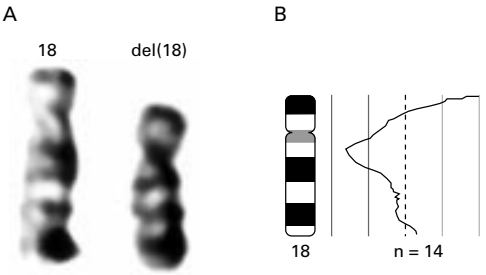

Figure 1 (A) G banded chromosomes 18 from the proband. The normal chromosome 18 is on the left and the deleted chromosome 18 on the right. Chromosomes are at the 550 band resolution. (B) CGH profile of chromosome 18 showing decrease in green to red fluorescence ratio (FR) over the region q11.2 to q12. The FR value over the q21 region was ~1.0 (not deleted). The ideogram used for CGH analysis is at the 400 band resolution. The CGH analysis provides molecular cytogenetic confirmation of the G band analysis.

Fluorescent genotyping analysis of microsatellite markers of the proband and both parents using a Pharmacia ALF DNA sequencer was informative for seven proximal chromosome 18 loci (table 1). A maternally derived deletion was detected at four loci, D18S36, D18S34, D18S67, and D18S535. This places the deletion breakpoints distal to the position of 52.86 cM and proximal to 66.66 cM on the sex averaged linkage map described by Broman *et al*.[10] The proximal breakpoint would map somewhere between D18S66 at 52.86 cM and D18S36 at 58.45 cM. The map position of 52.86 cM is close to the centromere since the locus D18S480, at linkage position 51.21 cM, is on the p arm. The molecular genetic analysis is therefore consistent with a more proximal breakpoint in band q11.2 and provides an initial set of reference loci for more precise mapping of deletion breakpoints in other cases.

This case represents the only case out of the 13 previously described cases[1–8] of proximal 8q deletion to have breakpoints defined at the molecular level. The majority of previously described cases of proximal 18q deletion were de novo in origin, as in the present case. Four affected subjects were identified in two generations of one family resulting from an insertional translocation.[3] Some of the more consistent phenotypic abnormalities in previously described cases included moderate to severe mental retardation, prominent forehead with a short nose and midfacial recession, deep set eyes, high arched palate, ear anomalies, a tendency to obesity, ataxia, and a risk of seizure. Hyperactivity, aggressive behaviour, and distractibility were common.

Table 1 Results of genotyping analysis at seven informative chromosome 18 loci

Locus	*Chromosome band position*	*Sex averaged genetic map position (cM)*	*Status in proband: maternal/paternal allele*
D18S66	q12.1	52.86	+/+
D18S36	q12.2-q12.3	58.45	−/+
D18S34	q12.2	62.29	−/+
D18S67	q12.2-q12.3	63.39	−/+
D18S535	18pter-qter	64.48	−/+
D18S65	q12.2-q12.3	66.66	+/+
D18S39	q21.2	77.36	+/+

Our patient falls into the milder end of the spectrum of phenotypes resulting from proximal 18q deletion. He did not have the typical dysmorphic facial features, but his hyperactive behaviour, distractibility, and mild to moderate mental retardation are consistent with descriptions of previous cases. Our result leads us to suggest that the typical facial dysmorphism associated with other proximal 18q deletion cases may be more associated with loss of band q12.3, which was not deleted in our case. Furthermore, the behavioural phenotype associated with proximal 18q deletion may be more a consequence of loss of the more proximal bands q11.2 and/or q12.1. Only more precise identification of deletion breakpoints in additional cases of proximal 18q deletion will allow us to determine if this hypothesis is correct.

