

*Case Report*

## Discrepancy between Non-invasive Prenatal Genetic Testing (NIPT) and Amniotic Chromosomal Test due to Placental Mosaicism: A Case Report and Literature Review

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We experienced a case of advanced maternal age in which a fetus was found to be positive for trisomy 18 at re-examination following indeterminate non-invasive prenatal genetic testing (NIPT), the amniotic fluid chromosomal test revealed a normal karyotype, and confined placental mosaicism (CPM) was observed in an SNP microarray analysis of the placenta. The child was born with no defects or complications. In the present case, the result of the original NIPT at week 15 of pregnancy was indeterminate and the subsequent re-examination result was positive; since the definitive normal diagnosis was not reported until the latter half of week 21, the pregnant patient was subjected to psychological stress for a long period of time. The problem with NIPT is that most of the fetus-derived cell-free DNA in the maternal blood is not derived directly from the fetus but from the villus cells of the placenta, leading to indefinite diagnoses; for that reason, the pregnant patient was subjected to psychological stress for a long period of time. Of the 18,251 cases undergoing NIPT in the past 2 years in Japan, 51 had indeterminate results; this was the second case in which a subsequent re-examination gave a positive result for trisomy 18.

**Key words:** non-invasive prenatal genetic testing, massively parallel sequencing, confined placental mosaicism, genetic counseling, trisomy 18

Non-invasive prenatal genetic testing (NIPT) is indeterminate when the fetus-derived cell-free DNA concentration in the blood is low [1-4], and the mechanism thereof has not been completely elucidated. We experienced a case in which a re-examination of an “indefinite” fetus gave a positive trisomy 18 reading, the amniotic fluid chromosomal test subsequently carried out for a definite diagnosis revealed a normal karyotype, and confined placental mosaicism (CPM) was observed in a microarray analysis of the placenta. We hereby report on the issues with NIPT as well as suggestions for resolving them.

### Case Report

The mother was a 39-year-old women, primigravida. There was nothing worthy of special mention regarding her family history and past history. Her husband was 46 years old. She became pregnant by intracytoplasmic sperm injection. Based on her older maternal age, she was referred as an outpatient to the Genetic Counseling Department in our hospital, where she underwent NIPT at 15 weeks and 2 days of pregnancy. At 17 weeks and 5 days of pregnancy, she underwent NIPT again following post-examination counseling. The false positive trisomy 18 test results

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were revealed at 19 weeks and 4 days of pregnancy, and chromosomal testing of her amniotic fluid was carried out the following day. A fetal ultrasound was also carried out at this time; however, no clear findings suggesting chromosomal abnormalities in the fetus were found. Upon probing the company that conducted the test as to why it took so long for the results to appear, they responded that the first test had been positive for trisomy 18, but was not informative because the target chromosomal concentration level was near the standard cut-off level. Upon re-testing by NIPT, one specimen was negative, but trisomy 18 positive was observed in other specimens and so it was ultimately determined as "positive." Fluorescence *in situ* hybridization (FISH) was returned at 20 weeks and 5 days of pregnancy, confirming that the 18th chromosome was normal. The final report may have extended beyond 22 weeks of pregnancy, and so the patient agreed to make a decision regarding continuing pregnancy after the interim report. At 21 weeks and 5 days of pregnancy, chromosomal testing of the patient's amniotic fluid revealed that the karyotype was 46,XX (Fig. 1), and the couple was told that CPM might be the cause thereof. CPM

causes placental insufficiency, leading to fetal growth restriction; therefore, perinatal period management was subsequently carried out at our hospital. Fortunately, fetal development and the course of pregnancy were both good. She went into labor at 39 weeks and 5 days of pregnancy, and delivered a 3,306 g baby girl whose Apgar scores were 8 and then 9 at 1 and then 5 min, respectively. The child was not observed with any external malformations, and the extracted placenta was subjected to pathologic and chromosomal testing. In the placenta pathology, marginal attachment of the umbilical cord, venous dilatation and partial angiitis of the placenta surface were observed (Fig. 2); however, villus growth corresponding to several weeks was observed with no clear observation of abnormal findings. At the same time, regarding chromosomal testing of the placenta, SNP microarray analysis was carried out and arr (18) $\times$ 2-3 was found (Fig. 3). The estimated percent mosaicism of cells with trisomy 18 is 40-50%. Because the previous amniocentesis on this pregnancy showed normal female results (46,XX), the trisomy 18 cells in this microarray analysis likely represent confined placental mosaicism.

