

Pregnancy Outcomes in Trisomy 16 Mosaicism Pregnancies Detected by NIPT, A Series of Case Reports

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Case Report

Keywords: born with low weight, CMA, mosaic trisomy 16 (MT16), Non-invasive prenatal testing (NIPT), prenatal diagnosis

Posted Date: April 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-429782/v1>

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Abstract

Trisomy 16 is often associated with a high risk of abnormal outcome. A retrospective analysis of 14 cases with T16 high risk in NIPT, and all had undergone prenatal diagnosis, including karyotype and CMA. NIPT had detected 11 of T16, 2 of T16 mosaicism, and 1 of more Chr. 16. Prenatal diagnosis confirmed 5 true positive cases and 9 false positive cases. In the 5 true positive cases, 3 out of 5 cases had ultrasound abnormality. In the 9 false positive cases, all the pregnancies continued. All the pregnancies were born with low weight (<2.5kg) except case 7. There were two pregnancies with premature, which suggested that CPM 16 pregnancies may be at higher risk for preterm delivery. NIPT could serve as a fast and early prenatal screening to provide guidance for pregnancy and termination of pregnancy is medically safer when it is performed in the earlier pregnancy.

Introduction

Since 2011, Noninvasive prenatal testing (NIPT) was introduced to clinic, the application of this technology has continuously evolved. In recent years, researchers start to focus on sharing their experience with expanded NIPT and discussing the outcomes of rare autosome trisomies, and they found trisomy 16 (T16) is one of the common rare autosome trisomies detected by NIPT. Complete T16 is generally thought to be incompatible with life, while viable mosaic trisomy 16 (MT16) has been reported extensively [1, 2]. Almost all MT16 pregnancies originate from a trisomy 16 zygote as a result of a maternal meiosis \times non-disjunction [3]. As with many trisomic conceptuses, trisomy 16 mosaicism can undergo rescue, with the risk of residual mosaicism and uniparental disomy (UPD) for chromosome 16 in the surviving fetus. Besides UPD, other factors may contribute to the pathogenesis of trisomy 16 mosaicism are (1) the degree of trisomy in various tissues of the placenta and fetal membranes; (2) the degree and distribution of trisomy in tissues of the fetus; and (3) the sex of the fetus [4].

T16 is the most possible associated with adverse perinatal outcome, such as high risk of abnormal outcome, intrauterine growth retardation (IUGR), fetal-death-in-utero, preeclampsia, preterm delivery, neonatal death, developmental delay, congenital heart defect, and many other anomalies [4]. Thus, a detailed account of the detection of trisomy 16 is essential for numerous prenatal testing modalities. In this paper, we report a series of new cases of T16 and to perform a careful prenatal cytogenetic diagnosis for patients and to give more knowledge and reference about T16 and prenatal diagnoses for clinicians.

Material And Method

Detection of NIPT

Whole blood samples of 5 to 10mL from pregnant women were collected in EDTA within 8h. Afterwards, cfDNA extraction, library construction, quality controlling, and pooling was performed by JingXin Fetal Chromosome Aneuploidy (T21, T18, and T13) Testing Kits (CFDA registration permit No. 0153400300). Then, using JingXin BioelectronSeq 4000 System (CFDA registration permit NO. 20153400309), a

semiconductor sequencer, to DNA sequencing. Sequencing reads were filtered and aligned to the human reference genome (hg19) [5]. Fetal and maternal chromosome copy number variations (CNVs) were classified with our modified Stouffer's z-score method as described previously [6]. Meanwhile, an absolute value of the Z-score greater than 3 was marked with chromosome aneuploidies or microdeletions/microduplications.

Prenatal diagnosis

NIPT high risk cases were advised to perform invasive prenatal diagnosis, including chromosome karyotype analysis and chromosomal microarray analysis (CMA). The metaphase chromosome G-banding karyotyping was performed at a level of 320 to 400 bands. CMA was performed for amniotic fluid or cord blood. Fetal genomic DNA was amplified labeled and hybridized by using CytoScan 750K array platform (Affymetrix, USA) according to the instruction from the manufacturer's protocol. Data were visualized by scanning with the CytoScan™ and analyzed with the Chromosome Analysis Suite software (Affymetrix, USA) based on the GRCH37 (hg19) assembly.

