Taiwanese Journal of Obstetrics & Gynecology 56 (2017) 238–242

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Case Report

Prenatal diagnosis and molecular cytogenetic characterization of low-level mosaic trisomy 12 at amniocentesis associated with a favorable pregnancy outcome

Chih-Ping Chen [a, b, c, d, e, f, *], Chen-Ju Lin [a, g], Schu-Rern Chern [b], Peih-Shan Wu [h], Yen-Ni Chen [a], Shin-Wen Chen [a], Chen-Wen Pan [a], Chien-Wen Yang [b], Wayseen Wang [b, i]

[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] Department of Medicine, MacKay Medical College, New Taipei City, Taiwan
[h] Gene Biodesign Co. Ltd., Taipei, Taiwan
[i] Department of Bioengineering, Tatung University, Taipei, Taiwan

ARTICLE INFO

Article history:
Accepted 12 January 2017

Keywords:
amniocentesis
mosaic trisomy 12
prenatal diagnosis
trisomy 12 mosaicism

ABSTRACT

Objective: We present prenatal diagnosis of low-level mosaic trisomy 12.
Case Report: A 40-year-old woman underwent amniocentesis at 18 weeks of gestation because of advanced maternal age, which revealed a karyotype of 47,XX,+12[5]/46,XX[24] consistent with 17.2% (5/29) mosaicism for trisomy 12. Repeat amniocentesis performed at 21 weeks of gestation revealed a karyotype of 47,XX,+12[4]/46,XX[6] consistent with 40% (4/10) mosaicism for trisomy 12. Interphase fluorescence *in situ* hybridization (FISH) on 112 uncultured amniocytes detected 23 cells with trisomy 12 consistent with 20.5% (23/112) mosaicism for trisomy 12. Polymorphic DNA marker analysis excluded uniparental disomy 12. Array comparative genomic hybridization (aCGH) on uncultured amniocytes revealed a result of arr 12p13.33q24.33 (230,451–133,773,499) × 2.2, 17p12 (14,191,925–15,442,037) × 1.0 consistent with 10–20% mosaic trisomy 12. The father carried the 17p12 microdeletion. The fetal ultrasound findings were unremarkable. A 3958-g female fetus was delivered at 37 weeks of gestation with no phenotypic abnormality. The cord blood had a karyotype of 46,XX. Postnatal interphase FISH on urinary cells revealed 7.14% (7/98) mosaicism for trisomy 12.
Conclusion: Low-level mosaic trisomy 12 at amniocentesis can be associated with a favorable pregnancy outcome. Interphase FISH and aCGH on uncultured amniocytes are useful for confirmation of low-level mosaic trisomy 12 at amniocentesis.

Introduction

Clinical reports of mosaic trisomy 12 detected by amniocentesis are very rare. We previously reported prenatal diagnosis and molecular cytogenetic characterization of low-level mosaic trisomy 12 with a favorable pregnancy outcome [1–3]. Here, we present an additional case with a similar result. Our experience may provide useful information for the clinicians, genetic counselors, and parents during genetic counseling of mosaic trisomy 12 at amniocentesis.

Case Report

A 40-year-old, gravida 2, para 0, woman underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Her husband was 48 years old. The woman and her husband were healthy, and there was no family history of congenital malformations. Amniocentesis revealed a karyotype of 47,XX,+12[5]/46,XX[24] consistent with 17.2% (5/29 colonies) mosaicism for trisomy 12. Among 29 colonies of cultured amniocytes, five colonies had a

* Corresponding author. Department of Obstetrics and Gynecology, MacKay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei 10449, Taiwan.
E-mail address: cpc_mmh@yahoo.com (C.-P. Chen).

