

# Noninvasive prenatal testing aids identification of tetrasomy 18p: A case report

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## ABSTRACT

Tetrasomy 18p syndrome (Online Mendelian Inheritance in Man 614290) is a rare chromosomal disorder that is seen in approximately 1 in every 180,000 live births. It is caused by the presence of isochromosome 18p, which is a supernumerary marker composed of two copies of the short arms of chromosome 18. Isochromosome 18p is one of the most commonly observed isochromosomes. We report tetrasomy 18p syndrome diagnosed prenatally after noninvasive prenatal testing (NIPT) was positive for trisomy 18. Tetrasomy 18p was finally diagnosed by G-banding and fluorescence *in situ* hybridization of chromosome 18p, before invasive confirmatory testing the karyotype findings by NIPT showed an increase in the DNA fragments from chromosome 18p, indicating duplication of chromosome 18p. NIPT can detect not only trisomy 13, 18, and 21, but also structural chromosomal anomalies, such as deletions and duplications. An NIPT report "positive for trisomy 18" indicates the possibility of tetrasomy 18p, and detailed analysis of NIPT data can reveal subchromosomal copy number variations, to a certain extent, before definitive diagnostic testing.

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## 1. Introduction

The utility and the clinical validity of cell-free fetal DNA screening for trisomies are well recognized all over the world. In Japan, clinical studies of screening for trisomy 13, 18, and 21 in high-risk pregnancies have been performed, though these studies have mostly involved older women. Moreover, sex-chromosome aneuploidies and genome-wide copy number variations have not been examined in such studies [1]. Genome-wide screening for copy number variations using cell-free DNA in maternal plasma is technically possible [2], and some companies are now offering genome-wide screening. However, the validity of genome-wide screening from cell-free fetal DNA has not yet been established.

In this case report, we describe a fetus with suspected tetrasomy 18p detected by noninvasive prenatal testing (<sup>1</sup>NIPT).

