Two cases of 5q deletions in patients with familial adenomatous polyposis: possible link with Caroli's disease

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Abstract

Two cases are reported of patients with deletions of chromosome 5q. Both have familial adenomatous polyposis (FAP) and mild mental retardation. In both, macroscopic polyposis was confined to the proximal colon in adult life (in their thirties) although microscopic adenomatosis was shown in the more distal colon with occasional single polyps. Both subjects had dermoid cysts, and congenital hypertrophy of the retinal pigment epithelium (CHRPE) was seen in case 2. Case 1 has gastroduodenal polyps and desmoid tumours; case 2 has a marfanoid habitus with an abnormal pectus, wasted calf muscles and clawing of the toes, and Caroli's syndrome. His deletion is cytogenetically more extensive than that in case 1. The paucity of adenomas in the left side of the colon suggests that FAP cannot always confidently be excluded by sigmoidoscopy alone. The expression of the disease in the colon in these cases could be milder than in the more usual autosomal dominant cases where nonsense mutations resulting from single base changes of small deletions rather than deletion of the whole gene are the usual finding.

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Familial adenomatous polyposis (FAP) is an autosomal dominant trait characterised by the development of large numbers (over 100 being diagnostic) of adenomatous polyps in the colon,[1] usually from the teenage years, and these polyps have a high risk of becoming malignant. Upper gastrointestinal polyps may also develop, but are less frequent. Extracolonic manifestations are variably seen in this condition, including intra-abdominal desmoid tumours, sebaceous cysts, odontiginous cysts in the jaw, and congenital hypertrophy of the retinal pigment epithelium (CHRPE), these latter occurring in over 80% of affected subjects. The condition was initially referred to as Gardner's syndrome when the extracolonic manifestations were pronounced, and considered to be distinct from FAP, but linkage studies have shown that the two conditions are allelic.[2]

There are five published cases of deletion of the long arm of chromosome 5 associated with mild mental deficiency and FAP; the first of these provided critical information localising the gene for this condition to within the region of the deletion.[3-5] The assumption was that the loss of one of the normal alleles at the FAP locus led to the development of the FAP phenotype. There have been other case reports of subjects with 5q deletions and dysmorphic features, so that a phenotype characteristic of this deletion is being recognised.[5-7] Some of these cases could have had FAP but were too young to have demonstrable colonic polyps at the time the case report was written. No other specific single gene disorder has been included in the characteristic 5q deletion syndromes delineated, and no liver abnormality has been noted to be associated with it.

We describe two cases of 5q deletions in subjects with FAP, in one of whom the detection of the deletion led to the diagnosis of FAP being made. In this case (case 2) Caroli's syndrome was also present, and the patient had a marfanoid habitus with wasting of the calf muscles and mild toe clawing.

Caroli's disease is a rare condition consisting of dilatation of distal intrahepatic bile ducts with intrahepatic stone formation.[8] While diagnostic criteria may vary from one centre to another there are links between this condition and the so called fibropolycystic diseases of the liver, which include biliary hamartomata (Von Meyenburg complexes), choledochal cyst, and autosomal recessive polycystic kidney disease (ARPKD).[9 11] It is not clear whether cases of autosomal dominant polycystic kidney disease (ADPKD) can also be associated with Caroli's disease.

It is suggested that the presence of several distinct abnormalities in case 2 might represent a contiguous gene syndrome,[12] owing to the loss by deletion of the normal alleles of several neighbouring genes.

Case reports

CASE 1

Case 1 was the only child of non-consanguineous parents. The proband's father was alive and well in his 70s; her mother had died aged 60 years of colon cancer. She was said to have been of slightly reduced intelligence, and had received some treatment for polyps in the bowel. Unfortunately further details were not available.

The proband worked as a clerk typist, but had a speech defect and was of low normal intelligence. She was admitted to hospital for investigation of iron deficiency anaemia at the age of 38 years. She was otherwise well, but had previously had two operations for removal of sebaceous cysts from her abdominal wall at the age of 27 years, and two sebaceous cysts

had been removed from her scalp when she was 34 years of age. On clinical examination she was of normal height and stocky build, with no obvious neurological problems other than her mild speech defect, but she had a thoracic kyphosis.

