The Egyptian Journal of Medical Human Genetics (2016) 17, 137–140

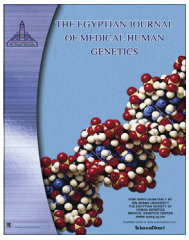
HOSTED BY

ELSEVIER

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



CASE REPORT

Cytogenetic diagnosis of Roberts SC phocomelia syndrome: First report from Kashmir

Tahir M. Malla, Arshad A. Pandith, Fayaz A. Dar, Mahrukh H. Zargar *

Advanced Centre for Human Genetics, Sher-i-Kashmir Institute of Medical Sciences, Srinagar 190011, India

Received 5 June 2015; accepted 18 June 2015
Available online 14 July 2015

KEYWORDS

Cytogenetics;
Roberts syndrome;
Premature centromeric separation;
Premature chromatid separation

Abstract There are several syndromes in which specific mitotic chromosomal abnormalities can be seen, like premature centromere separation, premature (sister) chromatid separation, and somatic aneuploidies. Identifications of such specific cytogenetic findings can be the key factor that leads towards the diagnosis of syndromes like Roberts SC phocomelia. The case presented here as Roberts SC phocomelia syndrome was identified as a child with multiple congenital anomalies and dysmorphic features. Conventional cytogenetic analysis of the case revealed premature sister chromatid separation. The premature centromeric separation was also confirmed by C banding analysis of the child. It is the first and the only case of Roberts SC phocomelia diagnosed from this part of the world. The present case report emphasizes the importance of conventional cytogenetics in the diagnosis of such syndromes.

1. Introduction

Roberts SC phocomelia syndrome is an autosomal recessive developmental disorder characterized by pre and postnatal growth retardation, microcephaly, craniofacial anomalies, mental retardation and tetraphocomelia in varying degrees of severity. Robert's syndrome was initially reported by John Roberts in a male child bearing cleft lip and tetraphocomelia [1]. Later, in four individuals from two families of European descent, Herrmann et al. reported similar, but milder malformations which were referred to as SC phocomelia [2]. These two syndromes had varying phenotypic expression and were later concluded as the same entity because of resemblance of thalidomide embryopathy with Robert's syndrome and were therefore termed as Roberts SC phocomelia syndrome [3]. The gene responsible for the syndrome called ESCO2 gene is located at 8p21.1 and was discovered by Hugo and Vega in 1995 [4]. This syndrome is rare with approximately 100 cases described in the literature [5]. Ours is the first report on Robert's syndrome from Kashmir, North India.

1.1. Clinical description

The proband, a two year old baby girl, is a product of non-consanguineous marriage, second in birth order and born to the parents of same geographical area of Kashmir valley. The patient had characteristic dysmorphic facies with defective

* Corresponding author. Tel.: +91 01942 401013x2477.
E-mail address: mhameedz@gmail.com (M.H. Zargar).
Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2015.06.006

development of all four extremities that was the main constituent of malformation complex. The craniofacial abnormalities include small low set ears, prominent frontal bones, prominent eyes, shallow orbits, hypertelorism, bilateral cleft lip and palate, micrognathia and short neck. She had severe fixed flexion deformities of all limbs. The limbs were short with hands and feet located closed to the body. The thumbs were absent bilaterally, with oligodactyly of upper and lower extremities and flexion deformity at knee joints. There was no visceromegaly and no cardio-vascular defects. The genitals were normal.

2. Methodology

2.1. G banding analysis

For G banding analysis 72 h peripheral blood lymphocyte cultures were set using RPMI 1640 medium (Sigma, St Louis, USA) supplemented with phytohemagglutinin (Himedia Labs, India) and fetal bovine serum (Himedia Labs, Mumbai, India). The cells were arrested in metaphase by colcemid treatment 2 h prior to the hypotonic shock with 0.57 M KCl solution. The cells were finally fixed with pre chilled Carnoy's fixative and slides were prepared the next day by air drop method [6]. GTG banding of one day old slides was carried out and the slides were stained using 4% Giemsa stain [7]. Thirty well spread metaphases were selected for the analysis.

2.2. C banding analysis

For C banding analysis three day old slides were dipped for 15 min in 0.2 N HCl and incubated in a saturated solution of barium hydroxide at 60 °C for 8–10 min. The slides were rinsed in distilled water for 30 s and excess barium hydroxide was removed by a rapid dip in 0.1 N HCl. The slides were reincubated in a coplin jar containing 2XSSC for one hour in a 60 °C water bath. The slides were briefly rinsed in distilled water and stained in 5% Giemsa stain [8].

