Detection of Fetal Trisomy 8 Mosaicism with Cell-free Fetal DNA in Maternal Plasma

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Case Report

Keywords: trisomy 8 mosaicism, cell-free fetal DNA, next-generation sequencing, noninvasive prenatal testing, rare autosomal trisomies, prenatal diagnosis

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Abstract

Background

Cell-free fetal DNA in the maternal plasma is widely used to identify the aneuploid, including trisomy 21, 18, 13 and sex chromosomes. While for the detection of rare autosomal trisomies, the outcome has not come to a consensus. Here we reported a case of trisomy 8 mosaicism (T8M) which was identified by cffDNA screening.

Methods

Noninvasive prenatal screening (NIPS), fetal karyotyping, single nucleotide polymorphism array (SNP array) and interphase fluorescence in situ hybridization (FISH) analysis were used to characterize etiology in the fetus.

Results

In this study, trisomy 8 was initially identified by cell-free fetal DNA in the maternal plasma. Ultrasound scans and magnetic resonance imaging observed fetal hydrenephrosis and irregular spine. Trisomy 8 mosaicism was diagnosed based on the SNP array and amniocentesis results. The couple decided to continue the pregnancy after genetic counseling and a male infant was delivered. Interphase FISH analysis in the uncultured neonatal cord blood cells confirmed trisomy 8 mosaicism, with a percentage of 10%. In the follow up, periodic fever as well as language retardation was observed, indicating a poor prognosis.

Conclusions

Our study provided an insight into the identification of low level rare autosomal trisomy mosaicism with cell-free fetal DNA.

1 Introduction

Cell-free fetal DNA (cffDNA) in maternal blood plasma was first discovered in 1997. It is the DNA fragments circulating in the maternal plasma mostly derived from the placenta. CffDNA is widely applied for the detection of fetal chromosome abnormalities, including trisomy 21, 13, 18 and sex chromosomes, which are under recommendation of American College of Medical Genetics and Genomics (ACMG) and the American Congress of Obstetricians and Gynecologists (ACOG).

Rare autosomal trisomies (RATs) refer to trisomies other than trisomy 21, 18 or 13. RATs are not included in the detecting scopes of the noninvasive prenatal screening (NIPS) due to lack of large-scale population data. The observations about identification of RATs and copy number variations (CNVs) with cffDNA are available recently. However, the detecting accuracy of RATs remains unsatisfactory. Therefore, more evidences are required to estimate the outcome of RATs detected by NIPS.

Here we reported a case of trisomy 8 mosaicism (T8M) identified by cffDNA screening. Hydrenephrosis and irregular spine were observed by fetal ultrasound scans (US) and magnetic resonance imaging (MRI). T8M was confirmed by fluorescence in situ hybridization (FISH) on the uncultured neonatal cord blood cells, with a percentage of 10%. Our study provided an insight into the identification of low level rare autosomal trisomy mosaicism with cell-free fetal DNA.

2 Materials And Methods

2.1 Case report

The 35-year-old healthy woman was at the 17th week of gestation. Abnormal copy numbers were identified in the chromosome 8 by cffDNA screening (Fig. 1). In addition, hydrenephrosis and irregular spine were observed in the fetal US and MRI at the 22th and 32th week, respectively (Fig. 2).

This study was approved by the Ethics Committee of Women's Hospital, School of Medicine Zhejiang University (IRB-20210170-R). The participants provided their written informed consents.

2.2 CffDNA screening

Maternal blood sample was collected in an EDTA-K2 containing tube. The blood plasma was separated by centrifugation at 1600g for 10min. Then, the supernatant was re-centrifuged at 14,000g for 10min to remove cell debris. All of the centrifugations were performed at 4°C. The subsequent procedures, including the isolation of cell-free DNA, library construction and sequencing, were performed as previously described.

2.3 Amniocentesis and fetal karyotyping
Amniocentesis was performed at the 23th week of gestation under real-time ultrasound guidance. A total of 30ml amniotic fluid was collected and the initial 5ml was discarded. Amniotic fluid cells were cultured with BIOAMF-2 Complete Medium (Biological Industries, Cromwell, CT) in a 5% CO₂ incubator at 37 °C. G-banding analysis at 320–400 band resolution was performed on cultured amniocytes according to the standard procedure.

2.4 Chromosome microarray (CMA)

Fetal Genomic DNA was extracted from the cultured amniotic fluid cells with the Gentra Puregene Kit (Qiagen, Hilden, Germany). CytoScan™ HD array (Affymetrix, SantaClara, CA) was used to analysis the copy number, according to the manufacturer's instructions. Chromosome Analysis Suite (ChAS) software (Affymetrix, Santa Clara, CA) was used to analyze the raw data and visualize the results based on the GRCh37/hg19 assembly.

