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Signs in dysmorphology

The importance of chromosome studies in Roberts syndrome/SC phocomelia and other cohesinopathies

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ABSTRACT

Roberts syndrome/SC phocomelia is a rare, autosomal recessive syndrome characterised by pre- and postnatal growth retardation, microcephaly, craniofacial anomalies, mental retardation, and tetraphocomelia in varying degrees of severity. The clinical diagnosis can be challenging in phenotypically mild cases. In the extremely mild case presented here, specific mitotic abnormalities were detected and proved to be very helpful, since Roberts syndrome/SC phocomelia could be diagnosed after finding premature centromere separation and somatic aneuploidy at routine karyotyping. We discuss these and other mitotic cytogenetic abnormalities that can be of significant diagnostic importance, but which will be missed if only array studies are performed. We also discuss the difference between premature centromere separation and premature (sister) chromatid separation.

1. Introduction

In the new era of array diagnostics, regular chromosomal analysis is gradually being abandoned. In some instances, however, chromosomal analysis can be of the utmost importance in the diagnostic process. This is obvious in the case of balanced structural aberrations like translocations and insertions, which will be missed on an array platform. The same applies for the so-called chromosome breakage syndromes, which require specific cytogenetic techniques to detect the increased chromosomal vulnerability that is pathognomonic for these entities.

There are several syndromes in which specific mitotic chromosomal abnormalities can be seen, like premature centromere separation, premature (sister) chromatid separation, and somatic aneuploidies. In clinically less obvious cases, such specific cytogenetic findings can be the factor that leads towards identification of the syndrome. We illustrate this by presenting a patient with a very mild clinical presentation of Roberts syndrome/SC phocomelia, in whom clinical features did not reveal a direct diagnosis, while the cytogenetically detected premature centromere separation and somatic variegated aneuploidy clearly did. Several mitotic cytogenetic abnormalities that can be of diagnostic importance will be discussed in relation to the corresponding syndromes.

2. Case report

The proband, a girl, is the first child of unrelated parents who originate from the same geographical area. She was born at 37^{+4} weeks of gestation. Birth weight was 2300 g (3rd–10th centile) and head circumference 31 cm (3rd centile). At the age of 1 month, we noted mild facial dysmorphisms: a naevus flammeus on the forehead, mild hypertelorism, upslanted palpebral fissures, and protrusion of the tongue (Fig. 1a). Both hands showed five digits with slight shortening of the index fingers and clinodactyly of the fifth fingers. The legs and feet could not be examined because of plaster treatment for bilateral pes equinovarus. Proportions seemed normal. Abdominal ultrasound showed a mildly enlarged pyelum of the left kidney.

No direct diagnosis was made and chromosomal analysis was performed because of intra-uterine growth retardation, pes equinovarus and the dysmorphic features. In metaphase spreads of peripheral blood cells, premature centromere separation of several chromosomes was seen (Fig. 2). Furthermore, 9 out of 26 cells analysed showed somatic aneuploidy, with monosomies and trisomies of various chromosomes (6 hypodiploid and 3 hyperdiploid cells). Parental karyotypes showed no premature centromere separation or aneuploidy.

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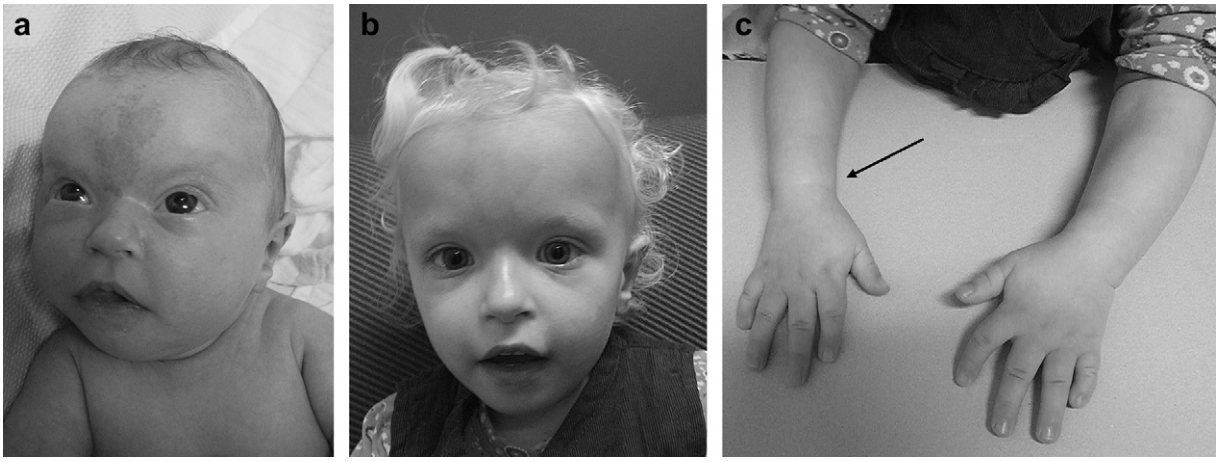