Investigations for the anaemia included a colonoscopy, which showed multiple adenomas (over 100 in number) in the ascending colon; the transverse and descending colon were apparently free of polyps. Histological examination of the adenomas showed that they were tubular adenomas. Liver function tests were normal. She underwent a colectomy and ileorectal anastomosis and pathological examination of the colon removed at surgery showed carpeting of the ascending colon up to the hepatic flexure with multiple mucosal polyps, some sessile and some pedunculated. The mucosa of the rest of the colon contained only occasional sessile polyps. Most of the polyps were tubular adenomas with mild epithelial dysplasia; a few were tubulovillous adenomas, and some microadenomas were present. No malignancy was found. These features confirmed the diagnosis of familial adenomatous polyposis (FAP).

She made a good postoperative recovery, but a year later she developed swelling and discomfort in the right iliac fossa, with subacute intestinal obstruction. This swelling was ascribed to a growing desmoid tumour in the abdomen, part of which was excised from the abdominal wound. Histological examination showed the characteristic elongated spindle shaped cells with a prominent collagenous stroma found in desmoids. Abdominal ultrasound and CT scans were performed, and these were consistent with an intra-abdominal desmoid; other findings were of a uterine fibroid. The liver, spleen, and kidneys had normal appearances and no dilatation of the biliary ducts was noted. Two years later two further intra-abdominal desmoids were delineated in the mesentery by ultrasound scans. Gastroscopy showed carpeting of the fundus of the stomach with fundic polyps showing mild cystic dilatation on histological examination; two duodenal adenomatous polyps were also seen. Three rectal polyps were removed, histologically benign tubular adenomas, and intramucosal adenomatosis was also seen.

CASE 2

Case 2 was born at term by normal delivery to a 31 year old mother and a non-consanguineous 34 year old father, both of whom were healthy. His mother had 10 pregnancies of which five resulted in spontaneous abortions. Of the five live births, two girls died in childhood, one from an accident at 5 years of age, and the other of sudden infant death syndrome at 3 months of age. The patient's two surviving brothers were well, of normal intelligence, and with none of the clinical features described in the proband. There was no family history of FAP, Caroli's syndrome, gastrointestinal cancer, or neurological disease.

At birth the proband was cyanosed and hypotonic, but recovered quickly without the need for long term neonatal care. His motor development was delayed and he did not crawl but 'bottom shuffled', and was very lax jointed and hypotonic. He walked and spoke in sentences only at 3 years. His gait was always slightly abnormal, and he would fall over without saving himself. Initially he attended school for children with special educational needs, but later he attended a normal school, with special coaching, until the age of 18 years, and established a wide vocabulary. He required psychiatric treatment when he was 20 years. Subsequently he has lived with his family because of his mild mental retardation, helping his parents in the family shop.

The proband was referred at the age of 31 years because of a two year history of weight loss and steatorrhoea (of 50 g/day) associated with bleeding because of vitamin K deficiency, and a diagnosis of Caroli's syndrome (with dilatation of the distal intrahepatic bile ducts with intrahepatic stone formation) had been made. Diarrhoea had initially developed at the age of 20 years and endoscopic removal of colonic polyps had been performed on intermittent surveillance until he was 25 years old.

At the referring hospital the following tests had been normal: SeHCAT (a test for malabsorption of bile acids), small bowel histology, pancreolauryl test, breath hydrogen, and pancreatic CT scan. Endoscopic retrograde cholangiopancreatogram (ERCP) had shown Caroli's syndrome with multiple common bile duct stones, some of which were removed following sphincterotomy. Pancreatic cannulation was not achieved.

On examination he was slim, weight 55 kg (10th centile), and was 1·8m tall (75th centile). He was sociable but had limited understanding. He had a long, thin face with large, prominent ears, a bossed, high forehead, a long philtrum (fig 1), and a high arched palate. There were freckles on his shoulders and arms but no café au lait patches. He had an asymmetrical chest shape with a pectus prominent on the left. He had long thin fingers and toes and increased joint laxity. There was a 3 cm cystic lesion on the medial border of the lower right thigh. The feet were highly arched with trophic changes on the soles and clawing of the toes. There was webbing between the second and third left toes. There was smooth, nontender hepatomegaly 5 cm below the costal margin. Position sense was reduced in the toes, but vibration sense and Romberg's test were normal. Coordination was slightly impaired in all four limbs. There was slight calf wasting of the 'inverted Champagne bottle' type bilaterally but reflexes were brisk, more so on the right, with an absent right ankle jerk and equivocal right plantar response. The remainder of the neurological examination was normal. Ophthalmology showed three areas of congenital hypertrophy of the retinal pigment epithelium (CHRPE) in the left eye.

