H ANTON-CULVER
University of California, Irvine, USA

Correspondence to: Dr Seltmann, Essen University Medical School, Virchow Strasse 173, Institute of Cell Biology, D-45147 Essen, Germany

1 Florida G, Piantanida M, Minelli A, *et al*. The same molecular mechanism at the maternal meiosis I produces mono- and dicentric 8p duplications. *Am J Hum Genet* 1996;**58**:785-96.
2 Guo WJ, Faith CD, Zapata MC, Miller ME. Clinical and cytogenetic findings in seven cases of inverted duplication of 8p with evidence of a telomeric deletion using fluorescence in situ hybridization. *Am J Med Genet* 1995;**58**:230-6.
3 Feldman GL, Weiss L, Phelan MC, Schroer RJ, Van Dyke DL. Inverted duplication of 8p: ten new patients and review of the literature. *Am J Med Genet* 1993;**47**:482-6.
4 Minelli A, Floridia G, Rossi E, *et al*. D8S7 is consistently deleted in inverted duplications of the short arm of chromosome 8 (inv dup 8p). *Hum Genet* 1993;**92**:391-6.
5 Kerangueven F, Essioux L, Dib A, *et al.* Loss of heterozygosity and linkage analysis in breast carcinoma: indication for a putative third susceptibility gene on the short arm of chromosome 8. *Oncogene* 1995;**10**: 1023-6.
6 Chuaqui RF, Sanz-Ortega J, Vocke C, *et al*. Loss of heterozygosity on the short arm of chromosome 8 in male breast carcinomas. *Cancer Res* 1995;**55**:4995-8.
7 Dib A, Adelaide J, Chaffanet M, *et al.* Characterization of the region of the short arm of chromosome 8 amplified in breast carcinoma. *Oncogene* 1995; **10**:995-1001.
8 Chaffanet M, Popovici C. t(6;8) and t(8;13) translocations associated with stem cell myeloproliferative disorders have close or identical breakpoints in chromosome region 8p11-12. *Oncogene* 1998;**16**:945-9.

J Med Genet 2000;**37**:71–75

Appendiceal carcinoma complicating adenomatous polyposis in a young woman with a de novo constitutional reciprocal translocation t(5;8)(q22;p23.1)

EDITOR—Familial adenomatous polyposis (FAP) is an autosomal dominant condition characterised by the presence of more than 100 adenomatous polyps in the colon and rectum. Polyps generally first appear in the second or third decade of life and are usually most numerous distally. Left untreated, colorectal cancer is virtually inevitable and generally arises in the fourth or fifth decade.[1] Adenocarcinoma of the appendix is an uncommon neoplasm and has only rarely been reported in association with FAP.[2]

The gene responsible for FAP, *APC*, was initially localised to the long arm of chromosome five (5q) by linkage.[3 4] This followed a case report describing carcinomas of the rectum and ascending colon, adenomatous polyposis, mental retardation, and various dysmorphic features in a 42 year old man with a constitutional deletion of 5q.[5] Most patients with FAP have normal karyotypes.[6] Mental retardation and dysmorphic features are unusual in such people but characterise those rare patients with cytogenetically visible 5q deletions and FAP.[5–12] The few reports detailing the clinical findings in patients with submicroscopic deletions of *APC* suggest that such people may be mentally normal.[13 14]

In this report we describe a patient with adenomatous polyposis, mental retardation, and an apparently balanced translocation t(5;8)(q22;p23.1) causing submicroscopic deletion of *APC* and *MCC*.

