RESEARCH LETTER

Discordant results between fetal karyotyping and non-invasive prenatal testing by maternal plasma sequencing in a case of uniparental disomy 21 due to trisomic rescue

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ABSTRACT

Uniparental disomy (UPD) is an uncommon chromosome condition, but UPD involving chromosome 21 is rarely reported. We reported here a case who had first trimester screening test for Down syndrome, chorionic villus sampling for fetal karyotyping, quantitative fluorescence polymerase chain reaction (QF-PCR), as well as non-invasive prenatal testing (NIPT) by maternal plasma sequencing. There were discordant results between fetal karyotyping and NIPT due to UPD 21combined with confined placental mosaicism of trisomy 21. This demonstrated that it is possible to detect placental mosaicism by NIPT, but further studies are required to confirm its sensitivity. Therefore, all positive NIPT results must be confirmed by conventional invasive test and karyotyping. QF-PCR has the additional benefit in diagnosing UPD. © 2013 John Wiley & Sons, Ltd.

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INTRODUCTION

Prenatal screening and diagnosis for fetal aneuploidies has become an obstetric routine in many countries. Full karyotyping of fetal tissue obtained through invasive procedures such as amniocentesis or chorionic villus sampling (CVS) remains the diagnostic gold standard. The two major limitations of karyotyping are the long reporting time and the risk of abortion associated with the invasive procedures. Quantitative fluorescence polymerase chain reaction (QF-PCR) has been proven to be a cost-effective, robust and accurate rapid prenatal test for common aneuploidies, providing a fast reporting time within 24–48 h. In addition, QF-PCR enables the determination of parental origin of chromosomes. To minimize the need for invasive tests, many sonographic and biochemical screening strategies have been developed to identify the high risk group for fetal aneuploidy, with a 60–90% detection rate at a false positive rate of 5%. Lately, many studies have consistently confirmed that non-invasive prenatal testing (NIPT) by maternal plasma sequencing (MPS) is an extremely efficient screening test for common aneuploidies, in particular trisomy 21 and 18, with both sensitivity and specificity of over 99%.[1] However, as a new test, there are still much to be learnt and explored.

Uniparental disomy (UPD) is an uncommon chromosome condition, in which either whole homologous chromosomes, or part of it, are inherited from only one parent. UPD could be isodisomy in which a single chromosome from one parent is duplicated, or heterodisomy in which the two copies of the homologous chromosomes from one parent are inherited. UPD could lead to clinically significant conditions by producing either homozygosity for recessive mutations or aberrant patterns of imprinting.[2] UPD cannot be detected by conventional karyotyping, nor can it be detected by the current method of NIPT.

We reported here a case of UPD with confined placental mosaicism (CPM), which resulted in discordant results between fetal karyotyping and NIPT.

CASE

The patient was 42 years old, gravida 4 parity 1 abortion 2. She had a 3-year-old healthy daughter, and two spontaneous first-trimester abortions. She initially had a first trimester combined sonographic-biochemical screening test for fetal Down syndrome. The fetal nuchal translucency was normal (1.7 mm), but both pregnancy associated plasma protein A and free beta-human chorionic gonadotropin were abnormal at 0.18 and 5.11 MoM, respectively. The risk of Down syndrome was estimated to be 1 : 5, solely because of the abnormal biochemical profile. She therefore had a CVS at 12 week of gestation.

Both karyotyping and QF-PCR were performed according to standard protocol. QF-PCR showed normal number of chromosomes 18 and 13. However, all of the seven short tandem repeat (STR) markers (including D21S1411, D21S1412, D21S1414, D21S1433, D21S1445, D21S11 and 21q11.2) for Chromosome 21 were non-informative, presented as a single peak. Final karyotyping of the cultured cells was 46, XX. Findings were highly suggestive of UPD (isodisomy type, iUPD21). Further molecular study of the couple's DNA confirmed that all STR markers of Chromosomal 21 present in the CVS sample were inherited from the mother (Figure 1).

Before the CVS result was available, the patient had attended another institution for the NIPT by MPS, which was performed in BGI-Shenzhen. Details of the test were as previously reported.[1] The NIPT was positive for Trisomy 21.

Because of the discrepancy between the CVS and NIPT results, the patient requested an amniocentesis that was performed at 16 weeks of gestation. Study of the amniotic fluid cells confirmed a fetal karyotype of 46, XX with iUPD21. No fetal structure anomaly was detected on ultrasound examination.

Although the patient was counselled that no phenotypic abnormalities have been reported to be associated with UPD 21, the couple decided to terminate the pregnancy. After abortion, four placental biopsies were obtained. QF-PCR of one of the samples showed the same pattern as the original CVS sample consistent with iUPD21, whereas that of the remaining three samples showed trisomy 21 (Figure 2). The additional peaks in the abnormal samples were all from paternal origin. However, final karyotyping by long-term culture in all four samples were 46, XX.

