

Loss of the *SHOX* gene associated with Leri-Weill dyschondrosteosis in a 45,X male

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Abstract

A male patient is reported with a 45,X karyotype and Leri-Weill dyschondrosteosis (LWD). FISH analysis with *SHOX* and *SRY* gene probes was carried out. One copy of both *SHOX* and *SRY* was detected in interphase nuclei, clarifying the origin of LWD and the male phenotype. Molecular results suggested that the 45,X karyotype arose through two independent events. The first occurred at paternal meiosis leading to an unequal crossing over between the short arms of the X and Y chromosomes. As a consequence, the *SRY* gene was translocated onto Xp, thereby explaining the male phenotype of the patient. The second event probably occurred at maternal meiosis or at the early stages of the zygote resulting in the loss of the maternal X chromosome.

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Leri-Weill dyschondrosteosis (LWD, OMIM 127300) is a dominant disease characterised by short stature and skeletal dysplasia including Madelung deformity.^{1,2} LWD in the homozygous form shows Langer syndrome characterised by severe short stature and hypoplasia or aplasia of the ulna and fibula.³ The association of LWD with unbalanced X;Y translocations allowed the disease to be mapped on the pseudoautosomal region of the X and Y chromosomes.^{4,5} LWD has recently been related to deletions and mutations of the *SHOX* gene, which maps on Xp22.3 in the pseudoautosomal region of the X and Y chromosomes.^{6,7} The *SHOX* gene is supposed to be a so called anti-Turner gene since its mutation or deletion is associated with growth failure. A *SHOX* point mutation has also been detected in one patient with idiopathic short stature.⁸

We report LWD in a 45,X male, which is a very rare condition and usually associated with Turner syndrome.

Materials and methods

CASE REPORT

The proband, a boy, is the third child of non-consanguineous parents. At birth the father was 35 years old and the mother 31. The boy was born by caesarean section at term after an uneventful pregnancy. Birth weight was 2800 g. No lymphoedema was noticed. The boy was first evaluated by us at the age of 14

years because of short stature and delayed puberty. Weight was 35 kg (<3rd centile), height 130 cm (<3rd centile), and OFC 53 cm (10th centile). Clinical evaluation showed a triangular face, synophris, downward slanting palpebral fissures, short forearms with bowing of the radius, subluxation of the distal ulna, wedging of carpal bones, and shortening of the tibia. X rays showed Madelung deformity with dorsal subluxation of the distal ulna and mild sclerosis of the proximal metaphyses of the phalanges. No delayed bone age was observed. Based on these clinical features, a diagnosis of LWS was made (fig 1). The patient also had a small penis and testes (volume 5 ml) whose site, morphology, and structure were normal as shown by ultrasound. Hormonal tests, including FSH, LH, testosterone, androstenedione, and DHEAS were normal for age. No features of X linked chondrodysplasia punctata were detected.

CYTOGENETIC AND MOLECULAR ANALYSES

Chromosome analysis was carried out on peripheral blood lymphocytes of the proband and his parents by GTG banding. FISH was performed according to Calabrese *et al*⁹ using a cosmid probe for the *SHOX* gene (34F5)⁸ and a YAC probe for the *SRY* gene (pHu14).¹⁰ PCR



Figure 1 Madelung deformity of the forearm with dorsal dislocation of the distal ulna.

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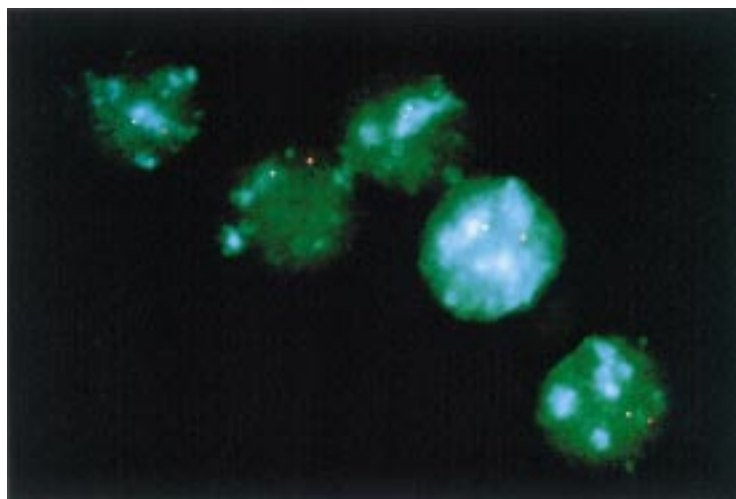


Figure 2 FISH analysis with specific probes for the *SRY* gene (red) and *SHOX* gene (yellow) showing a single copy of both genes in interphase nuclei.

analysis was performed on DNA from peripheral blood cells of the proband and his parents with specific primers for the pseudoautosomal boundaries of the X and Y chromosome (PABX and PABY),¹¹ the *SRY* gene,¹² the amelogenin gene on the X chromosome (*AMX*), the amelogenin-like sequence of the Y chromosome (*AMELY*),¹³ the loci sY254 and sY255 of Yq¹⁴, and the polymorphic sequence DXS52.¹⁵ In order to look for point mutations within the coding region of the *SHOX* gene of the proband and his father, we performed PCR reactions with specific primers for the five exons of *SHOX*.⁷ The amplification products were directly sequenced by means of a PCR products Sequencing Kit (Amersham, UK). Each exon was sequenced on both the sense and the antisense strand.

