Taiwanese Journal of Obstetrics & Gynecology 54 (2015) 592–596

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Short Communication

Prenatal diagnosis and array comparative genomic hybridization characterization of interstitial deletions of 8q23.3–q24.11 and 8q24.13 associated with Langer-Giedion syndrome, Cornelia de Lange syndrome and haploinsufficiency of *TRPS1*, *RAD21* and *EXT1*

Chih-Ping Chen [a, b, c, d, e, f, *], Ming-Huei Lin [a], Yi-Yung Chen [a], Schu-Rern Chern [b], Yen-Ni Chen [a], Peih-Shan Wu [g], Chen-Wen Pan [a], Meng-Shan Lee [a], Wayseen Wang [b, h]

[a] *Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan*
[b] *Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan*
[c] *Department of Biotechnology, Asia University, Taichung, Taiwan*
[d] *School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan*
[e] *Institute of Clinical and Community Health Nursing, National Yang-Ming University, Taipei, Taiwan*
[f] *Department of Obstetrics and Gynecology, School of Medicine, National Yang-Ming University, Taipei, Taiwan*
[g] *Gene Biodesign Co. Ltd, Taipei, Taiwan*
[h] *Department of Bioengineering, Tatung University, Taipei, Taiwan*

ARTICLE INFO

Article history:
Accepted 16 July 2015

Keywords:
8q23.3–q24.13 deletion
Cornelia de Lange syndrome-4
EXT1
Langer-Giedion syndrome
RAD21
TRPS1

ABSTRACT

Objective: The aim of this research was to present prenatal diagnosis of Langer-Giedion syndrome (LGS/TRPS type II) and Cornelia de Lange syndrome-4 (CDLS4).
Materials and methods: A 36-year-old woman underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. Conventional cytogenetic analysis of amniocentesis revealed an interstitial deletion of chromosome 8q or del(8)(q23.3q24.13). Level II prenatal ultrasound examination revealed craniofacial dysmorphism. The pregnancy was terminated, and a malformed fetus was delivered with characteristic craniofacial dysmorphism of LGS/TRPS type II and CDLS4. Whole-genome array comparative genomic hybridization (aCGH) on the DNA extracted from cultured amniocytes was performed.
Results: The analysis by aCGH revealed a result of arr 8q23.3q24.11 (116,087,006–118,969,399)×1, 8q24.13 (123,086,851–124,470,847)×1 (NCBI build 37) with a 2.88-Mb deletion of 8q23.3–q24.11 encompassing six OMIM genes, *TRPS1*, *EIF3H*, *RAD21*, *SLC30A8*, *MED30*, and *EXT1*, and a 1.383-Mb deletion of 8q24.13 encompassing four OMIM genes, *ZHX2*, *DERL1*, *ZHX1*, and *ATAD2*.
Conclusion: In the present case, the conventional cytogenetic analysis of cultured amniocytes revealed del(8)(q23.3q24.13), whereas aCGH analysis of cultured amniocytes showed the deletions of 8q23.3–q24.11 and 8q24.13 with the presence of the segment 8q24.12. Therefore, aCGH provides the advantage of better understanding of the nature of interstitial deletion and genotype–phenotype correlation in this case.

Introduction

An interstitial deletion of chromosome 8q including 8q23.3 and 8q24.11 has been associated with Langer-Giedion syndrome (LGS) and Cornelia de Lange syndrome-4 (CDLS4) [1–3]. LGS or trichorhinophalangeal syndrome (TRPS) type II (OMIM 150230) is a contiguous gene syndrome associated with loss of functional copies of the *TRPS1* gene (OMIM 604386) at 8q23.3 and the *EXT1* gene (OMIM 608177) at 8q24.11 [4]. CDLS4 (OMIM 614701) is associated with heterozygous mutations or deletion of the *RAD21* gene (OMIM 606462) at 8q24.11 [1–3,5,6]. We previously reported a 19-year-old male with laxity of the skin and joints, facial dysmorphism,

* Corresponding author. Department of Obstetrics and Gynecology, Mackay Memorial Hospital 92, Section 2, Chung-Shan North Road, Taipei, Taiwan.
E-mail address: cpc_mmh@yahoo.com (C.-P. Chen).

http://dx.doi.org/10.1016/j.tjog.2015.08.013

epilepsy, gastroesophageal reflux, cardiovascular defects, mental retardation, developmental delay, exostoses, scoliosis, an 8q23.3–q24.22 deletion, haploinsufficiency of *TRPS1*, *RAD21*, *EXT1*, and *KCNQ3*, LGS and CDLS4 [6]. Here, we present prenatal diagnosis of interstitial deletions of 8q23.3–q24.11 and 8q24.13 associated with LGS and CDLS4, and haploinsufficiency of *TRPS1*, *RAD21*, and *EXT1*.