Investigation showed a microcytic anaemia (Hb 9·4, MCV 65·8, MCH 20·1) because of iron deficiency (serum Fe 2 μmol/l, total iron binding capacity 69, ferritin 12 μg/l) which
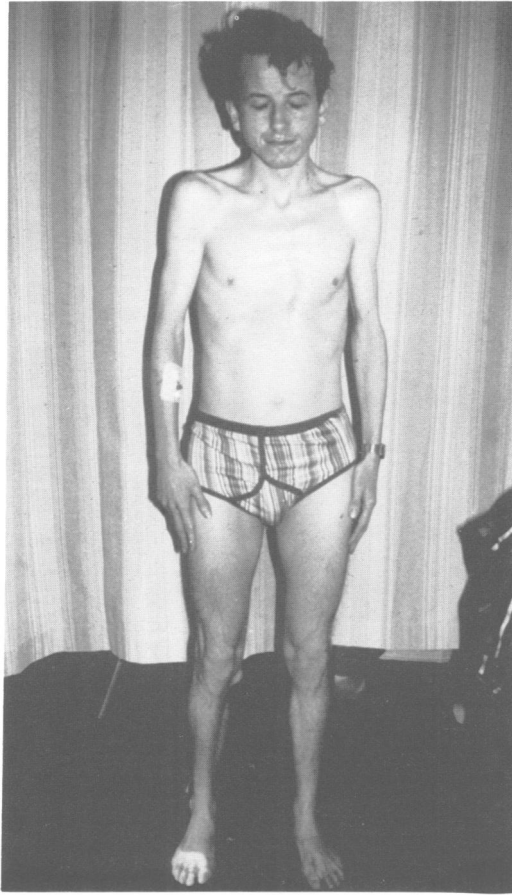
Figure 1   Clinical photograph of case 2.

was responsive to oral iron therapy. Other haematology, coagulation profile, plasma, and urine biochemistry were normal. At ERCP a dilated common bile duct was shown which contained two large stones and multiple smaller ones, especially in the upper duct. At the distal ends of the intrahepatic ducts, there were multiple saccular dilatations but no stones were seen within them (fig 2). A fine pancreatic duct of normal length was noted.
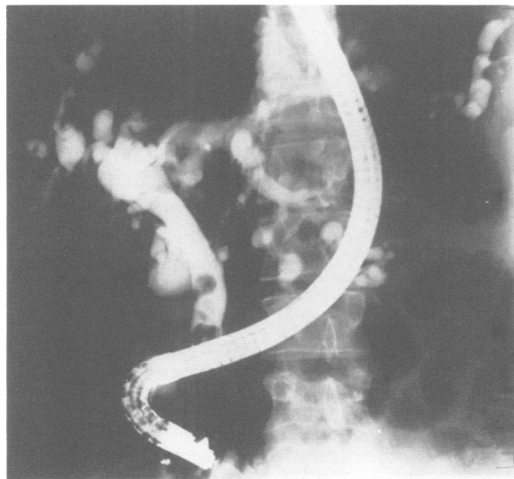

Figure 2   X ray of endoscopic retrograde cholangiogram of case 2, showing two stones in the common bile duct and multiple saccular dilatations of terminal intrahepatic bile ducts, characteristic of Caroli's syndrome.

Histology of small bowel mucosa obtained at ERCP was normal. Abdominal ultrasound showed cystic lesions in the liver communicating with the biliary tree and some stones within the common bile duct but no other abnormalities. Bile obtained at ERCP showed 35% deconjugated bile acids. At limited colonoscopy with inadequate preparation, there was an area of abnormal mucosa in the descending colon which on histology showed the typical changes of FAP with multiple submucosal microadenomata. Later full colonoscopy following standard preparation showed that these areas of abnormal mucosa continued up to the mid-transverse colon, beyond which there were increasing numbers of adenomatous polyps extending to the caecum. At least one of these in the ascending colon was rather large and shouldered. Well over 100 adenomatous polyps were seen; histology showed several further areas of moderate and also severe dysplasia with clear polyp formation.

