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Prenatal diagnosis of Roberts syndrome and detection of an *ESCO2* frameshift mutation in a Pakistani family

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Objectives We report two siblings with Roberts syndrome (RBS), and an attempt to delineate the underlying molecular mechanism leading to familial recurrence.

Methods Cytogenetic studies and direct sequencing of the *ESCO2* gene were carried out in the second affected fetus and the parents. Fetal DNA was obtained from amniocytes after amniocentesis. Parental DNA was obtained from peripheral blood samples.

Results Cytogenetic analysis of amniocytes revealed a normal male karyotype in 20 analyzed metaphases and chromosomal aneuploidies in 10 metaphases. All metaphases displayed premature separation of centromeres and puffing of heterochromatic regions near the centromere. A homozygous mutation leading to a frameshift in *ESCO2* was identified in the fetal DNA sample. Both parents are heterozygous carriers of the same mutation.

Conclusion The present case demonstrates the prenatal diagnosis of RBS associated with a frameshift mutation in *ESCO2*. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: prenatal diagnosis; Roberts syndrome; reduction defects; heterochromatin repulsion; *ESCO2* gene; frameshift mutation

INTRODUCTION

The Roberts syndrome (RBS) (OMIM #268 300) is a rare autosomal recessive disorder. The key features of RBS include tetraphocomelia, pre- and postnatal growth retardation, craniofacial abnormalities, and to a varying degree mental retardation (McDaniel *et al*., 2000). Additional features that are commonly seen are renal anomalies and heart defects (McDaniel *et al*., 2000). A characteristic cytogenetic phenomenon in RBS is premature centromeric separation. Additional cytogenetic findings include random chromosomal loss and sporadic aneuploidies involving different chromosomes (Jabs *et al*., 1991; Van Den Berg and Francke, 1993). Recently, mutations in *ESCO2* at 8p21.1 have been reported in RBS (Vega *et al*., 2005). The *ESCO2* gene product is a member of a conserved protein family that is required for the establishment of sister chromatid cohesion during S phase and has putative acetyltransferase activity. Here, we report the prenatal diagnosis of RBS in a family with a positive history. The clinical phenotype is associated with premature centromeric separation and a mutation in *ESCO2*.

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CASE REPORT

The parents of Pakistani origin are healthy and consanguine. The first pregnancy led to birth of a male infant with a birth weight less than 2000 g. All four proximal and distal limbs were extremely reduced with bilateral hypoplastic thumbs. In addition, dysmorphic features with widely spaced eyes, flat midface, hypoplastic nasal alae, ptosis and midface hemangioma were noted (Figure 1). During the subsequent years growth was severely retarded. No internal malformations were observed. He died at the age of four because of gastroenteritis.

During the second pregnancy, sonography revealed dysmelia in the 19th week (Figure 2(B)). An amniocentesis was performed. Cytogenetic analysis of amniocytes revealed a normal male karyotype in 20 analyzed metaphases and chromosomal aneuploidies in 10 metaphases. Specifically, in five metaphases one chromosome was missing, in four metaphases an additional chromosome was revealed, in one metaphase one missing chromosome 20 and an additional chromosome 13 were observed. All metaphases displayed premature separation of centromeres and puffing of heterochromatic regions near the centromere. After termination of the pregnancy, pathological examination of the fetus demonstrated symmetric limb reduction, absence of radii and ulnae and dysgenesis of thumbs (Figure 2(A)). Furthermore, abnormalities of the lower limbs with deflection of tibiae, absence of fibulae and flexion contractures of ankles were observed. Dysmorphic facial features

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Figure 1—Facial view of the first sibling. Reproduced with permission

included a flat profile with hypoplastic nasal bone. No internal malformations were observed.

Based on these morphological and cytogenetic findings, the siblings were diagnosed with RBS. Recently, mutations in *ESCO2*, which is localized at 8p21.1, have been reported in RBS. DNA was isolated from amniocytes and fibroblasts of the skin by standard techniques. The entire *ESCO2* coding region was screened for mutations. Polymerase chain reaction (PCR) was performed with approximately 200 ng DNA in a volume of 20 μL and primers specific for coding regions of the *ESCO2* gene. Primers are given in Table 1. Sequencing of PCR products was carried out using the DYEnamic ET Terminator Kit (Amersham Biosciences, Freiburg, Germany) and the Megabace 1000 (Amersham Biosciences, Freiburg, Germany) according to the manufacturer's instructions. Analysis of genomic DNA from amniocytes and fibroblasts revealed a homozygous mutation at exon 4 (Figure 2(E)). Specifically, a 2 bp-deletion (p.R293fxX299) was found, which causes a frameshift with a predicted truncated protein. Analysis of genomic DNA from blood samples revealed heterozygosity for this mutation in each parent (data not shown).

