

Research Letter

Unexplained shortening of the long bones in the third trimester as the only prenatal feature in a male fetus with 45,X/46,X,r(Y) mosaicism

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A 20-year-old, woman (gravida 2 para 0) was referred to the hospital at 32 weeks of gestation because of shortening of the long bones and a tentative diagnosis of achondroplasia. The pregnancy was uneventful until 28 weeks of gestation, when shortening of the long bones was noted. Level II ultrasound at 32 weeks of gestation revealed a normal amount of amniotic fluid and a singleton male fetus with a biparietal diameter of 8.1 cm (32 weeks), abdominal circumference of 24 cm (28 weeks), femur length of 4.6 cm (25 weeks), tibial length of 3.5 cm (23 weeks), fibular length of 3.7 cm (22 weeks), humeral length of 3.8 cm (23 weeks), and normal male external genitalia. The woman underwent cord blood sampling for molecular analysis of mutations of the *FGFR3* gene, array comparative genomic hybridization (aCGH) analysis of genomic imbalance, and cytogenetic analysis of chromosome abnormalities. *FGFR3* analysis revealed no mutations in the *FGFR3* gene and thus excluded achondroplasia, hypochondroplasia, and thanatophoric dysplasia. Oligonucleotide-based aCGH analysis of cord blood using CytoChip Oligo array

(BlueGnome, Cambridge, UK) revealed a 14.62-Mb duplication of Yp11.31-q11.221 and a 40.06-Mb deletion of Yq11.221-q12, or $\text{arr Yp11.31q11.221 (2,716,409 - 17,336,279 base pairs)} \times 2$, $\text{Yq11.221q12 (17,375,731 - 57,440,839 bp)} \times 0$ (Fig. 1). Cytogenetic analysis of cord blood revealed a karyotype of 46,X,idelic r(Y)(p11.31q11.221) in 20 cord blood lymphocytes. The parental karyotypes were normal. At 33 weeks of gestation, the woman underwent amniocentesis, which revealed a karyotype of 46,X,r(Y)(p11.31q11.221)[20]/46,X,idelic r(Y)(p11.31q11.221)[2]/45,X[2] (Fig. 2). Multicolor banding (MCB; MetaSystem, Altusheim, Germany) analysis of ten metaphase amniocytes showed that the marker chromosome originated from the short arm of the Y chromosome (Fig. 3). Fluorescence *in situ* hybridization (FISH) analysis was done with Vysis SRY/CEPX probes (Abbott, Abbott Park, IL, USA) and subtelomere-specific probes of Xp/Yp and Xq/Yq pseudoautosomal region subtelomeres (Cytocell, Adderbury, Oxfordshire, UK). The SRY (Yp11.31) and CEPX (Xp11.1-q11.1) probes correspond to the *SRY* gene and the α -satellite of the X chromosome, respectively. The Xp/Yp and Xq/Yq probes correspond to the homologous loci of DXYS129 and DXYS61, respectively. FISH analysis showed that the r(Y) chromosome contained the *SRY* gene (Fig. 4). FISH analysis showed that the X chromosome contained Xp and Xq signals,

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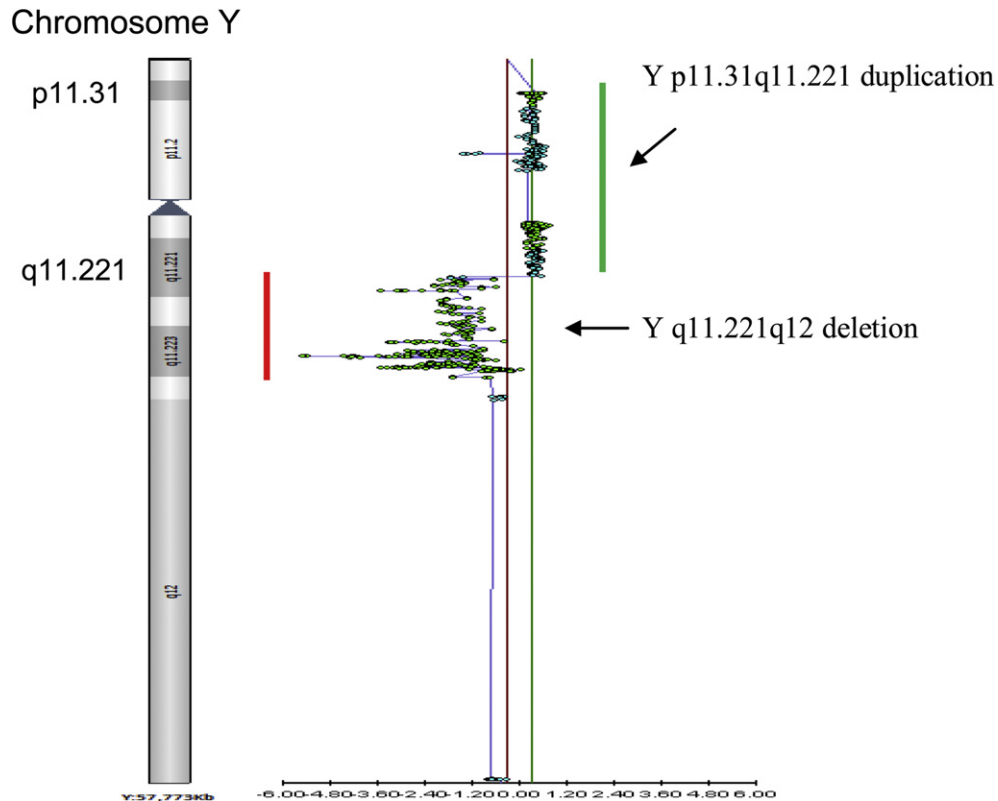