Fig. 1. Frontal view of the proband at ages 1 month (a) and 25 months (b) and forearms at age 25 months (c). a) At age 1 month a naevus flammeus on the forehead, mild hypertelorism and upslanted palpebral fissures can be noted. b) At age 25 months, apart from the mild hypertelorism, hypoplasia of the alae nasi can be seen. c) Note the extra skin crease on the radial side of the forearm (→), just below the wrist, marking the radial hypoplasia. The thumbs appear normal except for slightly low implant caused by the shortened metacarpalia. There is shortening and clinodactyly of the second and fifth fingers (reproduced with permission).

The finding of premature centromere separation and somatic aneuploidy suggested the possibility of a mild form of Roberts syndrome/SC phocomelia. This was confirmed by analysis of the causative gene for Roberts syndrome, *ESCO2*, showing a homozygous c.879_880delAG mutation, leading to a premature stop codon (p.Arg293fsX7). This specific mutation has been described before in several cases of Roberts syndrome/SC phocomelia from different ethnic backgrounds [4,14,16,17].

On re-examination at 5 months of age the child appeared to have slight radial shortening and radial deviation of the hands. Radiological studies showed mildly shortened radii and ulnae, with bowing of the radius on the left side and bilaterally abnormal radial heads with subluxation (Fig. 3). The first metacarpals and metatarsals seemed shortened and broad. Unfortunately, no X-rays were available from the entire hands, so that the skeletal cause of the shortened index fingers could not be determined. X-rays of the legs were reported to be normal. Cardiac ultrasound and ophthalmologic examination were performed since corneal opacities and cardiac malformations can occur in Roberts syndrome. No abnormalities were found. Surgical correction of the pes equinovarus was performed at the age of 8 months and the girl was clinically re-evaluated by us at the age of 25 months. Her height was 79,5 cm (−2.6 SD), her weight 9000 g (−2.0 SD) and her head circumference 44.8 cm (−2.4 SD). Her psychomotor development was normal. There was mild hypoplasia of the alae nasi and mild hypertelorism (Fig. 1b). An extra skin crease was seen on the radial side of the forearm, just below the wrist, marking the radial hypoplasia. Supination was reduced in both arms. The thumbs appeared normal, except for slightly low implant caused by the shortened metacarpalia. There was shortening and clinodactyly of the second and fifth fingers (Fig. 1c). The mild abnormalities of the hands did not affect their function.

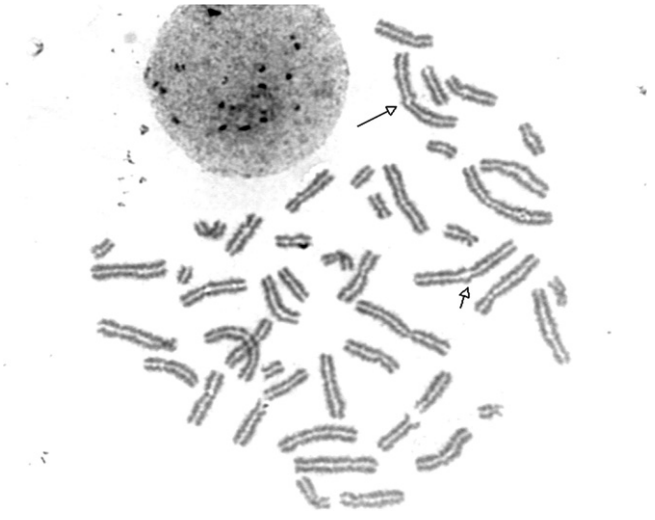

Fig. 2. Metaphase spread of a peripheral lymphocyte stained with Giemsa. Premature centromere separation, most markedly seen in chromosome 1 (see arrows).

