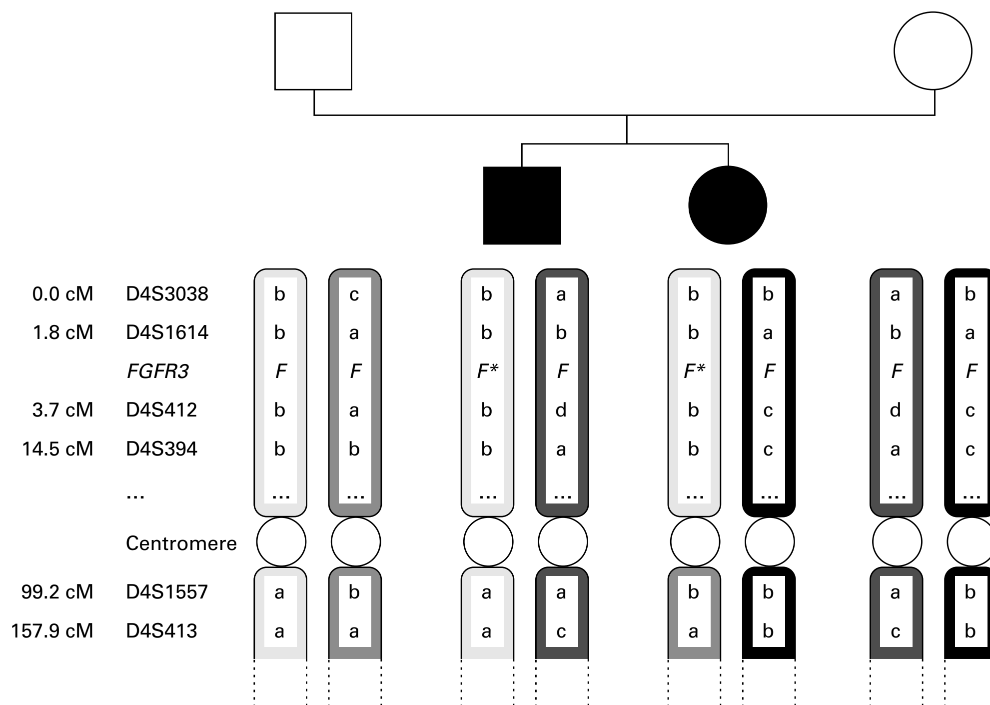

Figure 2 Inheritance of chromosome 4 markers in the nuclear family. The markers are indicated on the left together with their genetic distance in centimorgans from the 4p telomere according to the GB4 map (http://www.ncbi.nlm.nih.gov/genemap98). F indicates the position of the FGFR3 locus and the asterisk indicates the presence of the achondroplasia mutation; its assignment to the paternal haplotype is not experimentally proven (but see text). The children have inherited two different chromosome 4 haplotypes from their mother, while they share a paternal haplotype on 4p encompassing the FGFR3 locus.

somatic mosaicism for *FGFR3* mutations in spite of the high mutation rate in achondroplasia remains unexplained.

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Homozygous deletion of *SHOX* in a mentally retarded male with Langer mesomelic dysplasia

EDITOR—Langer mesomelic dysplasia (LMD) is a rare skeletal dysplasia characterised by severe short stature owing to shortening and maldevelopment of the mesomelic and rhizomelic segments of the limbs. Associated malformations are rarely reported and intellect is normal in all affected subjects reported to date.[1–5] The clinical observation has been made that the parents of subjects with LMD often have short stature, most commonly with the associated clinical and radiographic appearances of Léri-Weill dyschondrosteosis (DC).[6] This dominantly transmitted condition is characterised by moderate short stature predominantly because of short mesomelic limb segments. It is often associated with the Madelung deformity of the wrist, comprising bowing of the radius and dorsal dislocation of the distal ulna. This observation has led to the suggestion that LMD is the homozygous expression of the mutant gene for DC.[5 6]

Shears *et al*[7] and Belin *et al*[8] recently showed that the molecular defect in DC is mutation within or deletion of the *SHOX* gene located within the primary pseudoautosomal region of the X and Y chromosomes. Deletion or

