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*Case Report*

# Non-Invasive Prenatal Genetic Testing (NIPT) Leading to Prenatal Diagnosis of Trisomy 21 Mosaicism and 18q Deletion Syndrome: Two Cases

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**NIPT is non-definitive testing to estimate the possibility that fetuses have trisomy 21, trisomy 18, or trisomy 13. However, in NIPT-positive and indeterminate cases, rare chromosomal disease may become apparent, requiring advanced genetic considerations and counseling skills. We experienced two such cases, a trisomy 21 mosaicism case triggered by NIPT-positive status and 18q deletion syndrome triggered by NIPT-indeterminate status. These cases have two clinical implications for NIPT. First, it was revealed that trisomy mosaicism might be found in NIPT-positive cases that have lower Z-Scores than those inferred from the fraction of fetal cfDNA in the case of standard trisomy. Second, it is possible that microdeletion syndrome could be the reason for an indeterminate NIPT result. Today's genetic counseling requires more expertise in ethics and communication as well as genetic science because NIPT can lead to totally unexpected results.**

**Key words:** NIPT, massively parallel sequencing , trisomy 21 mosaicism, 18q-deletion syndrome, genetic counseling

As one of the screening tests routinely performed prior to birth, non-invasive prenatal genetic testing (NIPT) measures cell-free DNA in maternal blood in order to estimate the possibility that the fetus may be predominantly affected by trisomy 21, trisomy 18, or trisomy 13 [1,2]. As a screening test, it has clear advantages over amniocentesis, since it can be performed safely at an earlier stage from a simple blood draw. However, it is not generally known that rare chromosomal disorders, such as fetal microdeletion / duplication syndrome or trisomy mosaicism, may be found in NIPT-positive and -indeterminate cases. We herein report on 2 cases in which trisomy 21 mosaicism and 18q deletion syndrome were definitively diagnosed by the amniotic fluid chromosome test performed subsequent to obtaining abnormal NIPT results.

## Case 1

The mother was a 36-year-old woman, with 2 pregnancies and 0 births. The prior year, she had suffered spontaneous miscarriage at 10 weeks' pregnancy due to fetal trisomy 9. *In vitro* fertilization was performed this time and her pregnancy progressed without incident. No obvious fetal abnormalities were found on early pregnancy ultrasonography at 11 weeks and 2 days of pregnancy. She requested for NIPT after precise genetic counseling, and NIPT was performed at 11 weeks and 4 days of gestation. Consequently, her blood screened "positive" for trisomy 21. The concentration of chromosome 21 was 17.29% for the fraction of fetal cell-free

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DNA and 15.86% based on the Z-Score; this is half the concentration estimated from the fetal genome rate for the standard trisomy type (Fig. 1). An amniotic fluid chromosome test was performed at 17 weeks and 2 days of pregnancy and yielded a karyotype analysis of 47, XY, +21 [4] / 46, XY, [12] (Fig. 2). Of the 16 cells, 4 cells had trisomy 21, while 12 cells had normal chromosome counts, and trisomy 21 mosaicism was diagnosed. In mosaic trisomy it is believed that the phenotype is affected when the proportion of abnormal cells exceeds 20%; in the present case, over 20% cells sampled showed trisomy 21. Fetal ultrasonography at 20 weeks of pregnancy indicated no obvious abnormal findings. She was provided genetic counseling concerning the facts that it could not be said with certainty that the disease was mild, that no morphological abnormalities were observed by ultrasound and that the mosaic ratio in amniotic fluid cells was not always the same in the fetus, making it difficult to predict the severity of the phenotype. After much agonizing, a 400-g male fetus was selectively aborted at 21 weeks of pregnancy. A total of 5 genetic counseling sessions were performed.


**Fig. 1** Z-Score for chromosome 21: Although the Z-Score is clearly higher than the cutoff value, it does not reach the population average considered to be positive by the standard trisomy type, suggesting a fetal DNA concentration of ambiguous significance.