3. Discussion

Cytogenetic studies are of major value in diagnosing several syndromes that can be hard to recognise clinically. We here describe an example of a mild case of Roberts syndrome/SC phocomelia, where premature centromere separation and somatic aneuploidies were the clues that led to the final diagnosis.

3.1. Roberts syndrome and SC phocomelia: premature centromere separation

Roberts syndrome (OMIM #268300) is a rare autosomal recessive disorder characterised by pre- and postnatal growth retardation, microcephaly, craniofacial anomalies, mental retardation and tetraphocomelia in varying degrees of severity. The typical presentation of phocomelia is marked shortening of the long bones of the limbs with relatively normal hands and feet, although the thumbs are almost always affected. In the most severe form the hands or feet appear to be attached directly to the trunk. In Roberts syndrome, the upper extremities are in general more frequently and severely affected than the lower ones, while the forearms and -legs are more frequently affected than the upper arms and -legs.

SC phocomelia (OMIM #269000) is described as the combination of phocomelia, flexion contractures, multiple minor anomalies, including capillary haemangioma of the face, forehead and ears, hypoplastic cartilages of the ears and nose, micrognathia, scanty silvery-blond hair, cloudy corneas, growth retardation and possibly mental retardation. SC phocomelia is also an autosomal recessive inherited condition and has been shown to be a milder clinical variant of Roberts syndrome, rather than a distinct clinical entity [13,15].

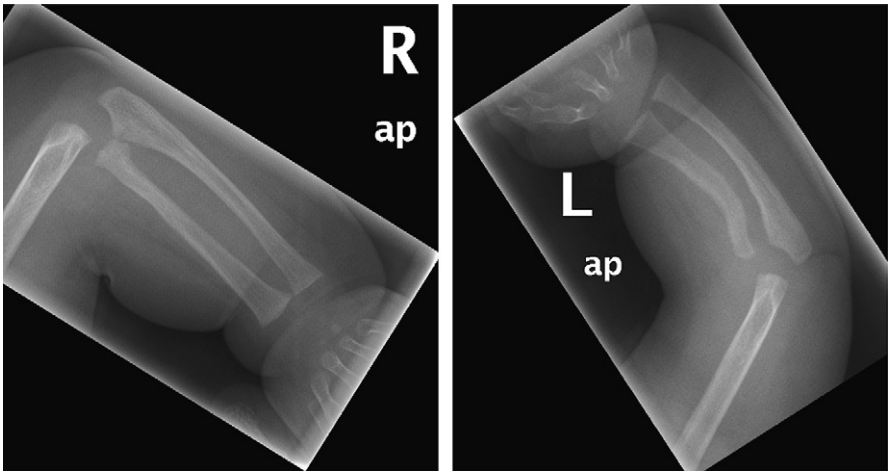

Fig. 3. X-rays of both forearms of the patient at age 5 months. Note the mildly shortened radii and ulnae, with bowing of the radius on the left side and abnormal radial heads with bilateral subluxation.

Most cases of Roberts syndrome/SC phocomelia show a recognisable, moderate to severe phenotype with tetraphocomelia. However, several mild cases have been reported, some of them even without detectable it limb malformations [15]. These cases could only be recognized because of the presence of premature centromere separation at karyotyping.