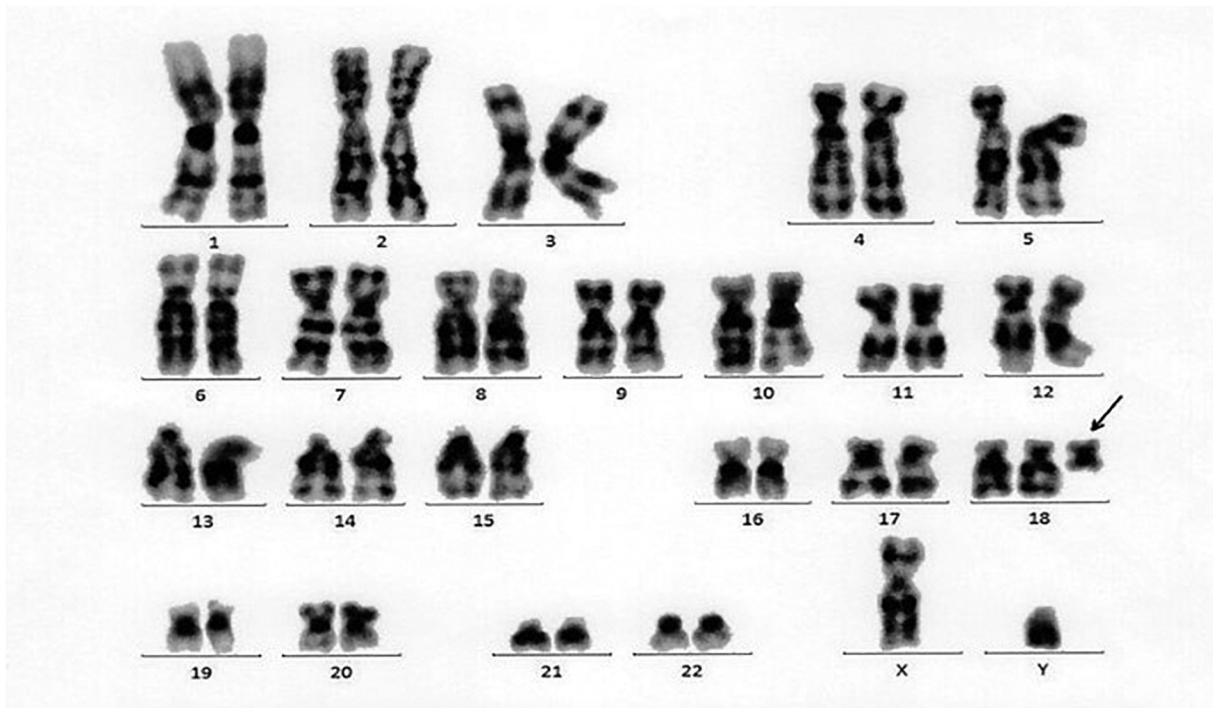
## 2. Case

A healthy, 41-year old, gravida 1 woman became pregnant after a single blastocyst transfer was made due to her husband with oligozoospermia. Her medical history was unremarkable. The woman's younger brother was known to have a ventricular septal defect. The woman opted for NIPT at 12+6 weeks of gestation because of an elevated nuchal translucency thickness (about 4 mm) and her age. We explained about the objectives and limitations of NIPT, and differences from invasive testing at genetic counseling. Peripheral blood was collected, DNA was extracted, and massive parallel sequencing [3,4] (MaterniT21 Plus) was performed using Illumina HiSeq 2000 (San Diego, United States). The sequencing data were used to calculate the Z-scores for trisomies 21, 18, and 13, which represent robust assessments of normalized chromosomal representation compared with the euploid genome. NIPT produced a positive result for chromosome 18, which was considered to indicate a high possibility of trisomy 18. Amniocentesis was carried out at 15+6 weeks of gestation. An ultrasound scan showed the biparietal diameter to be within normal range and no major anomalies. The result indicated 47,XY,+i(18)(p10)[20] (Fig. 1). Fluorescence *in situ* hybridization of chromosome 18p with a telomeric probe produced 4 signals, sufficient for a diagnosis of tetrasomy 18p. The pregnancy was terminated at 19+4 weeks on parental request.

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<sup>1</sup>noninvasive prenatal testing(NIPT)



**Fig. 1.** G-banding chromosomal analysis indicated a karyotype with an extra free marker chromosome in all examined cells. Fluorescence *in situ* hybridization of chromosome 18p with a telomeric probe showed 4 signals. The chromosomal analysis revealed 47,XY,+i(18)(p10)[20].

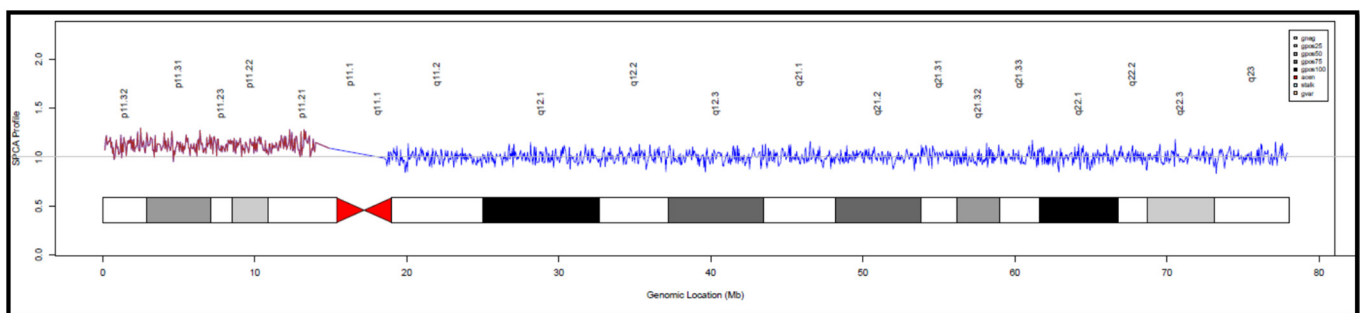
After the karyotype was revealed, the detailed NIPT data were confirmed. The fetal fraction rate was 12.76%. The Z-scores of chromosomes 13, 18, and 21 were 0.14792, 11.7663, and 0.07616, respectively. The Z-scores were within the normal range for chromosomes 13 and 21, but higher for chromosome 18 (Z-score > 3 is considered abnormal). The karyotype findings by NIPT showed an increase in the DNA fragments from chromosome 18p, indicating duplication of chromosome 18p (Fig. 2).