http://dx.doi.org/10.1016/j.tjog.2017.01.005

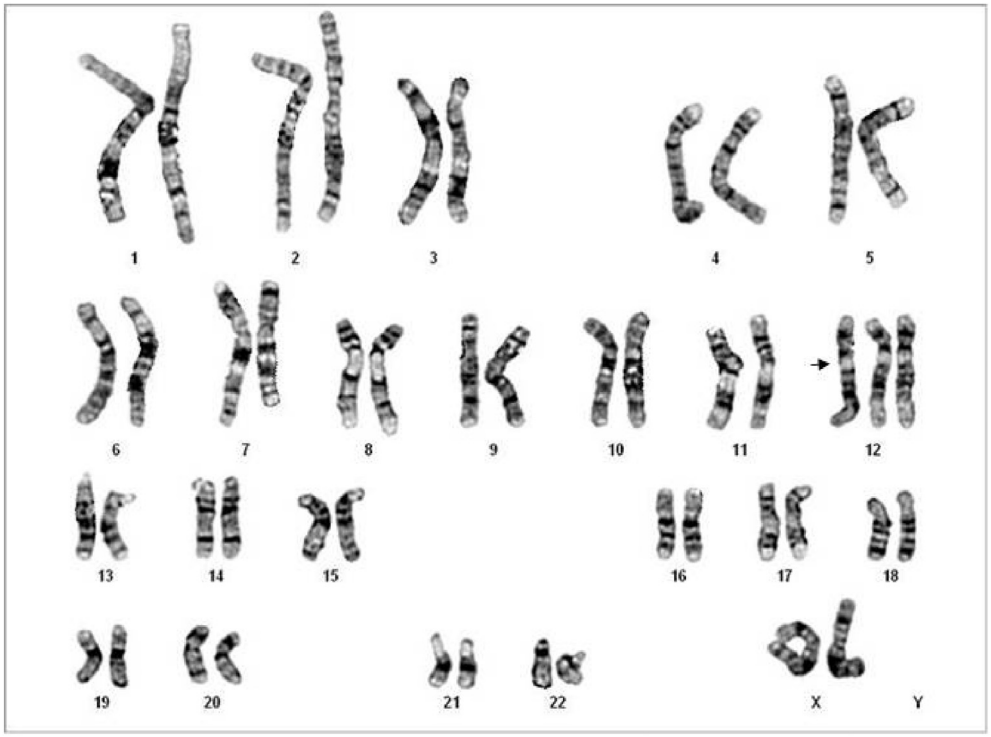


Figure 1. A karyotype of 47,XX,+12.

karyotype of 47,XX,+12 (Figure 1), whereas the rest, 24 colonies, had a karyotype of 46,XX. Prenatal ultrasound findings were unremarkable. The parental karyotypes were normal. Repeat amniocentesis was performed at 21 weeks of gestation. Simultaneous molecular cytogenetic analyses were performed on uncultured amniocytes using array comparative genomic hybridization (aCGH), interphase fluorescence *in situ* hybridization (FISH), and quantitative fluorescent polymerase chain reaction (QF-PCR) assays. Cytogenetic analysis of cultured amniocytes at repeat amniocentesis revealed a karyotype of 47,XX,+12[4]/46,XX[6] consistent with 40% (4/10 colonies) mosaicism for trisomy 12. QF-PCR analysis using the informative markers of D12S823 (12p12.1) and D12S302 (12q23.1) on the DNA extracted from the uncultured amniocytes and the parental peripheral bloods excluded uniparental disomy (UPD) 12 (Table 1). Interphase FISH analysis on 112 uncultured amniocytes using the bacterial artificial chromosome (BAC) probe of RP11-244D12 [12p11.22, fluorescein isothiocyanate (FITC), spectrum green] and RP11-627E5 (12q24.33, Texas Red, spectrum red) detected 23 cells with trisomy 12 consistent with a mosaicism level of 20.5% (23/112 cells) (Figure 2). The normal control amniocytes had a false positive rate of 1.9% (2/104 cells) for RP11-244D12 and a false positive rate of 3.9% (4/104 cells) for RP11-627E5. aCGH analysis of the DNA extracted from uncultured amniocytes using CytoChip ISCA Array (Illumina, San Diego, CA, USA) revealed a result of arr 12p13.33q24.33 (230,451–133,773,499) × 2.2, 17p12 (14,191,925–15,442,037) × 1.0 (Figure 3). The log2 ratio for 12p13.33q24.33 duplication was 0.12 consistent with 10–20% mosaicism. The 1.25-Mb 17p12 microdeletion contained 15 genes including three Online Mendelian Inheritance in Man (OMIM) genes of *HS3ST3B1*, *PMP22*, and *TEKT3*. aCGH analysis of the DNAs extracted from parental bloods revealed no genomic imbalance in the maternal blood and a result of arr 17p12 (14,111,802–15,442,037) × 1.0 in the paternal blood (Figure 4). The father had a 1.33-Mb 17p12 microdeletion encompassing 17 genes including four OMIM genes of *COX10*, *HS3ST3B1*, *PMP22*, and *TEKT3*. The father manifested no peripheral neuropathy. The parents elected to continue the pregnancy. At 37 weeks of gestation, a 3958-g (>97th centile) female baby was delivered smoothly with a body length of 51.5 cm (>97th centile) with neonatal brachial plexus palsy. The cord blood had a karyotype of 46,XX in 40/40 lymphocytes. At age 4 days, interphase FISH analysis on 98 uncultured urinary cells detected seven cells with trisomy 12 consistent with 7.14% mosaicism for trisomy 12. The normal control urinary cells had a false positive rate of 1.96% (2/102 cells) for the FISH probes. The neonate was phenotypically normal during follow-ups at age 1 month. Her body weight was 4.2 Kg (25–50th centile).

