Adenomatous polyposis coli and a cytogenetic deletion of chromosome 5 resulting from a maternal intrachromosomal insertion

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Abstract

We present the clinical and laboratory findings in an institutionalised adult patient originally referred for autism. A high risk of colorectal cancer was predicted when an interstitial deletion of the long arm of chromosome 5, del(5)(q15q22.3), was detected in her lymphocytes and deletion of the MCC and APC genes confirmed by molecular analysis. Adenomatous polyposis coli and carcinoma of the rectum were subsequently diagnosed in the patient. She was profoundly mentally retarded, autistic, and had minor dysmorphic features consistent with those of previous patients with similar deletions.

The deletion arose as a result of recombination within the small insertion loop formed at melosis by the direct insertion (dir ins(5)(q22.3q14.2q15)) found in the patient’s mother. This family further confirms the cytogenetic mapping of both MCC and APC genes to 5q22 and comparison with other recent cases suggests that both genes and their closely linked markers lie within the 5q22.1 subband.

Materials and methods

Lymphocytes were cultured by standard methods including semi-synchronisation with fluorodeoxyuridine and release with thymidine. Fibroblasts were also cultured by standard methods.

DNA was extracted from blood samples by standard methods.13 Samples were digested with the appropriate restriction endonuclease and size fractionated by agarose gel electrophoresis. DNA was transferred to either Gene Screen Plus (NEN, Dupont) or Hybond N Plus (Amer sham, UK) and hybridised according to the manufacturer’s specifications to DNA probes radiolabelled with [α-32P]dCTP (3000 Ci mmol-1) by the random hexanucleotide primer method.14 Membranes were washed to a stringency of 2 × SSC, 1% SDS at 65°C and autoradiographed at −70°C using Fuji RX x ray film.

In informative probe DNA clones used were the SW15 genomic subclone plS.71-3 (for the MCC gene)6 as a 3 kb HindIII fragment in pUC18, and FB54D, a 2-3 kb fragment of cDNA from the APC gene which recognises an MspI polymorphism.15

Results

CLINICAL FINDINGS

The proband was referred for chromosome analysis for autism at the age of 43 years. In addition, she had profound mental retardation, requiring institutional care since childhood.

She was born at term by normal delivery to parents both aged 34 years. She had three healthy younger sibs, one sister and two brothers. There was no family history of mental retardation or polyposis. At the age of 75 years the mother had an adenocarcinoma of the rectum excised. This was an early stage, moderately differentiated tumour arising in a villous adenoma.

The proband weighed only 2040 g at birth and had some difficulties with feeding and weight gain in the newborn period. Her early milestones were delayed. She never learnt to talk, never became continent, and was accepted into residential care at the age of 3 years. Her general health was good for most of her life. As an adult she was considered to have a mental age of 2 years and she exhibited autistic behaviour from before 3 years of age. She therefore met the ICD 10 criteria for profound mental retardation and childhood autism (persis tive developmental disorder).18 A right sided strabismus and episodes of conjunctivitis were also noted.

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After cytogenetic and molecular analyses were carried out at the age of 43 years, the proband developed diarrhea which was present for a few months before becoming blood stained. Colonoscopy showed hundreds of left sided polyps, confirmed on histological examination of a 5 cm polyp to be adenomatous. In addition, 6 cm from the anorectal margin there was a large fungating carcinoma. The examination was not continued to the right side of the colon so more proximal carcinomas may also have been present. Histology confirmed the diagnosis of adenocarcinoma of the rectum. In view of the probably inoperable nature of the tumour, it was decided that supportive care only would be given. The patient lost weight rapidly and died at the age of 45 years. Permission for necropsy was denied.

We examined the proband three months before her death (fig 1). She was 162 cm in height with a head circumference of 54 cm. Her head and face were broad with deep set eyes, a prominent forehead and jaw, and small ears of slightly increased thickness. The gums were thickened, the skin was pale, but no unusual skin lesions were detected. There were no gross neurological signs but mental retardation was profound.

Figure 1  Facial appearance of the proband at the age of 45.

Figure 2  Partial karyotypes of the proband and her mother with the normal chromosome on the left in each case. Upper pair: chromosomes 5 from the proband’s mother with arrows showing the position of band S514.3 on the normal and inserted chromosomes. Lower pair: chromosomes 5 from the proband with arrows showing the extent of the deleted material.

Figure 3  Idiograms of normal and rearranged chromosomes 5. Left: normal. Middle: the maternal inserted 5. Right: the deleted 5 from the proband. Black shading indicates what we have chosen to call the “inserted” segment which is retained in the proband. Snipping indicates the corresponding “interstitial” segment which is deleted in the proband.

