

# The Strategic Study in the Prenatal Diagnosis of a Chimeric Trisomy 15 Fetus

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**Abstract: Objectives:** To explore the utility of amniotic cell karyotype analysis in combined with chromosome microarray analysis in prenatal diagnosis. **Methods:** Chromosome microarray analysis (CMA) and amniotic cell karyotype analysis were accepted by a pregnant woman who carried a fetus with chromosome 15 aneuploidy suggested by non-invasive prenatal test (NIPT). Later on, the woman chose abortion because ultrasound detection had diagnosed that the fetus manifested hypospadias, long bone dysplasia, and abnormal vasa umbilicalis. The placenta, aborted fetus's tissues and organs were collected and verified by fluorescence in situ hybridization (FISH) technique to confirm the prenatal diagnosis. **Results:** Karyotype analysis of amniotic cell found only one 47, XY, +15 cell versus 49 normal cells. CMA of amniotic fluid sample indicated that there is a 75.9 Mb copy number gain on 15q11.2q26.3. FISH results of the placenta and fetal tissues from multiple organs showed that the proportion of trisomy 15 cells in placenta is significantly higher than which in other parts of the fetus. **Conclusion:** For fetuses with chromosome 15 aneuploidy indicated by NIPT, amniotic karyotype analysis in combined with chromosome microarray analysis could be a suitable solution for genetic diagnosis.

**Keywords:** NIPT, Chromosome Microarray Analysis, Karyotype Analysis, FISH, Chimeric Trisomy 15

## Introduction

As a consequence of abnormal karyokinesis during the embryo development, aneuploidy is an important factor of spontaneous abortion <sup>[1,2]</sup>. Trisomy 21, 18, 13 are the most commonly observed aneuploidies, which usually induce embryonic loss, mental retardation, facial dysmorphism, and delayed development. In many of cases it also induce cardiac abnormalities, kidney abnormalities, and umbilical bulging <sup>[3,4]</sup>. Here we present a chimeric trisomy 15 fetal case, whose chimeric ratio varied hugely between amniotic cell and placenta. By discussing these differences, we hope to explore the utility value of amniotic cell karyotype analysis in combined with chromosome microarray analysis in prenatal diagnosis.

## Clinical presentation

A 44 years old pregnant woman with singleton pregnancy came for genetic counseling. for non-invasive prenatal test (NIPT) had suggested an abnormal abundance of her fetus' chromosome 15 (Z value of Chromosome 15 is 11.4). At gestation 22 W2D she accepted amniocentesis for amniotic cell karyotype analysis and CMA test. At gestation 31W, routine ultrasound examination found that the fetus manifested genital, possible hypospadias, shortened long bone and abnormal vasa umbilicalis. therefore the woman chose abortion to end this pregnancy.

With the informed consent of the woman and the approval of the hospital ethics committee, a small part of the placenta and samples of various tissues and organs from the aborted fetus were collected for further detection.

## 1 Methods

### 1.1 NIPT detection

8ml of peripheral blood was taken from the pregnant woman and placed in the EDTA anticoagulation tube. The plasma was separated and free DNA was extracted. After constructing DNA library, DNA sequencing and bio-information analysis, the Z value of each chromosome was obtained. For pregnancies, the normal Z value range for autosome was -3 to 3.

### 1.2 Karyotype analysis

The amniotic fluid cells of the fetus are cultured at 37 ° C, and the KCl solution is hypotonic to the cells, causing the cells to swell. Then, ormaldehyde and glacial acetic acid solution was used for cell fixation. The immobilized cells were dropped at 25 ° C and 55% humidity, baked at 75 ° C, and finally stained with Giemsa stain. The number and morphology of chromosomes were observed under the microscope.