Case Reports

NIPT and prenatal diagnosis results

A total of fourteen T16 cases predicted by NIPT were included in this study. The age of the pregnant women was from 23–43 years old, and the gestational week was 13–25 weeks. The Z score of Chr. 16 was from 7.791–31.503, and the NIPT results predicted 11 of T16, 2 of T16 mosaicism, and 1 of more Chr. 16. All the high risk cases were recommended for prenatal diagnosis, including karyotype and CMA. Prenatal diagnosis confirmed 5 true positive cases (case 1–5) and 9 false positive cases (case 6–14). It is worth mentioning that case 1–5 were T16 predicted NIPT but prenatal diagnosis confirmed they were T16 mosaicism, and the CMA result of case 5 showed two loss of heterozygosity (LOH) with the deletion size fragments of 20.4 Mb and 6.1Mb, see Table 1.

Pregnancy outcomes

In the 5 true positive cases, case 1 insist on continuing pregnancy. Ultrasound examination suggested that case 1 was intra-uterine growth restriction, right umbilical vein is persistent, and the umbilical blood flow is abnormal. Case 1 was born with low birth weight, the weight was 1.9kg at birth, no other abnormalities in newborn screening. Both the karyotype and CMA showed a T16 mosaicism of case 2. A three-stage ultrasound scan was carried out at 30 GA weeks, which found small limbs and a cardiac defect and were inconsistent with gestational age. For a further echocardiography examination, it showed a total anomalous pulmonary venous drainage (TAPVD), ventricular septal defect (VSD), and left aortic arch with right descending aorta (Fig. 1). The parents determined to continue the pregnancy. A male newborn was delivered by cesarean section at 37 GA weeks. But, the infant died because of congenital heart disease 13 days after birth. Case 3 was confirmed of T16 mosaicism. A prenatal three-stage ultrasound scan showed a butterfly vertebra anomaly in T3. At 30 GA weeks, the pregnant woman

underwent an MRI scan, it showed the same abnormality as ultrasound result. The pregnancy was terminated at last. Case 4 was also confirmed of T16 mosaicism. The karyotype of parental peripheral blood was normal. In the end, the pregnancy was terminated. Case 5 was confirmed to have LOH in 16p13.3-p12.3 and 16q23.3-q24.3, and the fetus died in utero, Table 2.

Of these 9 false positive cases, all the pregnancies continued, and all babies were born with low weight (< 2.5kg) except case 7. There were two pregnancies with premature, which suggested that trisomy 16 pregnancies may be at higher risk for preterm delivery. In addition, case 8 suffered from cerebral edema and anemia during newborn screening, and the woman has preeclampsia. Case 13 and case 14 were premature babies, Table 2.

Discussion

Trisomy 16 is one of the most frequently encountered rare autosome abnormalities in first-trimester abortion. Full of trisomy 16 is not compatible with life, almost cases of trisomy 16 is mosaicism. Trisomy 16 mosaicism is viable and reported extensively in prenatal diagnosis, it is considered to be associated with adverse pregnancy outcome [4, 7]. And it is important for clinical counseling to exactly detected trisomy 16 mosaicism.

In the last few decades, cytogenetic technology is the main technique of prenatal diagnosis area. First trimester cytogenetic prenatal diagnosis on chorionic villi (CV) can be complicated by the detection of mosaicism, and chorionic villus sampling (CVS) is routinely used between 10–14 weeks of gestation. Trisomy 16 mosaicism is more often detected by CV analysis comparing amniocentesis. However, the result of chorionic villi analyze is not completely represented for fetus karyotype [8]. Presently, amniocentesis is the most widely used invasive prenatal diagnosis, and it is applicable at 16–18 weeks of pregnancy. Invasive prenatal diagnosis, including CVS and amniocentesis, exists risk of miscarriage (amniocentesis is 1.7% [9], CVS is about ~ 0.5-2% [10]). Amniocentesis may bring high anxiety during the period of waiting for results. Thus, a fast and non-invasive method will be very popular for patients. NIPT basing on cell-free DNA is a new sequencing technique. It is available for gestational week greater than 12, earlier than amniocentesis. In the present study, the earliest gestational of NIPT predicted high risk of T16 was 13 weeks, which meant NIPT could serve as a fast and early prenatal screening to provide guidance for pregnancy, and termination of pregnancy is medically safer when it is performed in the earlier pregnancy.