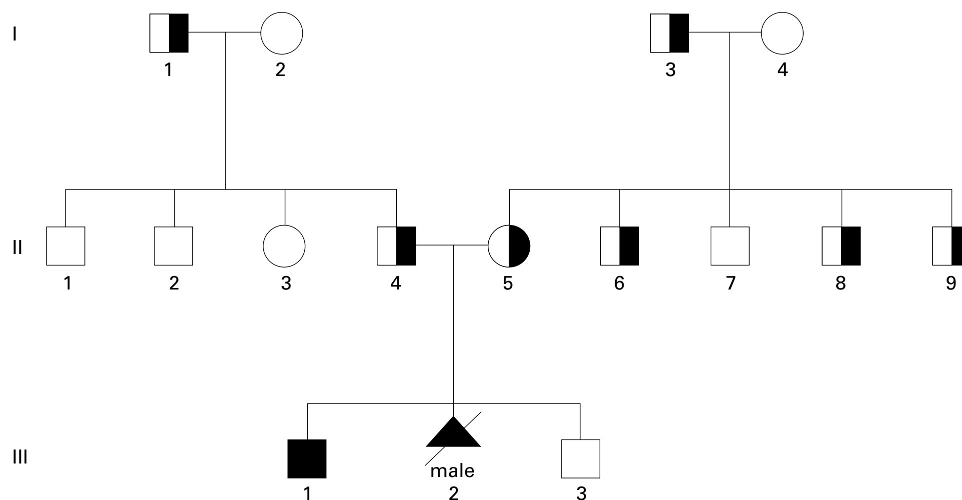

Figure 1 Pedigree of the family. Half filled symbols indicate subjects with dyschondrosteosis, filled symbols indicate Langer mesomelic dwarfism.

mutation of *SHOX* has also been shown to lead to short stature without overt bone dysplasia.[9 10] Belin *et al*[8] documented the molecular analysis of a 24 week fetus of a female patient with DC and a pseudoautosomal deletion encompassing *SHOX*. The fetus had a 45,X karyotype and the radiographic appearances of LMD. They showed that the Xp chromosomal haplotype at the *SHOX* locus retained by this fetus was the maternal one, encompassing the deleted *SHOX* gene, rendering the fetus nullizygous for *SHOX*. Shears *et al*[7] reported that deletions of *SHOX* alleles were derived from both parents in a 20 week fetus with radiographic appearances of LMD. These findings in fetuses were the first molecular evidence to support nullizygosity for *SHOX* as the cause of LMD; however, molecular confirmation in a living patient with LMD has not been previously described.

We present a description of a mentally retarded boy with LMD and his parents, both of whom have DC and normal intellect. Molecular analysis shows that each parent is hemizygously deleted at the *SHOX* locus and that the proband has inherited both alleles harbouring the deletions, confirming this as the aetiology of LMD.

The patient, a male, was the first born child to non-consanguineous parents both of whom had short stature (fig 1, III.1). He was delivered by caesarean section for fetal distress at 35 weeks' gestation. He was in good condition immediately after delivery. His birth weight was 1480 g (−2.6 SD), length was 33 cm (−4.3 SD), and OFC was 28 cm (−3 SD). Severe rhizomelic and mesomelic shortening of all four limbs was immediately obvious on delivery and subsequent radiographic findings were consistent with LMD (fig 2). He required gavage feeding for two weeks and developmental progress in the first two years of life was appreciably delayed with expressive language being particularly slow to develop. At review aged 12 years he remained severely mentally retarded. He had no speech, was not toilet trained, and could only assist in dressing by raising his hands and feet. He was ambulatory but walked with a swaying gait and with flexion at the hips to retain balance. In the year before review he had developed generalised seizures which were satisfactorily controlled with valproate. He was 97 cm tall (−7.2 SD) with a weight of 17 kg (−3.6 SD) and OFC of 49.5 cm (−3.0 SD). There was marked rhizomelic and mesomelic shortening of all limbs (fig 3). The hands and feet were of normal size, although there was partial soft tissue syndactyly and camptodactyly of digits 2-5, and clinodactyly of the fifth digits bilaterally in the hands. Both hands showed ulnar deviation at the wrists and both big toes were proximally placed. There were no other anomalies present except for a large gap between the central maxillary incisors and a mild pectus excavatum deformity. There was no mandibular hypoplasia. Pubertal status was Tanner stage 3.