2.5 Interphase FISH analysis

Neonatal cord blood cells were analyzed by trio-FISH with the Tel Vysion 8q Spectrum Red, Vysis CEP 4 Spectrum Auqa probe and TelVysion 2p spectrum Green. Interphase spread hybridization and washes were performed according to the manufacturer's instructions (Vysis, Downers Grove, IL). Interphase spreads were counterstained with DAPI (Vysis, Downers Grove, IL) and analyzed sing a Zeiss Imager. A2 microscope (Zeiss, Marly-le-Roi, France). Image acquisition was subsequently performed using a CCD camera with Isis (FISH Imaging System, Meta Systems, Altlussheim, Germany).

3 Results

3.1 Identification of fetal trisomy 8 by cffDNA screening

CffDNA screening showed that the copy numbers of chromosome 13, 18 and 21 were normal, the t-scores of which were fluctuating around 0 (Fig. 1A-C). While the t-score of chromosome 8 was as high as 4.5 (Fig. 1D), indicating that the fetus carry an extra copy number of chromosome 8. In addition, no sub-chromosomal deletion and duplication were found.

3.2 Determination of trisomy 8 mosaicism

As is Fig. 3A, CMA results showed that the SmoothSignal of chromosome 8 was 2.1, slightly higher than the normal range (~ 2.0). Then, karyotype analysis was performed on the cultured amniocytes. A total of 73 cells were analyzed and 1 cell was found to be trisomy 8 (Fig. 3B), indicating the existence of T8M. FISH analysis on the uncultured neonatal cord blood was subsequently performed to estimate the mosaic level of trisomy 8 (Fig. 3C-D). 50 interphase cells were counted and 5 cells were found to carry 3 red signals in the interphase FISH, suggesting that the percentage of T8M was 10%.

3.3 Follow-up

The proband was born at the 36th week of gestation. His birth weight was 3,110g and the 1-minute and the 5-minute Apgar score were both 10 points. The child was currently 3 years old. He suffered from periodic fever and language retardation, and manifested asymmetrical cheek and low-set ears.

4 Discussion

In the current observation, trisomy 8 was initially identified by cffDNA. Fetal US and MRI indicted hydronephrosis and irregular spine. T8M was suspected on the basis of the results of amniocentesis. After thoughtful considerations, the couple decided to continue the pregnancy and a male infant was delivered. FISH analysis on the uncultured neonatal cord blood cells verified the existence of T8M, with an percentage of 10%. Though the percentage was low, periodic fever as well as impaired speech was observed in the child in his follow up, indicating a poor prognosis. Our findings provided an insight into the identification of low level percentage of T8M with cffDNA screening.