### 1.3 CMA detection

Amniotic fluid DNA was extracted using QIAGEN's QIAamp DNA Blood Mini Kit, and samples were

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analyzed using a CytoScan 750K Array chip at 25 SNP/50 kb, 50 CNV/200 kb resolution. The specific experimental procedures refer to the standard experimental procedures associated with Affymetrix. The Affymetrix CytoScan 750K Array chip contains 200,000 SNP markers and 550,000 CNV markers, distributed on the entire genome of humans at an average density of approximately 1 marker / 4 kb. The chip scan data was analyzed using ChAS software.

#### 1.4 Fluorescence in situ hybridization detection

A part of placenta, tissues and organs of the aborted fetus were cutted, swelled, fixed, aged, pretreat. Probe denaturation, hybridization and DAPI counterstaining were performed subsequently, observing the hybridization signal with fluorescence microscope. The FISH probe was adopted D15Z1 (CEP15:15p11.2) probe by Vysis. Every sample was counted up to 100 cells, and the cells images were recorded and stored by Cytovision automatic workstation.

## 2 Results

### 2.1 Chromosome karyotype analysis results

The karyotype of fetal amniotic fluid cells is 47, XY, +15 [1] / 46, XY, [49], as it is shown in figure 1.



Figure 1. Trisomy 15 Figure 1. Cytogenetic karyotypes of Amniotic fluid cells occasionally found a 15 trisomy (where the red arrow is pointing)

### 2.2 CMA results

The CMA result of amniotic fluid sample was arr[hg19]15q11.2q26.3 (22,770,421-102,429,040)×2~3, suggesting a 75.9 Mb chimeric duplication in 15q11.2q26.3, as it is shown in figure 2.

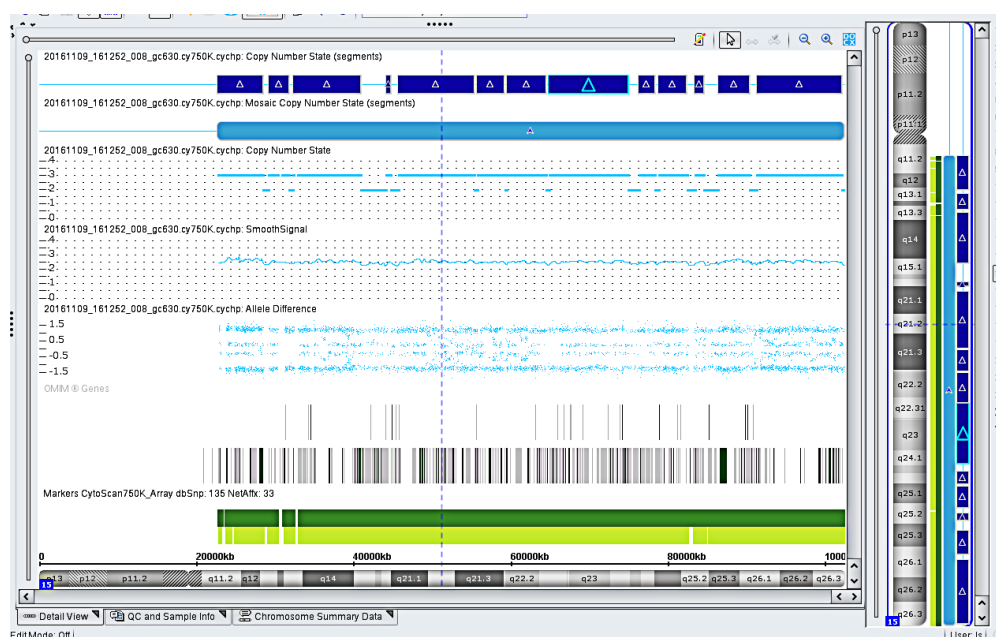


Figure 2. Genomic chip test results

### 2.3 FISH test results

The FISH results of the placenta, the tissues and organs of aborted fetus are shown in Table 1. The fluorescent signal is clearly identifiable. When the

proportion of abnormal cells is greater than 10%, indicating that the index is obviously anomalies; the proportion of abnormal cells is between 10% and 60%, suggesting chimera.