CYTOGENETIC AND MOLECULAR GENETIC FINDINGS

An interstitial deletion of the long arm of chromosome 5 with breakpoints at q15 and q22 was reported when the proband was first referred for chromosome analysis. This was confirmed in a later sample and the breakpoints refined to q15 and q22.3 (figs 2 and 3). The paternal karyotype was normal but an intrachromosomal insertion within the long arm of chromosome 5 was found in the proband’s mother (figs 2 and 3). Her karyotype is: 46,XX,dir ins(5)(q22.3q14.2q15) or, in full, 46,XX,dir ins(5)(p1ter→q14.2:q15→q22.3::q14.2→q15::q22.3→qter). One of the predictable outcomes of recombination between this inserted chromosome and its normal homologue was consistent with the deletion of q15 to q22.3 found in the proband and the proband’s karyotype is therefore formally: 46,XX,rec(5)del(q15q22.3),dir ins(5)(q22.3 q14.2q15)mat. As this region included the known cytogenetic location of the APC gene at that time, it was strongly suggested that the proband should be examined for evidence of colorectal and other neoplasms.

Southern analysis with probes for the MCC (probe SW15) and APC (probe FB54D) genes showed that only the paternal alleles had been inherited by the proband (fig 4) indicating that both loci lay within the deleted region. Other probes for this region, such as YN3.48(D5S81)
and MC5.61(D5S84), were only compatible with deletion, while probes 227(D5S377), C11p11(D5S71), ECB25(D5S98), EF5.44, MCC40, and L5.62 were homozygous in the proband and both parents and therefore uninformative.

As spontaneous chromosome instability has previously been reported in lymphocytes and low passage fibroblasts from patients with Gardner’s syndrome and normal chromosomes, 18–20 50 cells from each tissue were screened for such abnormalities in the proband. One of 50 cells from skin had an apparently balanced 2;15 translocation but there was no other evidence of structural rearrangements, rings, fragments, dicentrics, or other abnormal clones.

Cell lines from this family have been established at the European Collection of Animal and Cell Cultures under reference numbers DD134 and DD1079 (proband), DD136 (mother), and DD140 (father).

**Patients with interstitial deletion of 5q encompassing the APC/MCC gene**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Proximal deletions</th>
<th>Distal deletions</th>
<th>Large</th>
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<tbody>
<tr>
<td></td>
<td>1q12-q21.3</td>
<td>2q11-q22</td>
<td>3q</td>
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<tr>
<td>Age at examination (y)</td>
<td>13</td>
<td>42</td>
<td>33</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>APC/MCC loss</td>
<td>c</td>
<td>—</td>
<td>Mat</td>
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<tr>
<td>Parental origin</td>
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<tr>
<td>Subcutaneous lesions</td>
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<tr>
<td>CHRPE</td>
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<td>Mandibular osteoma</td>
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<td>Polyposis/adenoma</td>
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<td>Gastrointestinal cancer</td>
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<td>Abdominal/adrenal mass/dermoid tumour</td>
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<tr>
<td>Mental retardation</td>
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<td>Large/broad bone</td>
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<td>High prevalence of bore</td>
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<tr>
<td>Hypertension</td>
<td>+</td>
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<tr>
<td>Macro/megadactylia</td>
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<tr>
<td>High arched palate</td>
<td>—</td>
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<tr>
<td>Abnormal ears</td>
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<tr>
<td>Eye abnormalities (not CHRPE)</td>
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<td>Limb abnormalities</td>
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<tr>
<td>Receding hairline/early balding</td>
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<td>Hip abnormalities</td>
<td>—</td>
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<td>Hypotonia as infant</td>
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1 Lindgren et al. 11 Patient SD. 2 Herrera et al. 13, 4 Hockey et al. 8 Present case. 6 Hodgson et al. 12 Case 2. 7 Lindgren et al. 11 Patient EC. 8 Hodgson et al. 12 Case 1. 9, 10 Cross et al. 10
11 Kobayashi et al. 12 de Michaelena et al. 16
* Positive finding: +, slightly affected
* + Marked positive finding
* — Absent or not investigated
* c Confirmed by molecular methods
12 Limited phenotypic information available to Lindgren et al. 11
13 Brothers
14 Father, aunt and nephew
connected with the transition from adenoma to carcinoma may be located within the region proximal to the MCC/APC genes between bands q15 and q21.3.

Phenotypic information was available in six of seven patients with proximal deletions including the subject of this report (patients 1 to 6). Mild dysmorphic features including a large or broad head (3/6), high or prominent forehead (5/6), hypertelorism (4/6), macro-or prognathia (5/6), and a high arched palate (4/6) were found. Case 2 of Hodgson et al.² was unique in having the additional features of a marfanoid habitus and Caroli's syndrome, which have not been reported in any other patient with a similar deletion. Although all were mentally retarded, four had only mild to moderate mental retardation and the brothers reported by Hockey et al.⁵ were living semi-independent lives. By contrast, our patient and the patient of Herrera et al.⁶ were severely retarded and institutionalised from an early age.

