

Clinical Report

SHOX Mutations in a Family and a Fetus With Langer Mesomelic Dwarfism

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Léri–Weill dyschondrosteosis (LWD) and Langer mesomelic dysplasia (LMD) are caused by mutations in the SHOX gene. LWD results from haploinsufficiency and is dominantly inherited, while the more severe LMD results from the homozygous loss of SHOX. We describe a family and fetus with two SHOX mutations. Several relatives carry an approximately 200 kb interstitial deletion that includes the whole SHOX gene. Their condition is mild, with no Madelung deformity, and was originally diagnosed as hypochondroplasia (HCH). This deletion was also transmitted to a female fetus. However, unlike her carrier relatives, the ultrasound scan of the fetus and subsequent autopsy were consistent with LMD. The fetus inherited an additional Xp deletion (Xpter–Xp22.12) that also included the SHOX gene from her chromosomally normal father. This represents a unique molecular condition for LMD: the fetus is a compound heterozygote with two independent deletions, one inherited and one arising from a de novo event. © 2004 Wiley-Liss, Inc.

KEY WORDS: SHOX; Léri–Weill dyschondrosteosis; Langer mesomelic dysplasia; Xp deletion

INTRODUCTION

Langer mesomelic “dysplasia” (LMD) (OMIM 249700) is a rare condition characterized by severe short stature and mesomelic and rhizomelic dysgenesis of the limbs involving hypoplasia/aplasia of the ulna and fibula. Other malformations are rare, and intellectual development is usually normal, though a recent publication documents mental retardation in a boy [Robertson et al., 2000]. LMD is the homozygous manifestation of Léri–Weill dyschondrosteosis (LWD) (OMIM

127300), a dominantly transmitted disorder of short stature due to mesomelic shortness. LWD often, but not always, comprises radial bowing and dorsal distal ulna dislocation (Madelung deformity).

LWD is caused by haploinsufficiency of the SHOX gene (short stature homeobox-gene) and LMD is caused by the loss of both copies of SHOX [Belin et al., 1998; Shears et al., 1998]. SHOX is located within the Xp–Yp pseudoautosomal region (PAR 1). Thus, in males there are functional copies of the SHOX gene on both the X and Y chromosomes and in females the gene escapes X inactivation [Rao et al., 1997]. SHOX is expressed exclusively in the developing limbs and pharyngeal arches of human embryos [Clement-Jones et al., 2000]. SHOX haploinsufficiency causes the skeletal manifestations of Turner syndrome and its characteristic short stature [Ogata et al., 2001; Ross et al., 2001]. Mutations of the SHOX gene have also been implicated in the cause of up to 3% of patients with idiopathic short stature [Rappold et al., 2002].

We report on the clinical, cytogenetic, and molecular findings in a family and fetus with two forms of SHOX mutation. The female fetus had LMD while the grandmother, mother, and uncle all had SHOX haploinsufficiency due to a deletion of the entire gene. The fetus had an additional Xp deletion, detectable by the light microscope, which was shown to involve the paternally inherited X chromosome.

CLINICAL REPORT

The patient, a 29-year-old woman presented to the Fetal Medicine Department for routine ultrasonography at 20 + 1 weeks of gestation. The fetus had proximal and distal shortness of all four limbs, the distal bones being more severely affected than the proximal ones. Hands and feet appeared normal, but there was ulnae deviation of the hands at the wrist. Both ulnae were absent and fibulae were very short (Fig. 1). The radii and tibiae were shortened with some bowing of the radii. The rest of the fetal skeleton appeared normal; face and profile were also normal. No other ultrasound abnormalities were apparent.

The patient’s brother was diagnosed as having hypochondroplasia (HCH) (OMIM 146000) on the basis of short stature. The patient and her mother both had short stature (152 and 150 cm, respectively) but had not sought medical attention. Forearm radiographs were performed on the patient with normal results.

