Taiwanese Journal of Obstetrics & Gynecology 55 (2016) 863–866

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Case Report

Pallister–Killian syndrome: Cytogenetics and molecular investigations of mosaic tetrasomy 12p in prenatal chorionic villus and in amniocytes. Strategy of prenatal diagnosis

Francesco Libotte [a], Domenico Bizzoco [a], Ivan Gabrielli [a], Alvaro Mesoraca [a], Pietro Cignini [b], Salvatore Giovanni Vitale [c,*], Ilaria Marilli [d], Ferdinando Antonio Gulino [d], Agnese Maria Chiara Rapisarda [d], Claudio Giorlandino [b]

[a] Department of Genetics, Altamedica Fetal Maternal Medical Centre, Rome, Italy
[b] Department of Prenatal Diagnosis, Altamedica Fetal Maternal Medical Centre, Rome, Italy
[c] Department of Human Pathology in Adulthood and Childhood "G. Barresi", University of Messina, Messina, Italy
[d] Department of General Surgery and Medical Surgical Specialties, University of Catania, Catania, Italy

ARTICLE INFO

Article history:
Accepted 28 July 2016

Keywords:
cytogenetic analysis
isochromosome 12p
Pallister–Killian syndrome
prenatal diagnosis
tetrasomy 12p

ABSTRACT

Objective: Pallister–Killian syndrome (PKS) is a rare, sporadic genetic disorder caused by mosaic tetrasomy of the short arm of chromosome 12 (12p). Clinically, PKS is characterized by several systemic abnormalities, such as intellectual impairment, hearing loss, epilepsy, hypotonia, craniofacial dysmorphism, pigmentary skin anomalies, epilepsy, and a variety of congenital malformations. Prenatally, PKS can be suspected in the presence of ultrasound anomalies: diaphragmatic hernia, rhizomelic micromelia, hydrops fetalis, fetal overweight, ventriculomegaly in the central nervous system, congenital heart defects, or absent visualization of the stomach. In all these cases, a detailed genetic study is required. PKS is diagnosed by prenatal genetic analysis through chorionic villus sampling, genetic amniocentesis, and cordocentesis.

Case Report: We report two cases of PKS with prenatal diagnosis of isochromosome 12p made by cytogenetic studies. The first case is of a 36-year-old pregnant woman who underwent genetic chorionic villus sampling at 13th weeks of gestation after 1st trimester prenatal ultrasound revealed clinical features of PKS: flat nasal bridge and fetal hydrops. The second case is of a 32-year-old pregnant woman with genetic amniocentesis at 17th weeks of gestation that showed mos46,XX[21]/47,XX,+i(12p) associated to PKS.

Conclusion: New molecular cytogenetic techniques array comparative genomic hybridization and fluorescence *in-situ* hybridization in association with conventional karyotype are pivotal innovative tools to search for chromosomic anomalies and for a complete prenatal diagnosis, especially in cases such as PKS where array comparative genomic hybridization analysis alone could not show mosaicism of i(12p).

Introduction

Pallister–Killian syndrome (PKS) is a rare, sporadic genetic disorder caused by mosaic tetrasomy of the short arm of chromosome 12 (12p). Incidence is uncertain and is estimated around 1/20–25.000. It was first described by Pallister et al [1] in two adult patients and later by Killian and Teschler-Nicola [2]. Clinically, PKS is characterized by several systemic abnormalities, such as intellectual impairment, hearing loss, epilepsy, hypotonia, craniofacial dysmorphism (*coarse* face, hypertelorism, short nose, flat nasal bridge, long philtrum, cleft palate, and short neck), pigmentary skin anomalies, epilepsy, and a variety of congenital malformations [3,4].

PKS is cytogenetically characterized by a tissue-limited mosaicism for a supernumerary isochromosome for the short arm of

* Corresponding author. Department of Human Pathology in Adulthood and Childhood "G. Barresi", University of Messina, Via Consolare Valeria 1, 98125 Messina (ME), Italy.
E-mail address: vitalesalvatore@hotmail.com (S.G. Vitale).