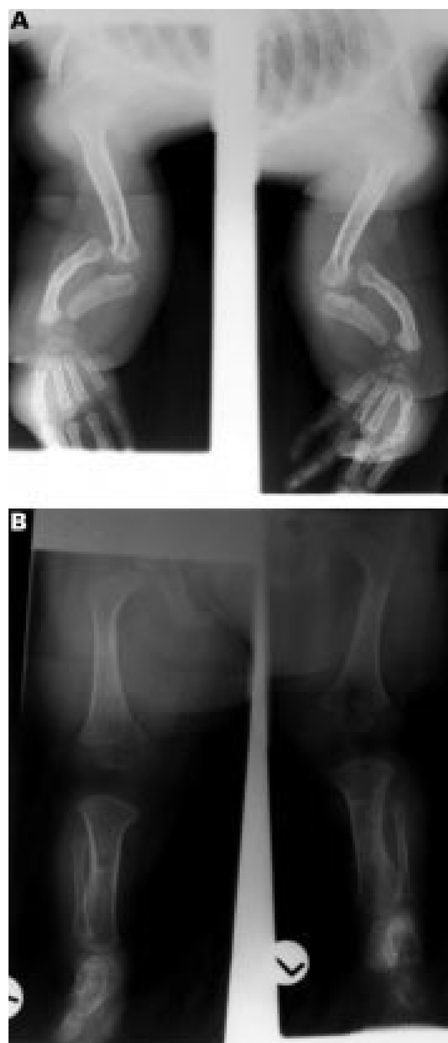

Figure 2 Radiographs of the proband. (A) AP view of the upper limbs. All of the long bones are shortened and undermodelled. The radius is bowed and there is distal ulnar hypoplasia. (B) The lower limbs. The long bones are short and undermodelled. Metaphyseal flaring and proximal fibular hypoplasia are present.

Figure 3 Clinical appearance of the proband, aged 12 years. Rhizomelic and mesomelic limb shortening is present. Syndactyly and camptodactyly is present in the hands. Proximal placement of the big toes is evident.

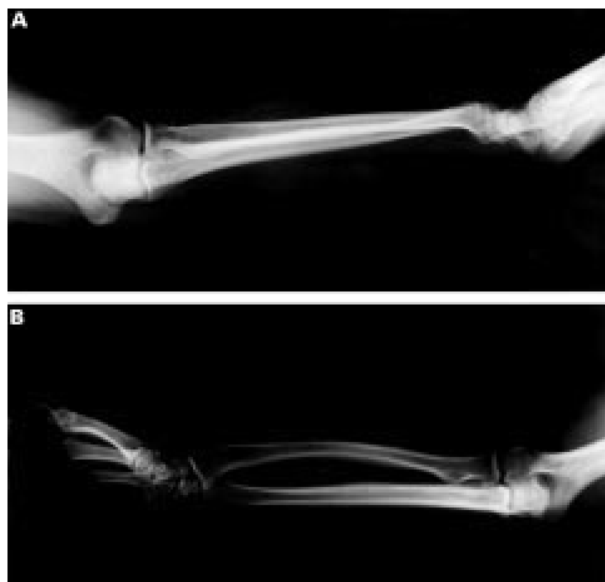

Figure 4 Radiographic appearance of the forearm of the parents of the proband. (A) Lateral view of the mother's forearm (II.5) showing dorsal dislocation of the ulna. (B) AP view of the paternal forearm (II.4) showing bowing and thickening of the midshaft of the radius.

Investigations have included a normal CT scan of the brain at 3 years and an interictal EEG performed at 13 years, which showed no frank epileptiform activity or asymmetry although there was a marked excess of theta activity and no well defined alpha rhythm. Results of a karyotype, fragile X molecular testing, and urine organic acid profile were normal.

The father of the proband (II.4) was 162 cm tall (−2.3 SD) and the mother (II.5) was 145 cm (−2.7 SD). Both parents were of normal intellect. They had short arms and legs, most pronounced in the mesomelic segments. A bilateral wrist deformity was clinically present in both, comprising a subluxation of the distal ulna and resulting in a mild limitation in supination. The mother's deformity was more pronounced than that of the father. Radiographs showed a Madelung deformity with bowing and thickening of the middle third of the radii (fig 4). The couple's second pregnancy, a male (III.2), had been terminated in the second trimester owing to detection of severely shortened limbs on ultrasound. No necropsy was performed. A brother of the proband (III.3), aged 6 years, was noted to be of normal intellect, 112.3 cm tall (−0.7 SD), and to have normal body proportions. Multiple members of the extended family exhibited short stature and the same

