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Confined placental mosaicism of trisomy 16 detected by non-invasive prenatal testing and multiple abnormalities

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Summary

This study aimed to investigate a case of confined placental trisomy 16 mosaicism (CPM16) with abnormal amniotic fluid, placental lake, and other abnormalities. Maternal serum screening was performed to assess the risk of foetal aneuploidy, and massively parallel sequencing was used to detect cell-free fetal DNA. The abnormalities of the fetus, amniotic fluid, and placenta were detected by ultrasonic inspection. Maternal serum screening indicated a high risk for trisomy 21, and the non-invasive prenatal testing (NIPT) result was positive for trisomy 16. The pregnancy was terminated and karyotype analysis of fetal heart blood revealed a 46, XX karyotype. Copy number variation (CNV) sequencing of placental tissues indicated that CPM16 is the main cause of false-positive NIPT results and intrauterine growth retardation (IUGR) diagnoses. Combining molecular genetics technologies, such as CNV sequencing, can be complementary, and provide an effective strategy to determine the cause of such abnormalities.

Keywords: Confined placental mosaicism; Non-invasive prenatal testing; Multiple abnormalities.

Introduction

In the past quarter-century, the main approach to fetal aneuploidy screening has been invasive prenatal diagnosis, such as amniocentesis or chorionic villus sampling (CVS). Lo *et al.* discovered cell-free fetal DNA in the plasma of pregnant women in 1997 [1], which resulted in the realisation of non-invasive prenatal testing (NIPT) for screening of foetal aneuploidy [2]. However, in addition to fetal aneuploidy, foetal, placental, maternal or other abnormalities have been frequently detected using both prenatal invasive and non-invasive diagnosis [3, 4]. Some abnormalities are technically difficult to detect by prenatal diagnosis or remain undetected [5], whereas other abnormalities are indicators of certain diseases [6]. The current study examined a case of confined placental trisomy 16 mosaicism with intrauterine growth retardation (IUGR), apparent thickening of the placenta, placental lake and oligoamnios. This study also investigated the biological basis for these abnormalities and the effects of prenatal diagnosis.

Case Report

This study was approved by the Institutional Ethics Committee of Suzhou Hospital affiliated to Nanjing Medical University. Written informed consent was obtained from all participants of this study.

NIPT was performed following standard techniques [7]. Maternal peripheral blood was collected, and DNA libraries were constructed and subjected to massively parallel sequencing on the HiSeq2500 platform. The obtained sequencing reads and the human genomic sequence hg20 were aligned. Uniquely mapped reads for Chr13, Chr16, Chr18, and Chr21 were subsequently counted and normalised for the GC content. Data from the test samples were compared with those from the reference sample, and Z-scores were calculated with $-3.0 < Z < 3.0$ as the normal value.

Ultrasound screening was performed repeatedly between 19 and 22 weeks (W) of gestation using the 730 Expert system with a 2–5 MHz transabdominal convex transducer and a three-dimensional broadband curved array transducer (3D6-2, 2–6 MHz) in accordance with the routine fetal ultrasound scan guideline [8]. To estimate fetal biometry and well-being, the following sonographic parameters were used: biparietal diameter, head circumference, abdominal circumference, femur diaphysis length, and humerus length. Conventional ultrasound scanning was also performed to evaluate the development of the fetal head, face, spine, chest and abdomen, internal organs., and limbs. The placenta, umbilical cord, and amniotic fluid were also examined.

Copy number variation (CNV) sequencing was performed as described previously [9]. Three duplicate biopsy samples from various regions of the placental tissue were collected. A representation of Chr16 in each sample was calculated using Chr16 sequence reads/total sequence reads. Levels of T16 mosaicism were subsequently determined using the following formula: CR Chr16 in the test sample/mean CR of Chr16 in the reference sample × 100%.

Haemothorax blood amounting to 3 ml was collected from the aborted fetus with heparinisation and then cultured in the medium containing phytohaemagglutinin. The G-banded karyotype was analysed in accordance with the International System for Human Cytogenetic Nomenclature (ISCN2013). The AI karyotyping image analysis system was used to count 60 metaphases, and 20 karyotypes were microscopically analysed in triplicate.