DISCUSSION

We reported here a case of CPM of chromosome 21, associated with fetal iUPD21. UPD is an uncommon but well-recognized condition, with a reported incidence of around 1.65/1000,[3] but UPD involving chromosome 21 is rarely reported. Similar to trisomies, increasing maternal and paternal age has been reported to be associated with an increased risk of UPD. Mechanisms leading to UPD include trisomy rescue, gamete complementation, monosomy duplication and post-fertilization errors,[2] some of which are common to the mechanism for the development of CPM.

The majority of trisomic cases were due to maternal meiotic non-disjunction, of which about 77.1% occurred at meiosis I, whereas 22.9% at meiosis II. Early post-zygotic mitotic loss of the trisomic chromosome, a phenomenon called trisomic

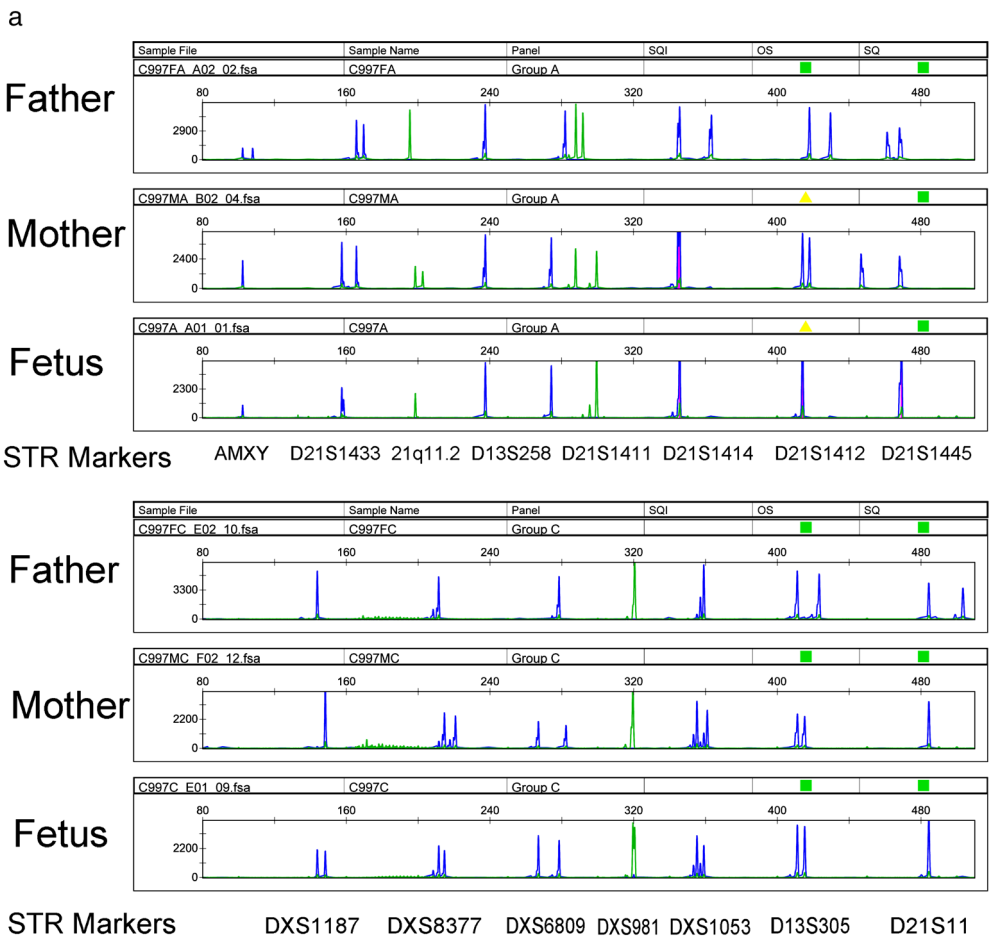

Figure 1 Comparison of quantitative fluorescence polymerase chain reaction (QF-PCR) results between the chorionic villus sample and parental samples. All seven short tandem repeat (STR) markers for chromosome 21 of the chorionic villus sample demonstrated single peaks. None of the peaks for chromosome 21 in the chorionic villus tissue was of paternal origin