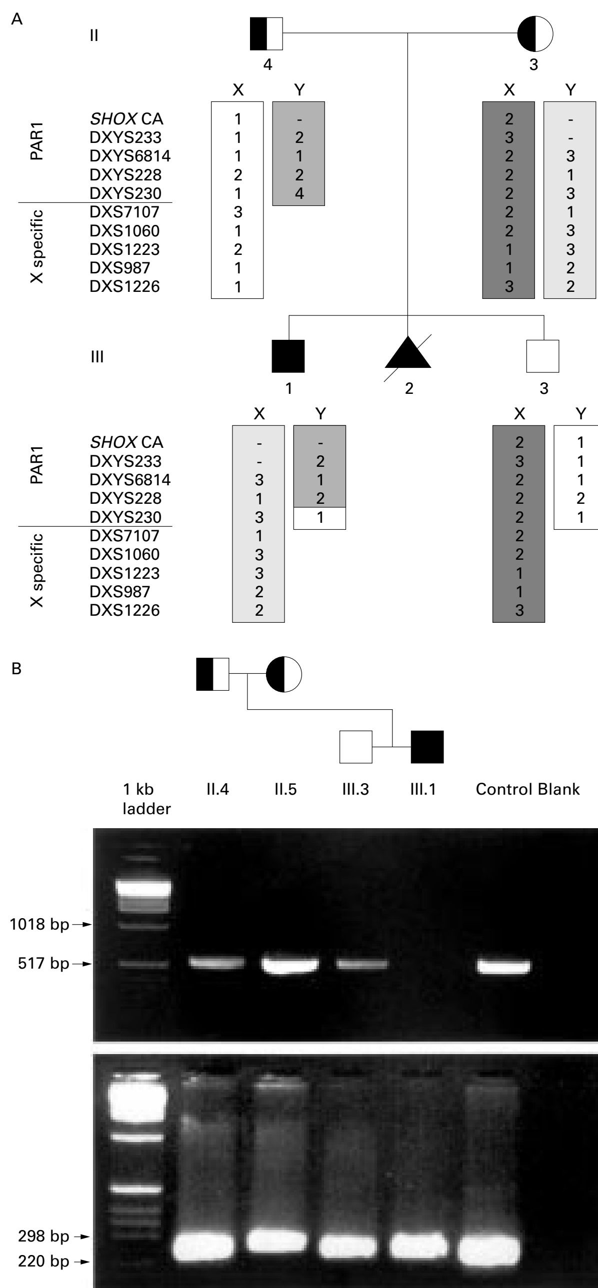

Figure 5 (A) Haplotypes of markers on Xp/Yp showing the maternal deletion and compatible with a paternal deletion. The obligate crossover in male meiosis is seen. (B) PCR of SHOX exon 2 (band at 517 bp) showing that no PCR product was obtained with DNA from the proband (III.1). No PCR product was obtained in the proband for any of the SHOX exons or for the SHOX CA repeat (data not shown). The lower panel shows that PCR product is obtained in the proband with control primers at the locus DXS230 (band between 220 bp and 298 bp).

appearance at the wrist although none of them was available for clinical examination. No other member of the extended family has intellectual impairment.

Blood samples were obtained from II.4, II.5, III.1, and III.3, and microsatellite analysis, fluorescence in situ hybridisation (FISH), and Southern blotting were performed to examine the region around the *SHOX* locus. Fluorescent labelled primers were used to amplify the pseudoautosomal microsatellite markers DXYS233, DXYS228, DXYS6814, DXYS230, and the *SHOX* CA repeat in the 5'UTR of *SHOX* exon 1.[7] Microsatellite analysis of the X specific markers DXS7107, DXS1060, DXS1223, DXS987, and DXS1226 was also undertaken.