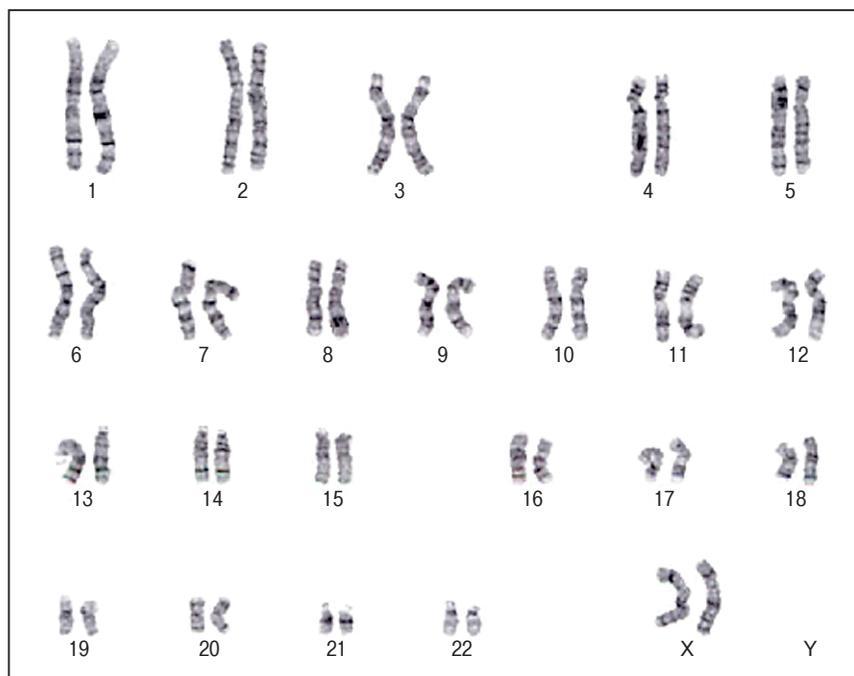


Fig. 1 Conventional karyotype analysis of cultured amniocytes shows the fetal karyotype as 46,XX.

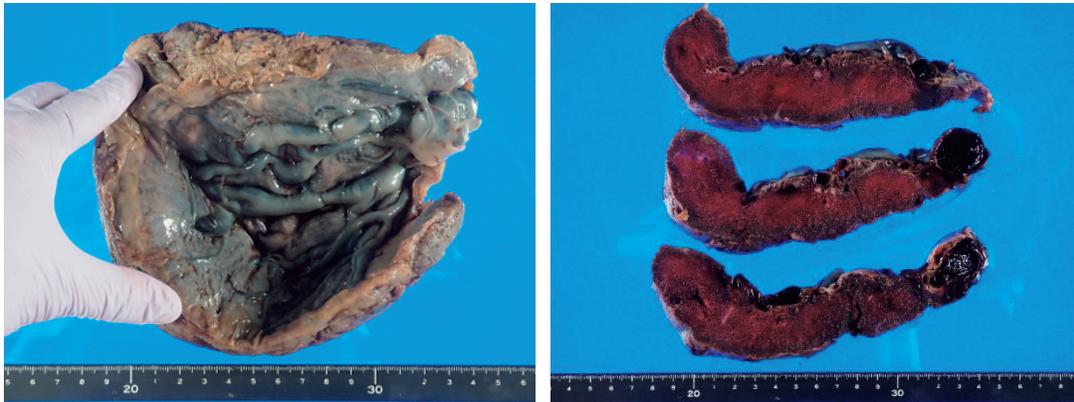


Fig. 2 Placenta pathology, marginal attachment of the umbilical cord, venous dilatation and partial angiitis of the placenta surface were observed.