### 3. Discussion

This case report demonstrates two important clinical issues. First, in addition to trisomy 13, 18, and 21, NIPT can detect structural chromosomal anomalies, such as deletions and duplications. Second, an NIPT report “positive for trisomy 18” does not rule out the possibility of tetrasomy 18p and the detailed NIPT data can show subchromosomal copy number variations, to a certain extent, before definitive diagnostic testing.

Regarding the first issue, NIPT is not a confirmatory test but a screening test, and from the amount of cell-free DNA of chromosome 13, 18, and 21 NIPT can detect not only trisomies but also structural chromosomal anomalies and the probability of tetrasomy 18p. There are some case reports of deletions and duplications of chromosome 18 detected by NIPT (Table 1) [5–9]. In two cases, NIPT could not identify a fetus positive for chromosome 18 anomalies, but in the others it could identify the possibility of chromosome aberrations. NIPT using genome-wide massive parallel sequencing could screen for the possibility of deletions and duplications of chromosome 18. Considering this, genetic counseling about deletions and duplications is necessary before NIPT.

Regarding the second issue, an NIPT report “positive for trisomy 18” can indicate not only trisomy 18 but also the possibility of tetrasomy 18p, and the detailed NIPT data can show subchromosomal copy number variations to a certain extent, before definitive diagnostic testing. Tetrasomy 18p has the usual number of copies of chromosome 18, plus an isochromosome 18p. As a result, each cell has four copies of the short arm of chromosome 18. The size of the gene involved in tetrasomy 18p is 15 mega base pairs (Mb). The four copies of the



**Fig. 2.** This ideogram was made by sequencing of cell-free DNA in maternal plasma. The chromosome 18 ideogram shows duplication of chromosome 18p.

**Table 1**  
Reports of deletions and duplications of chromosome 18 following noninvasive genetic testing (NIPT).

No.	Age	NIPT methodology	Indications of NIPT	Results of NIPT	Gestational weeks of confirmatory invasive test	Prenatal chromosomal test	Deletions or duplications	Reference
1	41	Genome-wide massive parallel sequencing (VeriSeq)	Increased risk (1:99) after combined first trimester screening test for trisomy 21	Trisomy 18 NCV value for trisomy 18 was 4.3 (>5SD)	16 weeks	Amniocentesis/Microarray	Tetrasomy 18p	5
2	35	NA	Screening (despite of low-risk results of the combined test)	Trisomy 18	After birth (16 months old)	Not performed	Tetrasomy 18p	6
3	20	Genome-wide massive parallel sequencing (Next-Seq CN500, Illumina, USA)	Screening	Deletion on chromosome X. The Z-score for chromosome X was -3.91.	20 weeks	Amniocentesis/ Karyotype analysis and sequencing (Next-Seq CN500, Illumina, USA)	Normal karyotype. 7.56 Mb deletions at 18p11.32-11.23	7
4	31	NA	Increased nuchal translucency	Low risk for trisomies of 13, 18, 21	18 weeks	Amniocentesis/Karyotype analysis and SNPmicroarray (Affymerix 750K)	6.9Mb deletions at 18p11.32-11.31 and 7.5 Mb deletions at 18p11.23-11.21	8
5	36	Genome-wide massive parallel sequencing	Screening	Duplications in 18p The t-score for chromosome 18 was 2.03.(T-score above 2.0 is high.)	16 weeks	Amniocentesis/ aCGH	14Mb duplications at 18p11.32-11.21	9
this case	41	Genome-wide massive parallel sequencing	Increased nuchal translucency	Trisomy 18 The Z-score for chromosome 18 was 11.7.	15 weeks	Amniocentesis/Karyotype analysis and FISH	Tetrasomy 18p	

Note: SNP single nucleotide polymorphism, aCGH array-based comparative genomic hybridization, FISH fluorescent *in situ* hybridization, NA not available.

short arm of chromosome 18 represent an approximately 40% increase in DNA compared with that in full trisomy 18 [10]. Some commercial laboratories use cell-free DNA screening for genome-wide gains and losses of 7 Mb or more, and so should be able to detect tetrasomy 18p. Improving cell-free DNA screening technology will increase accuracy in the near future.