At this point, the differential diagnosis included the femur, fibula, ulna (FFU) dysostosis, Reinhardt–Pfeiffer mesomelia (RPM), and Langer mesomelic “dysplasia” (LMD). FFU was considered unlikely due to the symmetry of the findings whilst RPM was a possibility but it had only been reliably reported in one family. LMD was considered the most likely diagnosis

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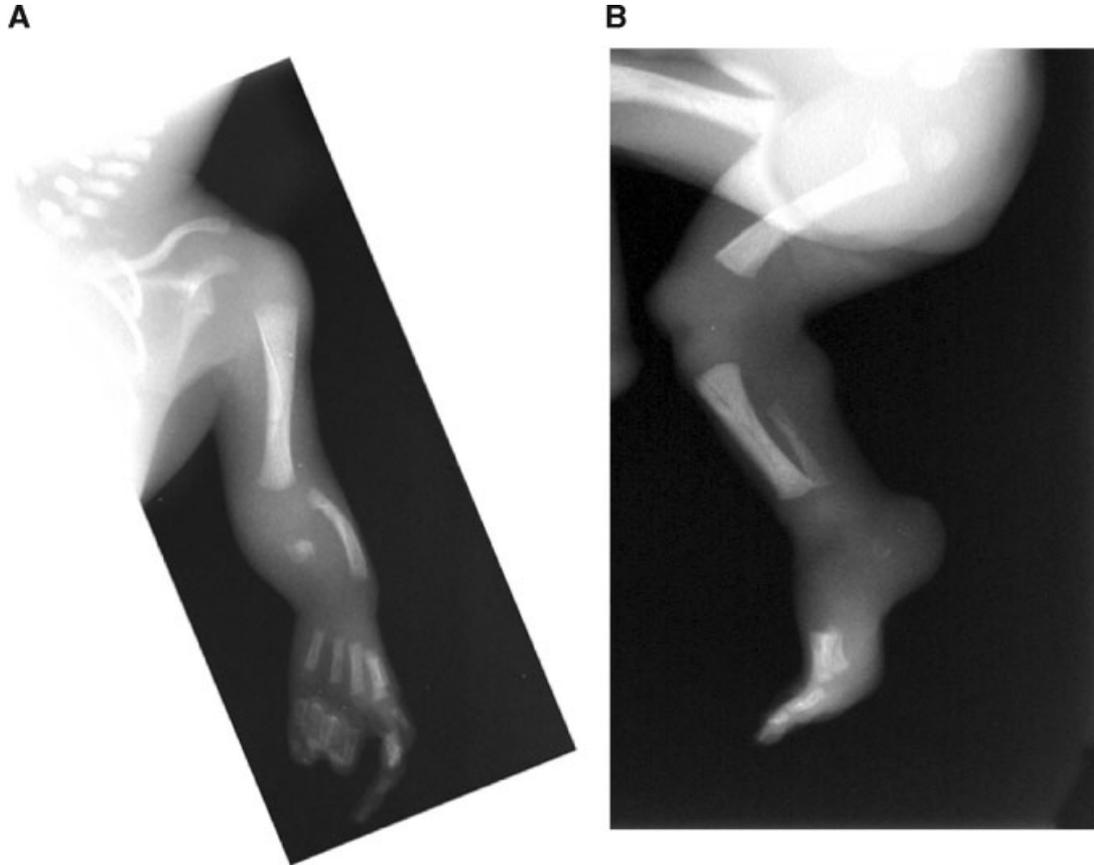


Fig. 1. **A:** Left forearm with severe hypoplasia of ulna and bowing of the radius. **B:** Left leg with hypoplasia of the fibula.

ultrasonographically but the parental phenotypes did not support this.

The patient and her husband were not offered a specific diagnosis; an amniocentesis was performed after consultation with orthopedic colleagues regarding post-natal prognosis. A

decision was made to terminate the pregnancy on the grounds of severe functional morbidity. After termination, an autopsy was performed with the parents' consent.

A skeletal survey confirmed the ultrasound findings of shortening of the humeri and more severe shortening and