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1 Chudley AE, Kovnats S, Ray M. Recognizable behavioral and somatic phenotype in patients with proximal 18q interstitial deletion. *Am J Med Genet* 1992;**43**:535-8.
2 Poissonnier M, Turleau C, Oliver-Martin M, Milleret-Proyart MJ, Prieur M, Dubos M, Cabanis MO, Magneret F, Blanc P, Noel L. Interstitial deletion of the proximal region of the long arm of chromosome 18, del (18q12). A distinct clinical entity? *Ann Genet* 1992;**35**:146-51.
3 Chudley AE, Bauder R, Ray M, McAlpine PJ, Pena SDJ, Hamerton JL. Familial mental retardation in a family with an inherited chromosome rearrangement. *J Med Genet* 1974;**11**:353-63.
4 Krasikov N, Thompson K, Sekhon S. Monosomy 18q12.1-q21.1: a recognizable aneuploidy syndrome? Report of a patient and review of the literature. *Am J Med Genet* 1992;**43**:531-4.
5 Schinzel A, Binkert F, Lillington DM. Interstitial deletion of the long arm of chromosome 18, del(18)(q12.2q21.1): a report of three cases of autosomal deletion with a mild phenotype. *J Med Genet* 1991;**28**:352-5.
6 Wilson GN, Al Saadi A. Obesity and abnormal behaviour associated with interstitial deletion of chromosome 18 (q12.2q21.1) *J Med Genet* 1989;**26**: 62-3.
7 Wilson GN, Towner JW, Forsman I, Siris E. Syndromes associated with deletion of long arm of chromosome 18 [del(18q)]. *Am J Med Genet* 1979; **3**:155-74.
8 Surh LC, Ledbetter DH, Greenberg F. Interstitial deletion of chromosome 18 [del(18)(q11.2q12.2 or q12.2q21.1]. *Am J Med Genet* 1991;**41**:15-17.
9 Breen CJ, Barton L, Carey A, Dunlop A, Glancy M, Hall K, Hegarty AM, Khokhar MT, Power M, Ryan K, Green AJ, Stallings RL. Applications of comparative genomic hybridisation in constitutional chromosome studies. *J Med Genet* 1999;**36**:511-17.
10 Broman KW, Murray JC, Sheffield VC, White RL, Weber JL. Comprehensive human genetic maps: individual and sex-specific variation in recombination. *Am J Hum Genet* 1998;**63**:861-9.

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Submicroscopic deletions of the *APC* gene: a frequent cause of familial adenomatous polyposis that may be overlooked by conventional mutation scanning

EDITOR—Familial adenomatous polyposis (FAP) is an autosomal dominant colon cancer predisposition syndrome in which patients develop hundreds to thousands of precancerous colonic polyps that have a high risk of becoming malignant.

Despite the fact that deletions of the *APC* locus were originally used to map[1 2] and identify[3 4] the *APC* gene, most studies of mutations in *APC* have used techniques which would not detect deletions.[5–7] Molecular analysis of the *APC* gene in FAP patients has found the pathogenic mutation in 70% of cases,[8] but a substantial minority have no mutation identified.

Interstitial deletions of chromosome 5q, which include the *APC* locus, have been reported in patients with polypo-

Table 1 Primer sequences

Primer	*Sequence*	*Ref*	*Product size (bp)*	*pmol per reaction*
APC exon 8 for	ACC TAT AGT CTA AAT TAT ACC ATC	18	184	5
APC exon 8 rev	GTC ATG GCA TTA GTG ACC AG	18		5
APC exon 15A for	GTT ACT GCA TAC ACA TTG TGA C	18	371	2.5
APC exon 15A rev	GCT TTT TGT TTC CTA ACA TGA AG	18		2.5
APC exon 15D for	CTG CCC ATA CAC ATT CAA ACA C	18	382	2.5
APC exon 15D rev	TGT TTG GGT CTT GCC CAT CTT	18		2.5
APC exon 15F for	AAG CCT ACC AAT TAT AGT GAA CG	18	435	7.5
APC exon 15F rev	AGC TGA TGA CAA AGA TGA TAA TG	18		7.5
MPZ exon 1 for	CAG TGG ACA CAA AGC CCT CTG TGT A	Yau (pers com)	389	7.5
MPZ exon 1 rev	GAC ACC TGA GTC CCA AGA CTC CCA G	Yau (pers com)		7.5
MPZ exon 2 for	CTC ACT TCC TCT GTA TCC CTT ACT G	Yau (pers com)	393	10
MPZ exon 2 rev	GGA GGA CAA TGT AGT CAG GGT GAC A	Yau (pers com)		10
MPZ exon 3 for	TGA CAG CTG TGT TCT CAT TAG GGT C	Yau (pers com)	453	7.5
MPZ exon 3 rev	TCC GAG TGT ATG CCC TGC ATT GAG G	Yau (pers com)		7.5

sis coli and mental retardation.[9] Submicroscopic deletions have been reported in only a few cases.[10 11] In one family,[10] linkage analysis with flanking and intragenic markers followed by in situ hybridisation with intragenic cosmid clones showed that the deletion was approximately 200 kb and included more than the 3' half of the *APC* gene and the 3' adjacent D5S346 microsatellite. A recent report[11] described a quantitative PCR assay to detect submicroscopic deletions which included the entire *APC* gene and the adjacent D5S346 microsatellite in three unrelated Italian FAP families. These submicroscopic deletions do not appear to be associated with mental retardation.