Clinical data were obtained by review of medical records. In addition, the patient was interviewed and examined by two of the authors (JF and AS) before her death. Cytogenetic studies were performed using standard techniques on a 72 hour peripheral blood culture with GTG banding, as previously reported.[6]

Slides for fluorescence in situ hybridisation (FISH) were obtained using the cell suspension retained after routine cytogenetic harvest. RNAse treatment, probe and chromosomal denaturation, and hybridisation conditions were as previously described[15] with the stringencies adjusted after assessment of the optimal conditions for each probe combination. The biotinylated probes were detected with

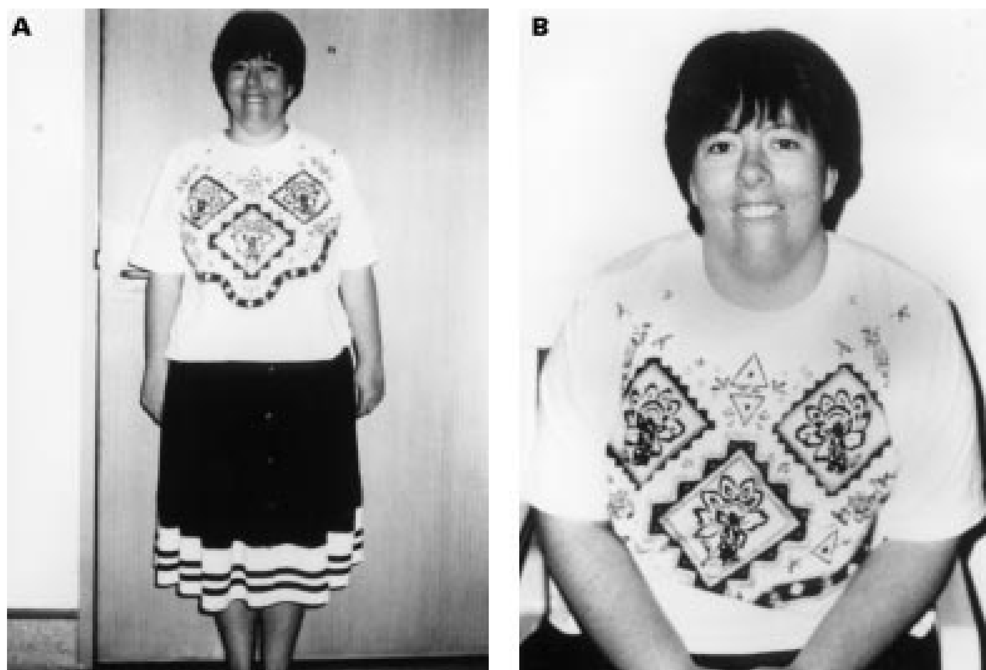

Figure 1 (A, B) The patient aged 26 years.

avidin-FITC (Calbiochem) followed by biotinylated anti-avidin (Vector) and finally avidin-FITC. The digoxigenin labelled probes were detected with mouse anti-digoxigenin followed by sheep anti-mouse Ig-rhodamine conjugated antibody (Boehringer Mannheim). Chromosomes, counterstained with DAPI (Sigma) and visualised by fluorescence microscopy (Zeiss), were captured using a computer image analysis system (Cytovision).

The following probes were used in FISH studies: MCC 40CI (partial *MCC* cDNA nucleotides 1634-3969), FB70B (partial *APC* cDNA nucleotides 2877-6452), and APC (full length *APC* cDNA) each kindly provided by Dr Bert Vogelstein (Johns Hopkins Oncology Center, Baltimore); D5S23 (chromosome 5p probe, Vysis); EGR-1 (chromosome 5q31 probe, Vysis); CHR8B/wcp8 (chromosome 8 library probe, Cambio); D8Z1 (chromosome 8 centromere probe, Oncor).

Genomic DNA was extracted from blood samples using the Instagene purification matrix (Biorad) according to the manufacturer's instructions. Each polymerase chain reaction (PCR) used 50-100 ng genomic DNA, 50 pmol/l of each oligonucleotide primer, 0.2 mmol/l of each dNTP (Pharmacia), 1.25 U Ampli*taq* DNA polymerase (Perkin Elmer) in Ampli*taq* reaction buffer (10 mmol/l Tris-HCl, 50 mmol/l KCl, MgCl optimised for each primer pair, pH 8.3) to a final volume of 25 µl. Thirty five amplification cycles were performed using an FTS-320 Thermal Sequencer (Corbett Research).