http://dx.doi.org/10.1016/j.tjog.2016.07.010

chromosome 12, i(12p). The karyotype in the cultured blood lymphocytes is normal in most cases, but one supernumerary isochromosome 12p is present at high percentage in cultured skin fibroblasts and bone marrow cells of patients [5]. The percentage is 0–2% in lymphocytes and 50–100% in fibroblasts. In children and adults, diagnosis of PKS usually requires a skin biopsy and/or a buccal smear and an analysis of fibroblasts [6]. Prenatally, PKS can be suspected in the presence of ultrasound anomalies: diaphragmatic hernia, rhizomelic micromelia, hydrops fetalis, fetal overweight, ventriculomegaly in the central nervous system, congenital heart defects, or absent visualization of the stomach. In all these cases, a detailed genetic study is required. PKS is diagnosed by prenatal genetic analysis through chorionic villus sampling, genetic amniocentesis, or cordocentesis [7]. Unfortunately, prenatal diagnosis of PKS remains problematic, mostly because of the difficulties to discriminate between the supernumerary isochromosome 12p and the duplication 21q, the variable level of mosaicism and the rapid decrease of the supernumerary isochromosome during amniocyte subculturing [8–10].

The mechanism of isochromosome i(12p) formation in PKS is still not completely clear. Hunter et al [11] proposed four hypotheses: (1) Meiosis I or II nondisjunction generating a disomic gamete that results in a trisomic zygote, then one of the chromosome 12 centromeric misdivision during mitosis; (2) isochromosome formation during meiosis in one parent, and simultaneous nondisjunction during meiosis in the other parent; (3) isochromosome formation associated with centromeric misdivision and nondisjunction during Meiosis I resulting in a gamete with both a normal chromosome 12 and an isochromosome 12p; and (4) normal gametes and zygote, isochromosome formation with postzygote mitotic nondisjunction, and centromeric misdivision. The purpose of this study is to underline the role of classic cytogenetic karyotype combined with the new molecular cytogenetic methods as fluorescence *in-situ* hybridization (FISH) and array comparative genomic hybridization (Array-CGH), for correct analysis in prenatal diagnosis of genetic disorders.

Case Reports

We report two cases of PKS with prenatal diagnosis of isochromosome 12p made by cytogenetic studies.

Case 1

The first case is of a 36-year-old pregnant woman who underwent genetic chorionic villus sampling at 13 weeks of gestation after 1st trimester prenatal ultrasound revealed clinical features of PK syndrome: flat nasal bridge and fetal hydrops (Figure 1). Cytogenetic analysis was carried out on chromosome spreads prepared from the direct preparation and from cultured chorionic villus. A total of 10 metaphases for direct analysis, and 16 metaphases from cell culture were analyzed. Metaphase chromosomes were prepared according to standard cytogenetic protocol. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature [13]. Array-CGH analysis was tested on patient DNA, extracted from chorionic villi. The genomic coverage of these arrays is up to 1 Mb resolution across the genome and ~100 kb resolution in 139 regions associated with constitutional disorders. DNA was hybridized to whole-genome BAC microarrays (Cyto Chip Focus Constitutional; BlueGnome, Cambridge, UK), according to the manufacturer's protocol (available at www.cytochip.com). A laser scanner InnoScan 710 AL (INNOPSYS, Carbonne, France) was used to excite the hybridized fluorophores, read, and store the resulting images of the hybridization. Scanned image quantification, array quality control, and aberration detection were performed by algorithm fixed settings in BLUEFUSE MULTI software (BlueGnome) [12]. Staining with the trypsin–Giemsa banding (G-banding) technique showed originally a mosaic karyotype for a supernumerary isochromosome (Figure 2) while the cell culture showed a normal karyotype. The chromosomal investigation of the parents using peripheral blood samples revealed a normal karyotype. The genome-wide copy number detection of DNA, extracted from chorionic villus, showed a gain, about 33.1 Mb, of the entire short arm of chromosome 12 in the fetus (Figure 3). In consideration of the ultrasonographic findings, flat nasal bridge and hydrops fetalis, G-banding karyotype of chorionic villus, Array-CGH analysis, and the result 46,XY (6)/47,XY,+i(12p)(4), we concluded that the presence of extra chromosome 12p mosaicism was the cause of the multiple congenital anomalies of fetus.