Methods

Clinical description

A 36-year-old, gravida 3, para 0, woman underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. The woman had experienced two spontaneous abortions. She and her husband were unrelated, and there was no family history of congenital malformations. Conventional cytogenetic analysis of amniocentesis revealed an interstitial deletion of chromosome 8q or del(8)(q23.3q24.13; Figure 1). Level II prenatal ultrasound examination at 21 weeks of gestation revealed craniofacial dysmorphism of bulbous nose, depressed nasal bridge, micrognathia, protruding upper lip, large protruding ears and large philtrum (Figure 2). However, the fetal biometry of the head circumference, femur length, and abdominal circumference were equivalent to 21 weeks of gestation. There was no short stature and no abnormalities of the long bones. The pregnancy was terminated at 22 weeks of gestation, and the 392-g malformed proband was delivered with characteristic craniofacial dysmorphism of LGS and CDLS (Figure 3).

Conventional cytogenetic analysis

Routine cytogenetic analysis by G-banding techniques was performed. Amniotic fluid, placental tissues, parental bloods, and cord blood were collected, and the samples were subjected to cell culture according to the standard cytogenetic protocol.

Array comparative genomic hybridization

Whole-genome array comparative genomic hybridization (aCGH) on the DNA extracted from cultured amniocytes was performed using NimbleGen ISCA Plus Cytogenetic Array (Roche NimbleGen, Madison, WI, USA). The NimbleGen ISCA Plus Cytogenetic Array has 630,000 probes and a median resolution of 15–20 kb across the entire genome according to the manufacturer's instruction. The DNA from cultured amniocytes was extracted first. It was carried out by following the manufacturer's protocol of a QIAamp DNA Mini kit (Qiagen, Inc., Valencia, CA, USA). Then, 0.5 μg of the extracted DNA was labeled with Cy5 dye and compared with an equivalent amount of normal female genomic DNA (gDNA, G1521; Promega, Madison, WI, USA) labeled in Cy3 dye to perform the aCGH experiment. The experiment was performed according to the procedures recommended from Roche NimbleGen ISCA plus Cytogenetic Array's user guide. The data were finally represented by using Nexus 6.1 (BioDiscovery, Hawthorne, CA, USA).

Results

G-banding chromosome analysis revealed a karyotype of 46,XY in the father, a karyotype of 45,X[3]/47,XXX[5]/46,XX[32] in the mother, and a karyotype of 46XY,del(8)(q23.3q24.13)dn in the amniotic fluid, cord blood, and placental tissues. The aCGH analysis revealed a result of arr 8q23.3q24.11 (116,087,006–118,969,399)×1, 8q24.13 (123,086,851–124,470,847)×1 (NCBI build 37) with a 2.88-Mb deletion of 8q23.3–q24.11 encompassing six OMIM genes—*TRPS1*, *EIF3H*, *RAD21*, *SLC30A8*, *MED30*, and *EXT1*—and a 1.383-Mb deletion of 8q24.13 encompassing four OMIM genes—*ZHX2*, *DERL1*, *ZHX1*, and *ATAD2* (Figure 4).

Discussion

Prenatal diagnosis of LGS has been previously described [7,8]. We additionally present prenatal diagnosis of interstitial deletion of 8q involving 8q23.3–q24.11 and 8q24.13 associated with LGS/TRPS