An *Rsa*I polymorphism in exon 11 of *APC*[16] was analysed by amplification of exon 11 and digestion of the PCR product with *Rsa*I (Boehringer). Reaction products were separated by electrophoresis in a 10% non-denaturing polyacrylamide minigel (Biorad) and visualised by ethidium bromide staining. The 255 bp amplification product yielded digestion fragments of 155 bp and 100 bp. A CA repeat polymorphism within *MCC* (CAMBC)[17] and a CA repeat polymorphism between *APC* and *MCC* (LNS-CA)[13 18] were labelled by [αS^{35}]dATP incorporation during PCR and characterised by electrophoresis in a 6% denaturing polyacrylamide gel and autoradiography as previously reported.[19]

The patient (fig 1) was the second child of a 32 year old mother and a 33 year old father. Her older brother was well and mentally normal. She was born prematurely at 29 weeks' gestation after an uncomplicated pregnancy. There were no major problems in the neonatal period but she was slow to speak and did not walk until the age of 2 years. At that time she began to have generalised convulsions. These were only partially controlled by medication and continued until the age of 18. Her performance at school was poor. Her hospital file notes an estimated IQ between 70 and 80 at the age of 10 although the method of assessment was not recorded. Physical examination at this time showed crowded dentition and she later required extensive dental work. The posterior hairline was noted to be low. The head circumference was normal (75th centile) while height was on the 10th centile. The third and fourth toes were short with the fifth toe longer than the fourth. Bilateral genu recurvatum was evident and the patient was mildly ataxic. There were no skin lesions and the fundi were normal.

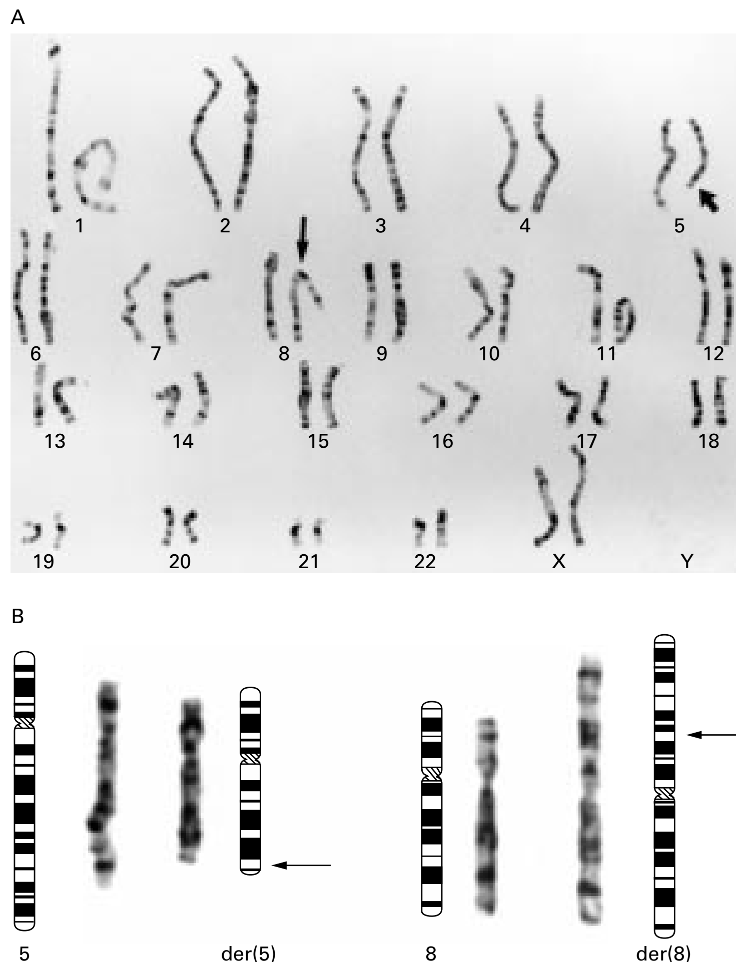

Figure 2 (A) GTG banded karyotype showing the translocation t(5;8)(q22;p23.1) in the patient. The arrows indicate the sites of the breakpoints. (B) Normal and derivative chromosomes 5 and 8.