While NIPT can be used to detect subchromosomal copy number variations, the American College of Obstetricians and Gynecologists does not recommend routine screening for genome-wide gains or losses with cell-free DNA [11], partly because of patient anxiety (the limitations and benefits of NIPT need to be discussed in genetic counseling), incomplete validation of NIPT for detecting copy number variations, clinical use, and cost-effectiveness. Although expanded cell-free DNA screening has been introduced in some clinical practices, research into positive predictive values (PPVs) and effective genetic counseling is still needed.

NIPT is valid for screening for trisomy 13, 18 and 21 with high sensitivity. Miranda J. et al. reported that in fetuses with increased nuchal translucency, NIPT would miss 12-19% of genetic anomalies [12]. NIPT would miss some genetic abnormalities in fetuses that harbored sex-chromosome aneuploidies, clinically relevant atypical chromosomal anomalies (trisomy 22, trisomy 21 mosaicism, and unbalanced translocation), and submicroscopic pathogenic variants. NIPT originally targeted only 3 anomalies, namely trisomies 13, 18, and 21, and not sex-chromosome aneuploidies. Further, when using trophoblast-derived cell-free DNA for NIPT, false positive results could be obtained because of placental mosaicism. Due to this limitation of target diseases and placental mosaicism, invasive testing is important when NIPT produces positive results.

Tetrasomy 18p syndrome is seen in approximately 1 in every 180,000 live births. The clinical phenotypes of this disease include neonatal feeding problems, developmental delays (mild to severe), microcephaly, abnormalities in muscle tone, dysmorphic features, and variants in brain MRI [13]. In the case presented here, prenatal evaluation by ultrasound showed an increased nuchal translucency at 12 weeks of gestation, while at 16 weeks of gestation there were no

abnormal findings. Tetrasomy 18p is difficult to detect or diagnose from fetal ultrasound scans. Isochromosome 18p is one of the most commonly observed isochromosomes. In the case presented here, a supernumerary marker in G-banding was thought to originate from chromosome 18 because of the NIPT result. Without the NIPT result, determining the origin of the supernumerary marker would have been more difficult.

Isochromosomes arise as a result of two independent events: non-disjunction and centromeric misdivision. An alternative mechanism for the formation of an isochromosome is a U-shaped exchange, which would typically produce a dicentric isochromosome [14]. Isochromosomes tend to originate from the maternal side, and nondisjunction, which occurs during maternal meiosis, suggests that advanced maternal age is a contributing factor.

In conclusion, in addition to detecting trisomy 13, 18, and 21, NIPT can be used to identify structural chromosomal anomalies. An NIPT result "positive for trisomy 18" does not exclude the possibility of tetrasomy 18p, and detailed NIPT data can show subchromosomal copy number variations and indicate the need for further testing.

Screening for genome-wide gains and losses with cell-free DNA is not recommended, and further research is necessary to improve detection of smaller copy number variations.

### Contributors

Yuko Tamaki contributed to conception and design of the study, acquisition of data, analysis and interpretation of data, and drafting the article.

Yukiko Katagiri contributed to critically revising the article for important intellectual content, and supervision.

Nahomi Umemura contributed to acquisition of data, and analysis and interpretation of data.

Naoki Takeshita contributed to critically revising the article for important intellectual content, and supervision.

Mineto Morita contributed to supervision, and final approval of the version to be submitted.

## Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this case report.

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## Patient Consent

Written informed consent was obtained from the patient for the publication of this case report. This report was approved by the Ethics Committee of Faculty of Medicine, Toho University (No. A19002\_A17077\_A16002\_25049).

## Provenance and Peer Review

This case report was peer reviewed.