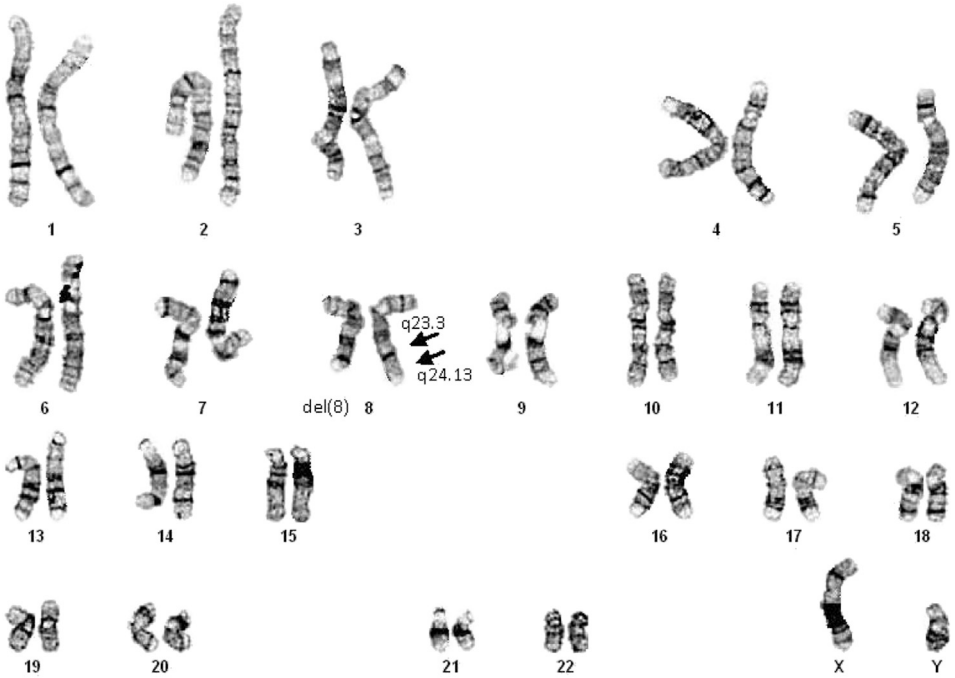

Figure 1. A karyotype of 46,XY,del(8)(q23.3q24.13). The arrows indicate the breakpoints.