Fig. 1. Oligonucleotide-based array comparative genomic hybridization analysis of cord blood shows a 14.62-Mb duplication of Yp11.31-q11.221 and 40.06-Mb deletion of Yq11.221-q12.

but the r(Y) chromosome did not contain Yp or Yq signals (Fig. 5). The parents chose to continue the pregnancy. At 34⁺6 weeks of gestation, intrauterine fetal death occurred, and a 1344g (<third centile) male fetus was delivered with a body length of 40 cm (<third centile) and normal male external genitalia. X-rays showed rhizomelic shortening of the long bones. Cytogenetic analysis of the extraembryonic tissues revealed a karyotype of 46,X,r(Y)(p11.31q11.221)[23]/45,X[17] in the placenta and a karyotype of 46,X,r(Y)(p11.31q11.221)[32]/45,X[8]/46,X,idic r(Y)(p11.31q11.221)[1] in the umbilical cord.

We have previously reported prenatal diagnosis and molecular cytogenetic characterization of a small marker chromosome derived from the ring Y chromosome associated with mosaicism for 46,X,r(Y)(:p11.31→q11.1::)[8]/46,XY[16] in a 3200 g male baby with normal male external genitalia [1]. Here, we have additionally reported prenatal diagnosis and molecular cytogenetic characterization of a small marker chromosome derived from the ring Y chromosome associated with short limbs and normal male external genitalia.

Short stature has been reported to be a prominent feature in male patients with 45,X/46,X,r(Y), 45,X/46,X,idic(Yp), 45,X/46,XY or 46,X,r(Y). Pezzolo et al [2] reported a 16-year-old male with 45,X/46,X,r(Y)/47,X,r(Y),+r(Y)/48,X,r(Y),+r(Y),+r(Y). The patient had a scrotal hypospadias and a normal sized penis at birth and short stature (143 cm) at 13 years of age. In a review of 25 cases with 45,X/46,X,r(Y) mosaicism, Hsu [3] found that 40% (n = 10) were phenotypic

males, 36% (n = 9) were phenotypic females, and 24% (n = 6) were intersex, and suggested that the 45,X cell lines determine sex differentiation. Hsu [3] also found that five of the ten phenotypic males with 45,X/46,X,r(Y) had short stature. Sher et al [4] reported two males with short stature since childhood, who both had 45,X/46,X,r(Y), *SRY*(+), presence of the Y short arm, and deletion of the Y long arm. One patient was born with a body length of 46 cm (<fifth centile), and both patients had normal male external genitalia. Kozstolanyi [5] reported a 2-year-old boy with 45,X/46,X,idic(Yp). The patient had hypospadias and a normal penis at birth and short stature (83.5 cm) at the age of 3 years and 5 months. Takihara et al [6] reported 45,X/46,X,idic(Yp) in a 41-year-old male with short stature (height 147 cm), small testes, and infertility. Yoshit-sugu et al [7] reported 45,X/46,X,idic(Yp) in a 68-year-old male with short stature (height 152.6 cm) and schizophrenia. Slim et al [8] reported prenatal diagnosis of 45,X/46,X,i(Yp) with i(Yp) in 76% of metaphase amniocytes at amniocentesis and i(Yp) in 96% of metaphase lymphocytes at cordocentesis at 27 weeks of gestation in a male fetus with short femur. The pregnancy continued normally to delivery, and the baby showed no sign of Turner syndrome except short stature (length 45.5 cm) and hypospadias. Richter-Unruh et al [9] reported six boys with a normal-appearing male phenotype and 45,X/46,XY mosaicism, four of whom were diagnosed postnatally because of short stature. Pohlschmidt et al [10] reported an 18-year-old male with 46,X,r(Y) and short stature (height 158 cm) with the bone age that was retarded by