Microsatellite analysis of families with FAP in our region showed one family with apparent non-maternity at D5S346 in one of the affected offspring of an affected mother, suggesting the possibility of an *APC* deletion. A quantitative PCR assay was therefore developed to detect submicroscopic deletions of the *APC* gene.

Previous analysis of 68 FAP families from the Yorkshire region found the pathogenic mutation in 46 cases (67%). These cases were referred for testing (with informed consent) from the Yorkshire Regional Clinical Genetics Service after clinical examination and counselling. All families with an unidentified mutation have been analysed by quantitative PCR; deletions have been identified in four families which is about 5% of the Yorkshire FAP families. In family 1, we studied three subjects affected with FAP. Cytogenetic analysis on one of the patients showed a normal female karyotype. In family 2, we studied four subjects affected with FAP. One of these patients, the father of the other three, did not develop the disease until he was 62 years old. In family 3, we studied two affected subjects. Only one sample was available from family 4. A patient with a cytogenetic deletion 46,XX,del(5)(q14.2q22.3) was also analysed by the quantitative PCR method.

Linkage analysis was performed using several flanking microsatellite markers: D5S299,[12] D5S82,[13] DS5122,[14] D5S346,[15] MCC,[16] and D5S318.[17] Products were analysed by GeneScan analysis on an ABI 373A DNA sequencer with 672 software.

The quantitative multiplex PCR contained amplimers for exons 8, 15A, 15D, and 15F of *APC*[18] with exons 1, 2, and 3 of *MPZ* (myelin protein zero) (S C Yau, personal communication) used as a control (table 1). The 5' end of one primer from each pair was labelled with the fluorescent phosphoramidite 6-FAM. The reaction was carried out in a total volume of 10 µl and contained 200-250 ng of template DNA, primers (table 1), 0.5 U of Promega *Taq* polymerase, 200 µmol/l of each dNTP, 1.5 mmol/l $MgCl_2$ in buffer (50 mmol/l tris, 15 mmol/l $(NH_4)_2SO_4$, 50 mmol/l KCl, 0.085 mg/ml BSA). The reactions were performed in an MJ Research thermal cycler. Cycling parameters were as follows: 95°C for five minutes, 18 cycles of 95°C for 30 seconds, 52°C for 30 seconds, 70°C for one minute, and a final extension at 72°C for five minutes. Products were analysed by GeneScan analysis on an ABI 373A DNA sequencer with 672 software. Fig 1 shows results from two of our families and a subject with normal dosage for *APC*. The amplification of the test sample was compared with the amplification of a control sample with a known point mutation in a different part of the *APC* gene. The peak height of each amplified exon was used to calculate dosage quotients, as described previously,[19] for all amplicons. For example, the dosage quotient (DQ) for exon 8 of *APC* and