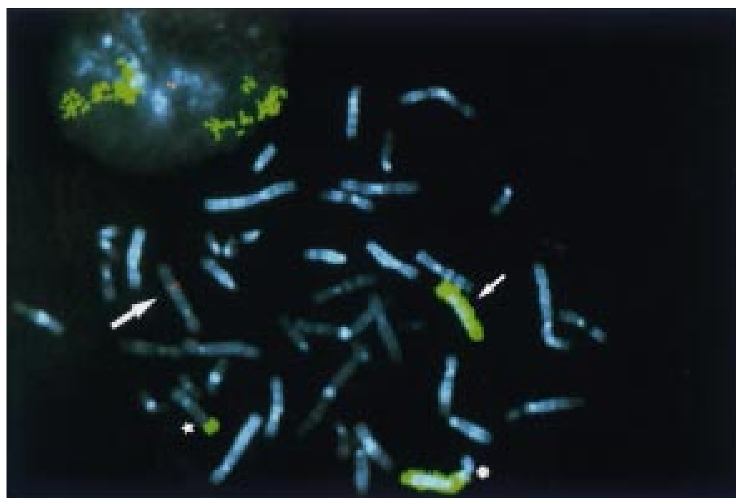

Figure 3 FISH study using the chromosome 8 library probe, CHR8B/wcp8, labelled green and the MCC gene probe, MCC 40CI, labelled red. An MCC signal is seen only on the normal chromosome 5 (large arrow) and not on either the der(8) (asterisk) or the der(5) (star) chromosomes. The normal chromosome 8 is indicated by a small arrow.

Visual acuity and hearing were unimpaired. An EEG at the age of 4 was abnormal with excessive generalised slow wave activity. A cerebral CT scan at 9 years showed mild ventricular dilatation and localised atrophy in the frontal areas and bordering the interhemispheric commissure. She attended a special school until the age of 17 and was actively involved in sports. Subsequently she worked successfully in a sheltered workshop while living independently with a group of mildly handicapped people.

She presented acutely at 26 years of age with a 24 hour history of right iliac fossa pain. She was febrile and had guarding and rebound tenderness at the site of her pain. A diagnosis of acute appendicitis was made and an appendicectomy performed. The appendix contained a mucin secreting carcinoma arising in a dysplastic villous adenoma and invading through the full thickness of the muscle wall. A right hemicolectomy was performed. The appendiceal stump had foci of adenomatous change but there was no residual carcinoma. Numerous small adenomatous polyps were noted throughout the right colon but the exact number was not recorded. Sigmoidoscopy subsequently showed left sided polyps but these were less numerous than had been found proximally. Eight months after hemicolectomy she presented again with a painful right iliac fossa mass. Laparotomy confirmed local tumour recurrence which could be only partially excised. She was treated with 5-fluorouracil and folinic acid but died approximately 12 months later. A post mortem examination was not performed.

Cytogenetic analysis showed a female karyotype with a translocation involving chromosomes 5 and 8 at breakpoints q22 and p23.1 respectively (fig 2). The translocation was cytogenetically balanced. Both parents had normal karyotypes. The patient's brother refused testing.

FISH studies were performed using several probe combinations: APC, D5S23, and EGR-1; FB70B and D5S23; FB70B and D8Z1; MCC 40CI and CHR8B/wcp8 (fig 3); and MCC 40CI and D8Z1. These studies confirmed the translocation and showed submicroscopic deletion of both *APC* and *MCC*. The *APC* and *MCC* probes hybridised only to the normal chromosome 5 and not to the der(5) or der(8) chromosomes.

