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 meiosis 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.

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An interstitial deletion of chromosome 5 reported in 1985¹ led to the mapping of adenomatous polyposis coli (APC) and Gardner's syndrome to the central long arm of chromosome 5.^{2,3} Two genes 150 kb apart have subsequently been identified in this region: MCC which is mutated in sporadic colon cancer⁴ and APC which is mutated in sporadic⁵ as well as the inherited forms of colon cancer found in familial adenomatous polyposis (FAP) families.⁶ Gardner's syndrome was previously distinguished from APC and FAP by the occurrence of extracolonic neoplasms but the two conditions are now thought to result from mutations of the same gene.⁷

Since the original report by Herrera *et al*,¹ a small number of further cytogenetic deletions of this region associated with APC or the Gardner's phenotype have been presented⁸⁻¹² and it has been further suggested that two distinct 5q syndromes exist.¹¹

We report the phenotypic, cytogenetic, and molecular genetic findings in the second family in which an otherwise rare intrachromosomal insertion has given rise to a 5q deletion and predisposition to APC.

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 (Amersham, UK) membrane and hybridised according to the manufacturer's specifications to DNA probes radiolabelled with [α^{32} P]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.

Informative probe DNA clones used were the SW15 genomic subclone pL5.71-3 (for the MCC gene)⁴ as a 3 kb *Hind*III fragment in pUC18, and FB54D, a 2.3 kb fragment of cDNA from the APC gene which recognises an *Msp*I 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 (pervasive developmental disorder).¹⁶ A right sided strabismus and episodes of conjunctivitis were also noted.

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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 5p14.3 on the normal and inserted chromosomes. Lower pair: chromosomes 5 from the proband with arrows showing the extent of the deleted material.

After cytogenetic and molecular analyses were carried out at the age of 43 years, the proband developed diarrhoea 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 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. Stippling 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)(pter→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)(q22.3q14.2q15)mat. As this region included the known cytogenetic location of the APC gene at that time,¹⁷ 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 YN5.48(D5S81)

Figure 4 Inheritance of alleles of the SW15 probe for the "MCC" gene (upper figures) and the FB54D probe for the "APC" gene (lower figures). The proband has inherited only one paternal allele in each case. The shading represents the polyposis, mental retardation, and unbalanced karyotype found in the proband, and the balanced chromosomal rearrangement found in her mother.

and MC5.61(D5S84), were only compatible with deletion, while probes π 227(D5S37), C11p11(D5S71), ECB27(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 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).

Discussion

To the best of our knowledge, only one previous family has been reported in which predisposition to the development of colorectal cancer has been inherited as a result of a balanced cytogenetic rearrangement giving rise to a deletion of chromosome 5 with constitutional loss of heterozygosity for the MCC and APC genes.¹⁰

Including the subject of this report, we are aware of 12 persons with cytogenetic deletions which encompass the MCC/APC region (table).¹⁸⁻¹² Molecular confirmation of deletion of either or both genes has been obtained in the present patient and four others.¹⁰⁻¹² Ten of 12 of these patients had adenomatous polyposis coli, while of the remaining two, one boy had had subcutaneous lesions removed from his legs by the age of 13¹² and only the 1 year old infant had no evidence of APC.¹⁹ Extracolonic manifestations including subcutaneous lesions (especially epidermal cysts), mandibular osteomata, desmoid tumours, or an abdominal or adrenal mass were found in seven of 10 patients with APC. Thus the proportion of patients with the Gardner's phenotype is similar in families with cytogenetic deletions and in the bulk of APC kindreds who have other mutations (for example, those reported to the Johns Hopkins Hospital Colon Polyposis Registry²⁰).