## References

- [1] O. Samura, A. Sekizawa, N. Suzumori, A. Sasaki, S. Wada, H. Hamanoue, et al., Current status of non-invasive prenatal testing in Japan, *J. Obstet. Gynaecol. Res.* 43 (2017) 1245–1255, <https://doi.org/10.1111/jog.13373>.
- [2] R.B. Lefkowitz, J.A. Tynan, T. Liu, Y. Wu, A.R. Mazloom, E. Almasri, et al., Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants, *Am. J. Obstet. Gynecol.* 215 (2016) 227, <https://doi.org/10.1016/j.ajog.2016.02.030>.
- [3] G.E. Palomaki, E.M. Kloza, G.M. Lambert-Messerlian, J.E. Haddow, L.M. Neveux, et al., DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study, *Genet. Med.* 13 (2011) 913–920, <https://doi.org/10.1097/GIM.0b013e3182368a0e>.
- [4] G.E. Palomaki, C. Deciu, E.M. Kloza, G.M. Lambert-Messerlian, J.E. Haddow, L.M. Neveux, et al., DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study, *Genet. Med.* 14 (2012) 296–305, <https://doi.org/10.1038/gim.2011.73>.
- [5] D.L. Lildballe, I. Vogel, I.C.B. Lund, I. Stornes, M.W. Jørgensen, E.M. Vestergaard, Non-invasive prenatal testing offered as part of a combined first-trimester screening program identifies tetrasomy 18p in a high-risk pregnancy, *Prenat. Diagn.* 36 (2016) 1112–1114, <https://doi.org/10.1002/pd.4943>.
- [6] G. Tolva, R. Silipigni, A. Quarenghi, P. Vergani, S. Gueneri, D. Milani, Tetrasomy 18p: The challenges of noninvasive prenatal testing and combined test, *J. Obstet. Gynaecol. Res.* 45 (2019) 705–708, <https://doi.org/10.1111/jog.13873>.
- [7] G. Zhao, P. Dai, S. Gao, X. Zhao, C. Wang, L. Liu, et al., A case of prenatal diagnosis of 18p deletion syndrome following noninvasive prenatal testing, *Mol. Cytogenet.* 12 (2019) 53–58, <https://doi.org/10.1186/s13039-019-0464-y>.
- [8] H. Qi, J. Zhu, S. Zhang, L. Cai, X. Wen, W. Zeng, et al., Prenatal diagnosis of de novo monosomy 18p deletion syndrome by chromosome microarray analysis. Three case reports, *Medicine(Baltimore)* 98 (2019), e15027, <https://doi.org/10.1097/MD.000000000015027>.
- [9] T.K. Lau, F.M. Jiang, R.J. Stevenson, T.K. Lo, L.W. Chan, M.K. Chan, et al., Secondary findings from non-invasive prenatal testing for common fetal aneuploidies by whole genome sequencing as a clinical service, *Prenat. Diagn.* 33 (2013) 602–608, <https://doi.org/10.1002/pd.4076>.
- [10] D.W. Bianchi, L.D. Platt, J.D. Goldberg, A.Z. Abuhamad, A.J. Sehnert, R.P. Rava, et al., Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing, *Obstet. Gynecol.* 119 (2012) 890–901, <https://doi.org/10.1097/AOG.0b013e31824fb482>.
- [11] Committee on Genetics and the Society for Maternal-Fetal Medicine, Committee Opinion No. 682: Microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools in obstetrics and gynecology, *Obstet. Gynecol.* 128 (2016) e262–e268, <https://doi.org/10.1097/AOG.0000000000001817>.
- [12] J. Miranda, Y. Paz, F. Miño, V. Borobio, C. Badenas, L. Rodriguez-Revenga, M. Pauta, A. Borrell, Should cell-free DNA testing be used in pregnancy with increased fetal nuchal translucency? *ISUOG.* 55 (2020) 645–651, <https://doi.org/10.1002/uog.20397>.
- [13] C. Sebold, E. Roeder, M. Zimmerman, B. Soileau, P. Heard, E. Carter, et al., Tetrasomy 18p: Report of the molecular and clinical findings of 43 individuals, *Am. J. Med. Genet. Part A* 152A (2010) 2164–2172, <https://doi.org/10.1002/ajmg.a.33597>.
- [14] D. Kotzot, G. Bundscherer, F. Bernasconi, L. Brecevic, I.W. Lurie, S. Basaran, et al., Isochromosome 18p results from maternal meiosis II nondisjunction, *Eur. J. Hum. Genet.* 4 (1996) 168–174, <https://doi.org/10.1159/000472191>.