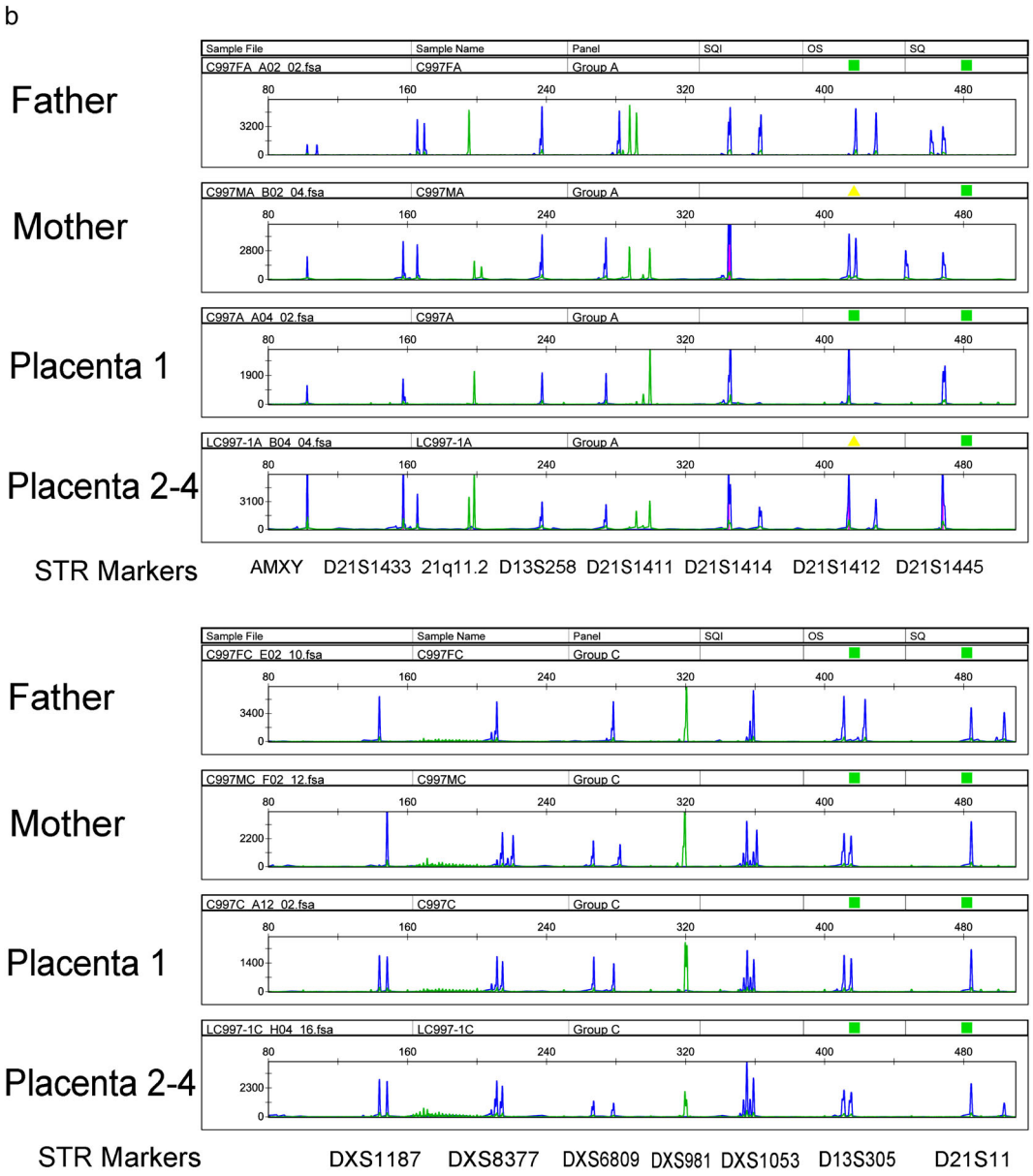

Figure 2 QF-PCR results of parental and four biopsies of placental samples. In placenta 1, all seven short tandem repeat (STR) markers for chromosome 21 of the placental sample demonstrated single peaks. None of the peaks for chromosome 21 in the placental tissue was of paternal origin. In placenta 2–4, six of seven STR markers for chromosome 21 of the placental samples showed 1:2 or 2:1 ratio diallelic, and the extra peaks of chromosome 21 were of paternal origin

rescue, could lead to the development of a diploid fetus and a mosaic placenta.[4] If trisomic rescue results in the preservation of two chromosomes originated from the same parent, the fetus will have UPD.

Therefore, in this case, the most likely mechanism was a maternal meiosis II non-disjunction error followed by trisomy rescue. Meiosis II non-disjunction resulted in the production of an oocyte with two identical copies of chromosome 21, whereas the extrusion of the paternal chromosome 21 during trisomic rescue resulted in a disomy embryo with iUPD and mosaic trisomic placental tissue.

