MOSAIC TRISOMY 7 AT AMNIOCENTESIS: PRENATAL DIAGNOSIS AND MOLECULAR GENETIC ANALYSES

Chih-Ping Chen1,2,3,4,5,6*, Yi-Ning Su7, Schu-Rern Chern2, Yuh-Ming Hwu1, Shuan-Pei Lin2,8, Chyong-Hsin Hsu8, Fiu-Jen Tsai4,9, Tao-yeuan Wang10, Pei-Chen Wu1, Chen-Chi Lee1, Yu-Ting Chen2, Li-Feng Chen1, Wayseen Wang2,11

Departments of 1Obstetrics and Gynecology, 2Medical Research, 8Pediatrics, and 10Pathology, Mackay Memorial Hospital, 3Institute of Clinical and Community Health Nursing, 4Department of Obstetrics and Gynecology, School of Medicine, National Yang-Ming University, 2Department of Medical Genetics, National Taiwan University Hospital, 11Department of Bioengineering, Tatung University, Taipei; 3Department of Biotechnology, Asia University, 4School of Chinese Medicine, College of Chinese Medicine, China Medical University, and 7Departments of Medical Genetics and Medical Research, China Medical University Hospital, Taichung, Taiwan.

SUMMARY

Objective: To present prenatal diagnosis and molecular genetic analyses of mosaic trisomy 7.

Materials, Methods and Results: A 38-year-old primigravid woman underwent amniocentesis at 19 weeks of gestation because of her advanced maternal age. Amniocentesis revealed a karyotype of 47,XY,+7[26]/46,XY[16]. Repeated amniocentesis at 21 weeks of gestation revealed a karyotype of 47,XY,+7[20]/46,XY[17]. Simultaneous cordocentesis revealed a karyotype of 46,XY in 100/100 cultured lymphocytes. Polymorphic DNA marker analyses of uncultured amniocytes and cord blood revealed a diallelic pattern with seemingly equal biparental inheritance of chromosome 7. Repeated cordocentesis and chorionic villus sampling at 23 weeks of gestation revealed a karyotype of 47,XY,+7[2]/46,XY[66] in cord blood and a karyotype of 47,XY,+7 in 24/24 cultured chorionic villi cells. Level II ultrasonography was normal. At 40 weeks of gestation, a 2,708 g normal male baby was delivered. The peripheral blood had a karyotype of 46,XY in 100/100 lymphocytes. Molecular analyses of placenta, urine, buccal swab, and peripheral blood revealed a diallelic pattern and seemingly equal biparental inheritance of chromosome 7 in all tissues. At 3 months of age, he manifested hypopigmented skin and inguinal hernia, but showed normal growth and mental development. Fluorescence in situ hybridization analysis of inguinal hernia sac tissue revealed that 19/100 (19%) of nuclei had three chromosome 7 signals.

Conclusion: Mosaic trisomy 7 at amniocentesis may be derived from a cell culture artifact from an undetected low level of trisomy 7 mosaicism in uncultured amniocytes, and can be associated with favorable fetal outcome if the blood has a normal karyotype or a very low level of mosaicism and if uniparental disomy for chromosome 7 is excluded. [Taiwan J Obstet Gynecol 2010;49(3):333–340]

Key Words: amniocentesis, mosaicism, mosaic trisomy 7, trisomy 7, uniparental disomy for chromosome 7

Introduction

Genetic counseling of mosaic trisomy at amniocentesis is difficult because of the phenotypic variability associated with the condition; some fetuses exhibit the typical phenotype, while others are normal [1–3]. Trisomy 7 mosaicism has variable and nonspecific clinical features [4–8]. Most patients with trisomy 7 mosaicism have a normal karyotype in blood lymphocytes, but mosaic trisomy 7 in fibroblasts derived from the skin show pigment abnormalities. Trisomy 7 mosaicism at amniocentesis has been reported to be associated with maternal uniparental disomy for chromosome 7 (UPD 7) and Silver-Russell syndrome (SRS) [9–11]. Here, we report the prenatal diagnosis and molecular
Materials, Methods and Results

A 38-year-old primigravid woman underwent amniocentesis at Mackay Memorial Hospital at 19 weeks of gestation because of her advanced maternal age. The woman had suffered from bilateral tubal occlusion and primary infertility. This was her first pregnancy that was conceived by in vitro fertilization and embryo transfer. Four embryos had been implanted and three survived. The triplet pregnancy had three gestational sacs. At 7 weeks of gestation, fetal demise occurred in two fetuses. The remaining singleton fetus developed well at the time of amniocentesis.