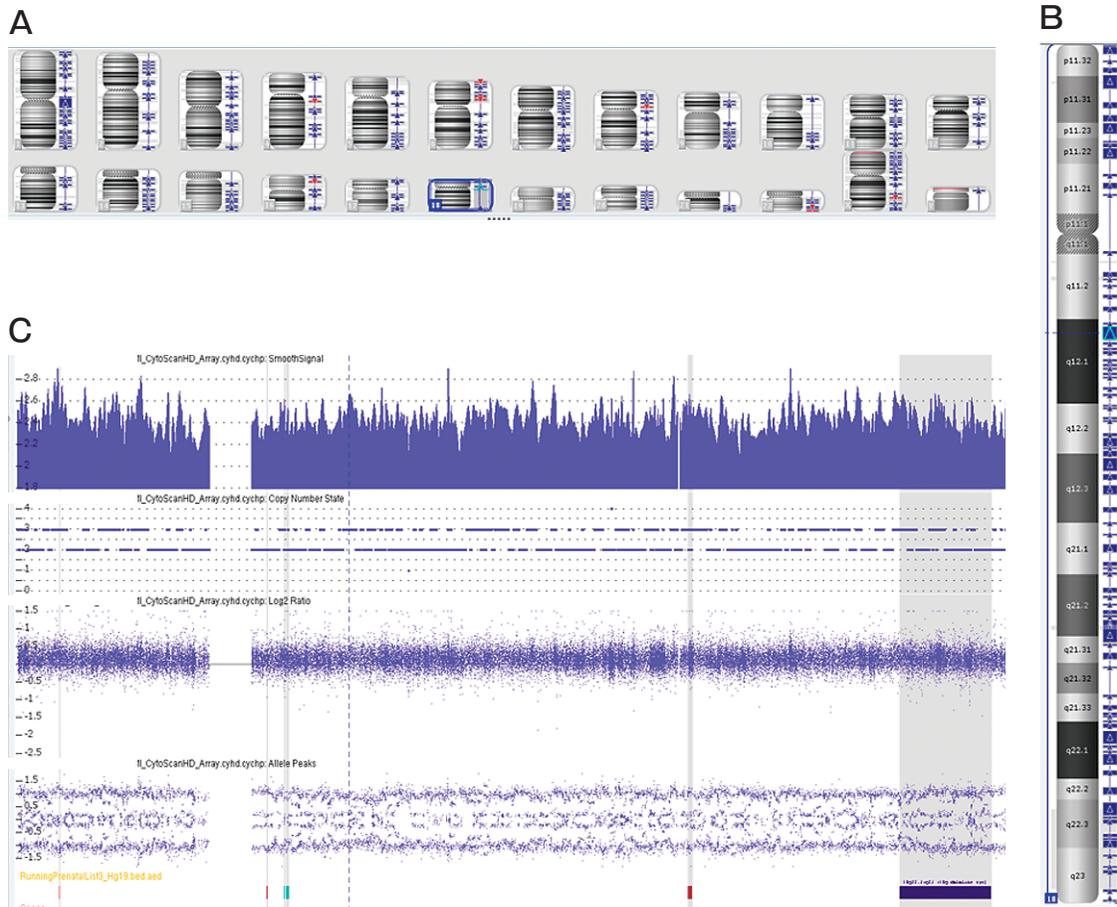


Fig. 3 SNP microarray result showing the presence of mosaicism of placental tissue. **A**, Summary of SNP microarray analysis showing a high density of short runs of allele homozygosity throughout the genome; **B**, A full view of chromosome 18; **C**, Detailed results of SNP microarray analysis, showing (from top to bottom) the smooth signal, copy number state, log2 ratio, and B allele frequency, identifying mosaicism for trisomy 18.

## Discussion

In the present case, the indefinite results of NIPT at week 15 of pregnancy and the false positive results of the subsequent re-examination resulted in the definite diagnosis being delayed until the latter half of week 21, such that the pregnant patient was subjected to psychological stress for a long period of time. The present case provides 2 clinical suggestions. Most fetus-derived cell-free DNA in the blood plasma of the maternal body is derived from trophoblastic cells of the placenta and not the fetus; such a source cannot provide a definite diagnosis via NIPT. Although CPM may be diagnosed (or inferred) prenatally by chorionic villus sampling (CVS) or NIPT [5, 6], these methods are not routinely performed and most CPM that occurs in pregnancy is never diagnosed. Since there are cases of normal development such as the present case, it is difficult to screen which cases should be suspected as suffering from CPM.

It is known that fetal cell-free DNA in maternal peripheral blood originates from trophoblasts and mainly consists of placental DNA [7, 8]. That is, NIPT is a test used to analyze placenta-derived DNA, allowing secondary results reflecting the pathology of the placenta to be observed [1, 2]. While the majority of NIPT results in Japan are generally "positive" or "negative," the result "not informative" is observed in 0.1% of cases [9]. The NIPT method adopted at our hospital is Massively Parallel Sequencing (MPS) using a next-generation sequencer; this method involves the detection of quantitative changes in each chromosome-derived component in cell-free DNA in the blood plasma of the maternal body to detect chromosome number abnormalities of the fetus [1-4]. Specifically, the base sequence of each fragment of cell-free DNA in the blood plasma of the maternal body is determined by a sequencer, with derived chromosomes determined via comparison with human genome information; however, samples with low fetal DNA in the blood plasma of the maternal body and samples with a low Z-score (index number of concentration changes of the concerned chromosome) are excluded, making results indeterminate. It is believed that these features may reflect CPM and related changes in the maternal body and fetus. Moreover, most CPM occurring during pregnancy is not diagnosed, making classification of cases difficult. Many reports have mentioned that the

placenta plays a major role in the onset of fetal diseases including abnormal fetal development accompanying pregnancy, and that CPM is present in approximately 10% of placentas associated with fetal growth restriction pregnancies (after excluding constitutional chromosomal abnormalities) [10-13]; research has also suggested that CPM is considerably more likely when placental insufficiency occurs in the presence of older maternal age [13]. However, the actual incidence of onset has not been clarified. If CPM is even suspected, careful perinatal management attending to the risk of fetal dysfunction and preeclampsia is required, but the prognosis of the child changes depending on the involved chromosome and the mosaic type, or the cause of onset. This makes the provision of accurate information during genetic counseling before and after pregnancy very important.

It has been suggested that the cause of false positive or not informative NIPT results may be as varied as CPM, submicroscopic chromosomal abnormalities, a vanishing twin, complications of tumors accompanying chromosomal aneuploidy of the maternal body, etc. [14]. However, the mechanism(s) thereof have not been completely elucidated. Elucidating false positives and the pathology of postponed determination is important in terms of inferring pregnancy prognoses; further development in our analytic capabilities would allow worried pregnant women and their physicians to be provided with reliable information. The experience of using NIPT in clinical practice confirms that abnormal results cannot be considered diagnostic. Pre-test genetic counseling should emphasize this. Diagnostic genetic testing such as amniocentesis should always be offered immediately after abnormal NIPT results are obtained.

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