The confirmation of CPM explained why the original first trimester biochemical profile was abnormal and why NIPT was positive for trisomy 21 in this case, because placenta is the major sources of both the biochemical markers and cell free fetal DNA in the maternal plasma.[5] Our case also demonstrated that NIPT, which studies DNA fragments coming from the whole placenta, is much more sensitive in detecting CPM then CVS, which only provide a limited sample. If the CPM were not known in the case, the NIPT result would have been considered to be a 'false positive' because the karyotyping of CVS was normal. Recently, Choi H, *et al.*[6] also reported a 'false positives' case of NIPT for high risk of Down syndrome at first trimester due to CPM. Because CPM is probably much commoner than we believe, occurring in at least 4.8% of the term placenta,[7] it is expected that more 'false positives' of NIPT due to CPM will be encountered when the use of NIPT becomes more widespread. This raises a

fundamental question of whether amniocentesis is a more appropriate and reliable follow up diagnostic test than CVS in case of positive NIPT, especially if there is absence of sonographic features in the fetus suggestive of trisomy. In any case, all NIPT-positive cases should not be considered diagnostic and must be confirmed by conventional invasive test and karyotyping.

In three of the four placenta biopsies, the QF-PCR showed trisomy 21 but karyotyping after long-term culture was normal. This is typical of type 1 CPM,[4] in which the trisomic cells are confined to the trophoblasts. This type of CPM is usually considered to be associated with a normal fetal outcome.

Uniparental disomy may lead to disorders by disrupting the balance of imprinted genes or by reduction to homozygosity for a recessive disorder. Clinically significant imprinting has been reported for some but not all chromosomes, such as UPD 6, 7, 11, 14, or 15.[8] In case of UPD 21, no abnormal phenotype has been reported so far.[9] There are only two case reports suggested that female carriers with UPD 21 could lead to recurrent pregnancies affected by trisomy 21.[10] Unfortunately, the couple did not want to take any possible risk of a fetus with UPD 21 and have requested termination of pregnancy.

The iUPD was suspected in this case because of the unusual pattern of QF-PCR. If the nondisjunction was meiosis I in origin, or if QF-PCR had not been performed, the UPD would not have been diagnosed. This would not have any clinical significant in this case, other than that the pregnancy would not have been terminated for UPD, but it could have major implications if other chromosomes such as 14 or 15 were implicated. When NIPT is more widely practice in the future, and if NIPT is extended to cover all 23 pairs of chromosome, it will provide valuable insight into the exact incidence of CPM and associated UPD.

In conclusion, we reported a case of confined placental mosaic trisomy 21 with fetal uniparental disomy, most likely due to trisomy rescue of an abnormal conceptus. This suggests that a positive NIPT may be caused by placental mosaicism and should be always confirmed by traditional chromosomal analysis. The supplementary QF-PCR seems useful in the diagnosis of UPD.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- The non-invasive prenatal testing (NIPT) by maternal plasma sequencing has been proven to be a safe and highly efficient screening method for fetal aneuploidy. Some studies suggested that these cell-free DNAs are from the placenta. Therefore, an abnormal NIPT result could be due to confined placental mosaicism.

WHAT DOES THIS STUDY ADD?

- We reported here a case of UPD with confined placental mosaicism, which resulted in discordant results between fetal karyotyping and NIPT.

REFERENCES

1. Dan S, Wang W, Ren J, *et al.* Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11 105 pregnancies with mixed risk factors. Prenat Diagn 2012; 32:1225–32.
2. Robinson WP. Mechanisms leading to uniparental disomy and their clinical consequences. Bioessays 2000; 22:452–59.
3. Engel E, DeLozier-Blanchet CD. Uniparental disomy, isodisomy, and imprinting: probable effects in man and strategies for their detection. Am J Med Genet 1991; 40:432–39.
4. Kalousek DK, Vekemans M. Confined placental mosaicism. J Med Genet 1996; 33:529–33.
5. Masuzaki H, Miura K, Yoshiura KI, *et al.* Detection of cell free placental DNA in maternal plasma: direct evidence from three cases of confined placental mosaicism. J Med Genet 2004; 41:289–92.
6. Choi H, Lau TK, Jiang FM, *et al.* Fetal aneuploidy screening by maternal plasma DNA sequencing: 'false positive' due to confined placental mosaicism. Prenat Diagn 2013; 33:198–200.
7. Artan S, Başaran N, Hassa H, *et al.* Confined placental mosaicism in term placentae: analysis of 125 cases. Prenat Diagn 1995; 15:1135–42.
8. Kotzot D. Prenatal testing for uniparental disomy: indications and clinical relevance. Ultrasound Obstet Gynecol 2008; 31(1):100–5.
9. Rogan PK, Sabol DW, Punnett HH. Maternal uniparental disomy of chromosome 21 in a normal child. Am J Med Genet 1999; 83:69–71.
10. Bán Z, Nagy B, Papp C, Beke A, Tóth-Pál E, Papp Z. Recurrent trisomy 21 and uniparental disomy 21 in a family. Fetal Diagn Ther 2003; 18:454–58.