## Case 2

The mother was 28 years old, with 1 pregnancy and 0 births. The pregnancy was natural. A nuchal translucency thickness of 5 mm was noted at 11 weeks of pregnancy and NIPT was performed at 13 weeks and 6 days of pregnancy, with the detection of early pregnancy ultrasound abnormalities as an indication. The result was indeterminate due to the deletion of the 18q22.1-q22.3 region (Fig. 3). Amniotic fluid chromosome testing was performed at 16 weeks and 0 days of pregnancy and yielded the karyotype 46, XY, r (18) (p11.2q21.3) (Fig. 4), *i.e.* a chromosome 18 ring. The SNP microarray analysis was arr 18p11.32p11.31×1 and 18q21.33q23×1 (Fig. 5). Partial deletions from the end of the short arm to 11.2 as well as from the end of the long arm 21.3 to the end were found on the chro-

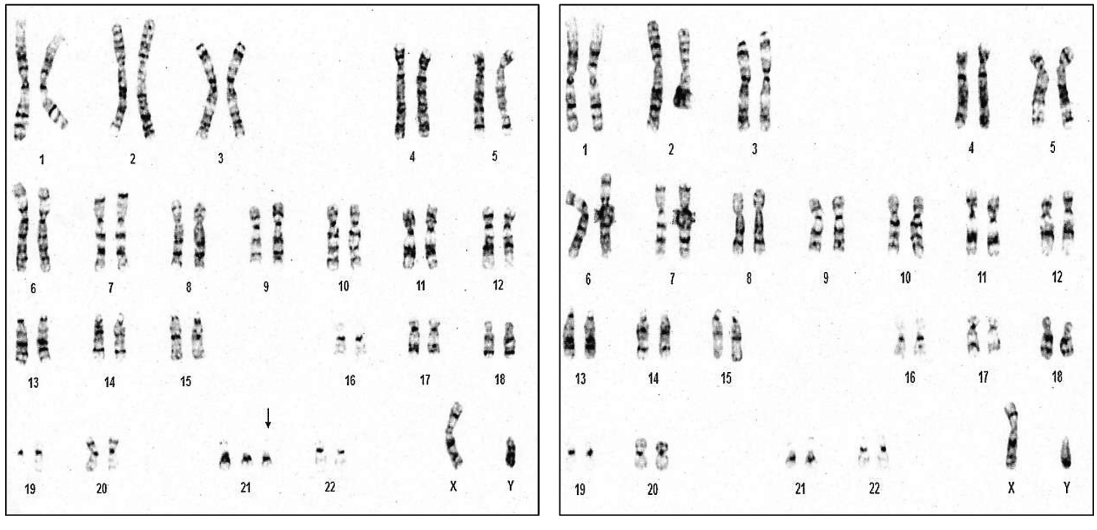

**Fig. 2** Conventional karyotype analysis of cultured amniocytes shows the fetal karyotype as 47, XY, +21 [4] / 46, XY [12].