Other investigations with normal results were Lundh meal test, ^{14}C bile acid breath test, duodenal biopsy, and faecal fat collection (while on pancreatic supplementation). Abdominal CT scan was consistent with other findings and showed no new pathology; there was no evidence of polycystic disease of the kidney. On EMG, myopathic features were present in the left deltoid and triceps muscles but not in more distal muscles. Nerve conduction was normal. Orthopantomogram was done, and showed a supernumerary unerupted tooth in the mandible but no other abnormalities. CT scan of the brain and skull was normal. Serum phytanic acid levels were normal. The proband therefore has features of Caroli's syndrome, FAP, and a marfanoid habitus with lax joints, and some evidence of hereditary motor and sensory neuropathy (HMSN). He was recommended to have panproctocolectomy with ileoanal anastomosis.

CYTOGENETIC AND DNA STUDIES

Cytogenetic studies of the probands and the parents of proband 2 were carried out on chromosome spreads obtained by blood lymphocyte culture and staining with trypsin-Giemsa banding techniques. They were found to have the following karyotypes: proband 1 46,XX,del(5)(q21.3q23.1) (fig 3A), proband 2 46,XY,del(5)(q15q22.3 or 23.1)de novo (fig 3B). Both parents of proband 2 showed a normal chromosome complement. It was not possible to karyotype either of the parents of case 1, and the proband herself was unwilling to donate further blood samples.

In case 2 fluorescence in situ hybridisation was used to confirm the deletion of the adenomatous polyposis coli (APC) gene locus within the deletion (fig 4). YAC A1010 (which maps to chromosome band 5q21 and encompasses the MCC ('mutated in colon cancer') and APC genes[13]) was used as a probe together with a probe specific for the centromere of chromosome 5. Both chromosomes 5 in all cells analysed from both parents showed paired signals from YAC A1010. In the proband, signal from
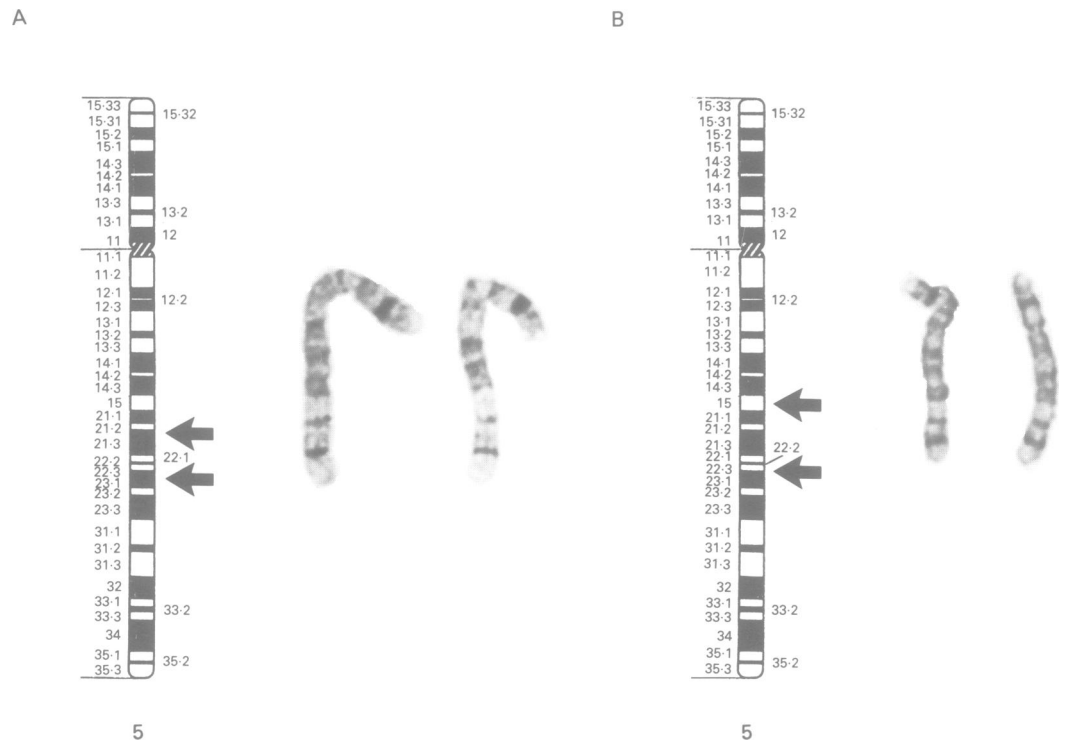
A

B

Figure 3   (A) Partial karyotype of case 1. (B) Partial karyotype of case 2.