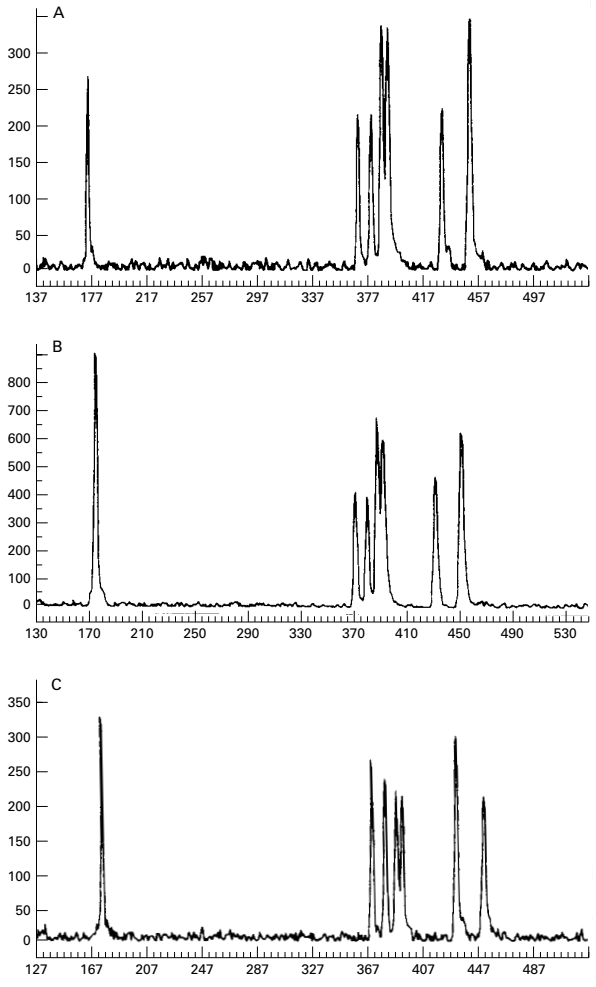

Figure 1 Electrophoretograms of quantitative PCR of exons 8 and 15 of APC and exons 1, 2, and 3 of MPZ. From left to right the peaks are exon 8 APC, exon 15A APC, exon 15D APC, exon 1 MPZ, exon 2 MPZ, exon 15F APC, and exon 3 MPZ. (A) Family 2 deleted for exons 8, 15A, 15D, and 15F of APC and D5S346. (B) Family 4 deleted for exons 15A, 15D, and 15F. (C) Normal (point mutation in APC identified).

Table 2 Dosage quotients (DQ) and standard deviation (SD) calculations for electrophoretograms shown in fig 1

Exon	Sample peak height	Control peak height	DQs ex1	DQs ex2	DQs ex3
Sample A Deleted for exons and 8 and 15A, 15D, and 15F					
APC8	270	494	0.50	0.51	0.48
APC15A	217	359	0.55	0.56	0.53
APC15D	219	415	0.48	0.49	0.46
APC15F	234	452	0.47	0.48	0.45
PO1	340	309			
PO2	342	319			
PO3	353	307			
	Mean DQ (exons 8 and 15)				
	0.50				
	SD				
	0.035				
Sample B Deleted for exons 15A, 15D, and 15F					
APC8	916	494	0.85	0.98	0.90
APC15A	414	359	0.53	0.61	0.56
APC15D	402	415	0.44	0.51	0.47
APC15F	465	452	0.47	0.54	0.50
PO1	675	309			
PO2	603	319			
PO3	634	307			
	Mean DQ (exon 8)			Mean DQ (exon 15)	
	0.91			0.51	
	SD			SD	
	0.067			0.052	
Sample C Normal					
APC8	375	494	1.00	1.15	1.10
APC15A	265	359	0.97	1.12	1.07
APC15D	249	415	0.79	0.91	0.87
APC15F	308	452	0.90	1.04	0.99
PO1	234	309			
PO2	210	319			
PO3	211	307			
	Mean DQ (exons 8 and 15)				
	0.99				
	SD				
	0.110				

exon 1 of *MPZ* is (sample *APC* exon 8 peak height/sample *MPZ* exon 1 peak height)/(control *APC* exon 8 peak height/control *MPZ* exon 1 peak height). Each *APC* exon was compared with each *MPZ* exon. A dosage quotient close to 1.0 indicated that two copies of the test gene are present; a dosage quotient close to 0.5 indicated only one copy. Dosage quotients and standard deviations of the mean dosage quotients were calculated using duplicate test and control sample loadings.

Dosage quotient and standard deviation results are shown in table 2. The values were close to expected values with standard deviations approximately 10% of the mean dosage quotient. Two clear groups of results emerged with no overlap seen between values close to unity or values close to 0.5. Results indicating deletions (dosage quotients close to 0.5) were seen in each case with more than one amplicon, excluding allelic dropout as a cause of reduced amplification.

Three of the four deletions identified were distinct. Family 1 showed apparent non-maternity with marker D5S346 between the affected mother and one of her affected daughters. Quantitative PCR showed the affected subjects to have only one copy of exon 8, exon 15A, exon 15D, and exon 15F of the *APC* gene and we concluded that the mutation in this family was a deletion which extended from at least exon 8 of *APC* to the 3' D5S346 microsatellite. A similar deletion was identified in family 2. In this family, all affected subjects were homozygous for the same MCC allele which suggests that the deletion could include this locus. In family 3, the deletion extended from at least exon 8 to exon 15F of *APC*. Microsatellite analysis showed the proband to be heterozygous at D5S346 and D5S82. In family 4 dosage quotients for exon 8 of *APC* showed that there were two copies of this exon present but only one copy of the regions of exon 15 examined. The proband was heterozygous at D5S346. We concluded that the mutation in this family was a deletion which included exons 15A, 15D, and 15F, but did not extend as far as D5S346. A summary of the microsatellite data and quantitative PCR is shown in table 3.