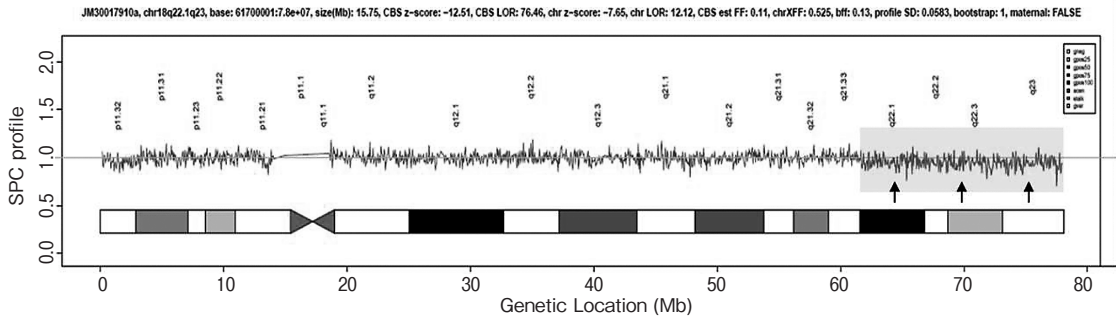


**Fig. 3** The MPS method result shows the deletion of the 18q22.1−q22.3 region.

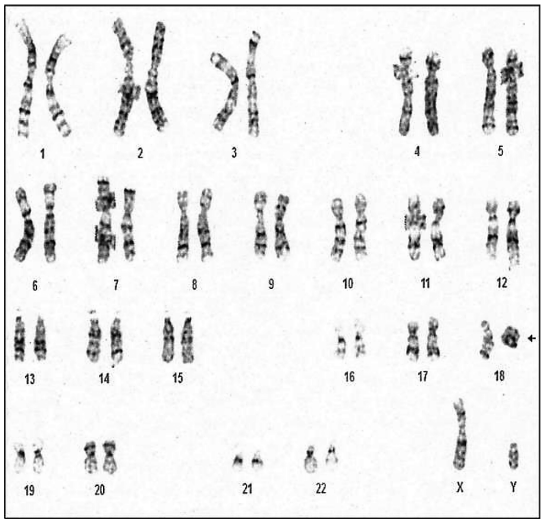

**Fig. 4** Conventional karyotype analysis of cultured amniocytes shows the fetal karyotype as 46, XY, r (18) (p11.2q21.3).

mosome. Because the MBP gene was present at the 18q22.3 site, this case was diagnosed as 18q deletion syndrome. Genetic counseling was provided to the couple, concerning the fact that the symptoms of 18q deletion syndrome would include growth failure, craniofacial malformation, urogenital abnormalities, limb abnormalities, neurological abnormalities, and cardiac construction abnormalities. After much agonizing, a 320-g male fetus was selectively aborted at 19 weeks and 6 days of gestation. A total of 4 genetic counseling sessions were performed.

In both cases, the subjects became pregnant again one year later and underwent NIPT. The results were negative and they continued their pregnancies uneventfully.

***Human rights statements and informed consent.*** All procedures followed were accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from both patients included in this report.

## Discussion

We experienced 2 cases with definitive diagnoses of trisomy 21 mosaicism and 18q deletion syndrome triggered by NIPT. Each case had 2 clinical implications. First, at the NIPT stage, it was revealed that trisomy mosaicism might be found in NIPT-positive cases that have lower Z-Scores than those inferred from the fraction of fetal cell-free DNA in the case of standard trisomy. Second, it is possible that microdeletion syndrome could be the reason for an indeterminate NIPT result.

Chromosome mosaicism is a state in which a cell population having a normal karyotype and a cell population having an abnormal (*e.g.*, trisomy) karyotype coexist in one individual. While mosaic chromosomal abnormalities are generally detected by G banding, they may also be detected by interphase nucleus FISH or array CGH testing. Compared to standard trisomy-type infants, the phenotype of mosaic-type infants is milder and the severity thereof is said to change depending on the prevalence of cells with chromosomal abnormalities [3]. Although it is said that the phenotype is affected when the proportion of abnormal cells exceeds 20%, the amniotic fluid cells analyzed by tests performed to diagnose mosaicism may grossly over- or underestimate this proportion depending on the tissues where the abnormality is prominent in the particular individual. Even if mosaicism is found in amniotic fluid cells, there is believed to be a 50% chance that the fetus will have a