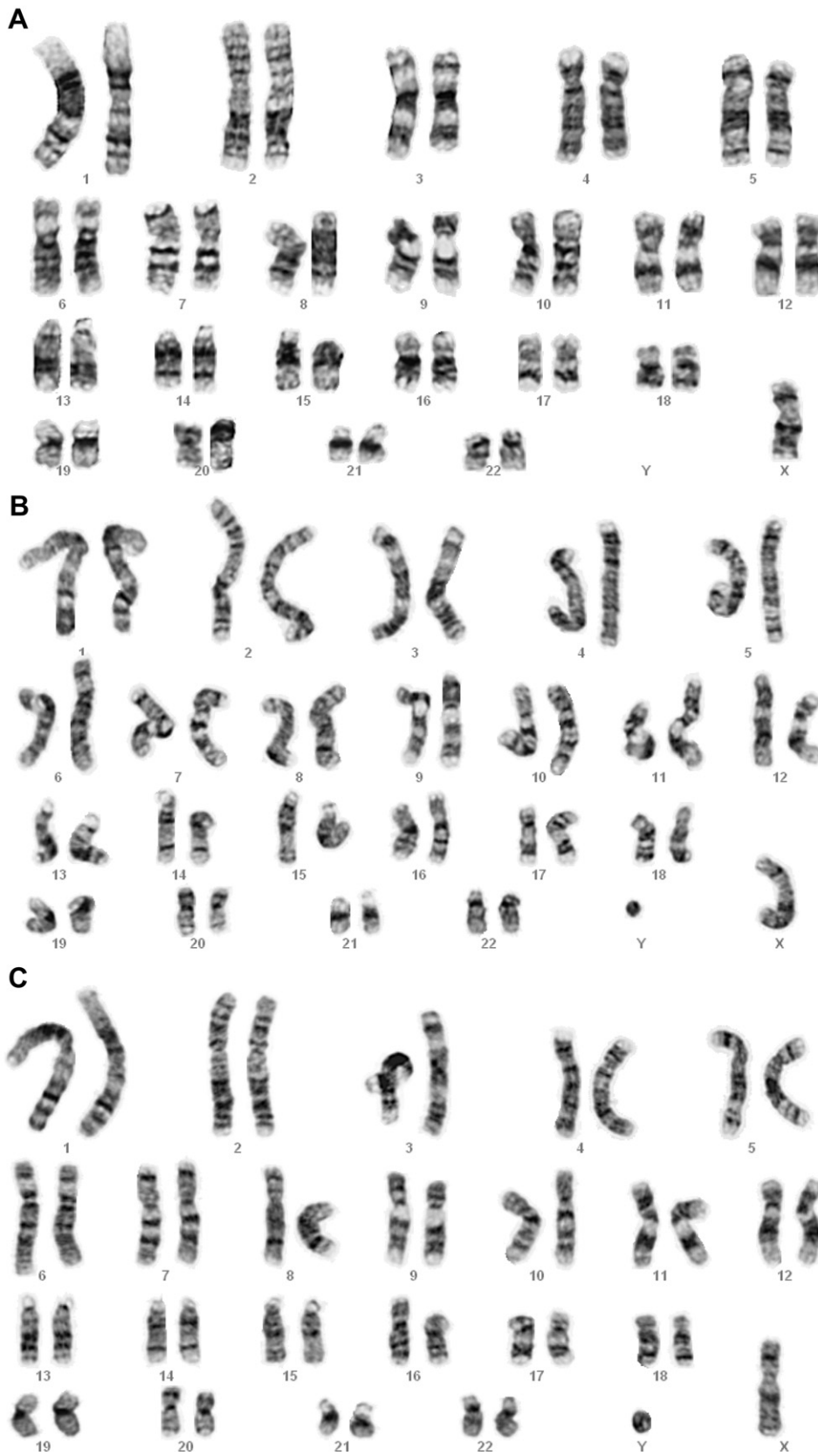


Fig. 2. (A) A karyotype of 45,X, (B) a karyotype of 46,X,r(Y) and (C) a karyotype of 46,X,idelic r(Y).