These 12 patients may be divided into three groups (table): those with proximal deletions extending to band q15 (patients 1 to 7), those with deletions which span or extend distal to band q22 (patients 8 to 11), and a single infant with a large deletion extending from band q15 to q31.2 (patient 12). Carcinoma of the colon or rectum or both was found in four of six patients over the age of 30 with proximal 5q deletions (patients 2 to 7) but in neither of the patients aged 38 and 54 with more distal 5q deletions (patients 8 and 10). If substantiated in further patients, this suggests that a factor

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

Patient Deletion	Proximal deletions							Distal deletions				Large
	1 q15q21.3	2 q15q22	3† q15q22	4† q15q22	5 q15q22.3	6 q15q22.3	7* q15q23.2	8 q21.3q23.1	9† q22q23.2	10† q22.1q23.2	11 q22.1q31.1	12 q15q31.2
Age at examination (y)	13	42	33	31	40	31	30	38	25	54	15	1
Sex	M	M	M	M	F	M	M	F	M	F	M	F
APC/MCC loss	c	—	—	—	c	c	—	c	c	—	—	—
Parental origin	—	—	Mat	Mat	Mat	—	—	—	Mat	Pat	—	—
Subcutaneous lesions	+	+	+	+	—	+	—	+	+	—	+	—
CHRPE	—	—	—	—	—	—	—	—	+	+	+	—
Mandibular osteomata	—	—	—	—	—	—	—	—	+	+	+	—
Polyposis/adenomata	—	+	+	+	+	+	+	+	+	+	+	—
Carcinoma colon/rectum	—	+	+	+	+	+	—	—	—	—	—	—
Abdominal/adrenal mass/desmoid tumour	—	+	—	—	—	—	—	+	—	+	—	—
Mental retardation	+	++	+	+	++	+	++	±	+	+	+	++
Large/broad head	—	—	+	+	—	—	—	—	—	—	—	—
High/prominent forehead	+	—	+	+	—	+	—	—	—	—	—	+
Hypertelorism	+	—	±	±	+	—	—	—	—	—	+	+
Macro/prognathia	+	+	+	+	+	—	—	—	—	—	—	+
High arched palate	+	—	+	+	—	+	—	—	—	—	+	+
Abnormal ears	+	—	—	—	+	+	+	—	—	—	+	+
Eye abnormalities (not CHRPE)	—	—	—	—	+	—	+	—	—	—	+	—
Limb abnormalities	—	+	+	—	—	—	+	—	—	—	—	—
Receding hairline/early balding	—	—	+	+	—	—	—	—	+	—	—	—
Hip abnormalities	—	+	—	—	—	—	—	—	—	—	—	+
Hypotonia as infant	—	—	—	—	+	+	+	—	?	—	±	+

1 Lindgren *et al.*¹¹ patient SD. 2 Herrera *et al.*¹ 3, 4 Hockey *et al.*⁸ 5 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.*⁹ 12 de Michaelena *et al.*¹⁹

+ Positive finding ± slightly affected

++ Marked positive finding

— Absent or not investigated

c Confirmed by molecular methods

* Limited phenotypic information available to Lindgren *et al.*¹¹

† Brothers

‡ Familial, 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.*¹² 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.*⁸ were living semi-independent lives. By contrast, our patient and the patient of Herrera *et al.*¹ 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.¹² The fourth case (patient 11), a Japanese boy of 15, had a large deletion, mental retardation, and a significant level of facial dysmorphism.⁹

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 dysmorphic features with an infant (reported by Lindgren *et al.*¹¹) 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 epicanthic 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.²¹ 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.*¹⁰ 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.*⁸ 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 in early adulthood of subarachnoid haemorrhage but was also mentally retarded and probably had an unbalanced insertion product, and the mother of case 1 of Hodgson *et al.*¹² 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 I, 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.*¹⁰ 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

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 Menko²¹ 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 al*¹¹ (patient 1, fig 6) whose distal breakpoint is given as q21.3 while, by contrast, the deletion in the family of Cross *et al*¹⁰ (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 al*¹¹ 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 HGM11.²³ LVB is supported by Quest Cancer Test and DME is supported by the Cancer Research Campaign.

Figure 6 Illustration of the extent of the deletions in the 12 subjects listed in the table. The area corresponding to band 5q22.1 is indicated by the stippled area and molecular confirmation of loss of the MCC or APC genes or both by the letter "c".

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