YAC A1010 was only found on both chromatids of the normal chromosome 5 and was absent from the deleted chromosome. The centromeric probe, used as a control and to assist chromosome identification, was observed on both the normal and deleted copies of chromosome 5.

In case 1 it was not possible to use in situ hybridisation on chromosome spreads from the parents. In this proband, signal from YAC A1010 was only seen on both chromatids of the normal chromosome 5, and no signal was seen on the deleted chromosome. Signal from the

Figure 4   Fluorescence in situ hybridisation on chromosome spread from case 2. Solid arrowheads indicate PGA16 centromere probe on chromosomes 5 and 19. Open arrowheads indicate probe A1010 on the normal chromosome 5.

centromeric probe was seen on both the normal and deleted chromosomes 5. From the results of the in situ hybridisation it is clear that both cases 1 and 2 have lost the region of DNA encompassing the MCC and APC genes.

To try and determine the boundaries of the deletion in case 2, DNA was prepared from his peripheral blood, and from his parents, and this was digested with the appropriate restriction enzymes, electrophoresed, and transferred to nylon membranes. These were then probed with radiolabelled polymorphic markers from the APC gene region to check for heterozygosity and inheritance of alleles. Case 2 was homo- or hemizygous for probes from the following loci: *D5S37, D5S85, D5S98, D5S134, D5S135, MCC, APC, D5S81,* and *D5S84* (fig 5). Further investigations using other polymorphic markers and quantitative densitometry are being carried out. He failed to inherit a paternal allele for *D5S135, D5S134,* and also for *D5S37,* suggesting that the deletion extends to this locus (fig 6). Paternity was confirmed using variable number tandem repeat (VNTR) probes from other chromosomes.

The fibrillin 2 dinucleotide repeat polymorphic marker described by Lee *et al*[14] was used to determine whether case 2 had one or two copies of this gene. PCR was carried out using their conditions but without radioactivity. The products were electrophoresed in a 4% Nusieve GTG (FMC)/agarose (3:1) gel. Human-hamster hybrids containing a single human chromosome 5 were also included in the assay. Single bands were seen for these hybrids but two bands could be seen in case 2 and his parents (fig 7).

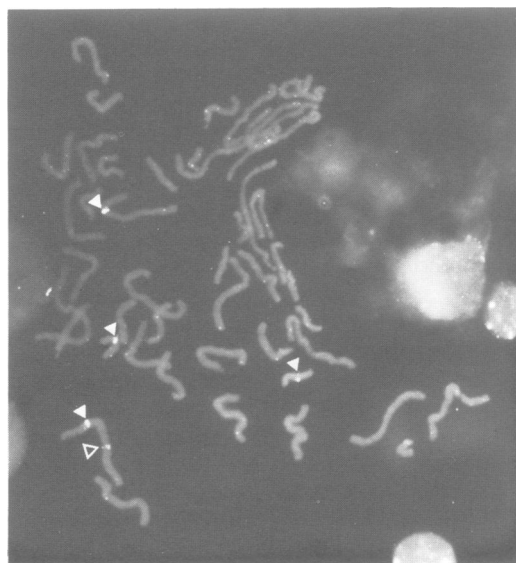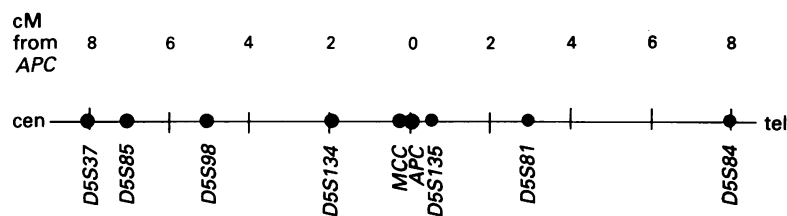An EBV transformed lymphoblastoid cell line has been established from case 2.

Figure 5 Positions of marker loci used in relation to the APC gene.