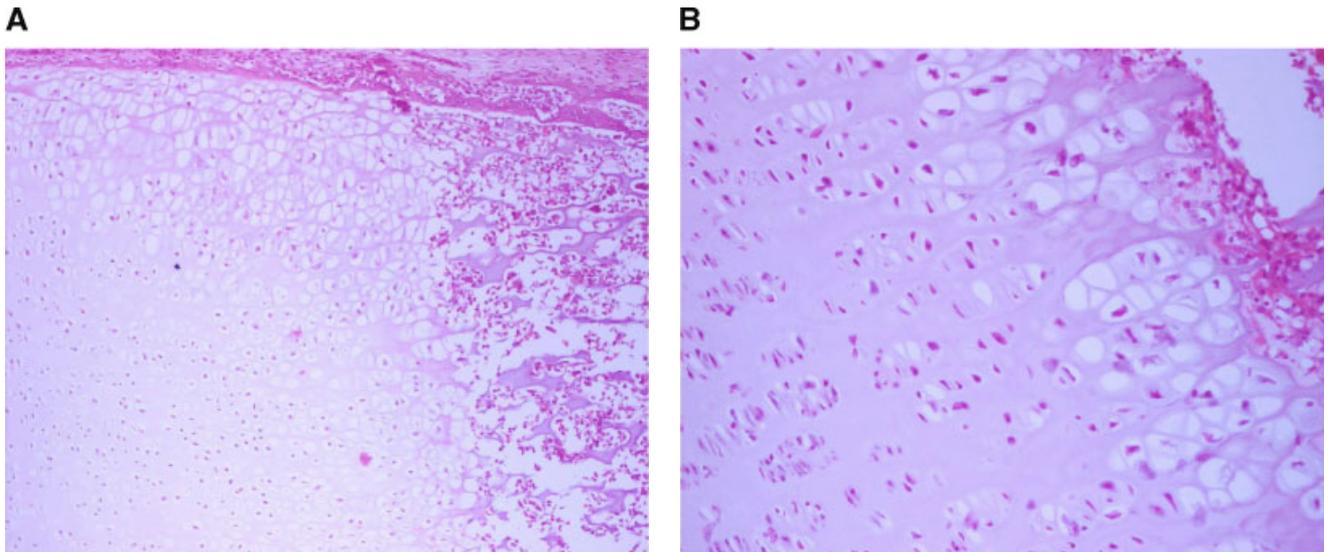


Fig. 2. **A:** Survey view (H + E) of rib costo-chondrial junction showing virtually no chondrocyte column formation. **B:** Higher power view (H + E) of rib costo-chondrial junction of a 37 week gestation fetus. Note conspicuous chondrocyte column formation.

bowing of the radii and tibia. Radiologically both ulnae were present but rudimentary. There was bilateral dislocation of the radial heads. No other skeletal abnormality was present. Post-mortem examination demonstrated a female fetus with non-dysmorphic facial features and normal mandible, shortening of the limbs, upper more than lower, and normal hands and feet. Internal examination revealed an ostium secundum atrial septal defect. All other organ systems were normal grossly and histologically. Histological examination of bone showed virtual absence of chondrocyte column formation in the rib costo-chondral junction (Fig. 2). Poor column formation was found in the femoral head. The severe disruption in growth plate architecture in the fetus could be caused directly by the absence of *SHOX* protein, which is normally expressed in the growth plate of the human embryo [Clement-Jones et al., 2000]. Alternatively, since the *SHOX* protein is a transcriptional activator, absence of *SHOX* could also affect the expression of

other physal genes required for normal growth plate development. Haploinsufficiency of *SHOX* is also associated with dysregulation of chondrocyte development and Munns et al. [2001] demonstrated reduced numbers of chondrocyte columns in two females with LWD. Specifically, Munns et al. [2001] noted that chondrocyte columns tended to be replaced by "nests" of chondrocytes with a side-by-side arrangement.

CONVENTIONAL CYTOGENETIC, MOLECULAR CYTOGENETIC, AND MOLECULAR ANALYSIS

Fetal amniocyte karyotype was 46,X,del(X)(p22.12). Both parents were cytogenetically normal, thus the deletion had arisen de novo. Microsatellite PCR analysis of fetal and parental DNA demonstrated that this deletion had arisen on the X chromosome transmitted from the father and defined the breakpoint between loci DXS8019 and DXS1053, correspond-