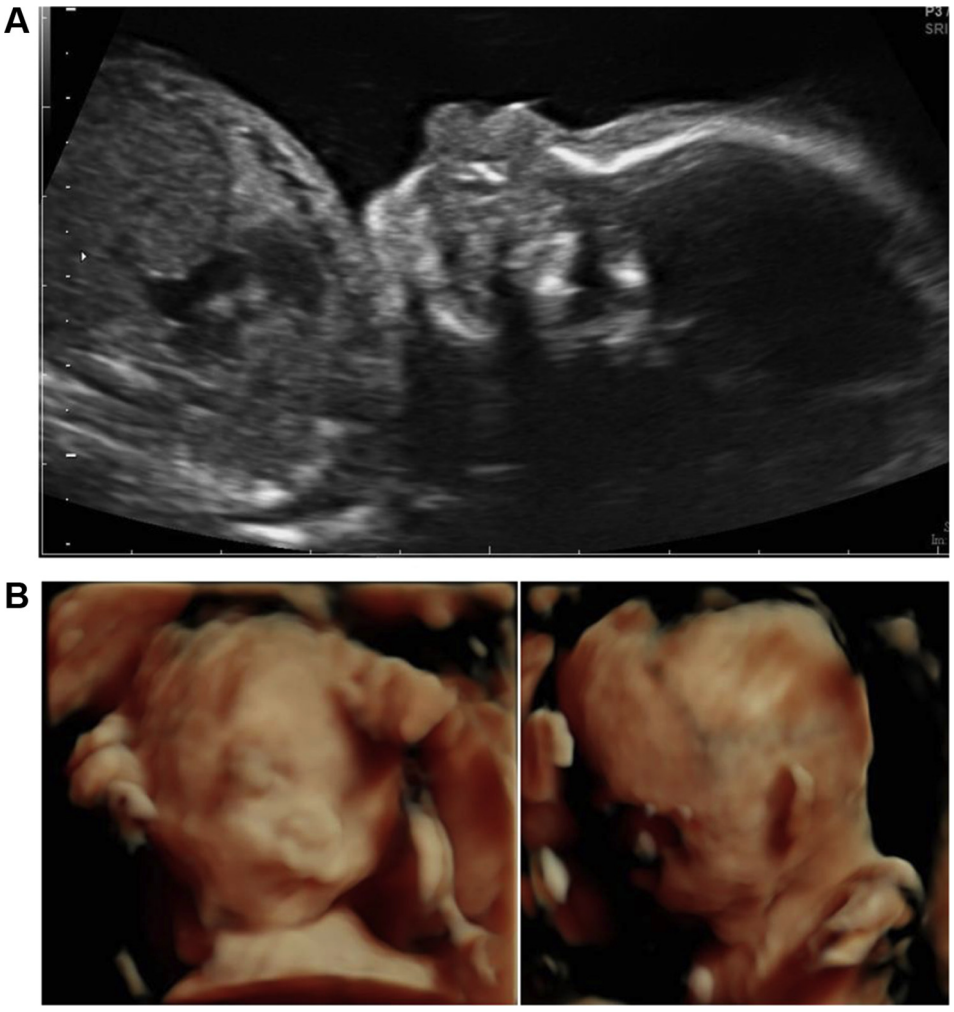

Figure 2. Craniofacial dysmorphism on (A) two-dimensional and (B) three-dimensional ultrasound.

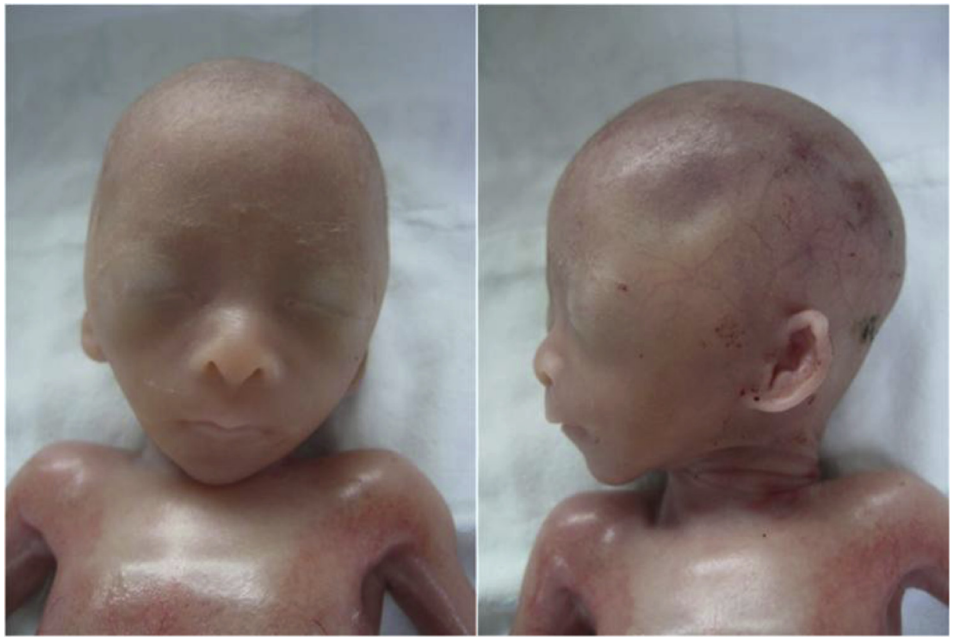

Figure 3. The proband at birth.