Table 1
Molecular results using polymorphic DNA markers specific for chromosome 12.

Markers	Locus	Father	Mother	Fetus (uncultured amniocytes)
D12S823	12p12.1	144, 152	124, 140	124, 144
D12S302	12q23.1	156, 156	152, 152	152, 156

Alleles (base pair sizes) are listed below each individual.

Discussion

The present case provides evidence that interphase FISH on uncultured amniocytes at repeat amniocentesis is very practical for determining the real mosaicism level of trisomy 12 at amniocentesis. In the present case, the first amniocentesis revealed 17.2% (5/29 colonies) mosaicism for trisomy 12 in cultured amniocytes, and the second amniocentesis revealed 40% (4/10 colonies) mosaicism for trisomy 12 in cultured amniocytes by conventional cytogenetic analysis. However, the second amniocentesis revealed 20.5% (23/112 cells) mosaicism for trisomy 12 by interphase FISH in uncultured amniocytes. The result obtained from interphase FISH on uncultured amniocytes is very useful for genetic counseling of inconsistent trisomy 12 mosaicism levels in different cultured amniocytes. Inconsistent trisomy mosaicism levels have been

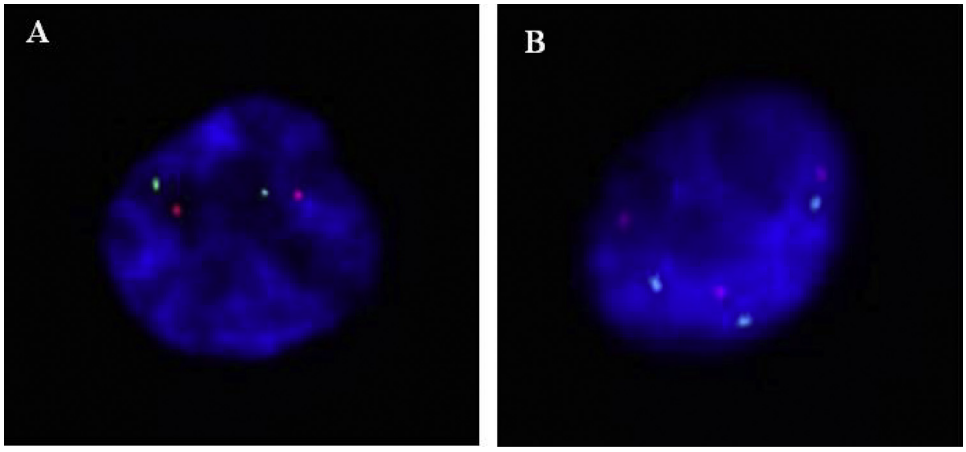


Figure 2. Interphase fluorescence *in situ* hybridization analysis on uncultured amniocytes using a 12p11.2-specific probe RP11-244D12 (spectrum green) and a 12q24.33-specific probe RP11-627E5 (spectrum red) shows (A) two green signals and two red signals in a disomy 12 cell and (B) three green signals and three red signals in a trisomy 12 cell.