To confirm that the translocation identified had resulted in deletion of the *APC* and *MCC* genes, intragenic polymorphisms were examined in DNA from the patient and her parents. The *Rsa*I polymorphism in exon 11 of *APC* was informative in the family. The patient's mother was homozygous for absence of the restriction site whereas her father was homozygous for presence of the restriction site. The patient had only a maternally derived allele, sug-

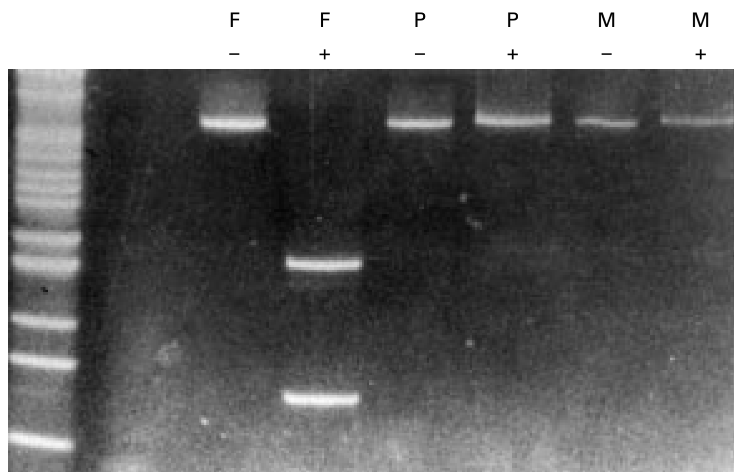

Figure 4 Exon 11 of APC was amplified from the patient (P), her father (F), and her mother (M). The PCR product from each source was electrophoresed in a 10% polyacrylamide gel either with (+) or without (−) preceding digestion with RsaI. The first lane shows a molecular weight marker (MspI digest of pBR322).

Table 1 *Clinical features in patients with cytogenetic abnormalities and adenomatous polyposis*

	Reference 5	7	7	8	9	9	11	10	10	12	14	14	This report
Cytogenetics	del(5)(q15q22)	del(5)(q15q22)		del(5)(q22.1q31.1)	del(5)(q22q23.2)		del(5)(q15q23.2)	del(5)(21.3q23.1)	del(5)(q15q22.3/ 23.1)	del(5)(q15q22.3)	t(5;10)(q22;q25)		t(5;8)(q22;p23.1)
APC gene	NR	NR		NR	Del		Del	Del	Del	Del	Dis/ partial del		Del
No of cases	1	2		1	2		1	1	1	1	2		1
Age(s)	42	31	33	15	25	54	30	38	31	43	32	47	26
Polyposis (distribution)	+ (Diffuse)	+ (NR)	+ (NR)	+ (Diffuse)	+ (Prox)	+ (NR)	+ (NR)	+ (Prox)	+ (Prox)	+ (NR)	+ (Diffuse)	+ (Prox)	+ (Prox)
Cancer (site)	+ (R,A)	–	–	–	–	–	–	–	–	+ (R)	–	+ (C)	+ (App)
ECMs	Des	Den, SC		Den, SC, RET, O	SC, RET, O, DP		NR	Des, SC, DP	Den, SC, RET	NR	DP		Den
Mental retardation	+ (Severe)	+ (Mod)	+	+ (NR)	+ (Mild/mod)	+	+ (Severe)	± (Low normal)	+ (Mild)	+ (Severe)	–	–	+ (Mild)
Dysmorphic features	+ (Multiple)	+ (Multiple)	+	+ (Multiple)	+ (Minor)	–	+ (Multiple)	+ (Minor)	+ (Multiple)	+ (Minor)	–	–	+ (Minor)

A: ascending colon; App: appendix; C: caecum; D: descending colon; Del: deletion; Den: dental abnormalities; Des: desmoid tumour; Dis: disruption; DP: duodenal polyp; ECMs: extracolonic manifestations of Gardner's syndrome; NR: not reported; O: osteoma; Prox: proximal; R: rectum; Ret: congenital hypertrophy of the retinal pigment epithelium; S: sigmoid colon; SC: skin cysts; T: transverse colon.

gesting that she was hemizygous for this region of *APC* and that the translocation had involved the paternally derived chromosome (fig 4). The *MCC* CA repeat polymorphism was not informative in this family but the patient amplified only one allele consistent with hemizygosity for *MCC*. Both parents also shared this allele. Analysis of LNSCA, between *APC* and *MCC*, was informative in the family. Again, the patient had inherited a maternal but not a paternal allele. Seven microsatellite polymorphisms from other chromosomes were examined in the three family members. The results did not suggest non-paternity.