Premature centromere separation (PCS) is a cytogenetic abnormality seen in metaphase cells. It is also referred to as heterochromatin repulsion (HR), because of the puffing or repulsion of the heterochromatic regions specifically around the centromere. This gives some chromosomes a "railroad track" appearance due to the absence of a constriction around the centromere. The chromosome puffing is most obvious at chromosomes 1, 9 and 16 because of their large heterochromatic regions, whereas the repulsion is evident at the acrocentrics and the long arm of the Y-chromosome, showing "splaying" of the Yq heterochromatin [15]. PCS/HR is most easily detected in C-band or Giemsa stained chromosome slides. PCS/HR is a pathognomonic sign of Roberts syndrome according to Schule et al. (2005) [13]. PCS/HR has been seen in cases with mild to severe phenotypic manifestations of Roberts syndrome. Hence, phenotypically mild cases that might be missed clinically can be diagnosed by finding this specific cytogenetic abnormality.

The discovery of the causative gene for Roberts syndrome and SC phocomelia, *ESCO2*, led to some understanding of the aetiology of PCS/HR. The ESCO2 protein is required for sister chromatid cohesion after DNA replication. *ESCO2* mutations only cause loss of cohesion at heterochromatic regions around the centromere, leading to the specific pattern of PCS/HR [17]. PCS/HR appears to be present in all cases of Roberts syndrome/SC phocomelia with proven mutations in the *ESCO2* gene found so far [4,13,16]. Vega et al. (2009) recently analysed genotype-phenotype correlations and phenotypic associations for a cohort of patients with proven *ESCO2* mutations [16]. They did not detect a correlation between the severity of clinical findings and specific mutations. The c.879_880delAG mutation found in our proband has been shown to cause marked intra- and interfamilial variation in severity, but no case with comparable mild symptoms has been described before. The absence of corneal and cardiac abnormalities in the proband is in accordance with the findings in other patients with the same c.879_880delAG mutation. A normal mental development was described in three out of six of the patients with this mutation. The pattern of limb anomalies in the proband represents a very mild form of the characteristic mesomelic reduction defects described in Roberts syndrome. It is remarkable that her thumbs are hardly affected, because almost 98% of the described patients with Roberts syndrome show hypoplasia or aplasia of the thumbs [16]. We would like to emphasise the importance of reporting mild and severe extremes in clinical presentation of syndromes related to their genotype, because it can add substantially to the knowledge of genotype-phenotype correlations.

Our patient not only showed PCS/HR, but also somatic aneuploidies in 9 out of 26 peripheral lymphocytes. 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. However, variegated aneuploidy is also seen in combination with premature (sister) chromatid separation in other cohesinopathies (Table 1).

3.2. Differential diagnosis: premature (sister) chromatid separation

Premature centromere separation (PCS/HR) should be differentiated from premature chromatid separation (Fig. 4), confusingly known under the same abbreviation (PCS). Premature chromatid separation is a condition with premature separation of not only the centromeric region but of the entire sister chromatids [7]. Other authors used the term premature *sister* chromatid separation (PSCS) for this cytogenetic abnormality [8,11], or premature centromere division (PCD). In 2004 Kajii and Ikeuchi proposed refraining from using the term premature centromere division (PCD), and to reserve it for the age-dependent disappearance of the X-chromosomal centromere [6]. In order to prevent confusion and to provide accurate differentiation, we suggest the use of separate terms and abbreviations for these distinct cytogenetic abnormalities. We prefer the abbreviation PSCS for the premature *sister* chromatid separation, because it most precisely describes the anomaly. The abbreviation PCS/HR can then be used for premature centromere separation, that is pathognomonic for Roberts syndrome.

Whereas PCS/HR is only seen in Roberts syndrome, PSCS has been described in several syndromes (Table 1), which have some overlapping clinical features with Roberts syndrome, like growth retardation, microcephaly and limb defects. The syndromes characterised by PCS/HR or PSCS are also known as cohesinopathies [9].

A high level of PSCS is associated with the rare, autosomal recessive *Mosaic variegated aneuploidy syndrome* (MVA syndrome, OMIM #257300), characterised by severe pre- and postnatal

Table 1
Overview of syndromes with PCS/HR, PSCS or somatic aneuploidies.