Results

The proband had a homogeneous 45,X karyotype on 150 consecutively examined lymphocyte metaphases. FISH analysis using probes specific for the *SHOX* and *SRY* genes showed the presence of one signal only on Xp. In addition, FISH analysis with *ZFX* and *SRY* probes on 300 nuclei from buccal and urinary epithelial cells also showed one signal each, excluding the presence of hidden mosaicism (fig 2).

The parents of the proband had two copies of *SHOX* on their sex chromosomes as expected. Sequencing analysis showed no point mutations or deletions of the *SHOX* gene. PCR analysis of the pseudoautosomal boundary (PABX/PABY) in the patient showed the band corresponding to PABY only, while the mother showed the band corresponding to PABX and the father both PABX and PABY specific bands. PCR amplification of the *SRY* gene showed the presence of this gene in the proband and in his father. Analysis of *AMX* and *AMELY* showed the presence of *AMX* in the proband and in his mother, while the father had both *AMX* and *AMELY* sequences. Analysis of the loci sY254 and sY255 showed no amplification products in the proband and his mother, while the father had bands of the

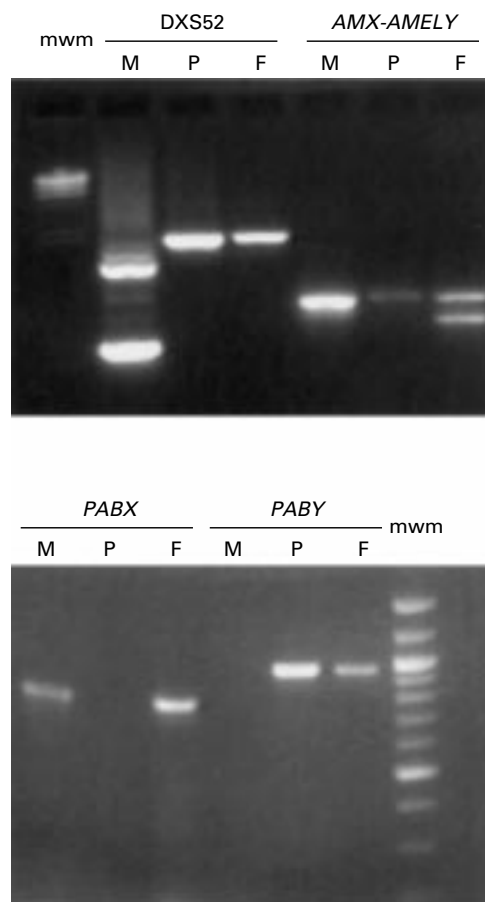


Figure 3 PCR amplification of loci DXS52, *AMX-AMELY*, *PABX*, and *PABY*. Mar=molecular weight marker, M=mother, P=proband, F=father.

expected size. PCR amplification of the polymorphic locus DXS52 in the patient showed a single band corresponding to the paternal allele (fig 3).

Discussion

LWD has been associated with loss or mutation of a copy of the *SHOX* gene.^{6,7} In the present report we describe the first case, to our knowledge, of LWD in a 45,X male. FISH analysis with a specific *SHOX* probe disclosed the presence of only one copy of the *SHOX* gene in both metaphases and nuclei. Since point mutations were found in some patients with LWD, we tested exons 1 to 5 of the *SHOX* gene by sequencing. No point mutation was detected in the single copy of the *SHOX* gene, confirming that haploinsufficiency of this gene is associated with LWD. Moreover, the patient was a 45,X male which is a very rare condition,¹⁶ since a 45,X karyotype is usually associated with a female phenotype. A "pure" 45,X male condition does not exist.¹⁷ In most cases hidden or undetected mosaicism is present,¹⁷⁻¹⁹ while in other cases a translocation of Yp onto an autosome or, more rarely, a translocation involving the p arms of X and Y chromosomes may be found.¹⁹⁻²⁵ In all these cases, FISH has shown the presence of *SRY* on an autosome or X chromosome. In the present case, FISH analysis showed the translocation of *SRY* onto

Xp explaining the male genitalia and the male development of the proband which resembled that observed in 46,XX males.²⁵

Regarding the mechanism of origin of the 45,X karyotype, in the majority of patients the missing X is lost through non-disjunction at paternal meiosis.²⁶ However, in the present case, the 45,X complement probably arose as a consequence of two different mutations, as evidenced by FISH and PCR data. The first mutation occurred during paternal meiosis and consisted of an abnormal exchange between the pseudoautosomal regions of the X and Y chromosomes following the obligatory crossover in *PAR1*.²⁷ In fact, FISH showed the presence of *SRY* on the sole X chromosome, and PCR resulted in the amplification of *PABY*, *SRY*, and *AMX* and absence of *PABX* and *AMELY* in the patient, suggesting that the breakpoint on the Y chromosome occurred between *SRY* and *AMELY*, within interval 3, as in 46,XX males.²⁵ The other mutation occurred during maternal meiosis leading to the formation of an egg without an X chromosome since PCR analysis of the polymorphic locus DXS52 showed the presence of the X chromosome of paternal origin only in the proband. Nevertheless we can not exclude a postzygotic non-disjunction although FISH analysis displayed no hidden mosaicism either in peripheral blood lymphocytes or in other tissues.

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