Discussion

There have been five previous reports of 5q deletions associated with FAP, the first of which[3] led to the localisation of the gene for FAP to this region of chromosome 5.[4] A subsequent case report was of two mildly mentally retarded brothers (reared apart) with FAP and a 5q deletion, whose mother had died of carcinoma of the colon with extensive polyposis in her early 40s. She was also mentally retarded. Details of the extent of the polyposis in these brothers were not given.[15] In an isolated case of FAP described by Kobayashi et al[7] over 500 adenomatous polyps were found throughout the colon at the age of 15 years. However, in a more recent report of an interstitial deletion of 5q22 with an interchromosomal rearrangement segregating with FAP and mental handicap in two related subjects, the proband presented at the age of 25 with anaemia, and multiple adenomatous polyps were found, these being predominantly on the right side of the colon. He also had multiple epidermoid cysts, CHRPE, mandibular osteomata, and a specific impairment of expressive speech. His affected maternal aunt was mentally retarded, had a spastic gait, and multiple colonic polyps were seen on colonoscopy at the age of 54

years.[16] The most recent report is of a 30 year old mentally retarded female (with a deletion 5(q15-q23.2) who was found to have over 300 adenomatous polyps in the colon, but the age at diagnosis and the distribution of the polyps within the colon was not stated. The same authors also reported a 13 year old boy with a deletion 5(q15-q21.3) who only had one benign polyp on colonoscopy.[5]

Features described in other subjects with 5q deletions which were found in our two cases are mild mental impairment, a speech defect in one of our cases, frontal bossing, epicanthic folds, a large head, a long philtrum, and a high arched palate. Kyphoscoliosis was noted in the case described by Herrera et al,[3] a feature found in our case 1 and in another case with a 5q deletion.[6] Club foot has been described in five reported cases,[5 7] and a spastic gait was noted in one of the cases reported by Cross et al,[16] so these may be part of the spectrum of features associated with deletions of this region of chromosome 5. Congenital contractures have been noted in a boy with a deletion of 5(q22.3-q31.1).[5] Our case 2 had an awkward gait with high arches to his feet, clawing of the toes, and brisk reflexes. He was of marfanoid build and had wasted calf muscles. He also had Caroli's disease. There was no evidence of liver or biliary dysfunction in case 1 or in any previous report of 5q deletions[5 7 17-19]; we are not aware of any previously reported association of Caroli's disease and FAP. It is, however, possible that the deletion in case 2 encompassed a gene involved in hepatobiliary fibropolycystic disease, and such a putative gene might be a candidate for other forms of polycystic disease. This would obviously not apply to the families with ADPKD in which
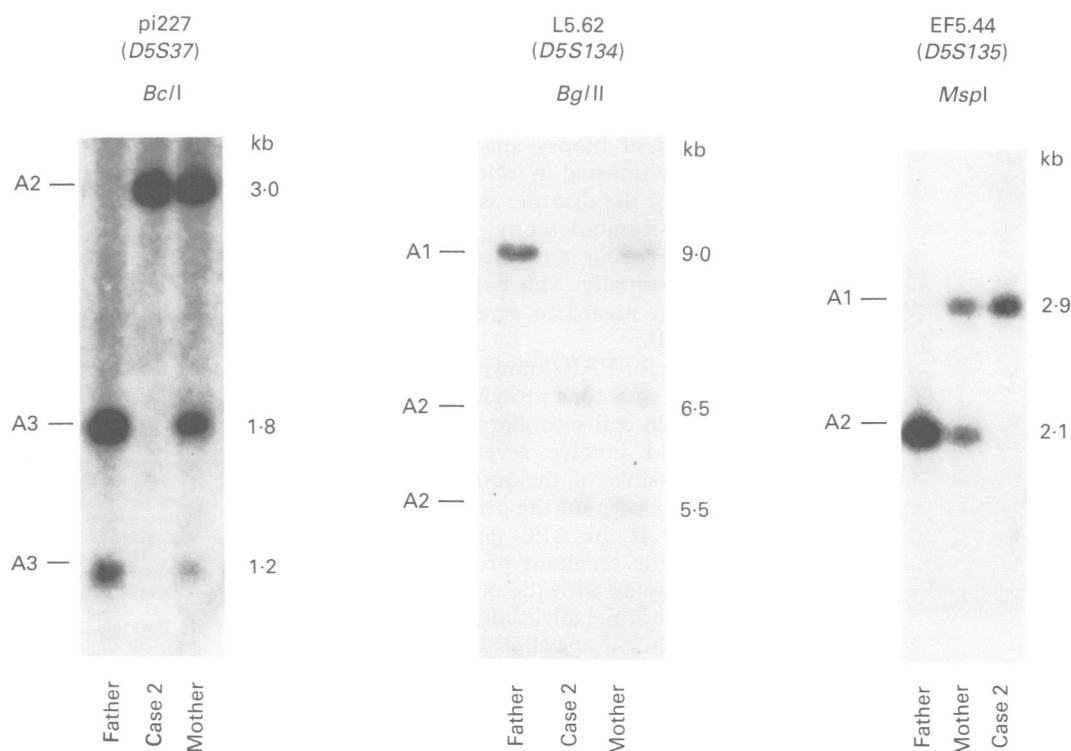

