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RESEARCH LETTER

Prenatal diagnosis of a ring chromosome 14 in a fetus with a severe skeletal dysplasia

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Ring chromosome 14 syndrome [r(14)] is a rare cytogenetic disorder associated with growth retardation, facial dysmorphism, hypotonia, seizures and retinitis pigmentosa (Van Karnebeek *et al*., 2002). Here we report on a new case of r(14) in a fetus with severe skeletal dysplasia and clinical features reminiscent of the phenotype described in paternal uniparental disomy of chromosome 14 [upd(14)pat] (Curtis *et al*., 2006). The clinical features observed in the present case are compared to those reported in previously published cases of r(14) and upd(14)pat (Jean *et al*., 1997; Van Karnebeek *et al*., 2002; McGowan *et al*., 2002; Sutton *et al*., 2003; Kagami *et al*., 2005; Curtis *et al*., 2006).

A 34-year old G4P2 woman was referred at 19 weeks' gestation because a cystic hygroma and intrauterine growth retardation (IUGR) were observed on ultrasound examination. No known drugs, radiation or teratogenic exposure were reported. The couple was not consanguineous. The woman's past obstetrical history revealed two early miscarriages and the birth of a healthy baby girl. Maternal serum screening performed at 16.4 week's gestation showed elevated human chorionic gonadotrophin (hCG) (3.45 MoM) and alpha fetoprotein (AFP) (3.30 MoM) levels.

Detailed ultrasound examination performed at 18 weeks revealed the presence of multiple abnormalities : ascites, cystic posterior cervical edema, narrow thorax with hyperechogenic lungs, short ribs, hepatomegaly. The biparietal diameter was 37 mm (10 percentile), the abdominal diameter was 43 mm (90 percentile) and the femur length was 17 mm (<2.5 percentile). All feet, tibia, humeri and radii measurements were below the 2.5 percentile.

The association of short and long bones, large extremities, femoral incurvation was suggestive of a severe fetal skeletal dysplasia.

After genetic counseling the parents elected to terminate the pregnancy. Amniotic fluid was sampled for karyotyping at termination of pregnancy (20 weeks' gestation). A female fetus was delivered and a necropsy was performed.

The fetus weighed 310 g and was 23 cm long. It showed generalized micromelia, cervical hygroma, a small bell-shaped thorax and a protruding abdomen. Facial features included a long prominent philtrum and retrognathism.

Autopsy revealed the presence of ascites, bilateral renal hypotrophy (combined weight : 1.12 g; expected weight : 2.4 ± 1.0 g) and cardiomegaly with right heart dilatation. The presence of tubular microcysts was noted in the cortical and medullar layers. Neuropathology examination revealed a small brain (<5th percentile) with abnormal organization of the cortical plate and of the subplate filled with heterotopic nodules. Longitudinal tracts were disorganized at the mesencephalic level.

X-ray examination confirmed the presence of short ribs and femora. The pelvis showed hypoplastic iliac bones. Mild platyspondyly was noted. Hands showed hypoplasia of distal phalanges. An absence of ossification of mid and distal phalanges of the 5th fingers was noted. (Figure 1(a) and (b))

Histological examination of the kidneys showed a normal cortico-medullar organization. Histological study of the epiphysal plate showed a regular cartilage-bone junction but poorly ossified cartilage columns (data not shown).

Cytogenetic analysis of 14 amniotic cells (RHG and GTG) showed a female karyotype. A ring chromosome 14 was observed in 50% of cells examined (Figure 1(c)). Seven metaphases showed 45 chromosomes with a missing ring 14 chromosome. The karyotype was interpreted as 45,XX,-14[7]/46,XX,r(14)[7]. Parental karyotypes were normal

FISH analysis was performed using BAC RP11-123M6 and BAC RP11-158A2 clones spanning respectively the *MEG3* locus and the subtelomeric region.