There have been 10 previously described cases in eight families of cytogenetically visible deletions encompassing 5q22 in association with adenomatous polyposis of the colon and rectum.[5 7–12] Varying degrees of mental retardation and a range of dysmorphic features have been described in these patients (table 1). Others have reported patients with similar deletions, mental retardation, and congenital anomalies in whom colonic disease was either not sought or had not yet developed.[11 20 21] Submicroscopic deletion of *APC* has been described in mentally normal subjects without dysmorphic features.[13] In the current report, a submicroscopic deletion encompassing *APC* and *MCC* has produced adenomatous polyposis in association with mild retardation and minor dysmorphic features. The mental retardation observed in this case may relate to the greater extent of the submicroscopic deletion. In our patient an area encompassing both *APC* and *MCC* was deleted and it is likely that other neighbouring genes have been either deleted or disrupted. In the family described by Mandl *et al*,[13] the deletion spanned a smaller region including the *APC* gene from exon 11 to the 3' untranslated region and the adjacent *DP1* gene. *MCC* was not involved.

We are aware of only one other report of FAP resulting from a chromosome 5 translocation.[14] In that report an affected mother and daughter are described with colonic polyposis and a translocation t(5;10)(q22;q25) resulting in disruption of the *APC* gene and an intragenic deletion. Neither mental retardation nor dysmorphic features were reported in these two patients.

In the current report, FAP was discovered after the patient presented with acute appendicitis. An appendiceal carcinoma was identified in the resection specimen. Appendiceal carcinoma has occasionally been described in patients with FAP but there is only one other published case of FAP first presenting as acute appendicitis with appendiceal carcinoma. Molecular genetic data were not reported in that case.[2] In the current report, appendiceal carcinoma has developed in association with a submicroscopic deletion of *APC* and *MCC* and polyps were most numerous in the right hemicolon. Similarly, in the report of van der Luijt *et al*[14] of a 5q translocation and polyposis, polyps were noted to be more numerous in the proximal colon of the mother and a caecal adenocarcinoma was found in her resection specimen. The reported distribution of colorectal polyps and carcinomas in patients with cytogenetically visible deletions of *APC* has been similar and more typical of attenuated adenomatous polyposis coli (AAPC) than of classical FAP. In AAPC, polyps generally develop later and tend to be more dense proximally than distally.[22] The reverse is true of classical FAP.[1] AAPC has been attributed in some cases to constitutional mutations at the 5' end of *APC* while in others mutations in exon 9 or at the 3' end of the gene have been reported.[22–25] It has been suggested that the 5' mutations result in very short protein products that, either through rapid degradation or lack of the necessary oligomerisation domains, do not compete with the residual wild type *APC* or bind to it, producing a dominant negative effect.[14 23–25] Mutations at the 3' end of *APC* may also produce null alleles although the explanation for this

remains unclear.[24] Deletions of *APC* produce a true null allele. In this respect they may be functionally comparable to the 5' and 3' *APC* mutations in AAPC and this may explain some of the phenotypic similarities noted above.

The contribution of *MCC* deletion to colorectal carcinogenesis in the current case remains unclear. Constitutional deletion of *APC* is presumably sufficient to explain the adenomatous polyposis in this patient and there are no reported cases of constitutional *MCC* mutation or deletion alone producing human colorectal disease. Somatic mutation of *MCC* has been reported in colorectal cancers but the role of *MCC* as an independent colorectal tumour suppressor gene has subsequently been questioned.[26 27]

We thank the patient and her parents for their cooperation, the patient's surgeon, Dr John Neophyton, and general practitioner, Dr John Cameron, for providing clinical details, Dr Dorothy Painter for reviewing the histopathology, Cathy Abbott for assistance in preparing DNA probes, and Nicole Chia for assistance in the cytogenetic studies.