CffDNA, originating from trophoblasts and circulating in the maternal plasma, is used to detect potential fetal chromosomal abnormalities. The analysis of cffDNA with the next-generation sequencing technology, also known as NIPS, provides a well-validated way to identify the aneuploid. CffDNA-based screening for trisomy 21 was superior to any other clinically available screening methods, with a detection rate (DR) of 99.2%. For other aneuploidies, such as trisomy 18, 13, monosomy X and other sex chromosome aneuploidies, the DRs are relatively lower compared with trisomy 21, approximately 96.3%, 91.0%, 90.3% and 93.0%, respectively. The observations about identification of RATs with cffDNA are available recently. However, large-scale population data of RATs for genetic counseling remain limited. Thus, whether the RATs identified by NIPS should be included in the final reports or not is still under debate. In fact, with an aggregate incidence of approximately 0.3%, RATs are not uncommon and associations between RATs and feto-placental diseases are increasingly reported. The ignorance of RATs may omit the increased risk of miscarriage, intrauterine growth restriction, small-for-gestational-age infants, uniparental disomy and NICU admission. Therefore, early identification of RATs is necessary to notice the risks of adverse pregnancy outcomes.
Trisomy 8, one of the RATs, was known for its associations with the acute myeloid leukemia (AML) \(^{23}\). T8M, also known as Warkany syndrome, is a rare chromosomal disorder, usually caused by a post zygotic non-disjunction. With an estimated prevalence ranging from 1:25000 to 1:50000, it is a disease affecting males more often than females\(^{24,25,26,27}\). The clinical manifestations associated with T8M are extremely variable, including central nervous, ocular, cardiac, gastrointestinal, genitourinary and musculoskeletal abnormalities \(^{25,28}\). Patients with T8M present personalized pattern of features, ranging from no phenotypes to a variety of birth defects (Table 1) \(^{28,29,30,31,32,33,34,35,36,37,38,39,40}\). Thus, prenatal detection for T8M is still a challenge in the clinical practice and more evidences are required to estimate the outcome of T8M.
Table 1  
Literature review and summary of the clinical manifestations in T8M patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient</th>
<th>mental retardation</th>
<th>dysmorphic facial feature</th>
<th>malformations of the kidneys</th>
<th>Developmental retardation</th>
<th>malformations of the bone</th>
<th>Fetual US abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Kuryka et al., 1988)</td>
<td>Patient 1</td>
<td>Yes</td>
<td>Micrognathia, low set of auricles, funnel-breast, hyper-trichosis on the arms, cloudy cornea in right eye, severe contractures in fingers, moderate extention impairment in joints, micrognathia, deep plantar skin furrows</td>
<td>bilateral moderate hydronephrosis</td>
<td>Yes</td>
<td>clinodactyly, bilateral absence patellae, V-shaped talipes valgus, narrow pelvis, right scoliosis of the thoracic segment of the vertebral column</td>
<td>Not mention</td>
</tr>
<tr>
<td>(Camurri et al., 1991)</td>
<td>Patient 2</td>
<td>Yes</td>
<td>slight contractures of finger joints, bilateral arachnodactyly in hands and feet, Deep palmar and plantar furrows</td>
<td>Not find</td>
<td>Not find</td>
<td>deformed sternum, Narrow pelvis, right thoracic scoliosis</td>
<td>Not mention</td>
</tr>
<tr>
<td>(Miller et al., 1997)</td>
<td>Not find</td>
<td></td>
<td>prominent forehead, bulbous nose with a depressed bridge, everted lower lip</td>
<td>Not find</td>
<td>Not find</td>
<td>Not find</td>
<td>Not mention</td>
</tr>
<tr>
<td>(de Pater et al., 2000)</td>
<td>Not find in 3 patients</td>
<td></td>
<td>4 patients had dysmorphic facial feature; 3 patients were normal; 3 were not mention</td>
<td>Not mention</td>
<td>Not mention</td>
<td>3 patients had skeletal anomalies; 4 patients were normal; 3 were not mention</td>
<td>Not mention</td>
</tr>
<tr>
<td>(Chen et al., 2011)</td>
<td>Not mention</td>
<td></td>
<td>the infant was grossly normal at birth</td>
<td>Not mention</td>
<td>normal at age 4 months</td>
<td>Not mention</td>
<td>polyhydramnios and fetal left-sided congenital diaphragmatic hernia (CDH)</td>
</tr>
<tr>
<td>(Iwatani et al., 2014)</td>
<td>Not mention</td>
<td></td>
<td>asymmetrical cranium, small lids, deep palmar, plantar furrows, hypospadias, and corpus callosum agenesis</td>
<td>Not mention</td>
<td>Not find</td>
<td>Not mention</td>
<td>Not find</td>
</tr>
<tr>
<td>(Ruland et al., 2017)</td>
<td>Not mention</td>
<td></td>
<td>Not mention</td>
<td>Not mention</td>
<td>Not mention</td>
<td>Not mention</td>
<td>agenesis of the corpus callosum (ACC)</td>
</tr>
<tr>
<td>(Sherer et al., 2017)</td>
<td>Not mention</td>
<td></td>
<td>Not mention</td>
<td>Not mention</td>
<td>Not mention</td>
<td>Not mention</td>
<td>ventricular septal defect (VSD) truncus arteriosus Type I</td>
</tr>
<tr>
<td>(Cassina et al., 2018)</td>
<td>Not find in 17 patients</td>
<td></td>
<td>Not find in 17 patients</td>
<td>Not find in 17 patients</td>
<td>Prenatal and post-natal growth retardation in patient 14</td>
<td>Clubfeet and clinodactyly of 3rd, 4th and 5th fingers in patient 17</td>
<td>Prenatal growth retardation in patient 14</td>
</tr>
<tr>
<td>(Sun et al., 2019)</td>
<td>Mild mental retardation</td>
<td></td>
<td>Not find</td>
<td>Not find</td>
<td>Not find</td>
<td>Not find</td>
<td>Not find</td>
</tr>
</tbody>
</table>
Here in, low level percentage of T8M was identified by cffDNA. Findings in his follow up were recorded before \(^41\), warning a poor prognosis. Therefore, the identification of RATs with cffDNA is essential for early diagnosis. In fact, some countries have embedded RATs into the detecting scope of cffDNA \(^42\). Our findings provided another confirmation for its sensitivity to identify low level percentage of RATs. What’s more, invasive prenatal test ought to be performed afterwards when RATs are identified in NIPS as to exclude the possible false positive results.

**Declarations**

**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Author Contributions**

MD conceived of the study; KY participated in its design and JH drafted the manuscript; CffDNA screening was carried out by YS and JH performed the CMA and FISH. PJ collected the samples and clinical data; YY helped to revise the manuscript. All authors have read and approved the final manuscript.

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**Acknowledgments**

We thank the patients and the relatives for their support during this research study.

**References**


Figures

Figure 1

Non-invasive Prenatal Screening (NIPS) results of fetal chromosomes 13, 18, 21 and 8. The horizontal axis represents genomic location (Mb) and the vertical axis represents t-score. NIPS revealed an extra copy of chromosomes 8 (D) and other chromosomes were normal (A-C).

Figure 2

Magnetic resonance imaging of this fetus. The fetus showed (A) normal brain, (B) hydromeephros, (C) irregular spine in sagittal section.

Figure 3
CMA, FISH and Karyotyping analysis of the fetus. (A)CMA results from cultured amniotic fluid. (B)Karyotyping from cultured amniocytes. (C,D) Chromosome 8 (Spectrum Red), chromosome 4 (Spectrum Blue) and chromosome 2 (Spectrum Green) probes on the neonatal cord blood. There are two red signals in the normal cell (D) and three red signals in T8 cell (C).