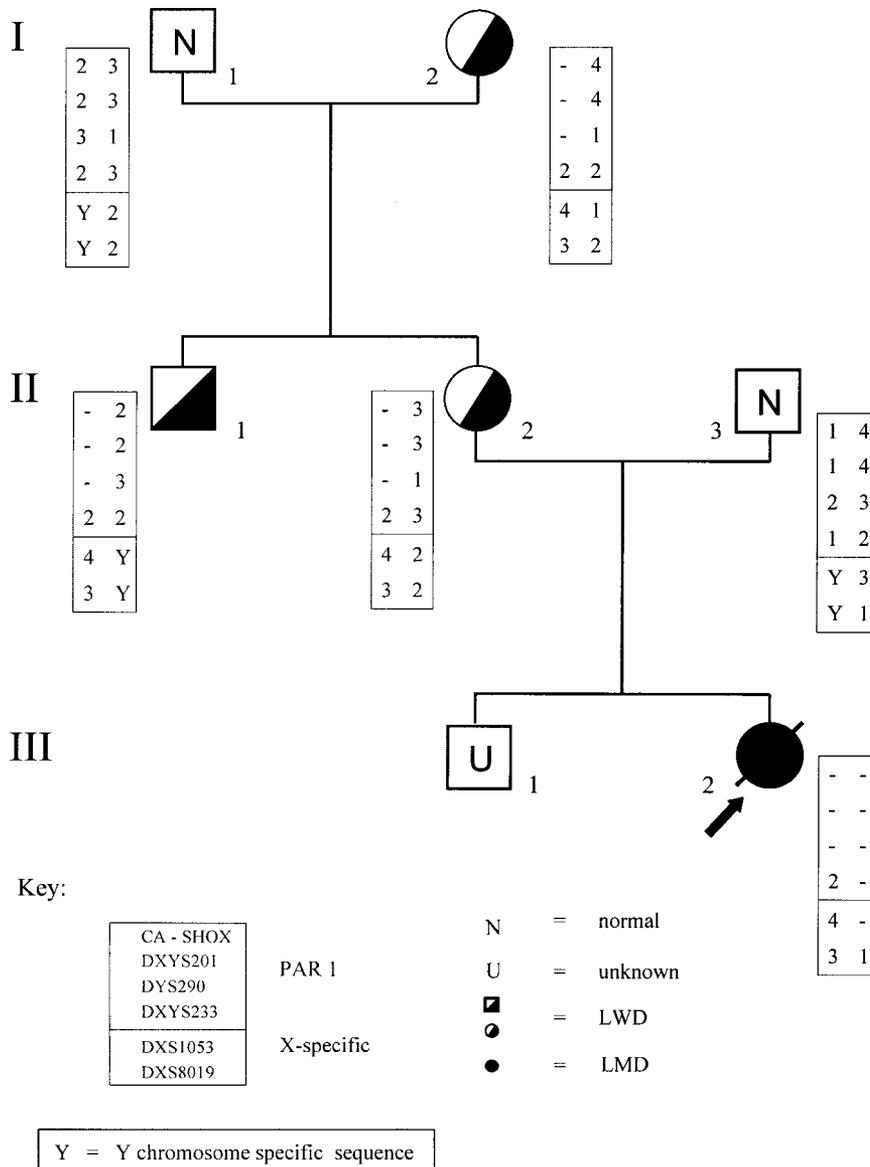


Fig. 3. Pedigree. Arrow: proband; the patient is individual II-2. PCR alleles of each individual are given in the boxes. The order of primers is from Xpter towards the centromere.

ing to approximately 16 Mb from Xpter (Fig. 3). This region includes the *SHOX* gene which is located approximately 0.5 Mb from Xpter.

In females, terminal deletions extending as far as Xp22.12 cause unilateral inactivation of the structurally abnormal X. Monosomy for this region of distal Xp typically results in short stature and the variable presence of some Turner syndrome manifestations [James et al., 1998; Zinn et al., 1998]. The ultrasound findings, therefore, were not consistent with the isolated loss of distal Xp. The skeletal abnormalities in the fetus and the family history of short stature and HCH in the mother and her brother, suggested that the fetus may have carried an additional mutation also present in other relatives. We undertook mutation testing of the *SHOX* gene and also the *FGFR3* gene. The presence of the common amino acid substitution N540K within the *FGFR3* gene, which accounts for 50% of patients with HCH [Prinster et al., 1998; Ramaswami et al., 1998] was excluded in the family. However, in the fetus a deletion was identified in the single maternal copy of the *SHOX* gene by FISH and confirmed by PCR. No product could be amplified by PCR from fetal DNA using the primers for the microsatellites *CA-SHOX* and *DYS290* which map to the 5' and 3' untranslated regions of the *SHOX* gene, respectively (Figs. 3 and 4). No hybridization signal could be detected on either X chromosome of the fetus using FISH with the overlapping cosmids 15D10 and 34F5 (Fig. 5A), which together contain the entire *SHOX* coding sequence [Rao et al., 1997]. Thus, the fetus carries a microdeletion which encompasses the whole maternal copy of the *SHOX* gene. Since the paternal copy of *SHOX* is also deleted the fetus is nullisomic for the *SHOX* gene and protein.

The mother of the fetus had a normal conventional karyotype but FISH analysis of the *SHOX* gene showed that she also carried the microdeletion. Cosmids 15D10 and 34F5 gave a signal on only one X chromosome in the mother (Fig. 5B). Microsatellite analysis also identified the microdeletion in the uncle and grandmother.