D KOOREY
N J BASHA
C TOMARAS
Departments of Gastroenterology and Molecular Genetics, Royal Prince Alfred Hospital, Sydney, Australia

J FREIMAN
Department of Medicine, Sutherland Hospital, Sydney, Australia

L ROBSON
A SMITH
Department of Clinical Cytogenetics, The New Children's Hospital, Sydney, Australia

Correspondence to: Dr Koorey, A W Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Missenden Road, Camperdown, Sydney 2121, Australia

1 Bussey H. *Familial polyposis coli*. Baltimore: The Johns Hopkins University Press, 1975.
2 Parker G, Stollman N, Rogers A. Adenomatous polyposis coli presenting as adenocarcinoma of the appendix. *Am J Gastroenterol* 1996;**91**:801-2.
3 Bodmer W, Bailey C, Bodmer J, *et al*. Localisation of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987;**328**:614-16.
4 Leppert M, Dobbs M, Scambler P, *et al*. The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science* 1987;**238**:1411-13.
5 Herrera L, Kakati S, Gibas L, Pietrzak E, Sandberg A. Gardner syndrome in a man with an interstitial deletion of 5q. *Am J Med Genet* 1986;**25**:473-6.
6 Koorey D, Smith A. Normal high resolution karyotypes in 26 unrelated individuals with hereditary colorectal neoplasia. *Am J Gastroenterol* 1992;**87**:1736-9.
7 Hockey K, Mulcahy M, Montgomery P, Levitt S. Deletion of chromosome 5q and familial adenomatous polyposis. *J Med Genet* 1989;**26**:61-2.
8 Kobayashi T, Narahara K, Yokoyama Y, *et al*. Gardner syndrome in a boy with interstitial deletion of the long arm of chromosome 5. *Am J Med Genet* 1991;**41**:460-3.
9 Cross I, Delhanty J, Chapman P, *et al*. An intrachromosomal deletion and familial adenomatous polyposis coli in two generations. *J Med Genet* 1992; **29**:175-9.
10 Hodgson S, Coonar A, Hanson P, *et al*. Two cases of 5q deletions in patients with familial adenomatous polyposis: possible link with Caroli's disease. *J Med Genet* 1992;**30**:369-75.
11 Lindgren V, Bryke C, Ozcelik T, Yang-Feng T, Francke U. Phenotypic, cytogenetic, and molecular studies of three patients with constitutional deletions of chromosome 5 in the region of the gene for familial adenomatous polyposis. *Am J Hum Genet* 1992;**50**:988-97.
12 Barber J, Ellis K, Bowles L, *et al*. Adenomatous polyposis coli and a cytogenetic deletion of chromosome 5 resulting from a maternal intrachromosomal insertion. *J Med Genet* 1994;**31**:312-16.
13 Mandl M, Caspari R, Jauch A, *et al*. Familial adenomatous polyposis: a submicroscopic deletion at the APC locus in a family with mentally normal patients. *Hum Genet* 1996;**97**:204-8.
14 Van der Luijt R, Tops C, Meera Khan P, *et al*. Molecular, cytogenetic, and phenotypic studies of a constitutional reciprocal translocation t(5;10)(q22; q25) responsible for familial adenomatous polyposis in a Dutch pedigree. *Genes Chrom Cancer* 1995;**13**:192-202.
15 James C, Jauch A, Robson L, Watson N, Smith A. A 3½ year old girl with trisomy 19q defined by FISH. *J Med Genet* 1996;**33**:795-7.
16 Kraus C, Ballhausen W. Two intragenic polymorphisms of the APC-gene detected by PCR and enzymatic digestion. *Hum Genet* 1992;**88**:705-6.
17 Van Leeuwen C, Tops C, Breukel C, *et al*. CA repeat polymorphism within the MCC (mutated in colorectal cancer) gene. *Nucleic Acids Res* 1991;**19**:5805.
18 Spirio L, Joslyn G, Nelson L, Leppert M, White R. A CA repeat 30-70 kb downstream from the adenomatous polyposis coli (APC) gene. *Nucleic Acids Res* 1991;**19**:6348.
19 Koorey D, McCaughan G, Trent R, Gallagher N. Dinucleotide repeat polymorphism at the D5S134 locus linked to the adenomatous polyposis coli (APC) gene. *Hum Mol Genet* 1992;**1**:655.
20 Pescia G, Gaide A, Juillard E. Syndrome dysmorphique, débilité mentale et délétion interstitielle des bras longs d'un chromosome 5. *Ann Genet* 1978; **21**:161-3.
21 Silengo M, Luzzatti L, Centerwall W, Costello J, Parslow M. Interstitial deletion of the long arm of chromosome no 5 in two unrelated children with congenital anomalies and mental retardation. *Clin Genet* 1981;**19**:174-80.
22 Lynch H, Smyrk T, McGinn T, *et al*. Attenuated familial adenomatous polyposis (AFAP). *Cancer* 1995;**76**:2427-33.
23 Spirio L, Olschwang S, Groden J, *et al*. Alleles of the APC gene: an attenuated form of familial polyposis. *Cell* 1993;**75**:951-7.
24 Van der Luijt R, Meera Khan P, Vasen H, *et al*. Germline mutations in the 3' part of APC exon 15 do not result in truncated proteins and are associated with attenuated adenomatous polyposis coli. *Hum Genet* 1996;**98**:727-34.
25 Soravia C, Berk T, Madlensky L, *et al*. Genotype-phenotype correlations in attenuated adenomatous polyposis coli. *Am J Hum Genet* 1998;**62**:1290-301.
26 Kinzler K, Nilbert M, Vogelstein B, *et al*. Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science* 1991;**251**:1366-70.
27 Curtis L, Bubb V, Gledhill S, Morris RG, Bird CC, Wyllie A. Loss of heterozygosity of MCC is not associated with mutation of the retained allele in sporadic colorectal cancer. *Hum Mol Genet* 1994;**3**:443-6.