3. Results

In most of the metaphases analyzed, chromosomes were found to have premature sister chromatid separation. Almost all the chromosomes of the metaphases observed had premature sister chromatid separation. The centromeric regions of chromosomes exhibited prominent separation of sister chromatids (Fig. 1). The sister chromatid separation was also confirmed by C-banding analysis (Fig. 2). The parental metaphases analyzed did not show any such separation of sister chromatids. Besides, G-banding of the child and his parents revealed no deletion, duplication or translocation. However, aneuploidy was recorded in 5% of the metaphases of the patient.

4. Discussion

Conventional cytogenetics has a crucial role in the diagnosis of some genetic disorders despite being gradually abandoned in the new era of array diagnostics. There are several syndromes in which specific mitotic chromosomal abnormalities can be seen, like premature centromere separation, premature (sister) chromatid separation, and somatic aneuploidies. Identifications of such specific cytogenetic findings can be the key factor that leads towards the diagnosis of syndromes like Roberts SC phocomelia [9]. Roberts SC phocomelia is one of the rare genetic disorders where cytogenetic analysis gives a primary insight of the chromosomal morphology and a firm

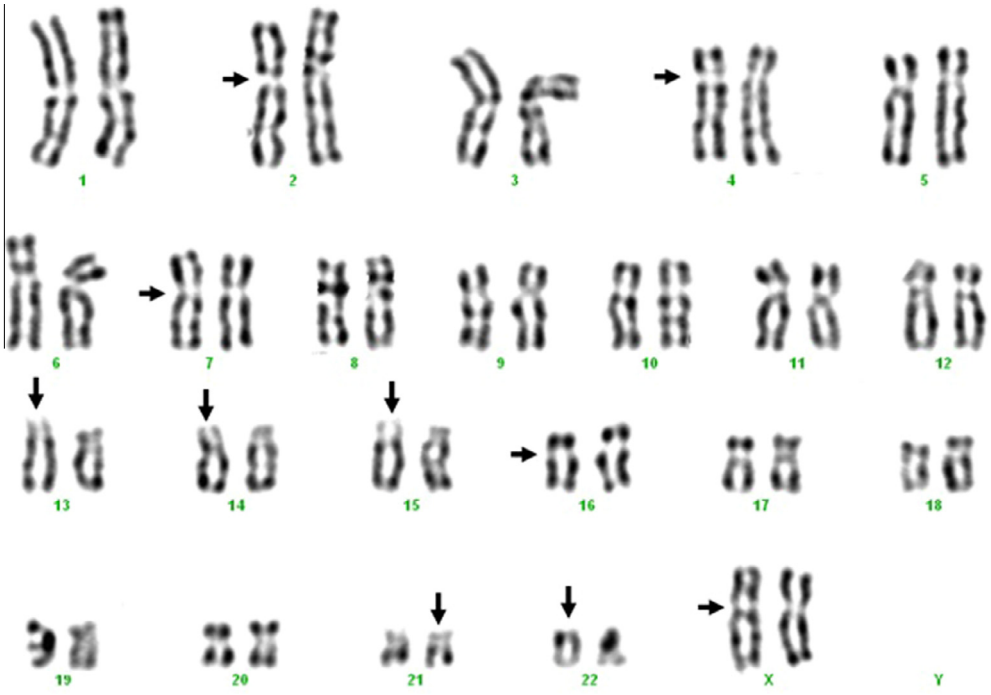


Figure 1 Karyotype of Robert's SC phocomelia syndrome showing sister chromatid separation and centromeric separation.

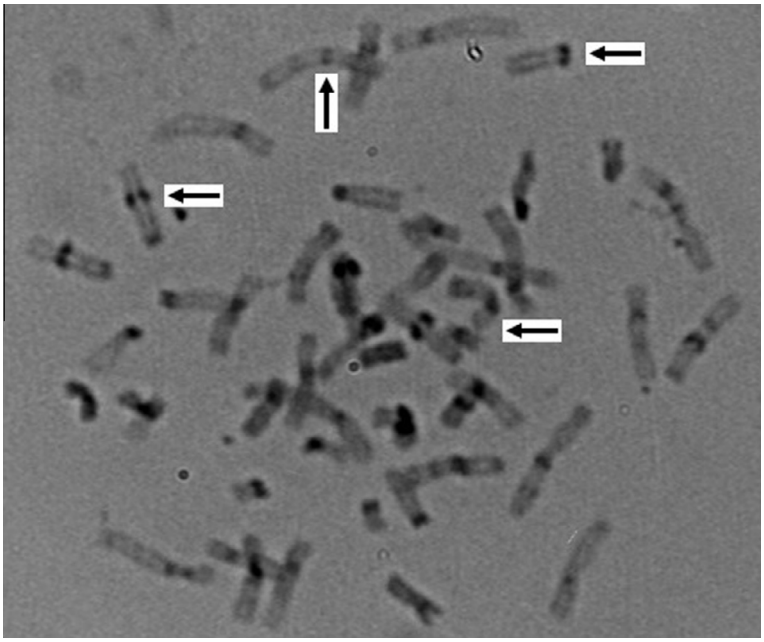


Figure 2 C-banded metaphase of Robert's SC phocomelia syndrome showing centromeric separation as indicated by arrows.

diagnosis of the syndrome is made based on the presence of early centromeric separation of the sister chromatids.