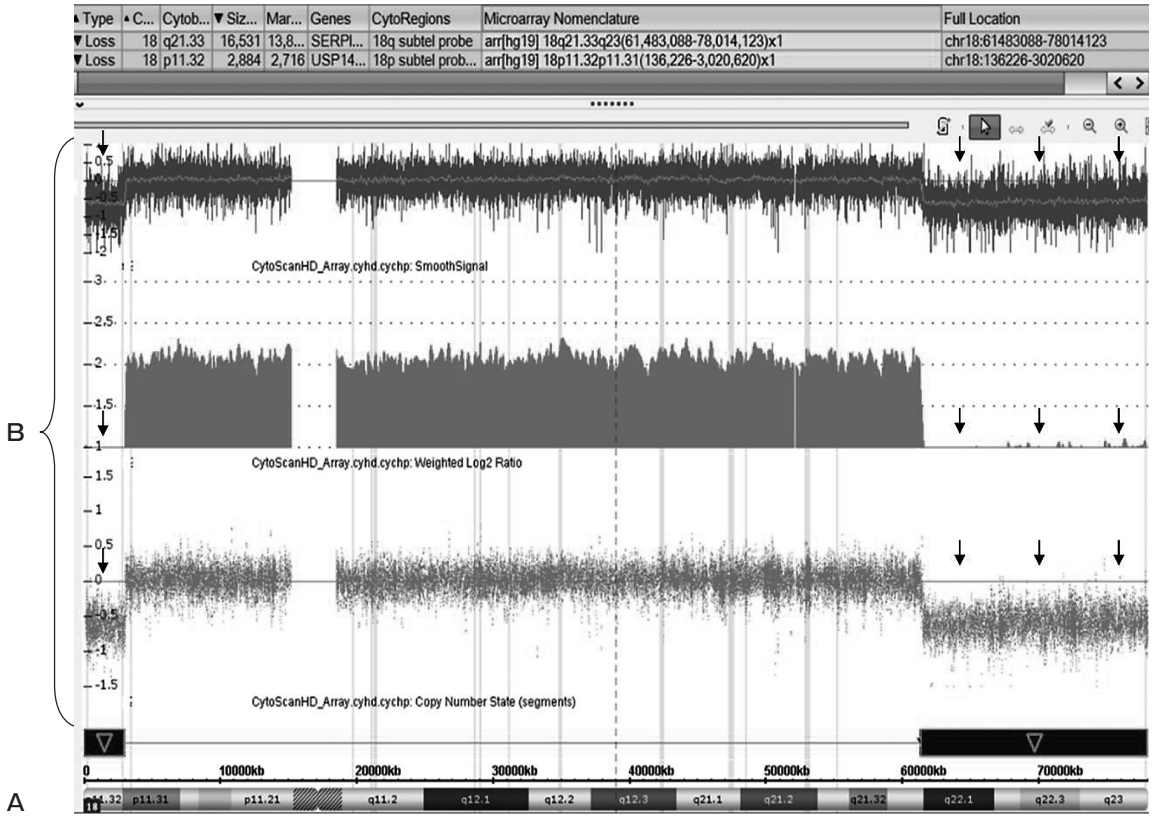


Fig. 5 SNP microarray result showing the presence of the 18q deletion syndrome. **A**, A full view of chromosome 18; **B**, Detailed results of SNP microarray analysis, showing (from top to bottom) smooth signal, log2 ratio, and copy number state, identifying the deletion of the 18p11.32p11.31 and 18q21.33q23 regions.

similar mosaic in the chromosome mosaicism, making it difficult to practically predict the severity of the phenotype [4,5]. Precise fetal ultrasonography is significantly unlikely to extract morphological abnormalities, as seen in the standard trisomy type; in fact, Trisomy 21 fetuses are said to exhibit findings comparable to those of a normal fetus [6]. Therefore, when chromosome mosaicism is found on amniocentesis, it is important to confirm the presence or absence of morphological abnormalities by precise fetal ultrasonography, estimate the phenotype including the result, and provide this information. Furthermore, it is difficult to investigate the karyotype of cells throughout the body, even when examined in detail after birth, as far as the phenotype and appearance of the baby can be understood. Although a detailed fetal ultrasonography was performed twice in our case, no morphological abnormalities were found, confirming that ultrasonography is of limited use in the diagnosis of trisomy mosaicism.

On the other hand, 18q deletion syndrome is a rare chromosomal aberration syndrome, with a frequency of 1/40,000 and a very diverse phenotype. Many cases are associated with growth failure, characteristic facial features, limb abnormalities, genitourinary malformations, and neurological abnormalities. Mental retardation has been observed as a neurological abnormality in almost all cases, with developmental delays accompanied by hypotonia, hearing loss, convulsions, and nystagmus also having been reported [7,8]. While it is reported that the degree of developmental delay varies (IQ 40-85) with the possibility of only mild developmental delays and learning disabilities, some reports indicate that more than half have IQs of 30-50 [9,10]. Although most of the chromosome deletion sites are at the ends of 18q21.2→qter, these clinical findings are associated with myelination failure in cerebral white matter, due to a defect in the myelin basic protein (MBP) gene present in 18q22.3 and cerebral atrophy

associated with white mass loss [11]. Despite these various abnormalities, the prognosis for life is relatively good. The phenotype of 18q deletion syndrome is diverse and follows the basic rule of "the larger the deletion region, the more severe the symptoms"; more precise relationships between phenotype and deletion site genes are still being clarified [12, 13].

