Roberts syndrome in an Indian patient with humeroradial synostosis, congenital elbow contractures and a novel homozygous splice variant in ESCO2

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Abstract
Roberts syndrome (also known as Roberts-SC phocomelia syndrome) is an autosomal recessive developmental disorder, characterized by pre- and postnatal growth retardation, limb malformations including bilateral symmetric tetraphocomelia or mesomelia, and craniofacial dysmorphism. Biallelic loss-of-function variants in ESCO2, which codes for establishment of sister chromatid cohesion N-acetyltransferase 2, cause Roberts syndrome. Phenotypic spectrum among patients is broad, challenging clinical diagnosis in mildly affected individuals. Here we report a 3-year-old boy with a mild phenotype of Roberts syndrome with bilateral elbow contractures, humeroradial synostosis, mild lower limb disparity, and facial dysmorphology. Trio whole-exome sequencing identified the novel biallelic splice variant c.1673+1G>A in ESCO2 in the patient. Aberrant ESCO2 pre-mRNA splicing, reduced relative ESCO2 mRNA amount, and characteristic cytogenetic defects, such as premature centromere separation, heterochromatin repulsion, and chromosome breaks, in patient cells strongly supported pathogenicity of the ESCO2 variant affecting one of the highly conserved guanine-thymine dinucleotide of the donor splice site. Our case highlights the difficulty in establishing a clinical diagnosis in individuals with minor clinical features of Roberts syndrome and normal intellectual and social development. However, next-generation sequencing tools allow for molecular diagnosis in cases presenting with mild developmental defects.

KEYWORDS
ESCO2, heterochromatin repulsion, humeroradial synostosis, Roberts syndrome, SC phocomelia

1 INTRODUCTION
Roberts syndrome (RBS; also known as Roberts-SC phocomelia syndrome; OMIM 268300) is characterized by the triad of mild to severe prenatal growth retardation, limb malformations including tetraphocomelia or mesomelia with upper limbs more severely affected than lower limbs, and craniofacial dysmorphism including bilateral cleft lip and/or palate, micrognathia, widely spaced eyes, exophthalmos, downsloped palpebral fissures, underdeveloped ala nasi, and beaked nose (Gordillo, Vega, & Jabs, 1993). Biallelic pathogenic variants in ESCO2 encoding establishment of sister chromatid cohesion N-acetyltransferase 2 cause RBS (Vega et al., 2005). Wide clinical variability has been...
recognized among affected individuals without any genotype–phenotype correlation (Schüle, Oviedo, Johnston, Pai, & Francke, 2005; Vega et al., 2010). The severity of the malformations determines survival. While severely affected individuals die during pregnancy or as infants, mildly affected individuals can reach adulthood (Gordillo et al., 1993). Clinical diagnosis of RBS is particularly challenging in individuals with a mild presentation, especially when associated with rare malformations. Karyotyping can help in establishing the diagnosis of RBS as premature centrosome separation and separation of the heterochromatic regions are observed as characteristic chromosomal abnormalities in lymphocytes of individuals with biallelic ESCO2 mutations (Goh et al., 2010; Gordillo et al., 1993; Sezer, Kayhan, Zenker, & Percin, 2019). Here we describe a 3-year-old boy with humeroradial synostosis, elbow flexion contractures, pre- and postnatal growth retardation and facial dysmorphism caused by a novel homozygous splice site variant in ESCO2. We confirmed the molecular diagnosis by ESCO2 transcript analysis and cytogenetic studies.

2 | CASE REPORT

The patient is the first child of nonconsanguineous parents. Antenatal records revealed intrauterine fetal growth retardation at 32 weeks and 6 days of gestation. There was no exposure to known teratogens in antenatal period, and there were no other perinatal complications. Birth was at full term by normal vaginal delivery. His birth weight was 2,800 g (−1 SD). Bilateral flexion deformities were noted across both elbows at birth. Developmental milestones were reached appropriate to age. He started walking at 1 year and 2 months and attained complete speech at 2 years of age. On physical examination at age 3 years and 8 months, he showed postnatal growth retardation, with weight of 9 kg (−4.5 SD), height of 83 cm (−4.5 SD) and occipital frontal circumference of 43 cm (−6 SD). He had elbow flexion contractures on both sides, bilateral elbow joint pterygium, and length disparity of the lower limbs (Figure 1a,b). Craniofacial dysmorphism included low-set ears, telecanthus, long palpebral fissures, underdeveloped nasal alae, and thin upper lip vermilion (Figure 1c). He showed bilateral clinodactyly of second and fifth fingers (Figure 1d). Radiographs of the elbows and hands revealed bilateral short middle phalanx of second and fifth fingers (Figure 1e) and humeroradial synostosis (Figure 1f).

Written informed consent was obtained from the parents of the patient in accordance with the Institutional Review Board of the University of Manipal. Permission to publish photographs was also provided. Methodological details are described in Supporting Information. By trio whole-exome sequencing and subsequent Sanger sequencing we found the homozygous splice variant c.1673+1G>A in the ESCO2 gene in the patient, and both parents were heterozygous carriers.