At the cytogenetic level, chromosomes of Robert's syndrome present with a rod-like morphology resulting in a 'railroad-track' appearance due to the absence of the primary constriction at the centromeric regions [10–13]. This phenomenon known as premature centromere separation (PCS) or heterochromatin repulsion (HR) constitutes the major diagnostic marker for RS. In this case, the centromeric regions of chromosomes exhibited prominent separation indicating the absence of establishment of cohesion between their sister chromatids. No direct diagnosis was made and chromosomal analysis was performed on the peripheral blood lymphocytes of the patient for the confirmatory diagnosis.

Aneuploidy has been found associated with PCS/HR most likely due to outlying, lagging or prematurely advancing chromosomes during mitosis [12]. The case in discussion not only showed PCS/HR, but also somatic aneuploidies in 6 out of 30 metaphases. Such somatic variegated aneuploidy has been reported before in several patients with Roberts syndrome [14,15] and is probably the direct consequence of the premature separation resulting in mal-segregation of both chromatids over the two daughter cells.

5. Conclusion

Recent advances in genetic diagnostics have enormous advantages over traditional karyotyping and have increased the yield of diagnoses in dysmorphology by approximately 15%. However, metaphase anomalies like PCS/HR and PSCS cannot be detected by techniques like array studies and therefore milder forms of syndromes like those of Roberts SC phocomelia may remain undetected. We therefore conclude from the present case study that routine cytogenetic analysis leads to the accurate diagnosis of Robert's phocomelia syndrome. Therefore, it is recommended in children with pre-and postnatal dysmorphic features and growth retardation.

Acknowledgment

The authors acknowledge the parents of the child for their cooperation.

References

[1] Herrmann J, Feingold M, Tuffli GA, Opitz JM. A familial dysmorphogenetic syndrome of limb deformities, characteristic facial appearance and associated anomalies: the 'pseudothalidomide' or 'SC-syndrome'. Birth Defects Orig Art Ser 1969;V:81–9.
[2] Temtamy SA, Ismail S, Helmy NA. Roberts syndrome: study of four new Rgyptian cases with comparison of clinical and cytogenetic findings. Genet Couns 2006;17:1–13.
[3] Vega H, Waisfisz Q, Gordillo M, Sakai N, Yanagihara I, Yamada M, et al. Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion. Nat Genet 2005;37:468–70.
[4] Gordillo M, Vega H, Trainer AH, Hou F, Sakai N, Luque R, et al. The molecular mechanism underlying Roberts syndrome involves loss of ESCO2 acetyltransferase activity. Hum Mol Genet 2008;17:2172–80.
[5] Judge C. A sibship with the pseudothalidomide syndrome and an association with Rh incompatibility. Med J Aust 1973;2:280–1.
[6] Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparation of leukocytes cultured from human peripheral blood. Exp Cell Res 1960;20:613–6.
[7] Seabright M. A rapid banding technique for human chromosomes. Lancet 1971;II:971–2.
[8] Schere JMJC. Production of C and T bands in human chromosomes after heat treatment at high pH and staining with ''Stains-All''. Human Genet 1974;23:311–4.
[9] Gerkes Erica H, Anne-Marie F, van der Kevie-Kersemaekers, Yakin M, Dominique FCM, et al. The importance of

chromosome studies in Roberts syndrome/SC phocomelia and other cohesinopathies. Eur J Med Genet 2010;53:40–4.
[10] German J. Roberts' syndrome I. Cytological evidence for a disturbance in chromatid pairing. Clin Genet 1979;16:441–7.
[11] Tomkins D, Hunter A, Roberts M. Cytogenetic findings in Roberts-SC phocomelia syndrome(s). Am J Med Genet 1979;4:17–26.
[12] Louie E, German J. Roberts's syndrome. II. Aberrant Y-chromosome behavior. Clin Genet 1981;19:71–4.
[13] Jabs EW, Tuck-Muller CM, Cusano R, Rattner JB. Studies of mitotic and centromeric abnormalities in Roberts syndrome: implications for a defect in the mitotic mechanism. Chromosoma 1991;100:251–61.
[14] Schulz S, Gerloff C, Ledig S, Langer D, Volleth M, Shirneshan K, et al. Prenatal diagnosis of Roberts syndrome and detection of an ESCO2 frameshift mutation in a Pakistani family. Prenat Diagn 2008;28:42–5.
[15] Van Den Berg DJ, Francke U. Roberts syndrome: a review of 100 cases and a new rating system for severity. Am J Med Genet 1993;47:1104–23.