J Med Genet 2000;**37**:75–77

NF2 gene deletion in a family with a mild phenotype

EDITOR—Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder that predisposes to bilateral vestibular schwannomas and other nervous system tumours. Two clinical subtypes have been proposed. The severe type (Wishart) has an onset before 25 years of age, a rapid course, and multiple nervous system tumours. The mild type (Gardner) has a later onset with a more benign course, often restricted to bilateral vestibular schwannomas.[1]

The *NF2* tumour suppressor gene is localised on chromosome 22q12 and encodes a protein called merlin or schwannomin, which is related to a family of cytoskeleton associated proteins.[2 3] Since the identification of *NF2*, various germline mutations have been identified,[4–6] as well as somatic mutations.[7] In general, germline mutations associated with a mild phenotype include missense mutations and small in frame deletions or insertions.[5]

In our study, the proband (III.2) is an 18 year old boy with a mild facial palsy. Slight enlargement of both vestibular branches of the eighth cranial nerve was observed after MRI scanning, but no other clinical features have been observed. His mother (II.4) was operated on at the age of 28 for bilateral vestibular schwannomas; she died at the age of 45 from breast cancer but with stable vestibular schwannomas. The grandmother (I.2) of the proband had progressive deafness followed by surgery for a right sided vestibular schwannoma at the age of 48 after which she died owing to postoperative complications. At necropsy a right and left sided vestibular schwannoma were found.

DNA from the proband and the available family members was prepared from peripheral blood according to standard procedures. Microsatellites were amplified from genomic DNA by the polymerase chain reaction (PCR). Eight polymorphic markers in the *NF2* gene region were studied in the family: nf2GAI (intron 1), D22S929 (intron 1), nf2CT3I (intron 1), nf2CAII (intron 3), nf2CAIV (intron 8), nf2CAV (intron 10), nf2CAVI (intron 13), and nf2GAII.[8]