Figure 6 DNA from case 2 and his parents hybridised to polymorphic probes near APC showing lack of inheritance of a paternal allele from these loci.
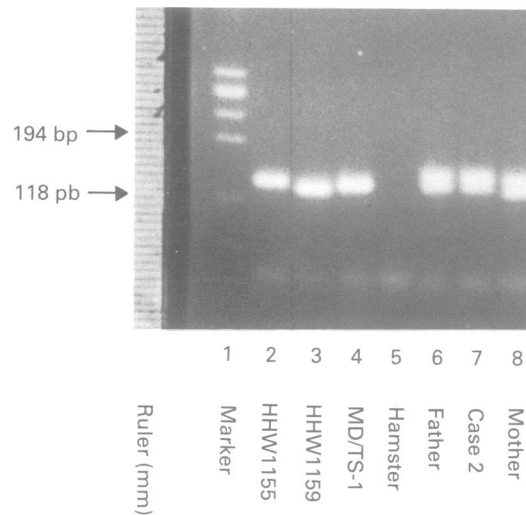
Figure 7 Products from the fibrillin 2 dinucleotide repeat assay. Lane 1 is a X174 RF DNA HaeIII digest (NEB) used as a marker, and lanes 2, 3, and 4 are human-hamster hybrids containing a single human chromosome 5.

the disorder is linked to markers on chromosome 16p[20 21] but the location of the PKD gene in other families, and in the autosomal recessive form, remains unknown.

We were able to show that it was unlikely that the fibrillin 2 gene was deleted in case 2, which made it unlikely that this was related to his marfanoid habitus, clawed toes, and wasted calf muscles, which are features of Beale's syndrome,[14 22 23] now thought to result from mutations in this gene (assigned to 5q23-31). However, we cannot rule out the possibility that the breakpoints in the deletion in case 2 could have caused an alteration in this gene.

It is of interest that in neither of our cases (nor in one of the published cases[16]) were florid multiple macroscopic polyps found in the descending colon. This meant that FAP was only diagnosed on colonoscopy with histological examination of biopsy specimens in case 2. This is very unusual in inherited FAP, where screening for the disorder is traditionally performed by sigmoidoscopy, since polyps virtually always occur in the sigmoid colon as well as more proximally. This has significant implications with regard to screening subjects at risk for FAP.

The gene for FAP (known as the APC gene) is known to code for a protein which is likely to form a coiled coil secondary helical structure, which could involve several different molecules, possibly of the product of the APC gene with or without the product of the MCC gene also.[24] If the APC gene is present but abnormal, the resultant protein could theoretically complex with the normal protein product of the normal allele and further impair its function, while if one allele is totally absent (as in our two deleted cases) such complexes would not develop and such impairment of the function of the normal protein would not occur. This may explain the relatively late onset and reduced distribution of polyps in our two cases, but not the presence of extracolonic

manifestations, which may have a different molecular pathogenesis.

Most cases of autosomal dominant FAP in which the mutation has been characterised are the result of point mutations leading to base changes, or frameshifts owing to small deletions or duplications causing nonsense or stop mutations in the APC gene, rather than large deletions.[25]

The intelligence of our two probands was not severely impaired and both had good verbal skills. This is remarkable in view of the large size of their chromosomal deletions. We hope that our two case reports can give some possible further insights into the pathogenesis of colonic polyps in FAP, and into the possible localisation of a gene involved in the pathogenesis of Caroli's syndrome.

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