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Table 1. — *Record of pregnancy follow-up after abnormal maternal serum screening.*

Sample	Gestational age	Prenatal/postnatal test	Result/diagnosis
Maternal peripheral blood	16 W	Maternal serum screen	1/190 risk of T21
cffDNA	18 W	NIPT	T13 negative (Z = 0.18), T18 negative (Z = -0.87) T21 negative (Z = -0.73), T16 positive
Established fetus	19 W	Ultrasound/sonogram	BDP=35 mm, FL=20 mm, nasal bone=3.0 mm
Amniocytes	21 W	aCGH	46, XX
Amniocytes/Maternal peripheral blood	21W	STR	No fetal DNA
Established fetus	21 W	Ultrasound/sonogram	IUGR, Severe oligoamnios
Established fetus	22 W	Ultrasound/sonogram	IUGR, Apparent thickening of the placenta Severe oligoamnios, Placental lake
	23 W	Ultrasound/sonogram	Terminate the pregnancy
Fetal heart blood	TOP	Karyotyping	46, XX
Multiple placental tissue	TOP	CNV sequencing	Confined placental trisomy 16 mosaicism (details in Table1 and Fig. 2)

W = weeks and TOP = termination of pregnancy. NIPT and calculation of Z-scores were performed with Z-scores > 3 or ≤ 3 defined as abnormal.

Table 2. — *The results of CNV sequencing.*

Region	Placental tissue	CNV sequencing result
Region A	Centre of placental-maternal side	47,XX,+16 [65%]/46,XX, [35%]
Region B	Middle of placental-maternal side	47,XX,+16 [60%]/46,XX, [40%]
Region C	Edge of placental-maternal side	46, XX
Region D	Centre of placental-fetal side	47,XX,+16 [63%]/46,XX, [37%]
Region E	Middle of placental-fetal side	47,XX,+16 [61%]/46,XX, [39%]
Region F	Edge of placental-fetal side	47,XX,+16 [64%]/46,XX, [36%]
Region G skin of fetus	46, XX region H umbilical cord	46, XX

A 29-year-old G1P0 mother with no family history of congenital anomalies, early infant deaths or consanguinity was referred for routine prenatal screening (Table 1). At 16W of gestation, maternal serological screening indicated a relatively high risk for T21. The mother subsequently received genetic counselling and selected NIPT on cell-free fetal DNA as a second screening test at 18W, which yielded a positive result for T16. Routine prenatal abdominal ultrasound assessment in the present centre at 19W revealed that one side of the fetal nasal bone was incomplete. Amniocentesis at 21W was conducted but failed because of low levels of amniotic fluid. Ultrasound assessment was repeated twice at 21W and 22W. The results showed signs of IUGR, including apparent thickening of the placenta and severe oligohydramnios with an amniotic fluid index of 4.8 cm (amniotic fluid index of < 5 cm is considered as oligohydramnios). Meanwhile, the placenta covered the intracervical mouth, accompanied with cystic organisations of different sizes and a thin placenta. The tissue was less than normal and showed a "boiling state" (Figure 1); hence, the patient was diagnosed with placental lake. The pregnant woman decided to terminate the pregnancy at 23W.

A haemothorax blood sample collected from the aborted fetus was analysed. The result showed a 46, XX karyotype, which was inconsistent with the result of NIPT. A previous study demonstrated that most cases of T16 are aborted spontaneously between 8W and 15W of gestation [10]. Given the discrepancy between the results of serological screening, NIPT, ultrasound screening, and karyotyping of fetal heart blood, the authors suspected a pregnancy with placental trisomy 16 mosaicism (CPM16). To investigate this case further, they analysed the aborted placenta by CNV sequencing of placental tissues (Tables 1 and 2). Six samples of placental tissue (three from the maternal side and three from the fetal side) combined with samples of the umbilical cord tissue and skin tissue were obtained to determine the relative levels and distribution of T16 mosaicism in the placental tissue (Tables 1 and 2). Three samples from the centre, middle. and edge of the fetal side of the placenta indicated average levels of 63%, 61%, and 64% for T16, respectively. However, only the centre and middle of the maternal side of the placenta showed average levels of 65% and 60% for T16, respectively; however, the edge of the maternal side of the placenta was normal for CNV sequencing analysis (Table 1).