DISCUSSION

RBS is a single disorder with a wide range of clinical manifestations. The hallmarks of RBS include symmetric limb reductions and craniofacial anomalies. Familial and sporadic cases have been reported consistent with an autosomal recessive mode of inheritance. Mitotic cells from many individuals with RBS display characteristic cytogenetic features including repulsion of heterochromatic regions near centromeres of chromosomes 1, 9, 16 and splaying the short arms of acrocentrics and of distal Yq (German, 1979; Louie and German, 1981). Schüle *et al.* proposed that heterochromatin repulsion (HR) and *ESCO2* mutations are tightly linked, causally related and allow to distinguish this particular type of phocomelia from other phocomelia syndromes with pre- and postnatal growth deficiency (Schule *et al*., 2005). This phenotype can now be delineated with confidence on the basis of *ESCO2* mutations. HR was seen with all *ESCO2* mutations detected so far (Schule *et al*., 2005; Vega *et al*., 2005).

A consistent finding in all patients is striking prenatal growth retardation. Most full-term infants weighed less than or equal to 2,100 g. Tetraphocomelia is a prominent characteristic of RBS. The phenotype varies from complete absence of arms and legs with rudimentary digits to mild growth reduction of the limbs. Most cases are characterized by a reduction in length or absence of humerus, radius or ulna. Reduction in the number or length of fingers is common with syndactyly or clinodactyly present. Similarly, reductions or absence of femur, tibia or fibula are frequent leg abnormalities. A qualitative analysis of the cases shows that the reduction in size of arm bones is often greater than that of leg bones. In addition, flexion contractures are common including contractures of the knees, ankles, wrists or elbows (Van Den Berg and Francke, 1993). However, neither the type nor the location of the *ESCO2* mutations predicts the severity of the phenotype.

We provide further evidence that RBS is caused by mutations in *ESCO2*. This gene encodes a protein with putative acetyltransferase activity that is believed to be required for establishment of sister chromatid cohesion during S phase. *ESCO2* is a human ortholog of a yeast gene involved in establishing sister chromatid cohesion at the time of replication. Thus, RBS is the first human disorder identified in which a chromatid-cohesion defect is associated with developmental abnormalities.

In our Pakistani family, we detected the p.R293fxX299 mutation which has also been reported in three consanguinous families of Turkish origin (Vega *et al*., 2005). Despite the different ethnic origin, a founder effect cannot be excluded. This point could be settled by haplotype analysis of the ESCO2 region in the Turkish and Pakistani families. In the case of founder effect a common haplotype would be expected.

Previously, prenatal diagnosis of RBS was only possible on the basis of ultrasonography in combination with chromosome analysis. However, cases with only minor malformations have also been described (Van Den Berg and Francke, 1993). Given the variable phenotypic expression of RBS, the final diagnosis of RBS in cases suspected by ultrasound or in families at risk depends on *ESCO2* gene mutation analysis. Thus, the present case demonstrates the example of prenatal diagnosis of RBS on the basis of ultrasonography, cytogenetic findings and mutation analysis.