Case 2

The second case is of a 32-year-old pregnant woman without ultrasound evidence and with unremarkable family history who underwent genetic amniocentesis at 17th weeks of gestation for maternal anxiety. Metaphase chromosomes were prepared according to standard cytogenetic protocol. The karyotype was described according to the International System for Human Cytogenetic Nomenclature (2013). FISH was performed on nuclei of the amniocytes using the CEP 12 DNA Probe Kit (Abbott Molecular, Des Plaines, IL, USA) according to the manufacturer's protocol. Prenatal cytogenetic analysis was performed with amniotic fluid using a culture cells according to standard G-banding techniques. The karyotype in the fetus showed 46,XX in 21 metaphase and 47,XX,+mar in 4 metaphase cells of 25 (Figure 4). A chromosomal investigation of the parents using peripheral blood samples revealed a normal karyotype. We carefully verified the marker chromosome because it was not easy to distinguish the marker chromosome from the long arm of chromosome 21 and another origin. First, FISH analysis using the CEP 12 DNA Probe Kit (Abbott Molecular) was performed to evaluate the origin of the marker chromosome. Two signals on both arms of the marker chromosome were noted (Figure 5). We concluded that the karyotype was: mos46,XX[21]/47,XX,+i(12p)[4], which is associated with PKS. We also performed Array-CGH analysis, but with negative results probably due to the low presence of supernumerary isochromosome in the tissue examined. After counseling, both couples decided to terminate the pregnancies.

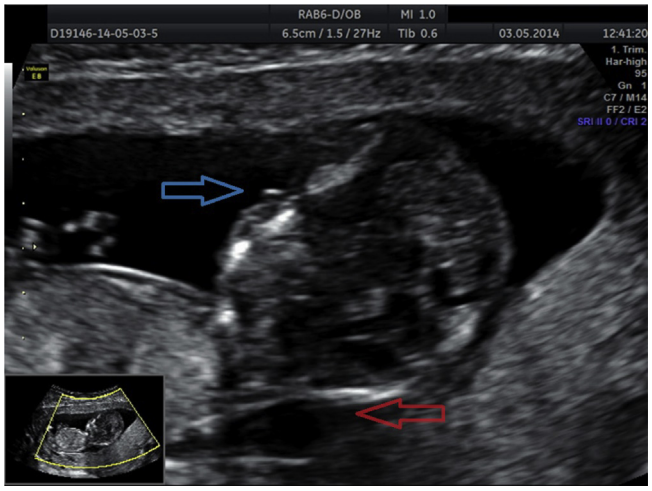

Figure 1. Fetus of 12^{+2} weeks, examined from a favorable position (the blue arrow show flat nasal bridge; the red arrow shows fetal hydrops).

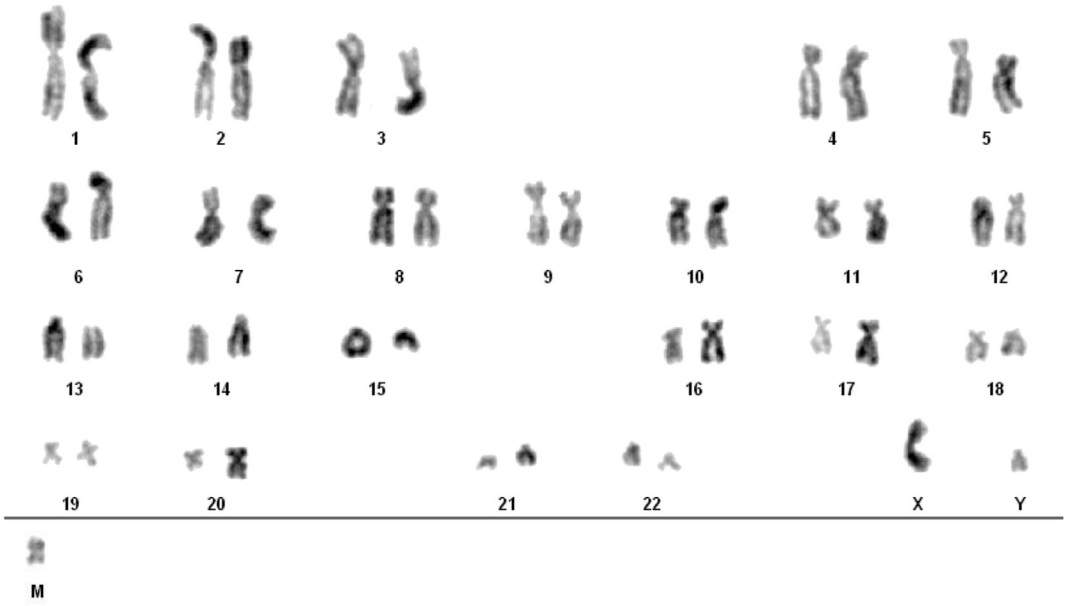

Figure 2. Conventional cytogenetic direct chorionic villus sample preparation analysis of the fetus showed 47,XY,+ mar in four cells and 46,XY in six cells.