Discussion

Numerous published data have suggested that NIPT exhibits high accuracy in detecting fetal trisomy 13, 18, and 21, with both sensitivity and specificity of > 99% [4]. However, several studies in recent years revealed discordant results between fetal karyotyping and NIPT; such results were attributed to false-positive results caused by confined placental mosaicism (CPM) [6, 7]. Thus, additional strategies should be proposed to confirm the results of NIPT. In the present study, a case of CPM16 was confirmed by CNV sequencing and karyotyping after NIPT. According to the specific placental cell lineages exhibiting the abnormal cell line, CPM could be categorised into three types. Placental mosaicism can only be found in trophoblast (type I) and chorionic stroma (type II); however, the condition can be confined to both cell lineages (type III) [11]. Type III is

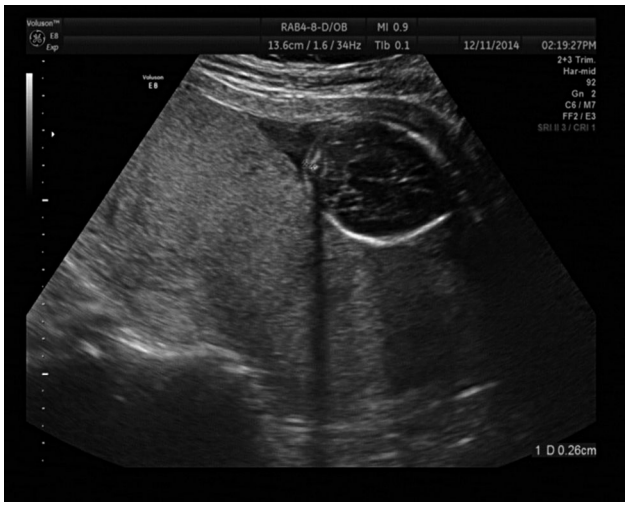

Figure. 1. — The image of ultrasound screening at 19W: apparent thickening of the placenta and severe oligoamnios are observed.

mostly meiotic in origin, and most reported cases with IUGR and intrauterine fetal death are associated with CPM16 [12]. On the basis of previous studies and the aforementioned data, CPM16 in the current study was categorised under type III.

The majority of IUGR cases are associated with placental insufficiency [13]. The placenta in the current study was thickened, and an obvious symptom of placental lake was observed (Table 1 and Figure 1). Therefore, the authors postulated that placental lake and other abnormalities may be the causes of IUGR in this study. Some studies demonstrated no apparent correlation between IUGR and CPM [14]. Regardless, T16 is the most common trisomy found in CPM associated with IUGR, and chromosome 16 was first identified as one of the candidate chromosomes related to IUGR [15]. Thus, IUGR is a potential indicator of CMP16 combined with positive NIPT results for T16.

Invasive diagnostic methods, such as amniocentesis or CVS, have long been regarded as the gold standard for aneuploidy confirmation [7]. However, placental abnormality and severe oligohydramnios may lead to failed amniocentesis, and some reported cases associated with severe oligohydramnios render amniocentesis technically difficult or failed [5]. Stagnation of fetal development and abnormal metabolism for the fetus may result in a small quantity of fetal cells in the amniotic fluid. Hence, amniocentesis remains limited with respect to aneuploidy detection, and several strategies should be combined to confirm the results.

Although challenges still exist for NIPT because of the false-positive results caused by CPM or occult maternal malignancies, NIPT for aneuploidy confirmation remains an effective strategy for prenatal screening. Meanwhile, the discordance between the fetal karyotype and NIPT caused by occult maternal malignancies revealed that presymptomatic detection of tumours in pregnant women undergoing routine NIPT may be realised [6], and further research should be proposed in the future. Hence, NIPT and other non-invasive detection strategies have more applications in disease detection and diagnosis.

In conclusion, the authors investigated a case of confined placental trisomy 16 mosaicism (CPM16) with abnormal amniotic fluid, placental lake, and other abnormalities. CNV sequencing analysis of the biopsy of the umbilical cord tissue and skin tissue could be complementary and provide an effective strategy to determine the cause of such abnormalities.

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