Using a quantitative PCR assay we found submicroscopic gene deletions of *APC* to be the pathogenic mutation in four unrelated FAP families in Yorkshire, accounting for about 5% of our FAP families. Taken together with the report of De Rosa *et al*[11] who found submicroscopic deletions in three Italian families with FAP (17% of their FAP pedigrees), these data suggest that submicroscopic deletions of the *APC* gene may be more common than is evident from published reports, perhaps because they would be undetected by conventional mutation scanning methods. For example, a recent study[7] found *APC* mutations in 106/190 FAP families but did not search for gene deletions. Our evidence suggests that gene deletions may be found in a substantial minority of FAP patients. That at least three of the four submicroscopic deletions were distinct, differing in the loss of D5S346 and exon 8, eliminates an undue bias in our sample because of a common founder mutation. As the assay is relatively quick and easy to perform, it will now form part of our initial diagnostic mutation screen for a new FAP patient.

A number of quantitative methods have been described to measure gene dosage, including Southern blotting,[20–22] oligonucleotide hybridisation, competitive PCR,[23–25] and more recently Taqman assays[26] and comparative genomic hybridisation (CGH) either using metaphase spreads[27] or arrayed targets.[28] Any useful dosage assay should meet criteria of specificity (absence of false positives), sensitivity (absence of false negatives), reproducibility, and be reasonably economical in time and material. Hybridisation assays using genomic DNA digests are somewhat more time consuming than PCR based methods and usually involve the use of radiolabelled probes. CGH is a promising alternative, but limitations in the size of deletions or duplications detectable by metaphase spreads must be overcome by the availability of arrayed DNA before the technique can be generally applicable. Since automated fragment analysis is already widely used in genetic diagnostic laboratories, the use of dosage quotient analysis offers a robust and accessible means to test for microdeletions and duplications.

Pathogenic duplications or deletions have long been reported, from α globin[23] and dystrophin[29] to tumour suppressor genes including *BRCA1*,[30] *BRCA2*,[31] and *hMSH2*.[32] It is therefore important that genetic testing includes the detection of altered gene dosage where appropriate as con-

Table 3 Summary of microsatellite and quantitative PCR results

	D5S299	D5S82	D5S122	APC exon 8	APC exon 15A	APC exon 15D	APC exon 15F	D5S346	MCC	D5S318
Family 1	Hom	Het	Hom	1 copy	1 copy	1 copy	1 copy	Hom	Het	Het
Family 2	Het	Het	Hom	1 copy	1 copy	1 copy	1 copy	Hom	Hom	Het
Family 3	Het	Het	Hom	1 copy	1 copy	1 copy	1 copy	Het	Het	Het
Family 4	Het	Het	Hom	2 copies	1 copy	1 copy	1 copy	Het	Hom	Het
Cytogenetic deletion	Hom	Hom	Hom	1 copy	1 copy	1 copy	1 copy	Het	Hom	Hom

Hom = homozygous at locus, Het = heterozygous at locus. The order of the markers and exons is 5' of *APC* to 3' of *APC*.

ventional mutation scanning methods may miss submicroscopic deletions.