In 26 of 42 separated amniocyte colonies, an abnormal karyotype of 47,XY,+7 was found (Figure 1), while the other 16 colonies had a karyotype of 46,XY. The cytogenetic result of amniocentesis was 47,XY,+7 [26]/46,XY[16]. The parental karyotypes were normal. Repeated amniocentesis at 21 weeks of gestation revealed 47,XY,+7[20]/46,XY[17]. Simultaneous cord blood sampling revealed a karyotype of 46,XY in 100/100 cultured lymphocytes. Polymorphic DNA marker analysis of the cord blood and uncultured amniocytes using microsatellite markers specific for chromosome 7 revealed a biparental inheritance of chromosome 7. There was a diallelic pattern and seemingly equal biparental inheritance of chromosome 7 in the cord blood and uncultured amniocytes (Figure 2). The molecular result excluded UPD 7.

At 23 weeks of gestation, the woman underwent repeated cord blood sampling and chorionic villus sampling at National Taiwan University Hospital. Cytogenetic analyses showed that the cord blood lymphocytes had a karyotype of 47,XY,+7[2]/46,XY[66], and the chorionic villi had a karyotype of 47,XY,+7 in 24/24 cultured chorionic villi cells. A methylation-specific polymerase chain reaction (PCR) assay was performed to identify the differential methylation of the imprinted PEG1/MEST locus on 7q32 and revealed biparental inheritance of chromosome 7 in the cord blood and chorionic villi (Figure 3). Polymorphic DNA

Figure 1. Karyotype of 47,XY,+7.
marker analysis of the chorionic villi cells revealed a
di allelic pattern with unequal biparental inheritance of
chromosome 7 with a dosage ratio of 1:2 (paternal
allele:maternal allele) (Figure 4).

Level II obstetric ultrasonography revealed normal
fetal growth biometry, a normal fetal craniofacial profile,
and no structural abnormalities. The parents decided
to continue the pregnancy. At 40 weeks of gestation, a
2,708 g male baby was delivered uneventfully at Mackay
Memorial Hospital. The baby was normal without pig-
ment abnormalities of the skin. Cytogenetic analysis of
the peripheral blood lymphocytes (100 cells) of the
neonate revealed a karyotype of 46,XY. Polymorphic
DNA marker analyses of placenta, urine, buccal swab,
and peripheral blood cells of the neonate revealed a dia-
allelic pattern with seemingly equal biparental inheri-
tance of chromosome 7 in all tissues examined (Figure 5).

Discussion

The present case provides evidence for a discrepancy
between the cytogenetic results of cultured amniocytes
and the molecular results of uncultured amniocytes in
mosaic trisomy 7 at amniocentesis. The high level of
trisomy 7 in cultured amniocytes might be derived from a cell culture artifact from an undetected low level of trisomy 7 mosaicism in the uncultured amniocytes. In the present case, although various tissues presented a diallelic pattern with seemingly equal biparental inheritance of chromosome 7, a very low level of trisomy 7 mosaicism cannot be completely excluded.

The quantitative fluorescent polymerase chain reaction (QF-PCR) assay shows limitations for the detection of very low levels of chromosomal mosaicism. The QF-PCR assay can detect mosaicism when the abnormal cell line contributes at least 15% of the whole sample [12]. In a cohort study performed to detect mosaicism of primary trisomies in prenatal samples by QF-PCR and karyotype analysis, Donaghue et al [12] found that the QF-PCR assay detected mosaicism when a meiotically derived abnormal cell line contributed 15% of the genotype, but mitotically derived mosaicism might not be detected at this low level.

At least 13 cases of mosaic trisomy 7 detected at amniocentesis have been reported (Table). The reported fractions of trisomy 7 cells or colonies range from 5% to 78%. Among those cases, nine had a normal phenotype and four had phenotypic abnormalities, of which two were associated with maternal UPD 7 and SRS [9–11]. Maternal UPD 7 is the mostly frequently reported UPD, second to UPD 15. Maternal UPD 7 is characterized by pre- and postnatal growth retardation, relative macrocephaly, micrognathia, a high arched palate, down-turned corners of the mouth, a triangular facial shape, and neuropsychological developmental delay [16,17]. Paternal UPD 7 is rare, and only three cases have been reported to date [18–20]. Paternal UPD 7 has been reported in several autosomal recessive disorders such as chloride diarrhea, cystic fibrosis, complete situs inversus, and immotile cilia. Paternal UPD 7 can be associated with developmental delay and overgrowth [20]. Maternal complete isodisomy 7 should be the result of a post-zygotic mitotic segregation error, whereas maternal heterodisomy 7 should be the result of trisomic rescue after a meiotic non-disjunction event at the first meiotic cell division [21,22].