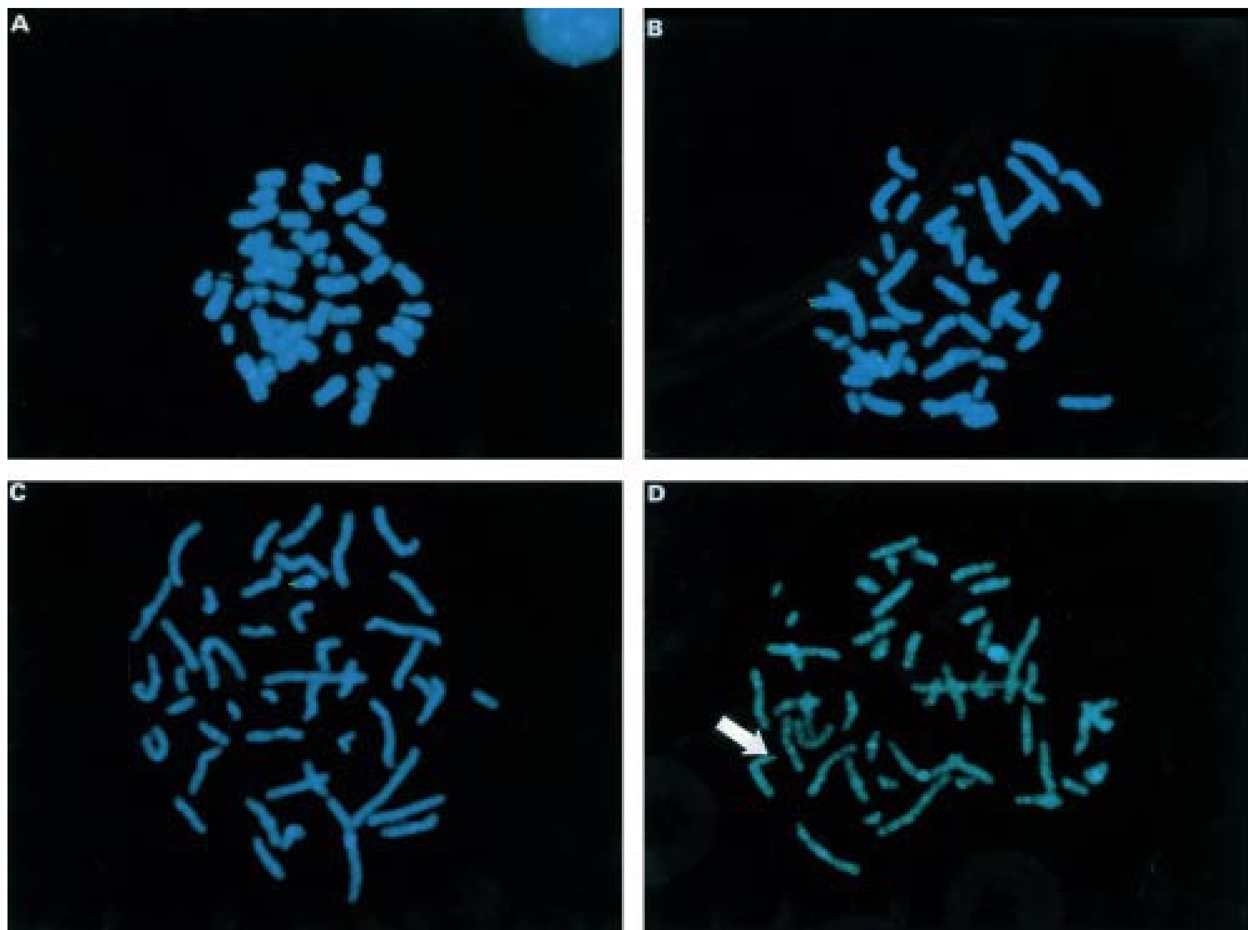

Figure 6 FISH studies with cosmid 34F5. (A) The mother of the proband (II.5), showing a deletion on one X chromosome. (B) The father of the proband (II.4), showing a signal on the X and the Y chromosomes. (C) The proband (III.1) showing a deletion on the X chromosome but a signal on the Y. (D) The maternal uncle of the proband (II.6) showing a deletion on the Y chromosome and a retained signal on the X (arrowed).

Results showed that the proband (III.1) did not inherit an allele at the DXYS233 locus from his mother (II.5), indicating a maternal PAR1 deletion (fig 5A). A paternally derived allele at DXYS233 was present. No PCR product was obtained with the proband's DNA for the *SHOX* CA repeat, and the parents could both be either hemizygous or homozygous at this locus. In addition, since the proband's DNA failed to amplify with primers for each exon of *SHOX* (fig 5B), we concluded that there was a homozygous deletion encompassing *SHOX* in the proband. Heterozygosity in the proband for the more proximal PAR1 microsatellite markers DXYS6814, DXYS228, and DXYS230 indicates that neither deletion extends into the X specific region, but both are confined to the pseudoautosomal region. The normal brother of the proband (III.3) has inherited the opposite maternal and paternal haplotypes to the proband.