Syndrome	Main features	Gene(s)	PCS/HR	PSCS	Somatic aneuploidies
Roberts syndrome/SC phocomelia	Mild to severe pre- and postnatal growth retardation and microcephaly, tetraphocomelia, craniofacial anomalies, cardiac and renal malformations, mild to severe mental retardation	*ESCO2*	++ [15]	–	+/++ [14,15]
Mosaic variegated aneuploidy syndrome	Severe pre- and postnatal growth retardation and microcephaly, brain abnormalities, childhood cancer, developmental delay	*BUB1B*	–	++, seen in 65.5% of patients [1]	++ [16]
Microcephalic osteodysplastic primordial dwarfism type II	Extreme pre- and postnatal growth retardation and microcephaly, bony abnormalities of arms and hands, Moyamoya disease, mild mental retardation	*PCNT*	–	+/−, low levels [11]	+/−, low levels [11]
Cornelia de Lange syndrome	Pre- and postnatal growth retardation and microcephaly, characteristic facial features, abnormalities of upper extremities, mild to severe mental retardation	*NIPBL, SMC3, SMC1A*	–	+/− [8]	-
Fanconi anaemia	Pre- and postnatal growth retardation and microcephaly, radial and thumb abnormalities, cardiac and renal malformations, anaemia/bone marrow failure, leukaemia, mental retardation	*FANCA-FANCN genes*	–	+/− [10,12]	–

– not a feature; +/− rarely described; ++ a frequent feature, references in square brackets.

growth retardation, developmental delay, microcephaly, brain abnormalities and childhood cancer [9]. Mosaic variegated aneuploidy is mandatory for this diagnosis, because an increased level of PSCS alone can be an isolated autosomal dominant finding without clinical significance, and has been seen in healthy heterozygous parents of MVA syndrome patients [2,7]. PSCS is not obligatory for the diagnosis of MVA syndrome as it was only found in 65.5% of MVA patients [1]. In 2004, Hanks et al. [5] identified compound heterozygous mutations in the *BUB1B* gene in several patients with MVA syndrome. The encoded protein, BUBR1, is a key protein in the mitotic spindle checkpoint. The mitotic spindle checkpoint ensures that the transition from metaphase to anaphase is delayed by blocking premature chromatid separation until all chromosomes have attached to the spindle. When the spindle checkpoint is defective, PSCS and aneuploidies may arise [5].

Recently, low-level mosaic variegated aneuploidy and PSCS have been observed in patients with *Microcephalic osteodysplastic primordial dwarfism type II* (MOPD II, OMIM #210720) [11]. The causative gene, pericentrin (*PCNT*), plays a role in chromosome segregation and mitosis and localizes to the centrosome. Rauch et al. suggested a role for PCNT in the spindle assembly checkpoint [11].

Kaur et al. reported the occurrence of PSCS in *Cornelia de Lange syndrome* (OMIM #122470) in 2005 [8]. They found PSCS in 41% of metaphase spreads from probands with or without mutations in the *NIPBL* gene, the only gene known to be associated with Cornelia de Lange syndrome at that time [8]. Like ESCO2, NIPBL is a regulator of the cohesin protein complex, involved in the cohesion of sister chromatids during mitosis until their coordinated segregation during anaphase. However, unlike ESCO2, the function of NIPBL does not seem to be restricted to the heterochromatic regions. Therefore, mutations in the *NIPBL* gene can lead to PSCS, whereas mutations in the *ESCO2* gene in Roberts syndrome, lead to PCS/HR. More recently, two other genes, *SMC1A* and *SMC3*, have been discovered to be involved in Cornelia de Lange syndrome. These genes encode proteins of the cohesion protein complex [3].

PSCS has also been reported in some patients with *Fanconi anaemia* and *Ataxia telangiectasia* [10,12].