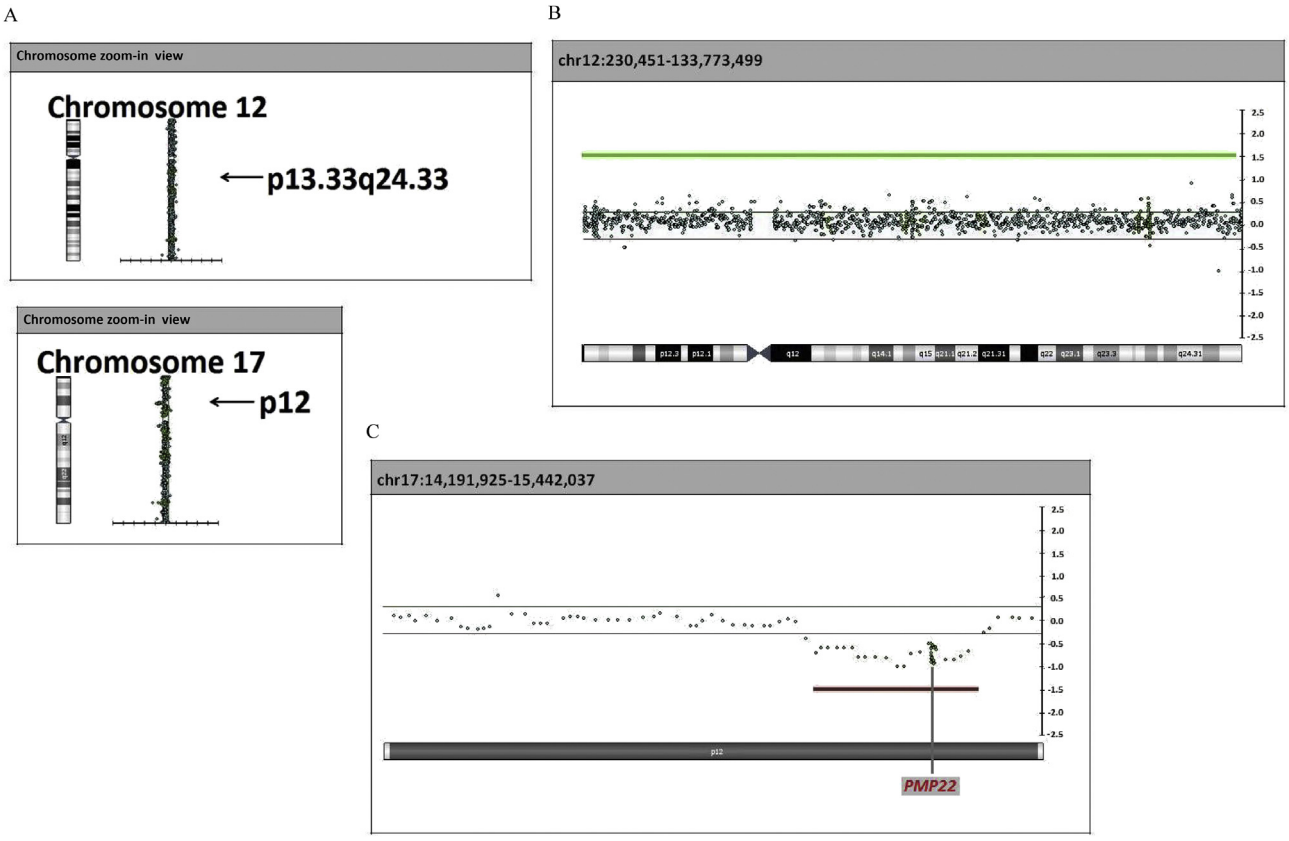
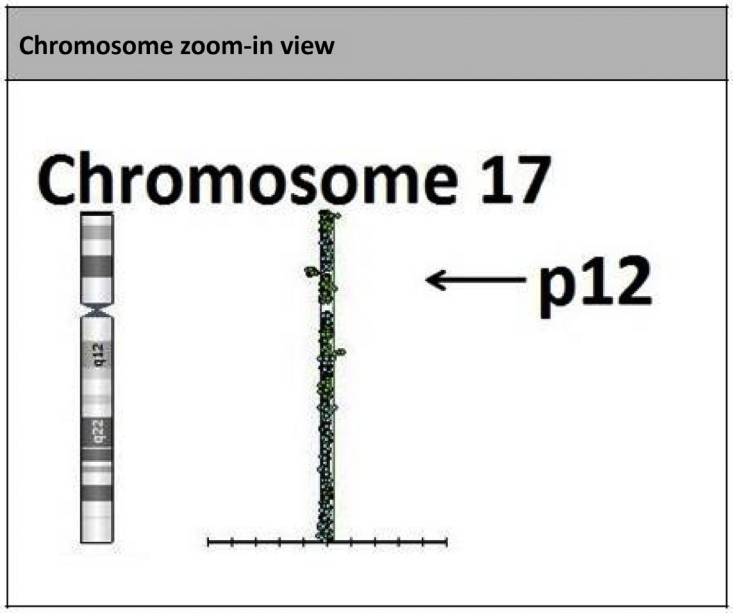