FIGURE 1  Photographs of the patient with the homozygous ESCO2 c.1673+1G>A variant at the age of 3 years and 8 months. Patient had bilateral flexion contractures across elbow joints (left side shown in a and right arm shown in b), lower limb length disparity (a), low-set ears, long palpebral fissures, underdeveloped ala nasi, thin vermilion of upper lip (c) and bilateral clinodactyly of second and fifth fingers (right hand shown in d). Radiographs demonstrate short middle phalanx of second and fifth fingers (e) and humeroradial synostosis (f)
shown). 47,XY,+7, 47,XY,+8, and 48,XY,+7,+22 (Figure S2e and data not different chromosomes (in 53% of analyzed cells), with the karyotypes GTG-banding in patient-derived fibroblasts revealed aneuploidy of lymphocytes with an average of 0.32 breaks per cell (Figure S2c,d). Interchange configurations in 10% of the analyzed metaphases from some breakage analysis indicated chromatid breaks and chromatid showed the typical chromosome (Figure S2a). Giemsa-stained metaphase chromosomes mere separation and widely separated heterochromatin of the Y phase chromosomes from lymphocytes revealed premature centro- (Figure S1d). Cytogenetic studies were performed on short-term lymphocyte and fibroblast cultures of the patient. C-banding of metaphase chromosomes from lymphocytes revealed premature centromere separation and widely separated heterochromatin of the Y chromosome (Figure S2a). Giemsa-stained metaphase chromosomes showed the typical “railroad track” appearance (Figure S2b). Chromosome breakage analysis indicated chromatid breaks and chromatid interchange configurations in 10% of the analyzed metaphases from lymphocytes with an average of 0.32 breaks per cell (Figure S2c,d). GTG-banding in patient-derived fibroblasts revealed aneuploidy of different chromosomes (in 53% of analyzed cells), with the karyotypes 47,XY,+7, 47,XY,+8, and 48,XY,+7,+22 (Figure S2e and data not shown).

3 | DISCUSSION

Here we document a patient with mild phenotype of RBS. His limb malformations comprised humeroradial synostosis, bilateral elbow flexion contractures and elbow joint pterygium, length disparity of the lower limbs, and bilateral hypoplastic middle phalanx of second and fifth fingers. He presented with growth retardation, but normal intellectual development, and craniofacial dysmorphism characteristic of RBS. Whole-exome sequencing uncovered the underlying genetic cause, the homozygous splice site mutation c.1673+1G>A in ESCO2. Aberrant splicing of the ESCO2 pre-mRNA, reduced ESCO2 mRNA amount in patient-derived fibroblasts and the RBS-characteristic chromosomal abnormalities, such as premature centromere separation and heterochromatin repulsion, in metaphase spreads of lymphocytes, and aneuploidy in fibroblast cultures confirmed pathogenicity of the ESCO2 variant affecting the highly conserved splice donor in intron 10.

Arm bone synostosis has been reported in 48% of cases with RBS, with femorotibial synostosis in 8% (Vega et al., 2010). Humeroradial synostosis has rarely been reported. Schüle et al. (2005) described a 31-year-old male with humeroradial synostosis and additional limb malformations, short stature, borderline intellectual disability, microcephaly, and craniofacial dysmorphism. Clinical presentation in our case was much milder, and molecular genetic analysis was necessary to determine the underlying cause and establish RBS in the boy. Similarly, in a 31-year-old male with cardiac malformation in childhood, apparently normal limbs, mild learning problems, and mild facial features cytogenetic studies revealed premature centromere separation and heterochromatin repulsion and gave the hint to RBS in the patient (Goh et al., 2010).

The majority of pathogenic ESCO2 variants are frameshift, nonsense, and splice site variants, all leading to loss-of-function (Gordillo et al., 1993), similar to the c.1673+1G>A variant identified in the patient reported here. A correlation of genotype with specific clinical features could not be established, suggesting other genetic and environmental factors to contribute to phenotypic expression (Gordillo et al., 1993). Our case highlights the difficulty in establishing a clinical diagnosis in individuals with minor clinical features of RBS and normal intellectual and social development. However, state-of-the-art next-generation sequencing tools allow for a rapid molecular diagnosis and subsequent establishment of a clinical diagnosis in these challenging cases. In addition, ESCO2 transcript and chromosome analysis provided strong evidence for pathogenicity of the intronic variant reported here.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Pauline E. Schneeberger evaluated the WES data, performed segregation and transcript analyses, and prepared Figure S1. Shalini S. Nayak and Katta M. Girisha performed clinical and radiologic evaluation, collected family details, summarized clinical data and prepared Figure 1. Sigrid Fuchs performed and evaluated the cytogenetics studies and prepared Figure S2. Pauline E. Schneeberger, Shalini S. Nayak, Sigrid Fuchs, Kerstin Kutsche, and Katta M. Girisha wrote the article. Kerstin Kutsche supervised the study. All authors read and approved the final version of the article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in article supplementary material. Whole-exome sequencing data are not publicly available due to privacy or ethical restrictions.
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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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