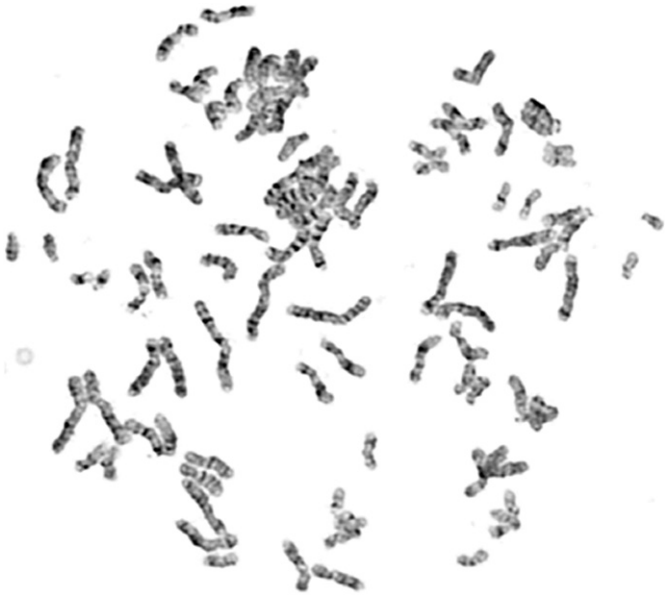

Fig. 4. Example of premature *sister* chromatid separation (PSCS) of almost all chromosomes. The result is a tetraploid cell with pair-wise ordering of the chromosomes, each composed of a single chromatid.

4. Conclusion

Application of an array platform in the diagnostic work-up of patients with mental retardation and/or dysmorphisms/malformations has enormous advantages over traditional karyotyping and has increased the yield of diagnoses in dysmorphology by approximately 15%. Karyotyping will therefore probably be abandoned in the near future as a routine primary investigation in dysmorphology. However, metaphase anomalies like PCS/HR and PSCS can not be detected by array studies and thus mild presentations of the syndromes outlined above may end up undetected. This is especially true for Roberts syndrome/SC phocomelia as demonstrated by our case report.

We therefore recommend performing routine karyotyping in children with pre- and postnatal growth retardation in combination with microcephaly or shortening of the long bones, especially the radius, if the array results prove to be normal.

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References

[1] P. Callier, L. Faivre, V. Cusin, N. Marle, C. Thauvin-Robinet, D. Sandre, T. Rousseau, P. Sagot, E. Lacombe, V. Faber, F. Mugneret, Microcephaly is not mandatory for the diagnosis of mosaic variegated aneuploidy syndrome. Am. J. Med. Genet. A 137 (2005) 204–207.

[2] A. Corona-Rivera, F. Salamanca-Gomez, L. Bobadilla-Morales, J.R. Corona-Rivera, C. Palomino-Cueva, T.A. Garcia-Cobian, E. Corona-Rivera, Cell cycle and centromere FISH studies in premature centromere division. BMC Med. Genet. 6 (2005) 33.

[3] M.A. Deardorff, M. Kaur, D. Yaeger, A. Rampuria, S. Korolev, J. Pie, C. Gil-Rodriguez, M. Arnedo, B. Loeys, A.D. Kline, M. Wilson, K. Lillquist, V. Siu, F.J. Ramos, A. Musio, L.S. Jackson, D. Dorsett, I.D. Krantz, Mutations in cohesin complex members SMC3 and SMC1A cause a mild variant of cornelia de Lange syndrome with predominant mental retardation. Am. J. Hum. Genet. 80 (2007) 485–494.

[4] M. Gordillo, H. Vega, A.H. Trainer, F. Hou, N. Sakai, R. Luque, H. Kayserili, S. Basaran, F. Skovby, R.C. Hennekam, M.L. Uzielli, R.E. Schnur, S. Manouvrier, S. Chang, E. Blair, J.A. Hurst, F. Forzano, M. Meins, K.O. Simola, A. Raas-Rothschild, R.A. Schultz, L.D. McDaniel, K. Ozono, K. Inui, H. Zou, E.W. Jabs, The molecular mechanism underlying Roberts syndrome involves loss of ESCO2 acetyltransferase activity. Hum. Mol. Genet. 17 (2008) 2172–2180.

[5] S. Hanks, K. Coleman, S. Reid, A. Plaja, H. Firth, D. Fitzpatrick, A. Kidd, K. Mehes, R. Nash, N. Robin, N. Shannon, J. Tolmie, J. Swansbury, A. Irrthum, J. Douglas, N. Rahman, Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nat. Genet. 36 (2004) 1159–1161.