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1 Herrera L, Kakati S, Gibas L, Pietrzak E, Sandberg AA. Gardner syndrome in a man with an interstitial deletion of 5q. *Am J Med Genet* 1986;**25**:473-6.
2 Bodmer W. Familial adenomatous polyposis (FAP) and its gene, *APC*. *Cytogenet Cell Genet* 1999;**86**:99-104.
3 Joslyn G, Carlson M, Thliveris A, Albertson H, Gelbert L, Samowitz W, Groden J, Stevens J, Spirio L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D, Abderrahim H, Cohen D, Leppert M, White R. Identification of deletion mutations and 3 new genes at the familial polyposis locus. *Cell* 1991;**66**:601-13.
4 Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisinger AC, Hedge P, McKenchie D, Finniear R, Markham A, Groffen J, Boguski MS, Altschul SF, Horii A, Ando H, Miyoshi Y, Miki Y, Nishisho I, Nakamura Y. Identification of FAP locus genes from chromosome 5q21. *Science* 1991;**253**:661-5.
5 Cottrell S, Bicknell D, Kaklamanis L, Bodmer WF. Molecular analysis of APC mutations in familial adenomatous polyposis and sporadic colon carcinomas. *Lancet* 1992;**340**:626-30.
6 Nagase H, Nakamura Y. Mutations of the APC (adenomatous polyposis coli) gene. *Hum Mutat* 1993;**2**:425-34.
7 Wallis YL, Morton DG, McKeown CM, Macdonald F. Molecular analysis of the APC gene in 205 families: extended genotype-phenotype correlations in FAP and evidence for the role of APC amino acid changes in colorectal cancer predisposition. *J Med Genet* 1999;**36**:14-20.
8 Bodmer WF, Bailey CJ, Bodmer J, Bussey HJ, Ellis A, Gorman P, Lucibello FC, Murday VA, Rider SH, Scambler P, Sheer D, Solomon E, Spurr N. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987;**328**:614-16.
9 Pilarski RT, Brothman AR, Benn P, Rosengren SS. Attenuated familial adenomatous polyposis in a man with an interstitial deletion of chromosome arm 5q. *Am J Med Genet* 1999;**86**:321-4.
10 Mandl M, Caspari R, Jauch A, Boker T, Raschke H, Sengteller M, Propping P, Friedl W. Familial adenomatous polyposis: a submicroscopic deletion at the APC locus in a family with mentally normal patients. *Hum Genet* 1996; **97**:204-8.
11 De Rosa M, Scarano MI, Panariello L, Carlomango N, Rossi GB, Tempesta A, Borgheresi P, Renda A, Izzo P. Three submicroscopic deletions at the APC locus and their rapid detection by quantitative-PCR analysis. *Eur J Hum Genet* 1999;**7**:695-703.
12 van Leeuwen C, Tops C, Breukel C, van der Klift H, Fodde R, Khan PM. CA repeat polymorphism at the D5S299 locus linked to adenomatous polyposis-coli (APC). *Nucleic Acids Res* 1991;**19**:5805.
13 Breukel C, Tops C, Vanleeuwen C, van der Klift H, Fodde R, Khan PM. A CA repeat polymorphism at the D5S122 locus tightly linked to adenomatous polyposis-coli (APC). *Nucleic Acids Res* 1991;**19**:6665.
14 Breukel C, Tops C, van Leeuwen C, van der Klift H, Nakamura Y, Fodde R, Khan PM. CA repeat polymorphism at the D5S82 locus, proximal to adenomatous polyposis-coli (APC). *Nucleic Acids Res* 1991;**19**:5804.
15 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.
16 van Leeuwen C, Tops C, Breukel C, van der Klift H, Deaven L, Fodde R, Khan PM. CA repeat polymorphism within the MCC (mutated in colorectal-cancer) gene. *Nucleic Acids Res* 1991;**19**:5805.
17 Wijnen J, Tops C, Breukel C, van Leeuwen C, Goverde A, van der Klift H, Fodde R, Khan PM. CA repeat polymorphism from YAC JW25 at the D5S318 locus, distal to adenomatous polyposis coli (APC). *Nucleic Acids Res* 1991;**19**:6965.
18 Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert, L, Albertson H, Joslyn G, Stevens J, Spirio L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D, Abderrahim H, Cohen D, Leppert M, White R. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991;**66**: 589-600.
19 Yau SC, Bobrow M, Mathew CG, Abbs SJ. Accurate diagnosis of carriers of deletions and duplications in Duchenne/Becker muscular dystrophy by fluorescent dosage analysis. *J Med Genet* 1996;**33**:550-8.
20 Cutting GR, Antonarakis SE, Youssoufian H, Kazazian HH Jr. Accuracy and limitations of pulsed field gel electrophoresis in sizing partial deletions of the factor VIII gene. *Mol Biol Med* 1988;**5**:173-84.
21 Chelly J, Marlhens F, Le Marec B, Jeanpierre M, Lambert M, Hamard G, Dutrillaux B, Kaplan JC. De novo DNA microdeletion in a girl with Turner syndrome and Duchenne muscular dystrophy. *Hum Genet* 1986;**74**: 193-6.
22 Mathew CG, Rousseau J, Rees JS, Harley EH. The molecular basis of alpha thalassaemia in a South African population. *Br J Haematol* 1983;**55**:103-11.
23 Borriello F, Weinberg DS, Mutter GL. Evaluation of gene deletions by quantitative polymerase chain-reaction - experience with the alpha-thalassemia model. *Diagn Mol Pathol* 1994;**3**:246-54.
24 Prior TW, Papp AC, Snyder PJ, Highsmith WE Jr, Friedman KJ, Perry TR, Silverman LM, Mendell JR. Determination of carrier status in Duchenne and Becker muscular dystrophies by quantitative polymerase chain-reaction and allele-specific oligonucleotides. *Clin Chem* 1990;**36**:2113-17.
25 Young P, Stogbauer F, Wiebusch H, Lofgren A, Timmerman V, Van Broeckhoven C, Ringelstein EB, Assmann G, Funke H. PCR-based strategy for the diagnosis of hereditary neuropathy with liability to pressure palsies and Charcot-Marie-Tooth disease type 1A. *Neurology* 1998;**50**:760-3.
26 Laurendeau I, Bahuau M, Vodovar N, Larramendy C, Olivi M, Bieche I, Vidaud M, Vidaud D. TaqMan PCR-based gene dosage assay for predictive testing in individuals from a cancer family with INK4 locus haploinsufficiency. *Clin Chem* 1999;**45**:982-6.
27 Perry A, Nobori T, Ru N, Anderl K, Borell TJ, Mohapatra G, Feuerstein BG, Jenkins RB, Carson DA. Detection of p16 gene deletions in gliomas: a comparison of fluorescence in situ hybridization (FISH) versus quantitative PCR. *J Neuropathol Exp Neurol* 1997;**6**:999-1008.
28 Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 1998;**20**:207-11.
29 Tuffery S, Chambert S, Bareil C, Sarda P, Coubes C, Echenne B, Demaille J, Claustres M. Mutation analysis of the dystrophin gene in southern French DMD or BMD families: from Southern blot to protein truncation test. *Hum Genet* 1998;**102**:334-42.
30 Petrij-Bosch A, Peelen T, van Vliet M, van Eijk R, Olmer R, Drusedau M, Hogersvorst FB, Hageman S, Arts PJ, Ligtenberg MJ, Meijers-Heijboer H, Klijn JG, Vasen HF, Cornelisse CJ, Van't Veer LJ, Bakker E, van Ommen GJ, Devilee P. BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. *Nat Genet* 1997;**17**:341-5.
31 Schmutzler RK, Bierhoff E, Werkhausen T, Fimmers R, Speiser P, Kubista E, Krebs D, Zeillinger R, Wiestler OD, von Deimling A. Genomic deletions in the BRCA1, BRCA2 and TP53 regions associate with low expression of the estrogen receptor in sporadic breast carcinoma. *Int J Cancer* 1997;**74**:322-5.
32 Wijnen J, van der Klift H, Vasen H, Khan PM, Menko F, Tops C, Meijers Heijboer H, Lindhout D, Moller P, Fodde R. MSH2 genomic deletions are a frequent cause of HNPCC. *Nat Genet* 1998;**20**:326-8.