Of the four patients with distal deletions (patients 8 to 11), three had deletions of relatively small size and were minimally dysmorphic (patients 8 to 10). Two of them had mild to moderate mental retardation (patients 9 and 10), and the third (patient 8) was described as having low normal intelligence and worked as a typist.⁷ The fourth case (patient 11), a Japanese boy of 15, had a large deletion, mental retardation, and a significant level of facial dysmorphism.⁸

There was little phenotypic overlap between the single infant with a large deletion (q14q13.1) (patient 12) and any of the adults in either group. By contrast, this patient shared most dysomorphic features with an infant (reported by Lindgren et al.⁹) who had a smaller distal deletion (q22.3q31.3) which did not include the APC/MCC locus. Their features included failure to thrive, short stature, high forehead, hypertelorism, marked epicantus folds, flat nasal bridge, abnormal ears, micrognathia, short neck, hip abnormalities, and severe mental retardation.

Intrachromosomal insertions are uncommon abnormalities with only 27 examples gathered in the recent review by Madan and Menkov.¹⁰ One third of these (9/27) were paracentric inversions confined to a single chromosome arm. When these nine are combined with the present insertion, the long arm of chromosome 5 is overrepresented with four out of 10 examples, two of which are the familial subjects of this report and that of Cross et al.¹¹. It is interesting in this context that the otherwise rare direct transmission of a deletion from a mother with an unbalanced karyotype to one or more offspring has occurred by implication in three of the families in the table; the mother of the two brothers described by Hockey et al.⁶ was mentally retarded and died of carcinoma of the colon in her 40s, the mother of the proband in the family of Cross et al.¹⁰ died of colon cancer in her 60s and was reported to have been of slightly reduced intelligence. It would seem that relatively large monosomies for chromosome 5 are therefore compatible not only with livebirth but with fertility and survival into late adult life. Thus, their familial occurrence, the relaxed selection against them, and their ascertainment through APC may account for their relative frequency.

With regard to the mechanism by which deletions are generated in balanced insertion carriers, our family strongly suggests that a double loop can be formed between an intrachromosomal insertion chromosome and its normal homologue at meiosis, even where the insertion is small in relation to the size of the chromosome. This is because a single recombination within the small insertion loop itself is the only established route by which the deletion found in the proband could have been generated from the direct insertion found in the mother (fig 5). This is in contrast with the family of Cross et al.¹¹, where it was the inserted segment itself which was deleted and where the same deletion could have resulted whether the insertion chromosome and its normal homologue formed a completely paired double loop at meiosis or whether the inserted

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Figure 5 Illustration of complete (left) and incomplete (right) pairing at meiosis of the normal and inserted chromosomes. A single crossover (as indicated) within the insertion loop (black shading) will result in deletion (or duplication) of the interstitial segment (stippled) as found in the present family. Recombination within the interstitial segment (stippled) of the incompletely paired chromosomes will only produce deletions or duplications of the inserted segment itself.
segment failed to pair with its normal homologue (fig 5). Chiasma frequencies are not evenly distributed along chromosome arms and it is therefore interesting that evidence from the single male meiosis from which the data were assembled suggests that a relatively high chiasma frequency is observed in the q14.2→q15 region which includes the small inserted region in our case.22

Madan and Menko22 have estimated that the risk of liveborn unbalanced offspring to carriers of intrachromosomal insertions is 15%, but caution that the risk within individual families may well exceed this. However, the surviving members of this family have not yet been willing to accept cytogenetic screening.

Comparison of the present patient with previously reported deletions underlines, with one exception, the assignment of the MCC and APC loci to band 5q22 (fig 6). The exception is patient SD of Lindgren et al11 (patient 1, fig 6) whose distal breakpoint is given as q21.3 while, by contrast, the deletion in the family of Cross et al10 (patients 9 and 10, fig 5) is thought to have a proximal breakpoint in the middle of 5q22. Loss of APC or MCC has been confirmed by molecular methods in both these deletions. Thus, either the cytogenetic interpretations are inaccurate, the rearrangements are more complex than “simple” deletions, MCC/APC may map to more than one location in different persons, or the deletions reported by both sets of authors extend a little further in each case giving a minimum region of overlap within 5q22.1. Examining the published photographs of the exceptional case of Lindgren et al11 suggests that band 5q22.2 is retained, in which case 5q22.1 would be the only cytogenetic region common to all reported cases. By implication, APC, MCC, and closely linked marker loci can be cytogenetically mapped to the same subband.

This family was previously briefly presented as family 2 in an abstract included in HGM11.12 LVB is supported by Quest Cancer Test and DME is supported by the Cancer Research Campaign.