Previously study reported that the mosaic trisomy 16 pregnancies birth weights for the live births were below the gestational age corrected mean birth weights in the general population [4, 11]. In our study, a true mosaic trisomy 16 pregnancy was continued, and the baby was born with the weight of 1.9kg which was a low weight infant. Of the 9 false positive cases, all the pregnancies were continued and all infants was born with low birth weight except case 7. This indicates that some level of below average growth is a nearly universal phenomenon in trisomy 16 mosaicism, and supports the hypothesis of undetected trisomy mosaicism as an aetiological factor in both severe and mild idiopathic intrauterine growth

restriction. In addition, there were 2 premature babies in this study, which suggests that trisomy 16 pregnancies may be at higher risk for preterm delivery. Further research is needed to determine whether there is truly a higher risk of preterm delivery in an unbiased population.

As we all know, cell-free DNA comes from the apoptosis cells of cytotrophoblast, so the NIPT result shows a high risk of trisomy 16 representing there was a certain extent trisomic cells in the placenta. It is recognized that the main source of false positive results is confined placental mosaicism (CPM). CPM is a type of chromosomal mosaicism in which the chromosome abnormality is present in chorionic villi/placenta, but not in the fetus itself. In our study, 9 cases were false positive. We highly suspected that it was caused by the CPM. But, this study was a retrospective study and all the pregnancies were finished, we couldn't obtain placenta samples to verify this point, which was a limitation of our study. Although the fetus whether it is involved or not can't be investigated accurately by NIPT, it is considered as a useful and valuable tool for predicting placental mosaicism [12]. In the Diane Van O with his colleagues' research, they found that cases with abnormal NIPT T16 results most likely caused by placental aberration mostly manifests IUGR or SGA [13]. Another research of Yi H et al showed IUGR could found in the 20% of CPM cases for T16 detected by NIPT with the evidence of placenta [14]. And in recently study, T16 was considered as the only individual RAT worth looking for and reporting through cfDNA screening, which associated with poor outcomes [15]. In our series of cases, all the false positive cases were low birth weight babies except case 7. We speculated low birth weight also related to CPM. But, it need more samples to verification, which will also be one of our near future research directions.

Case 5

had two LOH in 16p13.3-p12.3 and 16q23.3-q24.3. The deletion sizes of fragments were 20.4 Mb and 6.1 Mb. There is no evidence that the two LOH in this area is pathogenic, but if there is a recessive genetic disease carrying genes in this section, it will increase the risk of recessive genetic disease.

In conclusion, trisomy 16 mosaicism is complex, however, it could serve as a fast and early prenatal screening to provide guidance for pregnancy and termination of pregnancy is medically safer when it is performed in the earlier pregnancy. combination cytogenetic techniques and molecular methods can accurately detect trisomy 16 mosaicism. The pregnant outcome is diverse and hard to prediction, genetic counseling should be cautious.

Abbreviations

T16: Trisomy 16; MT16: mosaic trisomy 16; UPD: uniparental disomy; IUGR: intrauterine growth retardation; CNVs: copy number variations; FISH: Fluorescence in situ hybridization; TAPVD: total anomalous pulmonary venous drainage; VSD: ventricular septal defect; CVS: chorionic villus sampling; GA: gestational age; CV chorionic villu; LOH loss of heterozygosity; CPM confined placental mosaicism;

Declarations

Ethics approval and consent to participate

This study was performed with the approval of the Medical Ethics Committee of Guangdong Women and Children Hospital, and written informed consent was obtained from the patients.