SRS (OMIM 180860) is characterized by severe intrauterine growth restriction (IUGR), poor postnatal growth, craniofacial features of a triangular-shaped face with a broad forehead, down-turned corners of the
### Table. Reported cases of mosaic trisomy 7 at amniocentesis

<table>
<thead>
<tr>
<th>Author</th>
<th>Karyotype</th>
<th>Proportion of abnormal AF cells or colonies (total)</th>
<th>Confirmatory studies</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenkins et al [7]</td>
<td>47,XY,+7/46,XY</td>
<td>23% (39 cells)</td>
<td>46,XY (60 cells): blood 47,XY,+7 (26 cells)/46,XY (10 cells): skin</td>
<td>Developmental delay, facial asymmetry, epilepsy, hypomelanosis of Ito, bifid uvula, enamel dysplasia at 7 yr</td>
</tr>
<tr>
<td>Hsu et al [13]</td>
<td>47,XY,+7/46,XY</td>
<td>48% (25 colonies)</td>
<td>46,XY (100 cells): blood 47,XY,+7 (11 cells)/46,XY (11 cells): foreskin</td>
<td>Normal at 4½ yr</td>
</tr>
<tr>
<td>Benn, case VII-1</td>
<td>47,XY,+7/46,XY</td>
<td>36.6% (30 cells)</td>
<td>46,XY (203 cells): blood 47,XY,+7 (18 cells)/46,XY (20 cells): foreskin</td>
<td>Normal at 4yr</td>
</tr>
<tr>
<td>Bradshaw &amp; Jones, case VII-2</td>
<td>47,XY,+7/46,XY</td>
<td>18.2% (33 cells)</td>
<td>46,XY (100 cells): blood 47,XY,+7 (11 cells): foreskin</td>
<td>Normal at birth</td>
</tr>
<tr>
<td>Fadness, case VII-3</td>
<td>47,XX,+7/46,XX</td>
<td>5% (141 cells)</td>
<td>46,XX: blood 46,XX: placenta</td>
<td>Normal at birth</td>
</tr>
<tr>
<td>Neu, case VII-5</td>
<td>47,XX,+7/46,XX</td>
<td>31.3% (16 colonies)</td>
<td>46,XX (30 colonies): amniotic fluid 46,XX (30 cells): cord 46,XX (20 cells): villi</td>
<td>Normal at birth</td>
</tr>
<tr>
<td>Neu, case VII-6</td>
<td>47,XX,+7/46,XX</td>
<td>26.7% (15 colonies)</td>
<td>No</td>
<td>Normal at birth</td>
</tr>
<tr>
<td>Shaffer, case VII-7</td>
<td>47,XY,+7/46,XY</td>
<td>15% (20 cells)</td>
<td>46,XY (30 cells): cord blood</td>
<td>Normal at 25 mo</td>
</tr>
<tr>
<td>Van Dyke, case VII-8</td>
<td>47,XY,+7/46,XY</td>
<td>11.4% (44 cells)</td>
<td>No</td>
<td>Normal at 4 mo</td>
</tr>
<tr>
<td>Kivirikko et al [14]</td>
<td>47,XY,+7/46,XY</td>
<td>78% (9 colonies)</td>
<td>46,XY (100 cells): cord blood 46,XY (100 cells): blood 47,XY,+7 (36 cells)/46,XY (64 cells): pigmented skin 47,XY,+7 (24 cells)/46,XY (76 cells): hypopigmented skin</td>
<td>Mentally normal, hypopigmented skin, facial asymmetry, sparse hair, short left palpebral fissure, ptosis of the left eyelid, strabismus, enamel dysplasia, posterior-rotated low-set ears, undescended testes at 4 yr</td>
</tr>
<tr>
<td>Author</td>
<td>Karyotype</td>
<td>Proportion of abnormal AF cells or colonies (total)</td>
<td>Confirmatory studies</td>
<td>Phenotype</td>
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<tr>
<td>Chen et al [15]</td>
<td>47,XX,+7/46,XX</td>
<td>16.7% (18 colonies) 12% (25 colonies) (retap)</td>
<td>46,XX (40 cells): chorionic villi 46,XX (35 cells): cord blood, No UPD 7 46,XX (40 cells): amniotic membrane, No UPD 7 46,XX (40 cells): placenta, No UPD 7 46,XX (40 cells): cord, No UPD 7 46,XX (40 cells): liver, No UPD 7 46,XX (40 cells): lungs, No UPD 7 46,XX (40 cells): skin, No UPD 7</td>
<td>Normal at 22 wk of gestation</td>
</tr>
<tr>
<td>Flori et al [11]</td>
<td>47,XY,+7/46,XY</td>
<td>44% (26 colonies)</td>
<td>47,XY,+7 (27 cells)/46,XY (17 cells): placenta 46,XY (100 cells): blood Maternal UPD 7: blood 46,XY (100 cells): cord 47,XY,+7 (15 cells)/46,XY (85 cells): intestine 47,XY,+7 (100 cells): skin FISH analysis: trisomy 7 30/220 nuclei (13.6%): healthy colon 29/210 nuclei (13.8%): colon with hypoganglionosis 8/145 metaphases (5.5%): skin 13/300 nuclei (4.3%): skin</td>
<td>Prominent large forehead, prominent nasal bridge, low posterior-rotated ears, small and retruded chin, bilateral clinodactyl of fifth fingers and bilateral simian creases at birth Silver-Russell syndrome with growth retardation, hypotonia, a small triangular-shaped face, learning disability. Hirschsprung’s disease, short stature during infancy</td>
</tr>
<tr>
<td>Present case</td>
<td>47,XY,+7/46,XY</td>
<td>61.9% (42 colonies) 54.1% (37 colonies) (retap)</td>
<td>46,XY (100 cells): cord blood, No UPD 7 47,XY,+7 (2 cells)/46,XY (66 cells): cord blood (retap), biparental inheritance of chromosome 7 (methylation-specific PCR) 46,XY (100 cells): peripheral blood, No UPD 7, seemingly equal biparental inheritance (QF-PCR) Placenta: No UPD 7, seemingly equal biparental inheritance (QF-PCR) Buccal swab: No UPD 7, seemingly equal biparental inheritance (QF-PCR) Urine: No UPD 7, seemingly equal biparental inheritance (QF-PCR) FISH analysis: trisomy 7, 19/100 nuclei (19%): inguinal hernia sac</td>
<td>Normal at term. Mentally normal, hypopigmented skin on thighs and inguinal hernia at 3 mo</td>
</tr>
</tbody>
</table>