Additional PCR primers and FISH cosmids from PAR 1 [Rao et al., 1997] were used to determine the extent of the *SHOX* microdeletion (Fig. 4). No microsatellites were available distal to the *SHOX* gene but the proximal boundary of the deletion was determined by PCR to lie between the loci *DXS8032* and *DXS1449*. Using FISH, cosmid 43C11 gave one signal for the fetus and two for the mother demonstrating that the microdeletion is interstitial and defined the distal breakpoint between 43C11 and 15D10. Proximally the microdeletion

included cosmids M9E3, M11E6, and M29B11 but not M15G7. Thus, the size of the deletion is between approximately 170 and 250 kb.

DISCUSSION

We report on a family in which LWD is segregating with a microdeletion causing haploinsufficiency of the *SHOX* gene. The deletion, which was not visible cytogenetically, was transmitted to the fetus on the maternal X chromosome. However, rather than LWD, the ultrasound scan of the fetus and the autopsy findings were consistent with LMD, the homozygous form of LWD. The fetus had also inherited a distal Xp deletion on the paternal chromosome and thus is a compound heterozygote for deletions of the *SHOX* gene. The father was chromosomally normal, indeed the size of the deletion would make it lethal in male carriers, and so LMD in the fetus is caused by two independent deletions, one inherited and one arising from a de novo event. This represents a unique cause of LMD.

Although LMD is a rare disorder, the molecular analysis of a small number of cases has been reported. In the case most similar to ours, Belin et al. [1998] described a patient with a 45,X karyotype caused by loss of the paternal sex chromosome. The single X chromosome inherited from the mother carried a *SHOX* gene deletion. In three other reported patients, LMD was caused by the inheritance of two different mutations, one from each parent: a male infant [Ogata et al., 2002], a 20 week fetus [Shears et al., 1998], and a 12-year-old boy who was mentally retarded [Robertson et al., 2000]. Shears et al. [2002] described a consanguineous family in which transmission of the same *SHOX* point mutation caused both LWD and LMD.

Interstitial deletions, not usually detectable by conventional cytogenetic analysis, are the most common class of structural abnormality involving distal Xp [Ballabio and Andria, 1992]. In most cases, these deletions involve a region of approximately 2 Mb that includes the steroid sulphatase gene. Flanking the 2 Mb region are a family of low copy number repeat elements (LCRs) that predispose to recombination events and make the region susceptible to deletions. LCRs, varying in size between a few kilobases and several hundred kilobases [Ji et al., 2000], have been identified flanking many recurring microdeletions and microduplications throughout the genome [Lupski, 1998]. It is possible that microdeletions involving the *SHOX* gene, such as the one described here, could be formed through the same mechanism. PAR 1 contains many tandem and interspersed repeats [Rappold, 1993] and the region around the