Discussion

Variable tissue-specific mosaicism of isochromosome 12p is the cytogenetic feature of tetrasomy 12p syndrome. The variation of isochromosome 12p frequency does not correlate with the severity of congenital abnormalities in PKS fetus [10,14]. Isochromosome 12p is seen mainly in skin fibroblast cultures, in chorionic villus, and in amniotic fluid cell samples. In spite of such tissue-limited mosaicism, the extra chromosome can rarely be identified in blood lymphocytes during postnatal examination [15] or prenatal studies using chorionic villus sampling, genetic amniocentesis, and, rarely, cordocentesis [16]. Therefore, there is no strict limitation on the presence of extra isochromosome 12p in cell tissues, which are generally used in prenatal studies and postnatal cytogenetic examinations. Advanced maternal age and ultrasound abnormalities are the most common indications for prenatal investigation in reports of PKS. Prenatal diagnosis of PKS is difficult because of the rapid loss of the isochromosome 12p in the course of amniocyte subculturing [10]. In this paper we describe two cases of PKS. In the first case, ultrasonographic findings, flat nasal bridge, and hydrops fetalis, were the input for the research of the supernumerary isochromosome. G-banding karyotype of chorionic villus and Array-CGH analysis, confirmed the presence of extra chromosome 12p mosaicism, which was the cause of the multiple congenital anomalies of fetus (mos 46,XY [6]/47,XY,+i(12p)[4]). The difficulty in the second case was the low presence of the supernumerary isochromosome in the cultures during conventional cytogenetic analysis. FISH analysis confirmed the tetrasomy 12p (mos 46,XX[21]/47,XX,+i(12p)[4]).

These cases illustrate the importance of using the appropriate technique for correct analysis, suggesting that in prenatal diagnosis

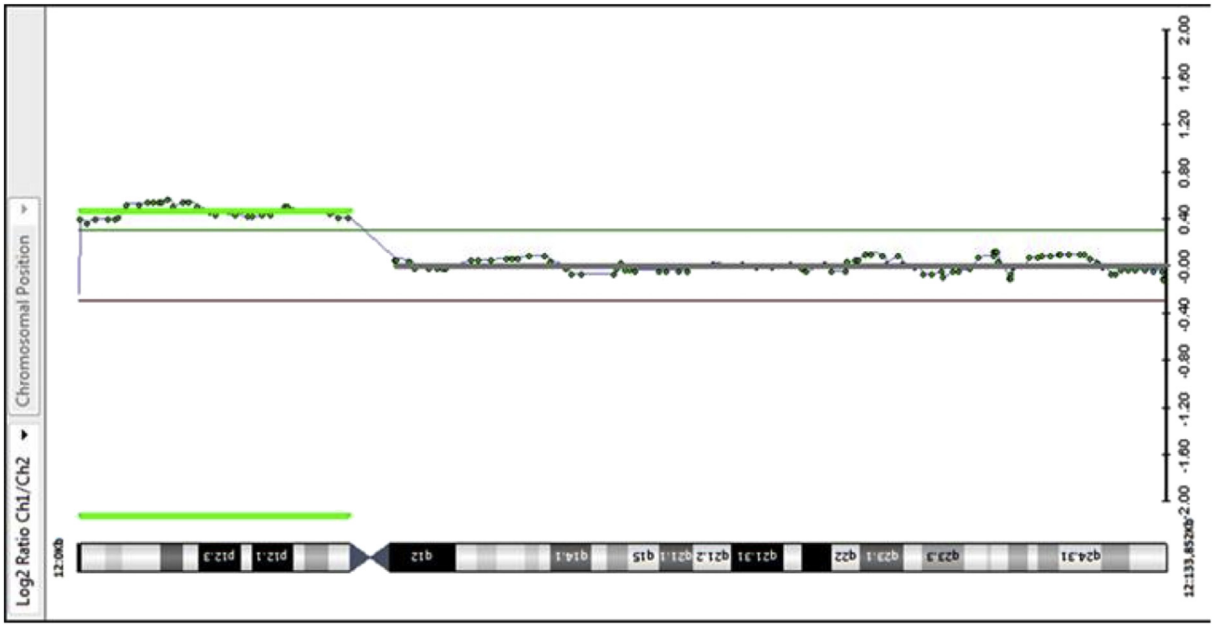

Figure 3. Microarray plot for a clinically significant 33-Mb gain at 12p13.33p11.

