An intrachromosomal insertion causing 5q22 deletion and familial adenomatous polyposis coli in two generations

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Abstract

We report familial adenomatous polyposis coli (FAPC) with epidermoid cysts, osteomata, and areas of congenital hypertrophy of the retinal pigment epithelium (CHRPEs) in a male patient and his maternal aunt, both of whom suffered a mild to moderate degree of mental handicap. Both had an interstitial deletion of the long arm of chromosome 5 (del(5)(q22q23.2)). Two other normal family members had the underlying direct insertion of chromosome 5 (dir ins(5)(q31.3q22q23.2)). Molecular genetic and fluorescent hybridisation studies have shown that loci *D5S37* and *D5S98* are outside the deletion whereas loci detected by probes EF5.44 and YN5.48 are lost. As expected, the molecular analyses indicate loss of one allele at the MCC and APC loci. The APC gene is located within band 5q22. Familial direct insertions should be considered as a cause of recurrent microdeletion syndromes.

In 1986, Herrera *et al*¹ reported a mentally retarded male with Gardner's syndrome and an interstitial deletion of the long arm of chromosome 5 (del(5)(q13q15) or (q15q22)). In 1989 an apparently identical deletion of 5q was

reported in two brothers with mild to moderate mental retardation, minor dysmorphic features, and FAPC.² In the intervening period, linkage of the APC gene to probes mapping in the region 5q21-q22 was shown^{3,4} and more recently the APC gene itself has been identified.^{5,6} To date, no evidence of genetic heterogeneity in this condition has been reported and the loss of heterozygosity for chromosome 5 in sporadic cancers has raised the possibility of a major role for this gene in colonic cancers.^{7,8} We report a family in which FAPC has presented in two generations in handicapped subjects as a result of a familial direct insertion. This unique family contributes to our knowledge of the microdeletion syndrome in the vicinity of the polyposis gene, refines the chromosomal localisation of that locus, and illustrates a little recognised mechanism for recurrent deletion.

Material and methods

Chromosome analysis was performed on preparations from cell cultures of peripheral blood with G banding using trypsin and Leishman's stain. In situ hybridisation was carried out using biotinylated cosmid probes C Beg-1, obtained by screening a human genomic library with the probe ECB27,⁹ which recognises the locus *D5S98*, and cL5.79.⁵ Detection was by a fluorescein avidin conjugate and fluorescence microscopy.¹⁰

High molecular weight DNA for restriction analysis was prepared from fresh or frozen blood by standard methods.¹¹ Informative probe DNA clones used were as follows: pi227

Figure 1 The proband aged 25 years. Note the epidermoid cyst below his right eye.

Figure 2 The colon removed from the proband at colectomy showing the large adenoma in the ascending colon.

Figure 3 The pedigree of the family, together with a diagrammatic representation of the molecular genetic analysis. Probes distal to ECB27 (*D5S98*) up to, and possibly including, MC5.61 (*D5S84*) are deleted. The genotypes for the APC intragenic polymorphism are not shown but both affected subjects showed molecular genetic evidence of allele loss.

Figure 4 (A) Normal chromosome 5 (left) and *del(5)(q22q23.2)*. (B) Normal chromosome 5 (left) and *dir ins(5)(q31.3q22q23.2)*.

(*D5S37*)¹² as a 900 bp *HindIII/EcoRI* fragment in piAN7, ECB27 (*D5S98*)⁹ as a 2.8 kb *SalI* fragment in phage lambda, SW15 genomic subclone pL5.71-3 (for the MCC gene)¹³ as a 3 kb *HindIII* fragment in pUC18, EF5-44¹⁴ as a 1.9 *EcoRI/HindIII* fragment in pUC18, YN5.48 (*D5S81*)¹⁴ as a 2.4 kb *TaqI* fragment cloned into the *AccI* site of pUC18, MC5.61 (*D5S84*)¹⁵ as a 3 kb *TaqI* fragment in pUC18 and FB54D, a 2.3 kb fragment of cDNA from the APC gene⁵ which recognises an *MspI* polymorphism.