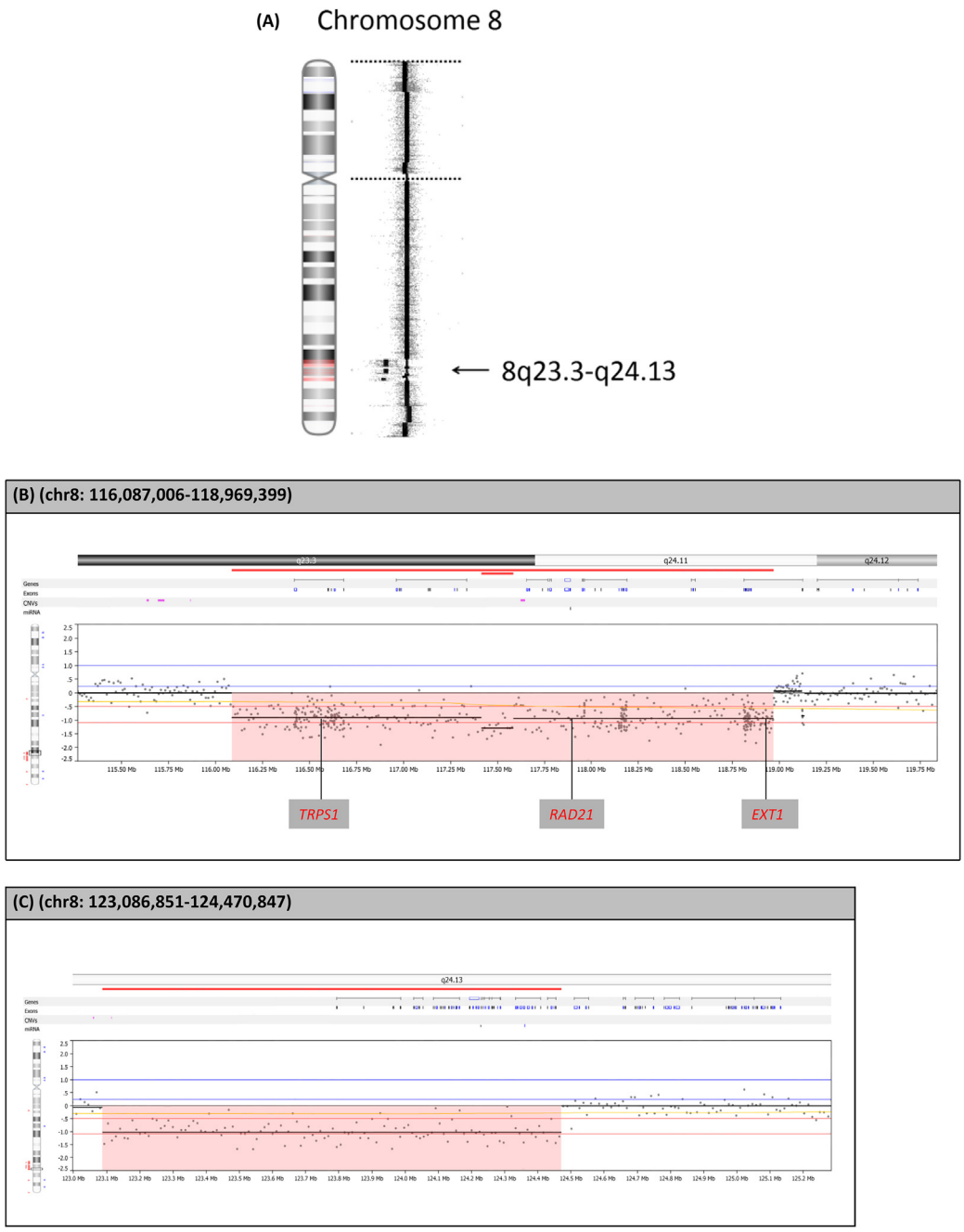

Figure 4. Array comparative genomic hybridization on cultured amniocytes shows a 2.88-Mb deletion at chromosome bands 8q23.3–q24.11 [arr 8q23.3q24.11 (116,087,006–118,969,399)×1] and a 1.383-Mb deletion of 8q24.13 [arr 8q24.13 (123,086,851–124,470,847)×1] (NCBI build 37). (A) Chromosomal view, and (B) and (C) zoom in view.

type II and CDLS4. LGS/TRPS type II is characterized by a peculiar face with bulbous nose and large protruding ears, loose redundant skin, lax joints, phalangeal abnormalities of the hands and multiple exostoses, mild growth deficiency, mild to severe mental retardation, microcephaly, and sparse scalp hair [4,6]. CDLS4 is characterized by a peculiar face with bushy eyebrows, synophrys, depressed nasal bridge, micrognathia, anteverted nares, microcephaly, prominent symphysis and spurs in the anterior angle of the mandible, growth deficiency, mental retardation, gastroesophageal reflux, duplication of gut, malrotation of colon, volvulus, pyloric stenosis, and occasional findings of seizures, congenital heart defects, and inguinal hernia [5,6]. The present case shows that fetuses with LGS/TRPS type II and CDLS4 due to haploinsufficiency of *TRPS1*, *RAD21*, and *EXT1* caused by 8q23.3–8q24.11 deletion may not present skeletal abnormalities and growth deficiencies on the second trimester ultrasound, but may present characteristic facial dysmorphism. The present case was prenatally diagnosed simply by routine amniocentesis because of advanced maternal age. Fairweather [7] first reported prenatal diagnosis of LGS/TRPS type II and del(8)(q24.1q24.3)dn in a fetus by amniocentesis due to abnormal prenatal ultrasound findings of a single umbilical artery, pulmonary stenosis with post-stenotic

dilation, pericardial effusion, and short femoral bones. Piotrowski et al [8] later reported prenatal diagnosis of LGS and 8q24 deletion at 32 weeks of gestation by amniocentesis due to fetal ascites with hydrometrocolpos presenting as an intraabdominal cyst.

