desmoid resected age 7 years and is free of recurrence at age 13 years. His father had a normal colonoscopy at age 50 years, but a paternal uncle and three further relatives were reported to have colorectal cancer, without polyposis.

### Molecular genetic analysis

Evidence of a similar protein truncating APC gene mutation between codons 1555 and 2256 was detected in families 1, 2 and 3 with the *in vitro* translation assay. Direct sequencing of genomic DNA revealed an identical 4 bp deletion at nucleotides 5844–5847 (codon 1962) of the APC sequence (Fig. 2). The deletion mutation was detected in all patients from families 1–3 for whom DNA was available (see Fig. 1). To determine if this recurrent mutation was likely to be the result of a founder effect or a 'mutation hot spot', a disease associated haplotype was

**A. Wild type APC sequence**

C C C C A G T C A T C C A A A G A C A T A C C A G A C A G A G G G G C A G C A

**B. Mutated APC sequence**

C C C C A G T C A T C C A A A G A C A T A C C A G A S G G G g G G G M A S t g

**Figure 2.** Sequence analysis of the mutated region in exon 15. (**A**) Normal chromatogram of the sequence between codons 1950 and 1980. (**B**) Mutated sequence between showing overlapping mutated and normal sequence between codons 1950 and 1980.

constructed at three polymorphic sites within or close to the APC gene (D5S82, D5S346 and APC-*Rsa*I) (3,24,25). The mutation associated haplotype in families 1, 2 and 3 was consistent with a common origin and this haplotype was not present in family 4.

## DISCUSSION

The identification of a germline APC gene mutation in our original kindred with FIF has confirmed our original suggestion that this disorder might be allelic with FAP. In addition, the identification of a further genotype–phenotype correlation provides more evidence that the APC protein has tissue-specific functions. The presence of an identical mutation in three kindreds was unexpected, in view of the inter-familial variations in phenotype with no evidence of colorectal polyposis in family 1 but up to ~200 polyps (at age 66) in an individual in family 2. Founder mutations are uncommon in classical FAP, and although the risk of colorectal cancer in the 3′ APC gene mutation families appears to be less than in classical FAP, early death from locally invasive desmoid tumours may occur. Recently, Eccles *et al.* (16) have reported a high frequency of desmoid tumours with very late onset of and incomplete penetrance of intestinal polyposis in a large kindred with an APC mutation at codon 1924. Thus it appears that APC mutations in the 3′ region of

exon 15 are associated with a high risk of desmoid tumour and a relatively low, but variable, incidence of colorectal adenomatous polyps. The inter-familial variations in phenotype observed between kindreds with identical mutations may result from modifier effects (17), although further studies will be needed to investigate this. We did not identify a mutation in family 4, but cannot exclude a germline APC gene mutation as the PTT we employed can, in some circumstances, fail to detect truncating mutations and would not detect missense mutations or small in-frame deletions (18).

Although the precise function of the APC protein is unknown, evidence suggests that it interacts with alpha and beta-catenins and appears to be associated with the microtubule cytoskeleton (19–21). Heptad repeat regions within the APC protein may form coiled coil structures and allow the APC protein to associate with itself and other proteins. The formation of a heterodimer between wild-type and truncated APC protein is thought to be more deleterious than a 50% reduction in APC protein levels. Hence 5′ APC mutations (<codon 157) which result in short truncated proteins and which are predicted not to dimerise with wild-type protein have been associated with a less severe phenotype than seen in classical FAP (10,22,23). Most APC mutations in classical FAP occur in the 5′ half of the coding sequence and mutations beyond codon 1597 are reported rarely (<2.5% of cases) (5). We have demonstrated that 3′

APC mutations which would be predicted to produce large truncated proteins lacking the tubulin-binding and basic domains in the carboxy region do not produce florid colorectal polyposis. Further studies are needed to (i) delineate the phenotype of 3′ APC gene mutations, (ii) determine the precise relationship between the position of 3′ APC gene mutations and severity of colonic polyposis and (iii) elucidate the effect of particular mutations on the stability and tissue specific functions of the APC protein. More information may lead to a better understanding of the molecular pathogenesis of desmoid tumours and provide insights into the structure–function relationships of the APC protein.

## MATERIALS AND METHODS

The *in vitro* protein translation assay was performed on high molecular weight genomic DNA as described previously (18). Exon 15 of the APC gene was divided into four overlapping sequences and PCR amplification was performed in a 25 µl reaction volume using 200 ng of genomic DNA as template under standard conditions. The samples were cycled at 94°C 30 s, 55°C 30 s, 72°C 2 min, 35 times. A 1.5 µl aliquot was then taken and used as the template in an *in vitro* transcription and translation reaction using a TNT T7 coupled reticulocyte lysate system (Promega, Madison, WI). For labelling purposes, 0.2 µl of EasyTag$^{35}$-S Methionine (NEN; Boston, Mass.) was included in each reaction. Labelled APC protein products were size fractionated on 12% sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE) gels (National Diagnostics, Atlanta, Georgia), fixed, dried and exposed to Biomax film (Kodak, Rochester NY).

Samples with detectable truncated proteins were investigated further by direct cycle sequencing using Thermo Sequenase (Amersham, Buckinghamshire) and a LiCor automated sequencer (LiCor, Lincoln, Nebraska). Primers were end labelled with IRD-41 (LiCor Lincoln, Nebraska) and used directly in the cycle sequencing reaction. Cycle sequencing parameters were 93°C 30 s, 55°C 30 s and 72°C 1 min, 21 times.

Haplotype analysis was performed at microsatellite loci D5S82 (24) and D5S346 (25), close to APC, and at the APC-*Rsa*I RFLP in exon 11 (3). For the microsatellite analysis, one primer of each pair was end-labelled with γ-$^{32}$P ATP using T4 polynucleotide kinase (New England Biolabs, Hitchin UK), and incorporated into the appropriate PCR reaction mix (final Mg$^{++}$ concentration of 1.5 mM, 60 ng each primer, approximately 300 ng DNA). Annealing temperatures of 48°C for D5S82 and 55°C for D5S346 were used. PCR products were electrophoresed on 6% denaturing polyacrylamide gels for 3–4 h, autoradiography was performed for 1–3 days. For the APC-*Rsa*I RFLP, approximately 300 ng DNA was amplified in a final volume of 25 µl at a final Mg$^{++}$ concentration of 2.5 mM, using 100 ng each of primers 11F (5′-GATGATTGTCTTTTTCCT-CTTGC-3′) and 11R (5′- CTGAGCTATCTTAAGAAATAC ATG-3′). Cycle conditions were 95°C 5 min, then 30 cycles of 57°C 45 s, 72°C 45 s, 96°C 45 s. Products were digested with *Rsa*I (Stratagene, Cambridge, UK), electrophoresed on 2% NuSieve/1% agarose gels, and scored for the presence or absence of the T→C polymorphism which creates an *Rsa*I site and thus allows digestion of the 215 bp PCR product to 85 bp and 130 bp.

## ACKNOWLEDGEMENTS

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