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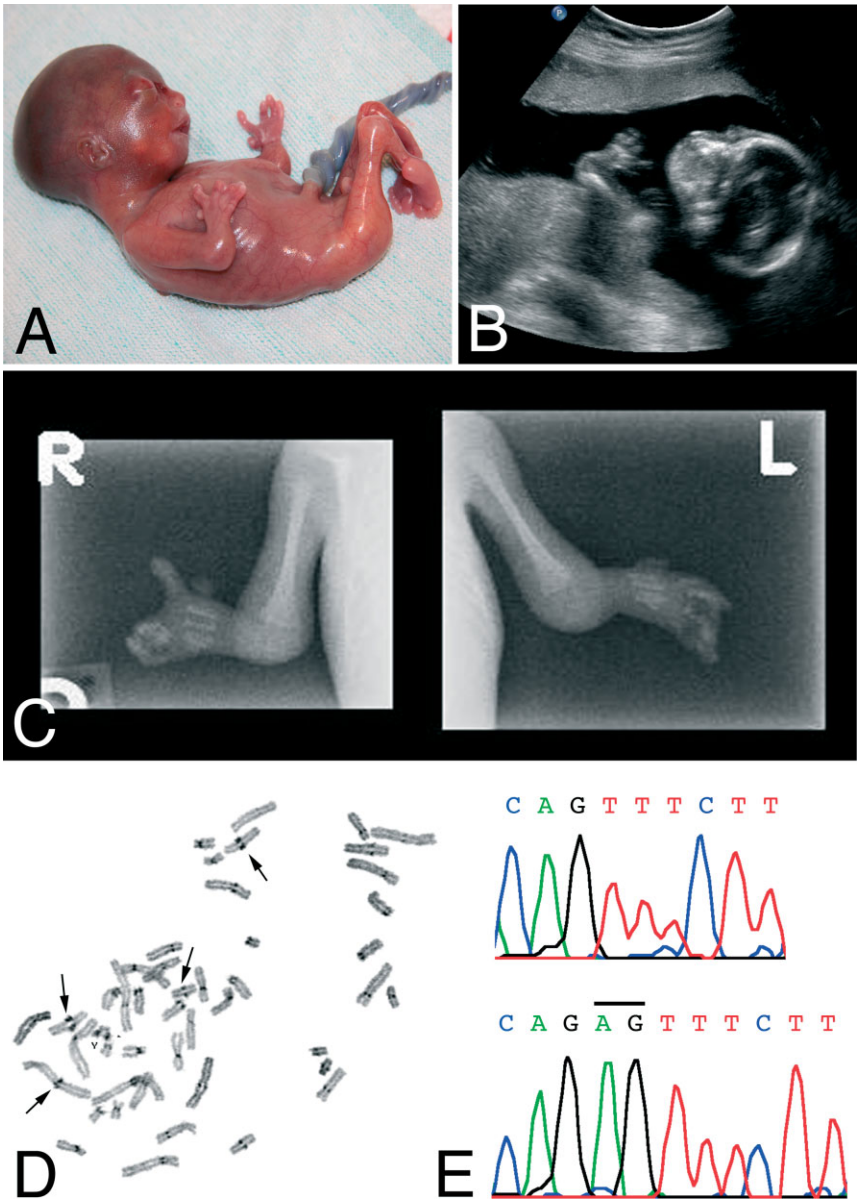

Figure 2—Main clinical, radiological, cytogenetic findings and sequence analysis. (A) Fetus with tetraphocomelia, including contractures of the knees and ankles. (B) Fetal ultrasound at 19 weeks revealed upper and lower limb reduction. (C) Radiographs of the left and right arm with missing radius and ulna, and hypoplastic appendage-like proximally placed thumb and humerus present. (D) C-banding of metaphase chromosomes. Arrows indicate premature centromere separation in several chromosomes. (E) Sequence analysis of the fetus (top) and control (bottom) demonstrates a 2 bp-deletion in exon 4 of the *ESCO2* gene

Table 1—Primers used for amplification and sequencing of *ESCO2* gene

Exon	Forward primer	Reverse primer	Product size (bp)	Annealing temperature (°C)
2	2F (5′-AGAGCAATGTCGAGGAAGACC-3′)	2R (5′-ACTGTAGCCACAAGGAGTAG-3′)	320	60
3.1	3.1F (5′-AATATGACCTACAAGTAGAGC-3′)	3.1R (5′-ACAATTGGCTTATAGATCACTC-3′)	567	55
3.2	3.2F (5′-AACTGCTAAGTATCAACCAAAG-3′)	3.2R (5′-ACACTATGCTATCTGGCTTTA-3′)	547	55
4	4F (5′-ATGTAATTCATAATAAACCAA-3′)	4R (5′-GACCCAGAAATTACAAGAATT-3′)	298	50
5	5F (5′-ATCTTGAGTATTATTTTGTC-3′)	5R (5′-TCAATATTATTCTCCTATTCC-3′)	295	50
6	6F (5′-AGTTAATGTCAGATAGAAAG-3′)	6R (5′-ATACCAAGTTCTGAACTGTC-3′)	325	50
7	7F (5′-GAATGAATGGTTATAGGAAC-3′)	7R (5′-GCTTGAAACATCATCATTAAA-3′)	331	50
8	8F (5′-ACTTTTAGTTGGATTTCATCTTC-3′)	8R (5′-AAGTGAATTATCTGTCAGCAAAG-3′)	327	55
9	9F (5′-AGTAATCATACATTCTGTGTAT-3′)	9R (5′-ATAACTTCAATTATCCACTAGG-3′)	378	50
10	10F (5′-ATTGTGTGACTAAAGGGATAAG-3′)	10R (5′-ACCTTCCTTAGCCTGACTTTA-3′)	474	55
11	11F (5′-AGGTTGATTTAAGTCAAGAGTA-3′)	11R (5′-GTATGAGTGTGAGTCTCGGTAT-3′)	344	55

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