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Homozygosity for a splice site mutation of the *COL1A2* gene yields a non-functional proα2(I) chain and an EDS/OI clinical phenotype

EDITOR—Type I collagen is the major structural protein of skin, bone, tendon, ligaments, and cornea. It is a heterotrimer of two α1(I) chains and one α2(I) chain. Mutations in one of its two structural genes (*COL1A1*, *COL1A2*) underlie the inherited disorders osteogenesis imperfecta (OI) and Ehlers-Danlos syndrome types VIIA and B (EDS VIIA, EDS VIIB).[1] Mutations which inhibit the processing of the protein precursor, procollagen, cause the ligamentous laxity and extreme joint hypermobility of EDS VII. Structural abnormalities and/or reduced production of type I collagen lead to the increased bone fragility, thin skin, blue sclerae, dentinogenesis imperfecta, and presenile deafness of osteogenesis imperfecta. The OI phenotype can vary from perinatally lethal to mild disease depending upon the nature of the mutation. Among the mild group are patients who are heterozygous for a non-functional *COL1A1* allele.[2–5] Homozygosity for a non-functional *COL1A1* allele appears to be incompatible with life.[6] Several years ago we described a male infant who was totally deficient in α2(I)chains owing to homozygosity for a non-functional *COL1A2* allele.[7–9] He had the severe, progressively deforming type of osteogenesis imperfecta (OI type III). His heterozygous third cousin parents, however, showed no overt clinical symptoms but