Table 1 Results of FISH test of placenta and different tissues and organs of induced labor

Numbering	Part	Normal cells	Abnormal cells	Abnormal ratio / (%)
01	placenta	63	37	39%
02	Umbilical cord	85	15	15%
03	Amniotic membrane	97	3	3%
04	Chorion	90	10	10%
05	Thigh skin	85	15	15%
06	Thigh muscle	86	14	14%
07	kidney	97	3	3%
08	Penis	93	7	7%
09	testis	89	11	11%
10	esophagus	87	13	13%
11	bladder	84	16	16%
12	stomach	95	5	5%
13	ureter	89	11	11%
14	Fetal blood	97	3	3%
15	Fetal cord blood	96	4	4%

### 3 Discussion

Chromosomal aneuploidy is a crucial cause of spontaneous abortion in early gestation and of birth defects. Aneuploidy embryos account for at least 10% of total pregnancies during human pregnancy, and in older women, this ratio can reach to 50%. Excessive stimulation of the ovaries also increases the probability of aneuploidy in the application of assisted reproductive technology<sup>[5,6]</sup>. This may be explained by the division and accumulation of female ovolutions throughout the development cycle. As women age, the probability of meiosis error increases<sup>[7]</sup>. Other studies have shown that the occurrence of aneuploidy is not only related to single factor, but more likely to multiple factors including drinking, smoking, using of exogenous hormones, interference with environmental secretions, and exposure to radiation<sup>[8,9,10]</sup>.

In this case, the NIPT test in the second trimester indicated that there was an abnormal abundance of the fetus' chromosome 15, and the amniocentesis was performed at gestation 22 w2d. Cytogenetic karyotype of amniotic fluid cells found only one 15 trisomy cell. CMA result suggested that the fetus had chimeric on 15 trisomy, indicating that NIPT also has certain sensitivity and specificity for other autosomal abnormalities.

As ultrasound examination discover no discernible abnormality during the first and the second trimester, the pregnant woman had doubt about the prenatal diagnosis and she required to continue the pregnancy.

At 31 weeks of gestation, ultrasound examination showed that the fetus had hypospadias, shortened long bones and abnormal vasa umbilicalis, aware of this new situation, the woman finally chose to abort the pregnancy. After induction of labor, the woman agreed to offer the induction child and its placenta. Researchers observed that the appearance of the induced child have no other abnormalities except for the hypospadias and short femur (7.5cm). FISH was performed to analyze the mosaic condition of chromosomal aneuploidy in different tissues, organs and placenta of induced labor. It was found that there were abnormal cell mosaics in the placenta and various parts of the body, but the chimeric ratio inconsistent, the proportion of placental mosaic is significantly higher than other parts. The literature reports that mitotic errors prevail in the early stages of human embryo implantation, but this error is "corrected" in the subsequent embryonic developmental stage. The presence of confined placental mosaicism also suggests a possible "self-correction" mechanism in chromosomal aneuploid chimeric fetuses<sup>[11,12,13]</sup>. In this report, the amniotic membrane, kidney, penis, stomach, fetal blood, fetal umbilical cord blood chimerism ratio is extremely low, less than 10%, and the placental chimeric ratio is significantly higher(39%). Chromosomal aneuploid cells preferred to distribute to the trophoblasts in human early embryo implantation. Further research of this field is still needed.

In summary, we combined karyotype analysis and chromosomal microarray analysis to detect a low proportion of chimeric fetuses on chromosome 15 and further verify after induction of labor. This indicates that amniotic fluid cell karyolysis combined with genomic microarray technology can more accurately assess fetal chromosome aneuploidy chimerism, and the study also accumulates clinical data for prenatal diagnosis of special chromosomal aneuploid chimeric fetuses.

There is no conflict of interest in the paper.

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