Figure 3. aCGH analysis on the DNA extracted from uncultured amniocytes shows a 10–20% genomic gain in chromosome 12 and a 1.25-Mb microdeletion at 17p12. (A) Chromosome zoom-in view; (B) chromosome 12; and (C) chromosome 17p12. aCGH = array comparative genomic hybridization.

reported in different amniocenteses, for example, between two different amniocenteses, Frohlich and Falk [4] reported 7% versus 0%, Cartolano et al [5] reported 26.7% versus 0%, Spiro et al [6] reported 7.5% versus 48%, and Gentilin et al [7] reported 17.6% versus 62.5% mosaicism for trisomy 12.

In a review of 32 cases of mosaic trisomy 12 at amniocentesis, Chen et al [1] reported that five out of nine cases with an apparently abnormal outcome had a mosaicism level > 34% in cultured amniocytes, whereas only three out of 23 cases with an apparently normal outcome had a mosaicism level > 34% in cultured amniocytes, indicating a correlation between a higher mosaic trisomy 12 level and an abnormal fetal outcome. The reported abnormal fetal ultrasound findings associated with mosaic trisomy 12 include polyhydramnios, intrauterine growth restriction, single umbilical artery, congenital heart defects, hydronephrosis and absence of stomach image, and the reported phenotypic abnormalities associated with postnatally detected mosaic trisomy 12 include developmental delay, pigmentary dysplasia, congenital heart defects, microcephaly, facial asymmetry, prominent ears, hypotonia, hemihyperplasia, intestinal malrotation, retinopathy,