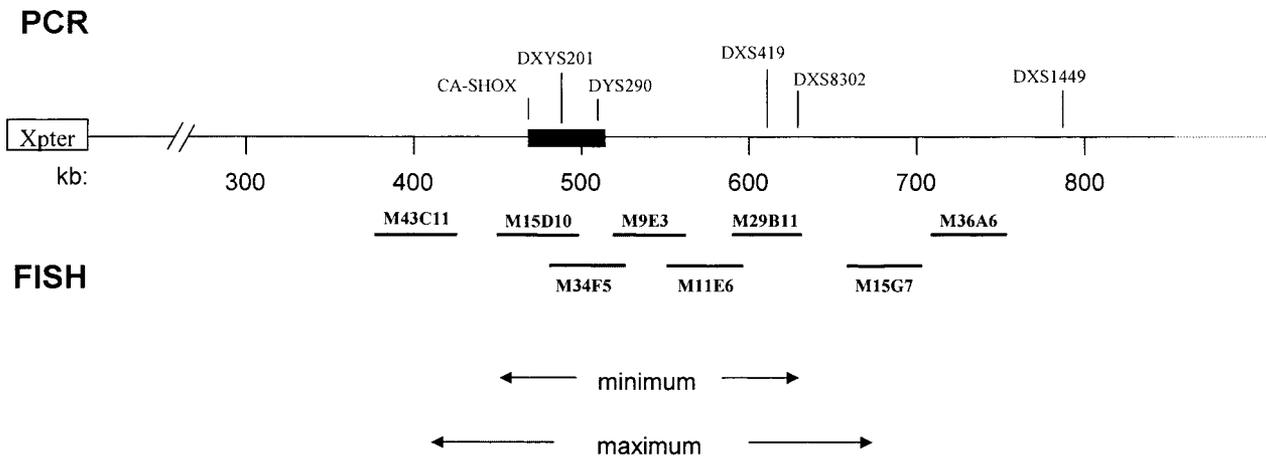


Fig. 4. Schematic map of distal Xp showing the approximate locations of PCR primers and FISH cosmids. The maximum and minimum sizes of the microdeletion are indicated by arrows. The *SHOX* gene is shown as a black box. Cosmid *M15D10* contains the PCR loci *CA-SHOX* and *DXYS201*, cosmid *M34F5* contains the PCR locus *DYS290* and cosmid *M29B11* contains *DXS419* and *DXS8302*. *DXS1449* is proximal to cosmid *M36A36*.

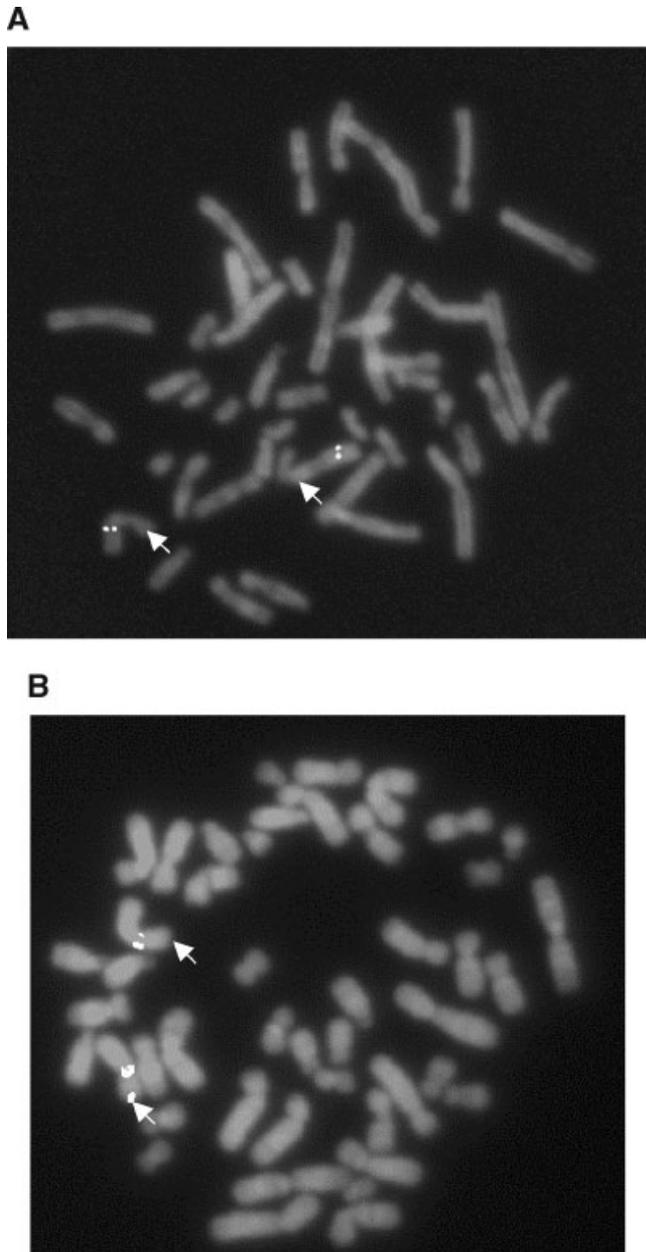


Fig. 5. **A:** FISH analysis of cosmid 34F5 on a metaphase chromosome spread of fetal origin. The white signal is the control cosmid 125A1 which maps to Xq22.2. The arrows indicate a deletion of the 34F5 cosmid on both X chromosomes. **B:** FISH analysis of cosmid 15D10 on a metaphase chromosome spread of maternal origin. The distal signal is the cosmid 15D10 and the proximal signal is the control probe DXZ2 which maps to the X centromere. The arrows indicate a normal signal on one X chromosome and a deletion of the 15D10 cosmid on the other.