[6] T. Kajii, T. Ikeuchi, Premature chromatid separation (PCS) vs. premature centromere division (PCD). Am. J. Med. Genet. A 126A (2004) 433–434.

[7] T. Kajii, T. Kawai, T. Takumi, H. Misu, O. Mabuchi, Y. Takahashi, M. Tachino, F. Nihei, T. Ikeuchi, Mosaic variegated aneuploidy with multiple congenital abnormalities: homozygosity for total premature chromatid separation trait. Am. J. Med. Genet. 78 (1998) 245–249.

[8] M. Kaur, C. DeScipio, J. McCallum, D. Yaeger, M. Devoto, L.G. Jackson, N.B. Spinner, I.D. Krantz, Precocious sister chromatid separation (PSCS) in Cornelia de Lange syndrome. Am. J. Med. Genet. A 138 (2005) 27–31.

[9] J. Liu, I.D. Krantz, Cohesin and human disease. Annu. Rev. Genomics Hum. Genet. 9 (2008) 303–320.

[10] K. Mehes, E.M. Buhler, Premature centromere division: a possible manifestation of chromosome instability. Am. J. Med. Genet. 56 (1995) 76–79.

[11] A. Rauch, C.T. Thiel, D. Schindler, U. Wick, Y.J. Crow, A.B. Ekici, A.J. van Essen, T.O. Goecke, L. Al Gazali, K.H. Chrzanowska, C. Zweier, H.G. Brunner, K. Becker, C.J. Curry, B. Dallapiccola, K. Devriendt, A. Dorfler, E. Kinning, A. Megarbane, P. Meinecke, R.K. Semple, S. Spranger, A. Toutain, R.C. Trembath, E. Voss, L. Wilson, R. Hennekam, F. de Zegher, H.G. Dorr, A. Reis, Mutations in the pericentrin (PCNT) gene cause primordial dwarfism. Science 319 (2008) 816–819.

[12] N. Sari, C. Akyuz, D. Aktas, F. Gumruk, D. Orhan, M. Alikasifoglu, B. Aydin, Y. Alanay, M. Buyukpamukcu, Wilms tumor, AML and medulloblastoma in a child with cancer prone syndrome of total premature chromatid separation and Fanconi anemia. Pediatr. Blood Cancer 53 (2009) 208–210.

[13] B. Schule, A. Oviedo, K. Johnston, S. Pai, U. Francke, Inactivating mutations in ESCO2 cause SC phocomelia and Roberts syndrome: no phenotype-genotype correlation. Am. J. Hum. Genet. 77 (2005) 1117–1128.

[14] S. Schulz, C. Gerloff, S. Ledig, D. Langer, M. Volleth, K. Shirneshan, P. Wieacker, Prenatal diagnosis of Roberts syndrome and detection of an ESCO2 frameshift mutation in a Pakistani family. Prenat. Diagn. 28 (2008) 42–45.

[15] D.J. Van Den Berg, U. Francke, Roberts syndrome: a review of 100 cases and a new rating system for severity. Am. J. Med. Genet. 47 (1993) 1104–1123.

[16] H. Vega, A.H. Trainer, M. Gordillo, M. Crosier, H. Kayserili, F. Skovby, M.L. Giovannucci Uzielli, R.E. Schnur, S. Manouvrier, E. Blair, J.A. Hurst, F. Forzano, M. Meins, K.O. Simola, A. Raas-Rothschild, R.C. Hennekam, E.W. Jabs, Phenotypic variability in 49 cases with ESCO2 mutations, including novel missense and codon deletion in the acetyltansferase domain, correlates with ESCO2 expression and establishes the clinical criteria for Roberts syndrome. J. Med. Genet. (2009).

[17] H. Vega, Q. Waisfisz, M. Gordillo, N. Sakai, I. Yanagihara, M. Yamada, D. van Gosliga, H. Kayserili, C. Xu, K. Ozono, E.W. Jabs, K. Inui, H. Joenje, 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. 37 (2005) 468–470.