Five to ten micrograms of purified DNA was digested with the appropriate restriction endonuclease: *TaqI* for the probe MC5.61, *BclI* for pi227, *BglII* for ECB27, and *MspI* for SW15, EF5.44, YN5.48, and FB54D. The DNA was then fractionated in 0.6 to 1.5% agarose gel and transferred to Hybond-N Plus nylon filters (Amersham) as recommended by the manufacturers. Before prehybridisation the DNA was cross linked to the membrane by exposing it to 254 nm UV light for three minutes. Prehybridisation was carried out in 0.5% SDS, 10% dextran sulphate, and 0.8 mol/l NaCl for 15 minutes to several hours at 65°C. Probes were radiolabelled with α -³²P-dCTP(3000 Ci/mmol⁻¹) by the random hexanucleotide primer method to a high specific activity,¹⁶ and hybridised to the filter at 65°C overnight in the presence of 10 μ g l⁻¹ salmon sperm DNA. Filters were washed to a stringency of 2 $\times$ SSC, 1% SDS and autoradiographed at -70°C using Fuji RX-L x ray film.

Figure 5 Metaphase from the proband after in situ hybridisation with C Beg-1, showing locus D5S98 to be present on both the normal and the deleted 5.

Case report

A 25 year old male with mild mental handicap presented with iron deficiency anaemia and positive occult faecal blood. Upper gastrointestinal endoscopy showed two severely dysplastic duodenal adenomata. Sigmoidoscopy was normal, but multiple adenomatous polyps were found at colonoscopy, predominantly on the right side of the colon. Clinical assessment suggested a particular impairment of expressive speech. He had a long midface (fig 1), a receding hairline, multiple epidermoid cysts, bilateral areas of congenital hypertrophy of the retinal pigment epithelium (CHRPEs), and multiple mandibular osteomata. Full thickness resection of the duodenal adenomata was performed followed six months later by colectomy and ileorectal anastomosis. The colon contained more than 100 adenomata with one large lesion in the ascending colon (fig 2). There were no visible lesions in the descending colon though microadenomata were found at histological examination.

Shortly afterwards, the proband's 54 year old mentally retarded maternal aunt presented with abdominal pain. She had no significant dysmorphic features or lipomas but had a spastic gait and severe obstructive airways disease. Gastroscopy was normal but colonoscopy again showed multiple colonic polyps. An abdominal ultrasound examination found bilateral adrenal masses; however, owing to the patient's frail state, no further invasive investigation was performed. Indirect fundoscopy showed multiple bilateral CHRPEs.

The proband's mentally retarded mother died in early adulthood from subarachnoid haemorrhage and only a limited necropsy was performed. His father is untraceable. All other living members of the family (fig 3) are of normal intelligence and showed no evidence of FAPC. Of possible interest is the observation

that all family members carrying the balanced insertion suffer from dyslexia.

LABORATORY RESULTS

The proband showed an abnormal 46,XY, del(5)(q22q23.2) karyotype (fig 4A) as did his mentally retarded aunt. A maternal aunt and a maternal uncle, both of whom were mentally normal with no evidence of FAPC, showed an intrachromosomal insertion of chromosome 5: dir ins(5)(q31.3q22q23.2) (fig 4B). The aunt had no evidence of CHRPEs on eye examination. All other members of the family so far investigated showed apparently normal karyotypes.

In situ hybridisation showed locus D5S98 to be present on both the normal and the deleted 5 in chromosome preparations from the proband (fig 5). Southern blot analysis of DNA extracted from blood of the retarded aunt with FAPC indicated heterozygosity at the D5S98 locus, confirming that this locus is outside the deleted segment. The cosmid probe cL5.79 hybridised to all four chromatids in over 90% of control metaphases, but was consistently seen on one chromosome only in the proband's cells. This indicates deletion of the locus recognised by this probe.