UPD = uniparental disomy; AF = amniotic fluid; FISH = fluorescence in situ hybridization; QF-PCR = quantitative fluorescent polymerase chain reaction.
mouth, a small mandible, prominent ears, fifth finger clinodactyly, asymmetry of the face, body and limbs, occasional genital anomalies, and café-au-lait spots [23]. Imprinting defects on 11p such as epimutations in 11p15 imprinting center region 1 represents 30% of the cases with SRS, and maternal UPD 7 represents about 5% of the cases with SRS [24,25]. Prenatal diagnosis of mosaic trisomy 7 should raise suspicion of UPD 7, although UPD 7 associated with prenatally diagnosed mosaicism is thought to be rare [26].

Prenatal diagnosis of UPD 7 can be achieved by QF-PCR by genotyping the fetus and the parents with microsatellite markers to identify the loss of one parental contribution, or with methylation-specific PCR [27–29]. Polymorphic DNA marker analysis requires samples from both parents and multiple polymorphic specific loci, whereas the methylation-specific PCR assay can specifically distinguish maternal and paternal alleles, and identify the differential methylation of the imprinted PEG1/MEST locus without requiring parental samples. The human PEG1/MEST gene is an imprinted gene on chromosome 7q32. PEG1/MEST is expressed from the paternal allele but not from the maternal allele [30]. Kaneko-Ishino et al [31] designated it as PEG1 (paternally expressed gene 1) because of its paternal expression, and Sado et al [32] referred to it as MEST (mesoderm-specific transcript) because of its predominant expression in the mesoderm and its derivatives.

The assessment of DNA methylation at specific sites can be performed by bisulfite treatment of DNA or by digestion with methylation-sensitive restriction enzymes. In CpG methylation, a methyl group can be attached to a cytosine base located at 5’ to guanosine. After bisulfite treatment, sodium bisulfite converts unmethylated cytosine to uracil, whereas methylated cytosine in the CpG dinucleotide is resistant to this chemical modification.

Flori et al [11] suggested that SRS patients with maternal UPD 7 might also result from an undetected low level of trisomy 7 mosaicism. SRS is well known to present with IUGR, short stature and limb anomalies on prenatal ultrasound [33–35]. Accordingly, prenatal diagnosis of mosaic trisomy 7 at amniocentesis should include detailed sonographic investigation of IUGR, short stature and asymmetry, and a molecular genetic analysis of UPD 7 in the cord blood.

In conclusion, mosaic trisomy 7 at amniocentesis may be derived from a cell culture artifact from an undetected low level of trisomy 7 mosaicism in the uncultured amniocytes, and can be associated with favorable fetal outcomes if the blood has a normal karyotype or a very low level of mosaicism and if UPD 7 is excluded.

Acknowledgments

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References