SHOX gene is thought to contain sequences which predispose to chromosome rearrangements [Rao et al., 1997]. An interstitial duplication of the *SHOX* gene was reported by Grigelioniene et al. [2001], which could have arisen as a reciprocal product of the deletion process. However, each microdeletion tends to have only a limited number of common breakpoints and among deletions causing LWD there is considerable variation in deletion size [Belin et al., 1998; Shears et al., 1998; Schiller et al., 2000; Ross et al., 2001; Rappold et al., 2002].

The phenotype of this fetus corresponds closely to that reported for LMD. In particular, the rudimentary/absent ulnae and fibulae and the thick and curved radii and tibiae are characteristic of LMD [Belin et al., 1998]. Thus, the phenotype of LMD appears to be consistent irrespective of the type(s) of mutation [Zinn et al., 2002].

In contrast, the phenotype due to *SHOX* haploinsufficiency is quite variable, both within and between families [Schiller et al., 2000]. The original diagnosis proposed in our family was HCH rather than LWD. Both disorders are skeletal dysplasias, characterized by disproportionate short stature, and mild cases can be difficult to distinguish from each other [Grigelioniene et al., 2000] particularly in families such as the above where Madelung deformity is absent.

Most authors conclude that there are no obvious phenotype/genotype correlations for *SHOX* mutations causing LWD. Clinical severity does not appear to correlate with the nature of the underlying mutation [Ross et al., 2001; Rappold et al., 2002] or, for deletion cases, with the amount of material lost [Belin et al., 1998; Grigelioniene et al., 2001]. However, it is possible that there may be a more subtle effect with whole gene deletions being less likely to cause Madelung deformity than mutations which disrupt only part of the gene [Musebeck et al., 2001; Rappold et al., 2002]. Ross et al. [2001] reported no difference in height between carriers of *SHOX* deletions and point mutations. However, Madelung deformity was present in 9/10 patients with intragenic mutations compared with 22/32 with *SHOX* deletions [Ross et al., 2001]. Madelung deformity was also absent in the family described here. Although not part of their conclusions, Rappold et al. [2002] demonstrated that 2% of patients with idiopathic short stature had whole gene deletions without obvious skeletal deformity.

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REFERENCES

- Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. 1992. Methylation of PvuII and Hha I sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 51:1229–1239.
- Ballabio A, and Andria G. 1992. Deletions and translocations involving the distal short arm of the human X chromosome: Review and hypotheses. *Hum Mol Genet* 4:221–227.
- Belin V, Cusin V, Viot G, Girlicj D, Toutain A, Moncla A, Vekemans M, Le Merrer M, Munnich A, Cormier-Daire V. 1998. *SHOX* mutation in dyschondrosteosis (Léri–Weill syndrome). *Nat Genet* 19:67–69.
- Clement-Jones M, Schiller S, Rao E, Blaschke RJ, Zuniga A, Zeller R, Robson SC, Binder G, Glass I, Strachan T, Lindsay S, Rappold DG. 2000. The short stature homeobox gene *SHOX* is involved in skeletal abnormalities in Turner syndrome. *Hum Mol Genet* 9:695–702.
- Grigelioniene G, Eklof O, Ivarsson SA, Westphal O, Neumeyer L, Kedra D, Dumanski J, Hagenas L. 2000. Mutations in short stature homeobox gene (*SHOX*) in dyschondrosteosis but not hypochondroplasia. *Hum Genet* 107:145–149.
- Grigelioniene G, Schoumans J, Neumeyer L, Ivarsson SA, Eklof O, Enkvist Tordai P, Fosdal I, Myhre AG, Westphal O, Nilsson NO, Elfving M, Ellis I, Anderlid B-M, Fransson I, Taoia-Paez I, Nordenskjold M, Hagenis L, Dumanski JP. 2001. Analysis of short stature homeobox-containing gene (*SHOX*) and auxological phenotype in dyschondrosteosis and isolated Madelung deformity. *Hum Genet* 109:551–558.
- James RS, Coppin B, Dalton P, Dennis NR, Mitchell C, Sharp AJ, Skuse DH, Thomas NS, Jacobs PA. 1998. A study of females with deletions of the short arm of the X chromosome. *Hum Genet* 102:507–516.