FISH was performed on metaphase spreads of lymphocytes obtained from whole blood or from transformed lymphoblastoid lines.[11] Cosmid LLNOYCO3'M'34F5, which contains *SHOX* exons 1-5b, was obtained from the Lawrence Livermore Y chromosome specific library and was labelled by nick translation with biotin-16 dUTP (Gibco BRL BioNick Labeling System). A total of 15 metaphases were analysed using a Leitz DMLB fluorescence microscope and images were captured and stored using Applied Imaging software. A hemizygous deletion of *SHOX* was shown in the mother (fig 6A). However, in the father, signals were visible on both the X and Y chromosomes (fig 6B) and in the proband an X chromosome deletion was present but there was a signal on the Y (fig 6C). We therefore performed Southern blotting to show the paternally derived *SHOX* deletion.

A Southern blot of *Hin*dIII digested genomic DNA of II.4, II.5, III.1, and III.3 was hybridised with a probe for *SHOX* exons 3 to 4. The blot was then stripped and rehybridised with a dosage control probe from chromosome 22 (TBX1, IMAGE cDNA clone 1876034). Probe labelling and detection was performed using the Gene Images™ system (Amersham). There was no signal with the *SHOX* probe in the proband, III.1 (upper panel, fig 7), but the dosage control probe signal was present (lower panel, fig 7), confirming that the proband is homozygously deleted for *SHOX*. A dosage effect was shown with the *SHOX* probe in the parents of the proband, II.4 and II.5. These lanes both showed a weaker intensity signal than the normal sib, III.3. The dosage control probe showed equal signals in all lanes indicating that the parents are hemizygously deleted for *SHOX*.

A variety of dominantly transmitted disorders, such as achondroplasia, aniridia, and Waardenburg syndrome, express a more severe phenotype when present in the homozygous form.[12] Langer mesomelic dysplasia has long been considered the homozygous expression of DC on purely clinical grounds, but until the demonstration of the molecular defect underlying the disorder, definitive evidence has been lacking. This report is the first confirmation in a living patient that nullizygosity for *SHOX* causes LMD. Both parents had the clinical and radiographic appearances of DC and both harboured heterozygous deletions of one *SHOX* allele.

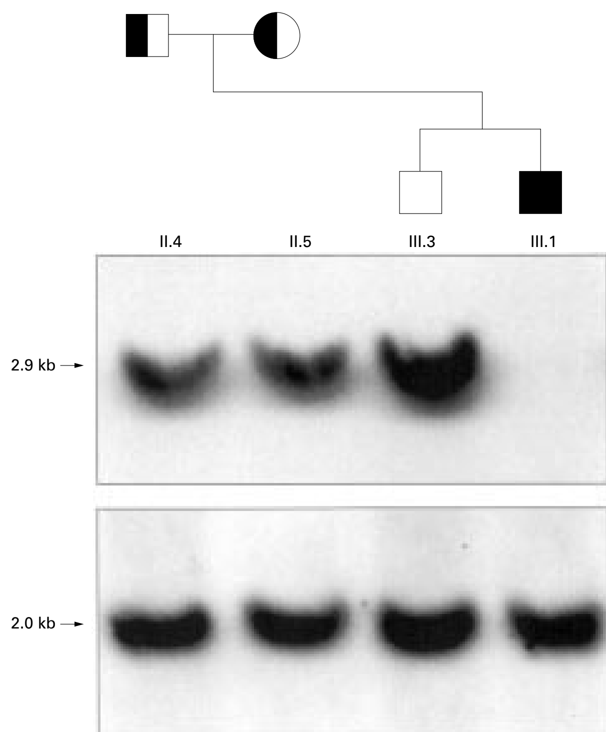

Figure 7 Southern blot of HindIII digested genomic DNA of II.4, II.5, III.1, and III.3 hybridised with a SHOX probe (genomic PCR product of SHOX exons 3 to 4). The probe detects a 2.9 kb band which is absent in III.1, indicating a homozygous deletion (upper panel). The band strength in III.3 is of greater intensity than in II.4 and II.5, whereas the control probe hybridises to a 2 kb band and shows equal signal intensity in all lanes (lower panel). This dosage effect shows that II.4 and II.5 are heterozygous for SHOX deletions.