Southern analysis of DNA extracted from blood of the proband and three of his aunts (fig 3) using seven informative DNA probes allowed molecular definition of the deletion. Probe data made possible deduction of parental haplotypes for the aunts. Comparison of the deleted and inserted chromosomes showed that loci D5S37 and D5S98 are outside the deletion whereas loci detected by probes SW15, EF5.44, and YN5.48 (D5S81) are deleted (fig 3). The results are compatible with D5S84 also lying within the deletion but are not conclusive. The smaller allele alone from the *MspI* polymorphism detected by the probe FB54D was present in the proband, whereas only the larger band was present in the affected aunt. Since they have the deleted chromosome in common these results provide evidence of allele loss at the APC locus.

Discussion

From the pedigree, it can be inferred that the proband's mother carried the familial abnormality of chromosome 5 in one form or another, and the fact that she was mentally handicapped suggests that she probably carried the deletion (del(5)(q22q23.2)). The proband's maternal grandparents were described as being of normal intelligence; it can be inferred that one of them carried the balanced insertion, dir ins(5)(q31.3q22q23.2). The interstitial deletion observed in members of this family affected by FAPC could have been produced by a single crossover event between the inserted chromosome 5 and the normal chromosome 5 in meiotic prophase. For complete meiotic pairing of the inserted 5 with its normal homologue, a double insertion loop must form (fig 6). A single crossover within the insertion loop for the region 5q23.2–5q31.3

Figure 6 Diagram to show the normal chromosome 5 and the dir ins(5) forming a double insertion loop at meiosis. A single crossover within the region 5q23.3-5q31.3 gives rise to gametes carrying rec del(5) or rec dup(5).

Figure 7 Diagram to show the normal chromosome 5 and the dir ins(5) pairing at meiosis with the inserted region (5q22-q23.2) forming unpaired loops. A single crossover between the loops gives rise to gametes carrying rec del(5) or rec dup(5).

would then result in one daughter chromosome with the deletion (rec del(5)(q22q23.2) dir ins(5)(q31.3q23.2)) and one daughter chromosome with a duplication of the same region (rec dup(5)(q22q23.2) dir ins(5)(q31.3q22q23.2)).

Alternatively, meiotic pairing of the chromosome 5 homologues in subjects with the insertion may occur as in fig 7, with the inserted segment (5q22–5q23.2) forming unpaired loops. Again a single crossover between the two unpaired loops would produce one deleted daughter chromosome (rec del(5)(q22q23.2) dir ins(5)(q31.3q22q23.2)) and one duplicated daughter chromosome (rec dup(5)(q22q23.2) dir ins(5)(q31.3q22q23.2)).

A recent review of cases of intrachromosomal insertions¹⁷ suggested that duplication recombinants from insertions are more frequently encountered than deletion recombinants such as have occurred in this family. The proband did not display overt dysmorphic features though his long midface and receding hairline resembled the features of the brothers described by Hockey *et al.*²

The fact that in situ hybridisation showed ECB27 to be present on both the normal and deleted chromosome 5 in the proband placed the proximal breakpoint of the deletion between that marker and cL5.79 which is deleted.

Southern analysis showed that the deletion encompassed loci telomeric to cL5.79 detected by the probes SW15 (MCC gene), FB54D (APC gene), EF5.44, and YN5.48. Using information available from published molecular genetic studies,⁵ our results suggest that the deletion is from 2 to 5 Mb in size.

Previous linkage mapping studies^{4,15} have suggested that the FAPC gene is located in band 5q22 close to the junction with band 5q21, whereas the most recent publications state that the locus is in 5q21 without presenting further data. The proximal breakpoint of the deletions in this family appears to be at approximately the middle of the band 5q22, indicating that the gene for FAPC is located in the distal half of band 5q22.

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