The present case had haploinsufficiency of *TRPS1*, *EXT1*, and *RAD21*. *TRPS1* is located at 8q23.3 and encodes a GATA-type transcription factor that has nine zinc-finger motifs involved in the development and differentiation of bones, kidneys, and hair follicles [9]. Mutations or haploinsufficiency of *TRPS1* causes TRPS type I and TRPS type III [10–14]. TRPS type I (OMIM 190350) is characterized by craniofacial dysmorphism of sparse scalp hair, bulbous tip of the nose, long philtrum, protruding ears, cone-shaped epiphyses at the phalanges, hip malformations, and short stature [10]. TRPS type III (OMIM 190351) is similar to TRPS type I but is characterized by severe short stature, severe brachydactyly and more severe shortening of all phalanges and metacarpals [12]. *EXT1* is located at 8q24.11 and encodes exostosin 1 which is essential in the regulation of chondrocyte differentiation, ossification, and apoptosis [15]. *EXT1* is a tumor suppressor gene [16–18]. Mutations or haploinsufficiency of *EXT1* will cause multiple exostoses type I (OMIM 133700) and chondrosarcoma (OMIM 215300) [19]. An interstitial deletion of 8q23.3–8q24.11 involving loss of the functional copies of *TRPS1* and *EXT1* causes LGS/TRPS type II. *RAD21* is located at 8q24.11 and encodes nuclear matrix protein 1 which is essential for sister chromatid cohesion during mitosis and meiosis [1,20]. Haploinsufficiency or heterozygous loss-of-function mutations of *RAD21* cause CDLS4 [1].

The peculiar aspect of the present case is the discrepancy between the conventional cytogenetics and aCGH. The aCGH analysis has the advantage of detecting microdeletion syndromes in the fetuses with a normal karyotype by conventional cytogenetic analysis [21,22] or an apparently balanced translocation by conventional cytogenetic analysis [23]. In the present case, the conventional cytogenetic analysis of cultured amniocytes revealed del(8)(q23.3q24.13), whereas aCGH analysis of cultured amniocytes revealed deletions of 8q23.3–q24.11 and 8q24.13 with the presence of the segment 8q24.12. Therefore, aCGH provides the advantage of better understanding of the nature of interstitial deletion and genotype–phenotype correlation in this case.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

This work was supported by research grants NSC-101-2314-B-195-011-MY3 and MOST 103-2314-B-195-010 from the Ministry of Science and Technology and MMH-E-104-04 from Mackay Memorial Hospital, Taipei, Taiwan.

References

[1] Deardorff MA, Wilde JJ, Albrecht M, Dickinson E, Tennstedt S, Braunholz D, et al. *RAD21* mutations cause a human cohesinopathy. Am J Hum Genet 2012;90:1014–7.

[2] Pereza N, Severinski S, Ostojić S, Volk M, Maver A, Dekanić KB, et al. Third case of 8q23.3–q24.13 deletion in a patient with Langer-Giedion syndrome phenotype without *TRPS1* gene deletion. Am J Med Genet 2012;158A:659–63.

[3] Pereza N, Severinski S, Ostojić S, Volk M, Maver A, Dekanić KB, et al. Cornelia de Lange syndrome caused by heterozygous deletions of chromosome 8q24: comments on the article by Pereza, et al. [2012]. Am J Med Genet 2015;167A:1426–7.

[4] Jones KL. Langer-Giedion syndrome. In: Jones KL, editor. Smith's recognizable patterns of human malformation. Philadelphia: Elsevier Saunders; 2006. p. 324.

[5] Jones KL. Multiple exostoses syndrome. In: Jones KL, editor. Smith's recognizable patterns of human malformation. Philadelphia: Elsevier Saunders; 2006. p. 500.

[6] Chen C-P, Lin S-P, Liu Y-P, Chern S-R, Wu P-S, Su J-W, et al. An interstitial deletion of 8q23.3–q24.22 associated with Langer-Giedion syndrome, Cornelia de Lange syndrome and epilepsy. Gene 2013;529:176–80.

[7] Fairweather P. Postmortem findings at 36 weeks gestation in trichorhinophalangeal syndrome: a case report. Pathology 2006;38:170–2.