The *SHOX* gene is located within the primary pseudoautosomal region (PAR1) at the telomere of the short arm of the X and Y chromosomes. This region escapes X inactivation in females and participates in obligate recombination during male meiosis. Consequently, DC segregates as an apparently "autosomal" dominant disorder. This is illustrated by the normal statured male sib of the proband who has inherited the two intact *SHOX* alleles, the paternal Y chromosome having undergone meiotic recombination with a breakpoint centromeric to the *SHOX* locus. The obligate requirement for crossing over in PAR1 may predispose to a higher rate of mispairing with resultant deletions in this very localised chromosomal region. An accurate estimation of the frequency of such events is difficult since it is likely that DC is underdiagnosed within the general population owing to the wide clinical spectrum. Analysis of the small number of patients with DC examined with molecular methods so far has indicated that deletion is a common mode of mutation in this disorder. The studies of Shears *et al*[7] and Belin *et al*[8] showed that 12 out of 14 cases were the result of deletions encompassing the *SHOX* locus, the remaining two being point mutations.

It is interesting to note that there are no previous reports of an association of mental retardation with LMD, although it has been described previously in association with DC. Shears *et al*[7] noted minor dysmorphic features and learning disabilities in a pair of female monozygotic twins with DC. They postulated that this might be explained by deletion of contiguous genes; however, in this case the responsible deletion encompassing the *SHOX* locus extended into the X specific region and was large enough to be visible on G banding. Spranger *et al*[13] reported a mother-son pair with DC, the son manifesting in addition mental retardation, myoclonic epilepsy, and chondrodysplasia punctata. Molecular mapping studies showed the extent of the maternally derived deletion to include not only *SHOX*, but also *ARSE*, the gene mutated in X linked chondrodysplasia punctata, and the putative mental retardation locus *MRX49*. The normal intelligence and seizure free status of the mother suggests that the phenotype of her son was attributable to heterozygosity for the *SHOX* deletion in addition to nullizygosity for *ARSE* and possibly *MRX49*.[14] Thus in both these cases the deletion extended well into the X specific region and could include established MRX loci. Microsatellite and cytogenetic analysis of the deletions in the present case have indicated that the maternal deletion is more extensive than the paternal deletion, but is still confined within PAR1 and does not extend into the X specific region. Several MRX loci map close to PAR1,[15] but to our knowledge none has been reported within PAR1. It is still possible that deletion of contiguous genes may contribute to the mental retardation in the proband of this family if one postulates the existence of a further MRX/Y locus within the pseudoautosomal region. It is worthwhile noting that the parents and other relatives affected by DC do not have intellectual impairment suggesting that the MR resulting from this putative pseudoautosomal gene follows recessive inheritance in this family. In addition, none of the patients from other DC families studied with microdeletions confined to PAR1 have mental retardation. It is likely that most LMD patients without mental retardation are homozygously deleted for *SHOX* but that one or both of the deletions do not encompass the putative MRX/Y locus. They could also carry one or more point mutations which would not be expected to cause mental retardation.

The study of this patient shows that nullizygosity for *SHOX* causes Langer mesomelic dysplasia and confirms it as the homozygous form of Léri-Weill dyschondrosteosis. The association of LMD with mental retardation in this patient also suggests the existence of a recessively acting pseudoautosomal mental retardation locus.

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Tandem triplication of chromosome 13q14 with inverted interstitial segment in a 4 year old girl

EDITOR—Application of chromosome painting has enabled confirmation that an additional segment in a presumed tandem duplication originates from the rearranged chromosome. More recent studies to determine more accurately the exact amount of duplication with cosmid FISH probes have, in a few instances, shown that some of the presumed duplications were in fact triplications of smaller segments.[1–7] In some of these patients, unequal distances between the FISH signals showed that the middle segment of the tandemly arrayed three segments was in the opposite orientation to the two flanking segments,[1 3 4] thus providing clues to the possible mechanism of formation.

Here we report on the clinical, cytogenetic, and molecular analysis of a patient displaying the same type of triplication for segment 13q14 including the retinoblastoma gene, again with opposite orientation of the middle segment.