Consent for publication

The authors declares that there are no financial or other relationships that might lead to a conflict of interest of the present article. The manuscript is approved by all authors for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This study was funded by National Key Research and Development Program of China (2016YFC100703).

Authors' contributions

All authors have materially participated in the study and manuscript preparation. H.-s. P. and J.-x. Y. collected all clinical data and drafted the manuscript; D.-m.W., F.-f. G. carried out all the molecular genetic analyses; Y.-p. H. participated in the data analysis; A.-h. Y. designed the work revised the manuscript. All authors have approved the final article.

Acknowledgments

None.

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Due to technical limitations, table 1 and 2 is only available as a download in the Supplemental Files section.

Figures

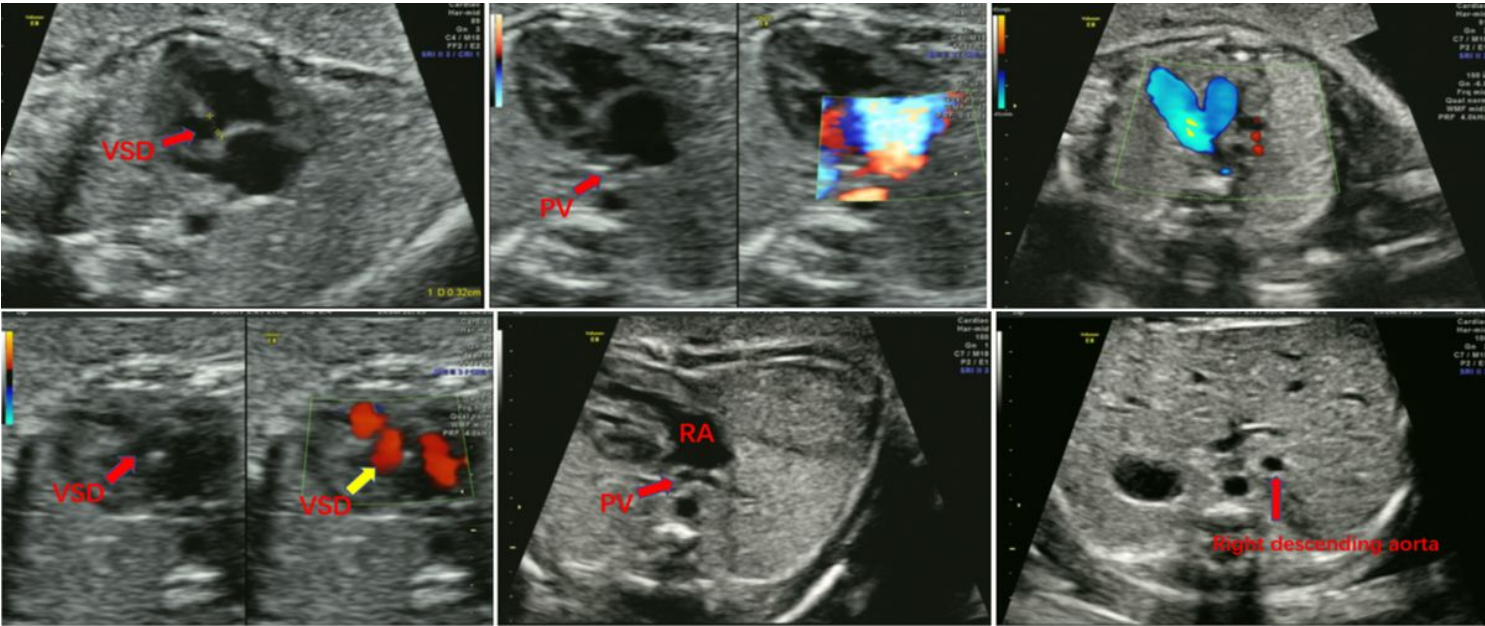


Figure 1
Echocardiography examination of case 2.

Supplementary Files

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- [tables.xlsx](#)