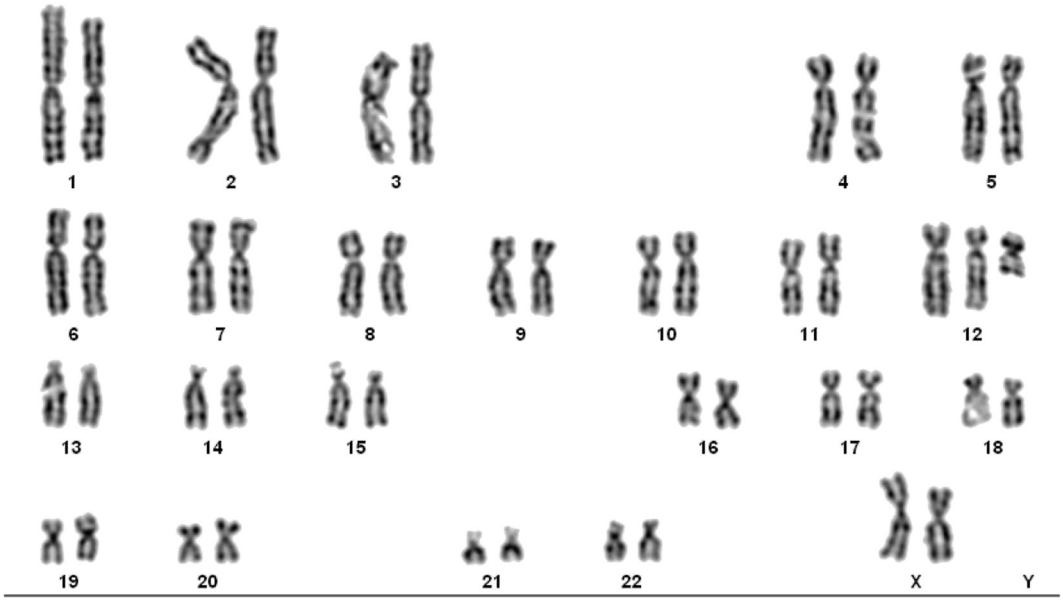

Figure 4. Trypsin–Giemsa-banded chromosomes with extra metacentric marker chromosome, 47, XX,+mar.

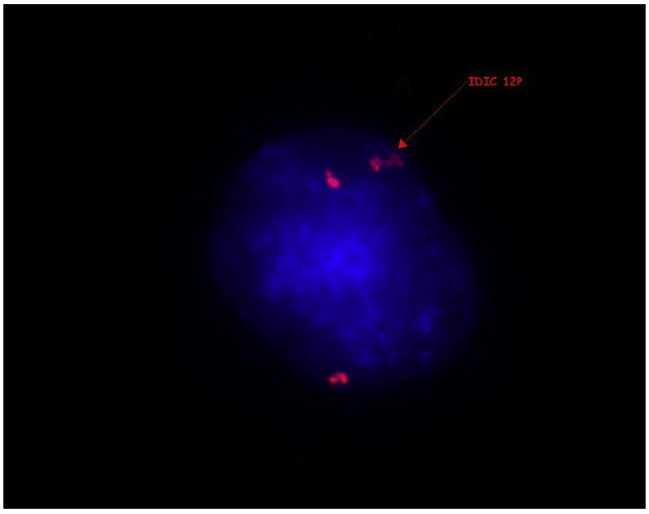

Figure 5. Fluorescence *in-situ* hybridization using painting probe CEP 12 DNA showed two red signals located symmetrically on both arms of the marker chromosome (arrow).

cytogenetic analysis, FISH, and Array-CGH are essential tools for a diagnosis of disease, in presence, as well as in absence, of ultrasonographic markers. In cases where G-banding is not sufficient, spectral karyotyping and multiplex-FISH, new molecular cytogenetic techniques allowing visualization of chromosomes in different colors using chromosome painting probes, may be useful tools for the identification of complex chromosomal abnormalities [17].