When NIPT is performed for the purpose of detecting numerical abnormalities on chromosomes 13, 18 and 21, the Massively Parallel Sequencing (MPS) method determines the number of DNA fragments derived from each chromosome, obtains the number of DNA fragments derived from each chromosome, and calculates the ratio of the number of DNA fragments of the target chromosome to the total amount of fragments detected. In order to ensure measurement accuracy, a cutoff value using the Z-Score is set as an index, by calculating the difference from the average of multiple normal karyotype samples, measured at the same time, to see how many fold off the values of the sample to be measured are from the standard deviation. Normally, trisomy 21 uses a Z-Score of 3 as the cutoff value, with those above this value considered trisomy 21 positive [14]. Although the relationship between the fetal DNA concentration and Z-Score resulting from the MPS analysis was clearly higher than the cutoff value in Case 1, as illustrated in Fig. 2, it did not reach the population average of standard trisomy fetuses, suggesting that our case was not a normal positive case. It is said in Japan that indeterminate results occur in 0.23% [15], with "vanishing twin" death in the early stages of pregnancy, fetal or maternal somatic cell mosaicism, placental mosaicism, maternal tumors, maternal autoimmune diseases, *etc*. having been reported as factors of indeterminate results, if the cell-free DNA concentration from the fetus in the maternal blood is less than 4% [16-19]. In Case 2, as illustrated in Fig. 3, the fragment amount of the entire chromosome 18 was abnormally low and it was already known via NIPT that it was not a normal case. Unfortunately, there is no specific criterion to determine whether a non-trisomy case is negative or indeterminate. This case was reported as indeterminate due to the partial deletion of chromosome 18, which has a different meaning from indeterminate status because of low fetal DNA blood concentration of a normal fetus. As far as we know, only one other case of 18p deletion syndrome [20] has been diagnosed with NIPT, namely the first report of 18q deletion syndrome. The present study is the first report on trisomy mosaicism detected by NIPT. It is difficult to describe to pregnant patients the possibility of facing such results before they undergo the NIPT test. Certainly, more consideration needs to be made as to how genetic counselors should deal with positive and indeterminate results.

When NIPT was first launched in the United States in 2011, it was a non-deterministic test that estimated the likelihood of having trisomy 21, trisomy 18, and trisomy 13. However, it is currently possible to test for the presence or absence of fetal single-gene disease by examining multiple genes in fetal cell-free DNA, contained in maternal blood, in addition to sex chromosome testing, chromosomal microdeletion, and duplication syndrome. Mutations in 25 genes that cause fetal bone system diseases, neuromuscular diseases, malformative syndrome, *etc*. have been confirmed from prenatal maternal blood samples, with 44 genetic diseases currently being tested [21]. This suggests that genetic testing, which was traditionally premised on postnatal testing, is now possible with prenatal testing using NIPT. With advances in prenatal diagnosis technology, genetic counseling is becoming more complex, both in terms of science and ethics, resulting in concerns as to whether the current system of genetic counseling is adequate.

## Conclusion

With NIPT, the Z-score is elevated to twice the fraction of fetal cell-free DNA, if the fetus is of the standard trisomy type, whereas the Z-Score is approximately half that of the standard trisomy type if it is a mosaic type, and significantly lower than the cutoff value if it is monosomy. Today's genetic counseling requires more expertise in communication and ethics as well as genetics because NIPT is not just a simple "yes-no" test; it can also present totally unexpected results. It is essential to ensure a high-quality genetic counseling system that enables a couple to be in an acceptable psychological state and to make autonomous decisions within a limited time, even if a rare chromosomal disease is found.

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