A



B

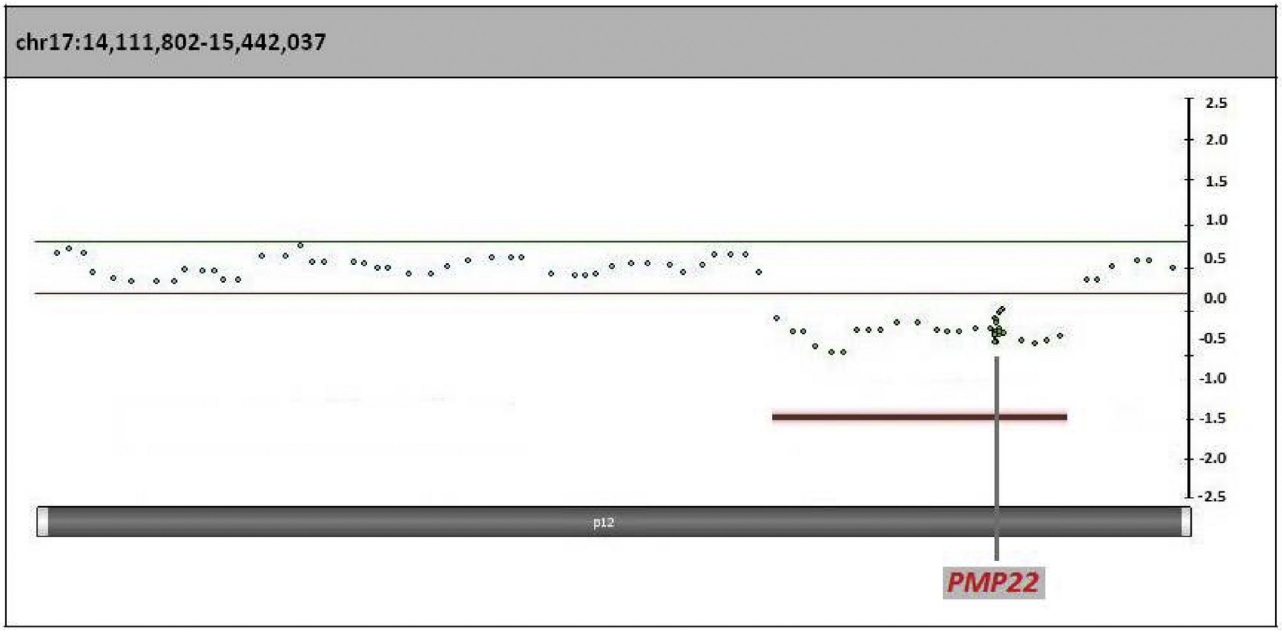


Figure 4. aCGH analysis on the DNA extracted from paternal peripheral blood shows a 1.33-Mb microdeletion at 17p12. (A) Chromosome zoom-in view; (B) chromosome 17p12. aCGH = array comparative genomic hybridization.

and sensorineural hearing loss [1]. Prenatal diagnosis of mosaic trisomy 12 should include a detailed ultrasound examination of fetal brain, heart, kidneys, and face.

The present case manifested fetal overgrowth which has been observed by our previously reports [2,3]. Congenital overgrowth have been described in cases with gene dosage increase in chromosome 12 such as mosaic tetrasomy 12p [8–10] and 12q duplication syndrome [11,12].

The peculiar aspect of the present case is the association of concomitant familial inheritance of 17p12 microdeletion encompassing *PMP22*. *PMP22* (OMIM 601097) encodes peripheral myelin protein 22, which is essential in all myelinated fibers in the peripheral nervous system [13]. Hereditary neuropathy with liability to pressure palsies (HNPP; OMIM 162500) can be caused by deletion of *PMP22*, whereas Charcot-Marie-Tooth disease Type 1A (CMT1A; OMIM 118220) can be caused by duplication of *PMP22* [14,15]. Korn-Lubetzki et al [16] reported HNPP 17p12 deletion in a family with inflammatory demyelinating polyneuropathy. In a study of 73 patients from 53 unrelated families with HNPP 17p12 deletion, Luigetti et al [17] reported that 56% of the patients showed an atypical presentation, and 10% of the patients were asymptomatic for neuropathic symptoms.

In summary, we present prenatal diagnosis and molecular cytogenetic analysis of low-level mosaic trisomy 12 at amniocentesis

associated with a favorable pregnancy outcome. Our presentation provides evidence for a correlation of low-level trisomy 12 mosaicism in uncultured amniocytes with a favorable fetal outcome under circumstances of no abnormal fetal ultrasound and no UPD 12. The present case also shows that in case of mosaic trisomy 12 at amniocentesis, interphase FISH on uncultured amniocytes is useful for rapid confirmation of low-level mosaicism, aCGH on uncultured amniocytes is useful for confirmation of the presence of mosaicism for trisomy 12, and QF-PCR assay on uncultured amniocytes is useful for rapid exclusion of UPD 12.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This work was supported by research grants MOST-104-2314-B-195-009 and MOST-105-2314-B-195-012 from the Ministry of Science and Technology and MMH-E-105-04 from MacKay Memorial Hospital, Taipei, Taiwan.