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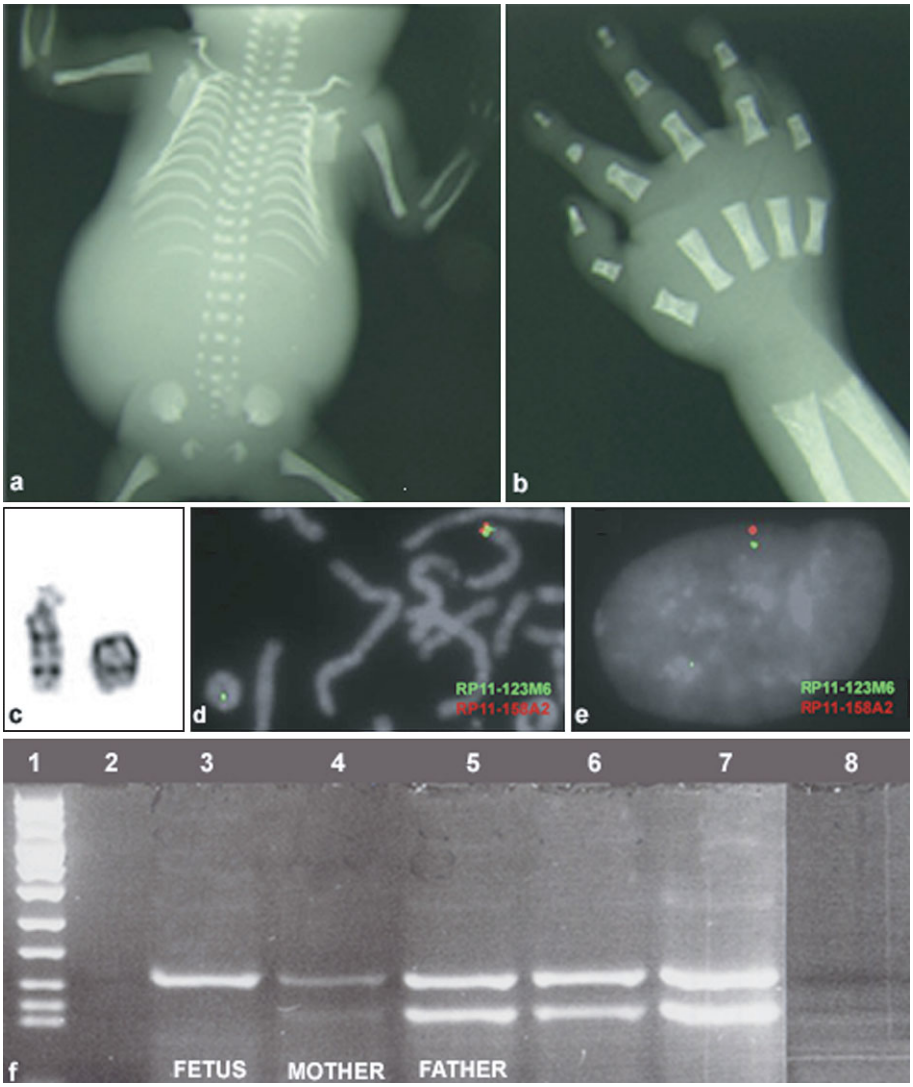

Figure 1—(a) Bell shaped thorax with abnormally curved ribs (b) Hypoplasia of distal phalanges, irregular length of the mild one and no ossification of mild and distal phalange of the fifth finger. (c) GTG partial karyotype showing a single r(14) (d) FISH analysis with BAC RP11-123M6 (14q32.2) and BAC RP11-158A2 (subtelomeric region of chromosome 14) on a metaphase : note the absence of signal with RP11-158A2 on the ring chromosome and an asymmetric signal with BAC RP11-123M6. (e) Same analysis on a nucleus : note the asymmetric signal with BAC RP11-123M6. (f) PCR of the differentially methylated region upstream of the *MEG3* promoter. Lane 1 molecular weight marker VIII (Roche Diagnostics, Mannheim, Germany); lane 2, control DNA without bisulphite modification; lane 3, patient sample; lane 4, patient's mother; lane 5, patient's father; lanes 6 and 7, normal controls; lane 8, Blank tube

In all metaphases, BAC RP11-158A2 and BAC RP11-123M6 were present on the normal chromosome 14. On the ring chromosome 14, BAC RP11-158A2 was deleted and the fluorescent signal of BAC RP11-123-M6 corresponding to the *MEG3* locus was present but weak. (Figure 1(d) and (e)).

We concluded that the breakpoint was located in BAC RP11-123M6 between the *MEG3* gene and the *DLK1* gene located proximally (UCSC genome browser database).

Therefore the karyotype of the fetus can be described as 45,XX,-14/46,XX,r(14)(p11q32.2).

DNA was obtained from cultured amniotic fluid cells and from each of the parents. The methylation pattern of the *MEG3* gene was studied (Murphy *et al*., 2003). PCR products were obtained using only paternally derived methylated allele specific primers. This result suggests that the maternal allele was absent (Figure 1(f)). Molecular analysis of eight polymorphic markers spanning the proximal part of chromosome 14 clearly indicated a biparental contribution (data not shown). We concluded that the ring chromosome 14 was maternal in origin and that the terminal deletion encompassed the *MEG3* gene.