In conclusion, according to our experience, new molecular cytogenetic methods Array-CGH and FISH in association with conventional karyotype are pivotal to search chromosomic anomalies and for a complete prenatal diagnosis, especially in cases such as PKS where Array-CGH analysis alone could not show mosaicism of isochromosome 12p.

Conflicts of interest

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

References

[1] Pallister PD, Meisner LF, Elejalde BR, Francke U, Hermann J, Spranger J, et al. The Pallister mosaic syndrome. Birth Defects Orig Artic Ser 1977;13:103–10.
[2] Killian W, Teschler-Nicola M. Case report 72: mental retardation, unusual facial appearance abnormal hair. Synd Ident 1981;7:6–7.
[3] Izumi K, Krantz ID. Pallister–Killian syndrome. Am J Med Gen C Semin Med Genet 2014;166C:406–13.
[4] Vermeesch JR, Melotte C, Salden I, Riegel M, Trifnov V, Polityko A, et al. Tetrasomy 12pter-12p13.31 in a girl with partial Pallister–Killian syndrome phenotype. Eur J Med Genet 2005;48:319–27.
[5] Mowery-Rushton PA, Stadler MP, Kochmar SJ, McPherson E, Surti U, Hogge WA. The use of interphase for prenatal diagnosis of Pallister–Killian syndrome. Prenat Diagn 1997;17:255–65.
[6] Kaur M, Isumi K, Wilkens A, Chatfield KC, Spinner NB, Conlin LK, et al. Genome-wide expression analysis in fibroblast cell lines from probands with Pallister Killian syndrome. PLoS One 2014;9:e108853.
[7] Langford K, Hodgson S, Seller M, Maxwell D. Pallister–Killian syndrome presenting through nuchal translucency screening for trisomy 21. Prenat Diagn 2000;20:670–2.
[8] Schaefer GB, Jochar A, Muneer R, Sanger WG. Clinical variability of tetrasomy 12p. Clin Genet 1997;51(2):102–8.
[9] Doray B, Girard-Lemaire F, Gasser B, Baldauf JJ, De Geeter B, Spizzo M, et al. Pallister–Killian syndrome: difficulties of prenatal diagnosis. Prenat Diagn 2002;22:470–7.
[10] Polityko AD, Goncharova E, Shamgina L, Drozdovskaja N, Podleschuk L, Abramchik E, et al. Pallister–Killian syndrome: rapid decrease of isochromosome 12p frequency during amniocyte subculturing. Conclusion for strategy of prenatal cytogenetic diagnostics. J Histochem Cytochem 2005;53:361–4.
[11] Hunter AG, Clifford B, Cox DM. The characteristic physiognomy and tissue specific karyotype distribution in the Pallister–Killian syndrome. Clin Genet 1985;28:47–53.
[12] Fiorentino F, Caiazzo F, Napolitano S, Spizzichino L, Bono S, Sessa M, et al. Introducing array comparative genomic hybridization into routine prenatal diagnosis practice: a prospective study on over 1000 consecutive clinical cases. Prenat Diagn 2011;31:1270–82.
[13] Simons A, Shaffer LG, Hastings RJ. Cytogenetic nomenclature: changes in the ISCN 2013 compared to the 2009 edition. Cytogenet Genome Res 2013;141:1–6.
[14] Shen JD, Liang DS, Zhou ZM, Xia Y, Long ZG, Wu LQ. Pallister–Killian syndrome: meiosis II non-disjunction may be the first step in the formation of isochromosome 12p. Chin Med J (Engl) 2010;123:3482–5.
[15] Leube B, Majewski F, Gebauer J, Royer-Pokora B. Clinical, cytogenetic, and molecular observations in a patient with Pallister–Killian-syndrome with an unusual karyotype. Am J Med Genet A 2003;15(123A):296–300.
[16] Chiesa J, Hoffet M, Rousseau O, Bourgeois JM, Sarda P, Mares P, et al. Pallister–Killian syndrome [i(12p)]: first pre-natal diagnosis using cordocentesis in the second trimester confirmed by *in situ* hybridization. Clin Genet 1998;54:294–302.
[17] Bint SM, Davies AF, Ogilvie CM. Multicolor banding remains an important adjunct to array CGH and conventional karyotyping. Mol Cytogenet 2013;6:55.