References

[1] Chen C-P, Su Y-N, Su J-W, Chern S-R, Chen Y-T, Chen L-F, et al. Mosaic trisomy 12 at amniocentesis: prenatal diagnosis, and molecular genetic analysis. Taiwan J Obstet Gynecol 2013;52:97–105.
[2] Chen C-P, Chang S-D, Su J-W, Chen Y-T, Wang W. Prenatal diagnosis of mosaic trisomy 12 associated with congenital overgrowth. Taiwan J Obstet Gynecol 2013;52:454–6.
[3] Chen C-P, Chern S-R, Su P-S, Chen Y-N, Chen S-W, Lee C-C, et al. Prenatal diagnosis of low-level mosaicism for trisomy 12 associated with a favorable pregnancy outcome. Taiwan J Obstet Gynecol 2016;55:900–1.
[4] Frohlich GS, Falk RE. Trisomy 12 mosaicism. Prenat Diagn 1991;11:881.
[5] Cartolano R, Guerneri S, Fogliani R, Galimberti A, Nicolini U. Prenatal confirmation of trisomy 12 mosaicism by fetal skin biopsy. Prenat Diagn 1993;13:1057–9.
[6] Spiro R, Rita D, Jazmines L, Jones C, Booth CW. Trisomy 12/monosomy X/normal female mosaicism: prenatal detection and confirmation in a liveborn. Prenat Diagn 1996;16:734–40.
[7] Gentilin B, Giardino D, Boschetto C, Guerneri S, Fortuna R, Rizzuti T, et al. Limited value of echography to predict true fetal mosaicism for trisomy 12. Prenat Diagn 2006;26:1186–9.
[8] Chiurazzi P, Bajer J, Tabolacci E, Pomponi MG, Lecce R, Zollino M, et al. Assisted reproductive technology and congenital overgrowth: some speculations on a case of Pallister–Killian syndrome. Am J Med Genet 2004;130A:315–6.
[9] Vora N, Bianchi DW. Genetic considerations in the prenatal diagnosis of overgrowth syndromes. Prenat Diagn 2009;29:923–9.
[10] Frković SH, Durisević IT, Trcić RL, Sarnavka V, Gornik KC, Muzinić D, et al. Pallister Killian syndrome: unusual significant postnatal overgrowth in a girl with otherwise typical presentation. Coll Antropol 2010;34:247–50.
[11] Wajntal A, Moretti-Ferreira D, De Souza DH, Koiffmann CP. Cytogenetic evidence of involvement of chromosome regions 15q12 and 12q15 in conditions with associated overgrowth. DNA Cell Biol 1993;12:227–31.
[12] Tyshchenko NA, Riegel M, Evseenkova EG, Zerova TE, Gorovenko NG, Schinzel A. Duplication of (12)(pter-q13.3) combined with deletion of (22)(pter-q11.2) in a patient with features of both chromosome aberrations. Eur J Med Genet 2007;50:128–32.
[13] Snipes GJ, Suter U, Welcher AA, Shooter EM. Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13). J Cell Biol 1992;117:225–38.
[14] Stogbauer F, Young P, Kuhlenbaumer G, De Jonghe P, Timmerman V. Hereditary recurrent focal neuropathies. Clinical and molecular features. Neurology 2000;54:546–51.
[15] Lupski JR, Garcia CA. Charcot–Marie–Tooth peripheral neuropathies and related disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Vogelstein B, Childs B, editors. The metabolic and molecular bases of inherited diseases. New York: McGraw Hill; 2001. p. 5759–88.
[16] Korn-Lubetzki I, Argov Z, Raas-Rothschild A, Wirguin I, Steiner I. Family with inflammatory demyelinating polyneuropathy and the HNPP 17p12 deletion. Am J Med Genet 2002;113:275–8.
[17] Luigetti M, Del Grande A, Conte A, Lo Monaco M, Bisogni G, Romano A, et al. Clinical, neurophysiological, and pathological findings of HNPP patients with 17p12 deletion: a single-centre experience. J Neurol Sci 2014;341:46–50.