Here we report on a case of mosaic ring chromosome 14 diagnosed prenatally. Molecular and FISH studies of the ring chromosome showed a distal deletion of the 14q32.2-14qter region of maternal origin

As shown in Table 1, the clinical features reported in the present case of ring chromosome 14 are unusual. The dysmorphic facial features classically described in patients presenting a ring chromosome 14 are absent. This can be explained by the early term of the pregnancy

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Table 1—Comparison of phenotypic features observed in the present case with cases of r(14), upd(14)pat

Clinical findings	r(14)[a]	Present case	Upd(14) pat[b]
Prenatal growth retardation	8/19	+	—
Cystic hygroma	1/1	+	—
Microcephaly	15/21	—	—
Downslanting palpebral fissures	10/16	—	—
Epi/telecanthi	14/19	—	—
Blepharophimosis	12/14		—
Retinis pigmentosa	9/19	—	—
Low set ears	9/10	—	—
Short neck	9/17	—	—
Seizures	22/23	—	—
Flat nasal bridge	13/16	—	11/11
Broad protruding philtrum	9/14	+	12/12
Micro/retrognathia	5/15	+	9/10
Short neck	9/17	—	11/11
Developmental retardation	17/17	—	9/9
Elevated alpha-fetoprotein	—	+	1/1
Ascites	—	+	—
Polyhydramnios	—	—	17/17
Small bell shaped thorax	—	+	15/15
Short abnormally curved ribs	—	+	5/5
Abdominal wall defect	—		14/14
Short limbs	—	+	7/12
Hypoplasia of distal phalanges	—	+	—
Right heart dilatation	—	+	—
Renal hypotrophy	—	+	—

[a] Jean *et al*., 1997; Van Karnebeek *et al*., 2002.
[b] McGowan *et al*., 2002, Sutton *et al*., 2003, Offiah *et al*., 2003, Kagami *et al*., 2005, Curtis *et al*., 2006.

since precise dysmorphic description is difficult at this term. In the unique case of prenatally diagnosed ring chromosome 14, revealed by a 8 mm cystic hygroma, the pregnancy was terminated at 14 weeks of gestation (Jean *et al*., 1997). The features described in this case cannot be attributed to the monosomic cell line since we do not know its distribution in the fetus. Nevertheless, in all cases of ring chromosome, whatever the chromosome implicated is, the presence of a monosomic cell line never affects the phenotype.

The narrow thorax with abnormally curved ribs and generalized skeletal dysplasia observed here have never been reported in cases of ring chromosome 14 or linear terminal deletion of chromosome 14. They are rather suggestive of paternal upd(14), which is associated with a severe musculoskeletal phenotype radiologically characterized by a bell-shaped thorax and 'coat-hanger' appearance of the ribs (Table 1). A cluster of reciprocally imprinted genes has been identified at 14q32.2. *MEG3*, also referred as *GTL2*, encodes for a nontranslated RNA and shows a maternal monoallelic expression and *DLK1* mapping 90 kb proximal to *MEG3,* which encodes for a transmembrane protein that contains epidermal growth factor (EGF)-like repeat motifs and shows a paternal monoallelic expression. This domain presents spatial, structural and epigenetic similarities with the well characterized IGF2/H19 domain on chromosome 11 (Wylie *et al*., 2000). In the present case, the maternal allele of *MEG3* has been deleted and the maternal allele of *DLK1* being preserved. We therefore postulate that the phenotype, particularly the radiological features observed in pat upd(14) and in the present case, results from a lack of expression of *MEG3*. Therefore, we can presumably explain the presence of features suggestive of pat upd(14) in this case of ring chromosome 14 by the extent of the deletion. It has been shown in effect that in cases of ring chromosome 14 or telomeric 14q32 deletions studied by FISH-mapping the breakpoints were always more telomeric than in the present case. The deletions never encompassed *MEG3/DLK1* region (Schlade-Bartusiak *et al*., 2005). We cannot explain why such extended deletions are not more often described, probably the severity of the phenotype impairs embryogenesis and fetal development and leads to early miscarriages.

In summary, a case of mosaic ring chromosome 14 was diagnosed prenatally in a fetus with a phenotype reminiscent of upd(14)pat. Molecular and FISH studies detected a maternal deletion of the 14q32.2–14qter region encompassing the *MEG3* gene. This suggests that some of the phenotypic features observed in upd(14)pat might result from absence of expression of the *MEG3* gene rather than over-expression of the paternally expressed *DLK1* gene.

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