The proband was born at term after an uneventful pregnancy to healthy, consanguineous, Turkish parents (third cousins). At the birth, the mother was 23 and the father was 28 years old. The patient's birth weight was 2700 g (<10th centile). At the age of 4 years, she was referred to a paediatric hospital for evaluation of developmental delay. Her weight (8 kg), height (85.0 cm), and head circumference (43.5 cm) were all far below the 3rd centile. Her cognitive abilities were estimated to be at the level of a 2 year old (IQ of about 50). Clinical assessment showed the following findings (fig 1): low frontal hairline, prominent antihelices and hypoplastic lobules of the ears, upward slanting palpebral fissures, deep set eyes, a capillary haemangioma next to the right eyebrow, a bulbous nasal tip, thin upper lip, pointed chin, excessive dental caries, right transverse palmar crease, diminished flexion in the metacarpophalangeal joints of both thumbs, and hypertrichosis of the legs.

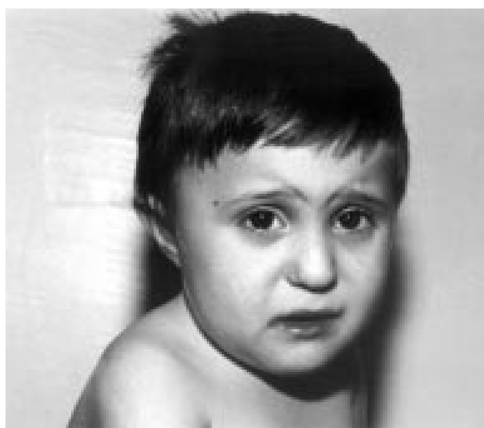

Figure 1 The proband aged 4 years. Note deep set eyes with upward slanting palpebral fissures and bulbous tip of nose.

Chromosome analysis was performed on GTG banded metaphase preparations of blood lymphocyte cultures of the patient and her parents by standard techniques.

FISH was carried out using probes from four loci on chromosome 13 according to standard protocols (Oncor® Inc) or as previously described.[8 9] Loci and their corresponding cosmid probes included D13S118 (c118), the retinoblastoma gene *RB1*, D13S319 (c319), and D13S25 (c25), all mapping to 13q14. Cosmid c118 maps to 13q14.1-q14.2 proximal to *RB1*, while cosmids c319 and c25 both map to 13q14.3 about 1 Mb distal to the *RB1* locus.[10]

FISH analysis was performed using a Zeiss Axioplan epifluorescence microscope and images were recorded either by conventional microphotography or by Photometrics CCD KAF camera (Tuscon, AZ, USA), controlled with Smart Capture imaging software (Vysis Inc, Downers Grove, IL, USA).

Genomic DNA was extracted from peripheral blood of the proband and her parents by standard methods. PCR analysis was performed using primers which amplify dinucleotide repeat polymorphisms. The following loci which map between bands 13q12 and 13q32-q34 were tested: D13S221, D13S218, D13S118, D13263, D13S155, D13S284, D13S153, D13S137, D13S124, D13S162, D13S170, and D13S173. PCR products were separated on a 6% polyacrylamide/urea gel and visualised by silver staining.

GTG banding analysis of the patient's metaphase spreads (resolution of about 400-500 bands/haploid karyotype) showed a female karyotype with 46 chromosomes in all 30 cells examined. One homologue 13 showed an approximate 1/4-1/3 increase in length owing to an additional segment in the middle of the long arm. Two narrow bands divided the additional segment into three equal parts, suggesting a triplication of band 13q14 (fig 2). The karyotypes of both parents were normal.

Hybridisation with the *RB1* gene probe showed one signal on the normal chromosome and three signals and thus three *RB1* copies on the rearranged homologue. The distances between the three signals appeared to be unequal; the distance between the first and second signals seemed to be shorter than that between the second and third signals. Cohybridisation of the *RB1* gene probe with differently labelled cosmids c25 or c319, as well as cohybridisation of cosmids c118 and c319, confirmed an interstitial triplication of the whole of band 13q14 (fig 2). In addition, dual colour FISH clearly showed that the middle segment was inverted in orientation; the order of signals was RG-GR-RG or GR-RG-GR (R stands for red; G stands for green). An order of RG-RG-RG or GR-GR-GR would be expected if the triplication had occurred in direct orientation. Unequal distance between three pairs of signals, with the first two in a row being very close, but both