[8] Piotrowski K, Halec W, Wegrzynowski J, Pietrzyk A, Henkelman M, Zajaczek S. Prenatal diagnosis of Langer-Giedion Syndrome confirmed by BACs-on-Beads technique. Ginekol Pol 2014;85:66–9.

[9] Gai Z, Gui T, Muragaki Y. The function of *TRPS1* in the development and differentiation of bone, kidney, and hair follicles. Histol Histopathol 2011;26:915–21.

[10] Momeni P, Glöckner G, Schmidt O, von Holtum D, Albrecht B, Gillessen-Kaesbach G, et al. Mutations in a new gene, encoding a zinc-finger protein, cause tricho-rhino-phalangeal syndrome type I. Nat Genet 2000;24:71–4.

[11] Hatamura I, Kanauchi Y, Takahara M, Fujiwara M, Muragaki Y, Ooshima A, et al. A nonsense mutation in *TRPS1* in a Japanese family with tricho-rhino-phalangeal syndrome type I. Clin Genet 2001;59:366–7.

[12] Lüdecke H-J, Schaper J, Meinecke P, Momeni P, Groβ S, von Holtum D, et al. Genotypic and phenotypic spectrum in tricho-rhino-phalangeal syndrome types I and III. Am J Hum Genet 2001;68:81–91.

[13] Hilton MJ, Sawyer JM, Gutiérrez L, Hogart A, Kung TC, Wells DE. Analysis of novel and recurrent mutations responsible for the tricho-rhino-phalangeal syndromes. J Hum Genet 2002;47:103–6.

[14] Piccione M, Niceta M, Antona V, Di Fiore A, Cariola F, Gentile M, et al. Identification of two new mutations in *TRPS1* gene leading to the tricho-rhino-phalangeal syndrome type I and III. Am J Med Genet 2009;149A:1837–41.

[15] Heinritz W, Huffmeier U, Strenge S, Miterski B, Zweier C, Leinung S, et al. New mutations of *EXT1* and *EXT2* genes in German patients with multiple osteochondromas. Ann Hum Genet 2009;73:283–91.

[16] Ahn J, Lüdecke H-J, Lindow S, Horton WA, Lee B, Wagner MJ, et al. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). Nat Genet 1995;11:137–43.

[17] Hecht JT, Hogue D, Strong LC, Hansen MF, Blanton SH, Wagner M. Hereditary multiple exostosis and chondrosarcoma: linkage to chromosome 11 and loss of heterozygosity for *EXT*-linked markers on chromosomes 11 and 8. Am J Hum Genet 1995;56:1125–31.

[18] Raskind WH, Conrad EU, Chansky H, Matsushita M. Loss of heterozygosity in chondrosarcomas for markers linked to hereditary multiple exostoses loci on chromosomes 8 and 11. Am J Hum Genet 1995;56:1132–9.

[19] Hecht JT, Hogue D, Wang Y, Blanton SH, Wagner M, Strong LC, et al. Hereditary multiple exostoses (EXT): mutational studies of familial EXT1 cases and EXT-associated malignancies. Am J Hum Genet 1997;60:80–6.

[20] Sumara I, Vorlaufer E, Gieffers C, Peters BH, Peters J-M. Characterization of vertebrate cohesin complexes and their regulation in prophase. J Cell Biol 2000;151:749–61.

[21] Chen C-P, Chang S-D, Wang T-H, Wang L-K, Tsai J-D, Liu Y-P, et al. Detection of recurrent transmission of 17q12 microdeletion by array comparative genomic hybridization in a fetus with prenatally diagnosed hydronephrosis, hydroureter and multicystic kidney, and variable clinical spectrum in the family. Taiwan J Obstet Gynecol 2013;52:551–7.

[22] Chen C-P, Lin C-J, Chern S-R, Liu Y-P, Kuo Y-L, Chen Y-N, et al. Prenatal diagnosis and molecular cytogenetic characterization of a 1.07-Mb microdeletion at 5q35.2–q35.3 associated with *NSD1* haploinsufficiency and Sotos syndrome. Taiwan J Obstet Gynecol 2014;53:583–7.

[23] Chen C-P, Lin S-P, Chern S-R, Wu P-S, Su J-W, Lee C-C, et al. A 1.37-Mb 12p11.22–p11.21 deletion coincident with a 367-kb 22q11.2 duplication detected by array comparative genomic hybridization in an adolescent girl with autism and difficulty in self-care of menstruation. Taiwan J Obstet Gynecol 2014;53:74–8.