- Ji Y, Eichler EE, Schwartz S, Nicholls RD. 2000. Structure of chromosomal duplicons and their role in mediating human genomic disorders. *Genome Res* 10:597–610.
- Lupski JR. 1998. Genomic disorders: Structural features of the genome lead to DNA rearrangements and human disease traits. *TIG* 14:417–422.
- Munns CFJ, Glass IA, LaBrom R, Hayes M, Flanagan S, Berry M, Hyland VJ, Batch JA, Phillips GE, Vickers D. 2001. Histopathological analysis of Len-Weill dyschondrosteosis: Disordered growth plate. *Hand Surgery* 6:13–23.
- Musebeck J, Mohnike K, Beyre P, Tonnies H, Neitzel H, Schnabel D, Gruters A, Wieacker P, Stumm M. 2001. Short stature homeobox-containing gene deletion screening by fluorescence in situ hybridisation in patient with short stature. *Eur J Pediatr* 160:561–565.
- Ogata T, Matsuo N, Nishimura G. 2001. *SHOX* haploinsufficiency and over-dosage: Impact of gonadal function status. *J Med Genet* 38:1–6.
- Ogata T, Muroya K, Sasaki G, Nishimura G, Kitoh H, Hattori T. 2002. *SHOX* nullizygosity and haploinsufficiency in a Japanese family: Implication for the development of Turner syndrome features. *J Clin Endocrinol Metab* 87:1390–1394.
- Prinster C, Carrera P, Del Maschio M, Weber G, Maghnie M, Vigone MC, Mora S, Tonini G, Rigon F, Beluffi G, Severi F, Chiumello G, Ferrari M. 1998. Comparison of clinical–radiological and molecular findings in hypochondroplasia. *Am J Med Genet* 75:109–112.
- Ramaswami U, Rumsby G, Hindmarsh PC, Brook CG. 1998. Genotype and phenotype in hypochondroplasia. *J Pediatr* 133:99–102.
- Rao E, Weiss B, Fukami M, Rump A, Neisler B, Mertz A, Muroya K, Binder G, Kirsch S, Winkelmann M, Nordsiek G, Heinrich U, Breuning MH, Ranke MB, Rosenthal A, Ogata T, Rappold GA. 1997. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nat Genet* 16:54–63.
- Rappold G. 1993. The pseudoautosomal regions of human sex chromosomes. *Hum Genet* 92:315–324.
- Rappold GA, Fukami M, Niesler B, Schiller S, Zumkeller W, Bettendorf M, Heinrich U, Vlachopadoulou E, Reinehr T, Onigata K, Ogata T. 2002. Deletions of the homeobox gene *SHOX* (short stature homeobox) are an important cause of growth failure in children with short stature. *J Clin Endocrinol Metab* 87:1402–1406.
- Robertson SP, Shears DJ, Oei P, Winter R, Scambler PJ, Aftimos S, Savarirayan R. 2000. Homozygous deletion of *SHOX* in a mentally retarded male with Langer mesomelic dysplasia. *J Med Genet* 37:959–964.
- Ross JL, Scott C, Martilla P, Kowal K, Nass A, Papenhausen P, Abboudi J, Osterman L, Kushner H, Carter P, Ezaki M, Elder F, Wei F, Chen H, Zinn AR. 2001. Phenotypes associated with *SHOX* deficiency. *J Clin Endocrinol Metab* 86:5674–5680.
- Schiller S, Spranger S, Schechinger B, Fukami M, Merker S, Drop SL, Troger J, Knoblauch H, Kunze J, Seidel Rappold GA. 2000. Phenotypic variation and genetic heterogeneity in Léri–Weill syndrome. *Eur J Hum Genet* 8:54–62.
- Shears DJ, Vassal HJ, Goodman FR, Palmer RW, Reardon W, Superti-Furga A, Scambler PJ, Winter RM. 1998. Mutation and deletion of the pseudoautosomal gene *SHOX* causes Léri–Weill dyschondrosteosis. *Nat Genet* 19:70–73.
- Shears DJ, Guillen-Navarro E, Sempere-Miralles M, Domingo-Jimenez R, Scambler PJ, Winter RM. 2002. Pseudodominant inheritance of Langer mesomelic dysplasia caused by a *SHOX* homeobox missense mutation. *Am J Med Genet* 110:153–157.
- Zinn AR, Tonk VS, Chen Z, Flejter WL, Gardner HA, Guena R, Kushner H, Schwartz S, Sybert VP, Van Dyke DL, Ross JL. 1998. Evidence for a Turner syndrome locus or loci at Xp11.2–p22.1. *Am J Hum Genet* 63:1752–1766.
- Zinn AR, Wei F, Zhang L, Elder FF, Scott CI, Martilla P, Ross JL. 2002. Complete *SHOX* deficiency causes Langer mesomelic dysplasia. *Am J Med Genet* 110:158–163.