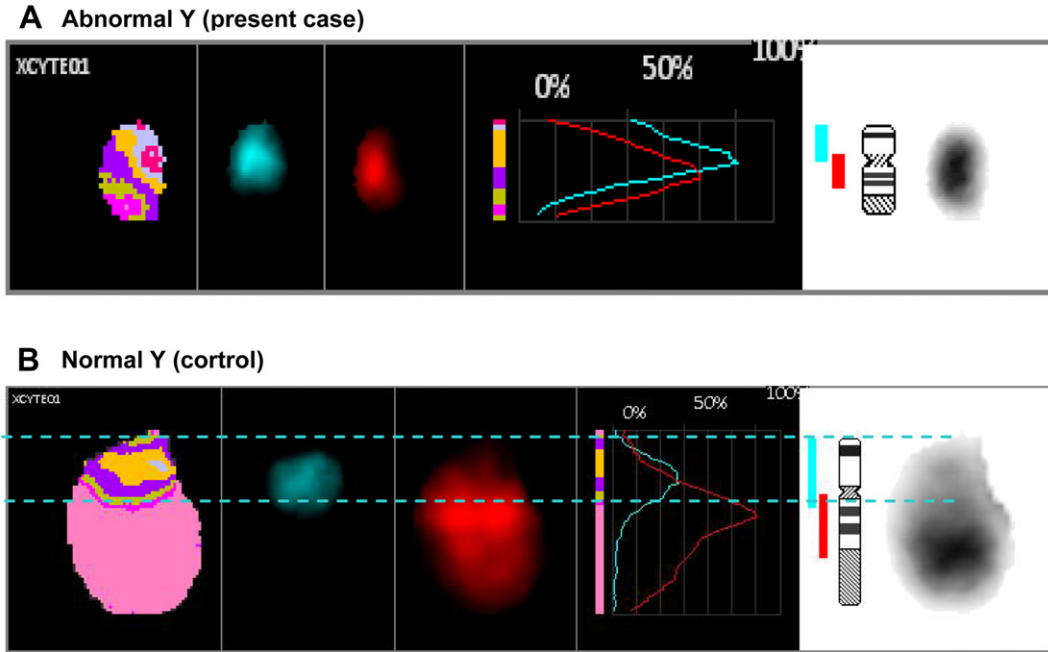


Fig. 3. (A) Multicolor banding analysis shows that the abnormal ring (r) Y chromosome is derived from the short arm and centromere of Y chromosome. (B) A normal Y chromosome for comparison.

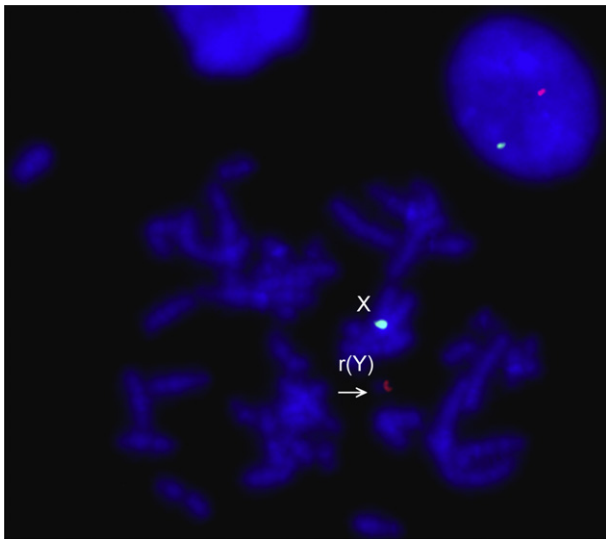


Fig. 4. Fluorescence *in situ* hybridization analysis on cultured amniocytes using CEPX (spectrum green) (Xp11.1-q11.1) and *SRY* (spectrum red) (Yp11.31) probes shows that the ring Y chromosome (arrow) contains *SRY* signal.

3 years. Daniel et al [11] reported an 11-year-old boy with 46,X,r(Y) *de novo* and short stature and hearing difficulties.

The present case provides evidence that unexplained shortening of the long bones in the third trimester can be a prominent feature in phenotypic males with 45,X/46,X,r(Y) mosaicism. We recommend cytogenetic analysis of the male fetuses with unexplained short limbs in the late gestation to detect 45,X/46,X,r(Y), 45,X/46,X,idic(Yp), 45,X/46,XY, or 46,X,r(Y).

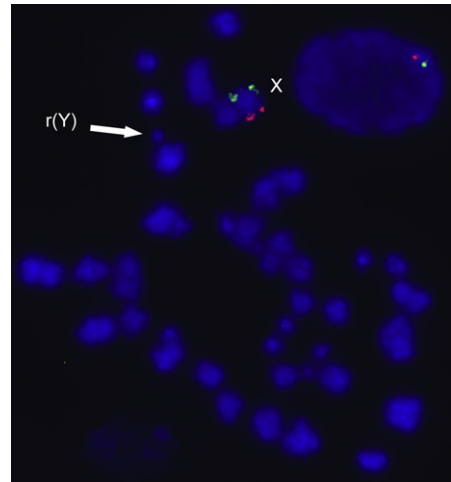


Fig. 5. Fluorescence *in situ* hybridization analysis on cultured amniocytes using Xp/Yp (spectrum red) (DXYS129; Xp/Yp pseudoautosomal region subtelomeres) and Xq/Yq (spectrum green) (Xq/Yq pseudoautosomal region subtelomeres) probes shows that the X chromosome contains both Xp and Xq signals, but the ring Y chromosome (arrow) contains neither Yp signal nor Yq signal.

Acknowledgments

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