ORIGINAL ARTICLE

Secondary findings from non-invasive prenatal testing for common fetal aneuploidies by whole genome sequencing as a clinical service

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ABSTRACT

Objective To report secondary or additional findings arising from introduction of non-invasive prenatal testing (NIPT) for aneuploidy by whole genome sequencing as a clinical service.

Methods Five cases with secondary findings were reviewed.

Results In Case 1, NIPT revealed a large duplication in chromosome 18p, which was supported by arrayCGH of amniocyte DNA, with final karyotype showing mosaic tetrasomy 18p. In Case 2, a deletion in the proximal long arm of chromosome 18 of maternal origin was suspected and confirmed by arrayCGH of maternal white cell DNA. In Case 3, NIPT was negative for trisomies 21 and 18. In-depth analysis for deletions/duplications was requested when fetal structural anomalies were detected at routine scan. A deletion in the proximal long arm of chromosome 3 was found and confirmed by karyotyping. In Case 4, NIPT correctly predicted confined placental mosaicism with triple trisomy involving chromosomes X, 7 and 21. In Case 5, NIPT correctly detected a previously unknown maternal mosaicism for 45X.

Conclusion Non-invasive prenatal testing is able to detect a wide range of fetal, placental and maternal chromosomal abnormalities. This has important implications on patient counseling when an abnormality is detected by NIPT.

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INTRODUCTION

Non-invasive prenatal testing (NIPT) for common fetal aneuploidies by massively parallel sequencing of maternal plasma DNA is a new technology detecting close to 100% of trisomy 21 pregnancies with a false positive rate of <0.1%.[1] There is much we do not yet know about this exciting new investigation, and there is much to learn and explore. Here, we report five cases of 'unexpected' secondary chromosomal abnormalities discovered after the implementation of this test in a clinical setting.

ABOUT THE TESTS

Full details about the NIPT test were as reported previously.[2] The NIPT test was offered to pregnant women carrying singleton pregnancies from 12 weeks of gestation or beyond. Before the blood test, each woman or couple had individual counseling by an obstetrician and an ultrasound scan to confirm the number of fetuses, fetal viability and fetal size and to exclude major fetal structural abnormalities. A written informed consent was obtained from all women. Five milliliters of maternal peripheral blood was collected into an ethylene diamine tetraacetic acid (EDTA) bottle. Samples were processed according to strict protocol. All subsequent procedures and molecular tests, including cell-free DNA isolation, library construction and sequencing, were performed at the clinical laboratory of BGI-Shenzhen, China, which had been ISO/IEC 17025 certified.

Whole genome sequencing was used for the NIPT. For aneuploidy detection, a binary hypothesis *t*-test and logarithmic

likelihood ratio (*L*-score) between the two *t*-tests were used to classify whether the fetus had aneuploidy.[1] This approach enabled not only the classification of pregnancies affected and unaffected by trisomies but also the identification of cases with mosaicism. The test report initially included risk assessment for trisomy 21 and trisomy 18 only, but was extended to include trisomy 13 since early 2012. However, bioinformatics analysis actually included aneuploidy detection of all 23 pairs of chromosomes, and detection for chromosomal deletions and duplications by a specially designed FCAPS (Fetal Copy Number Analysis through Maternal Plasma Sequencing, see later for further details) pipeline was routinely performed since July 2012. Before that, FCAPS was performed for cases with *t*-score above 2.0, aiming at the detection of partial trisomy. Referring clinicians were notified if any of these additional genome-wide abnormalities were suspected. Specifically, fetal sex was not reported, even on request, unless sex chromosomal abnormalities were suspected.

The FCAPS pipeline is a special algorithm for the detection of large deletions and duplications. In NIPT for aneuploidies, the relative proportion of DNA fragments from a specific chromosome is estimated and compared against the normal range. In FCAPS, the human genome was divided into a total of 308 789 sliding, 99%-overlapping basic observation units each has 84 000 expected unique reads. Potential breakpoints of deletions/duplications are localized by comparing the difference of read number, after correction for GC-bias, between the observational units on each side of the potential breakpoint. A binary segmentation algorithm with dynamic threshold determination is used to determine whether the potential breakpoint is significant or not. The detection power of FCAPS increases with increasing cell-free fetal DNA concentration and more sequencing reads. On the condition of 10% cell-free fetal DNA concentration and a sequencing read number currently obtained with NIPT for aneuploidies, FCAPS is able to detect close to 100% of deletions/duplications of 10 Mb or above. In-depth discussion on the FCAPS methodology can be found in relevant publication.[3]

Conventional cytogenetic studies, quantitative fluorescent polymerase chain reaction (QF-PCR) or arrayCGH (aCGH) studies were performed at the laboratory of the Department of Obstetrics and Gynaecology, Chinese University of Hong Kong, or the Department of Obstetrics and Gynaecology, Tsan Yuk Hospital, University of Hong Kong. The aCGH test offered by the Chinese University of Hong Kong was a custom-made panel for prenatal diagnosis that targeted at the loci of 100 common micro-deletion and micro-duplication syndromes of relevance in prenatal diagnosis at high resolution, plus whole genome coverage with a backbone resolution down to 100 kb.[4] The aCGH test offered by the University of Hong Kong was NimbleGen CGX-135K whole genome oligonucleotide microarray with resolution of 140 kb across the genome, and 40 kb or less in regions of clinical relevance. The array can evaluate over 245 genetic syndromes and over 980 gene regions of functional significance in human development.

CASE 1

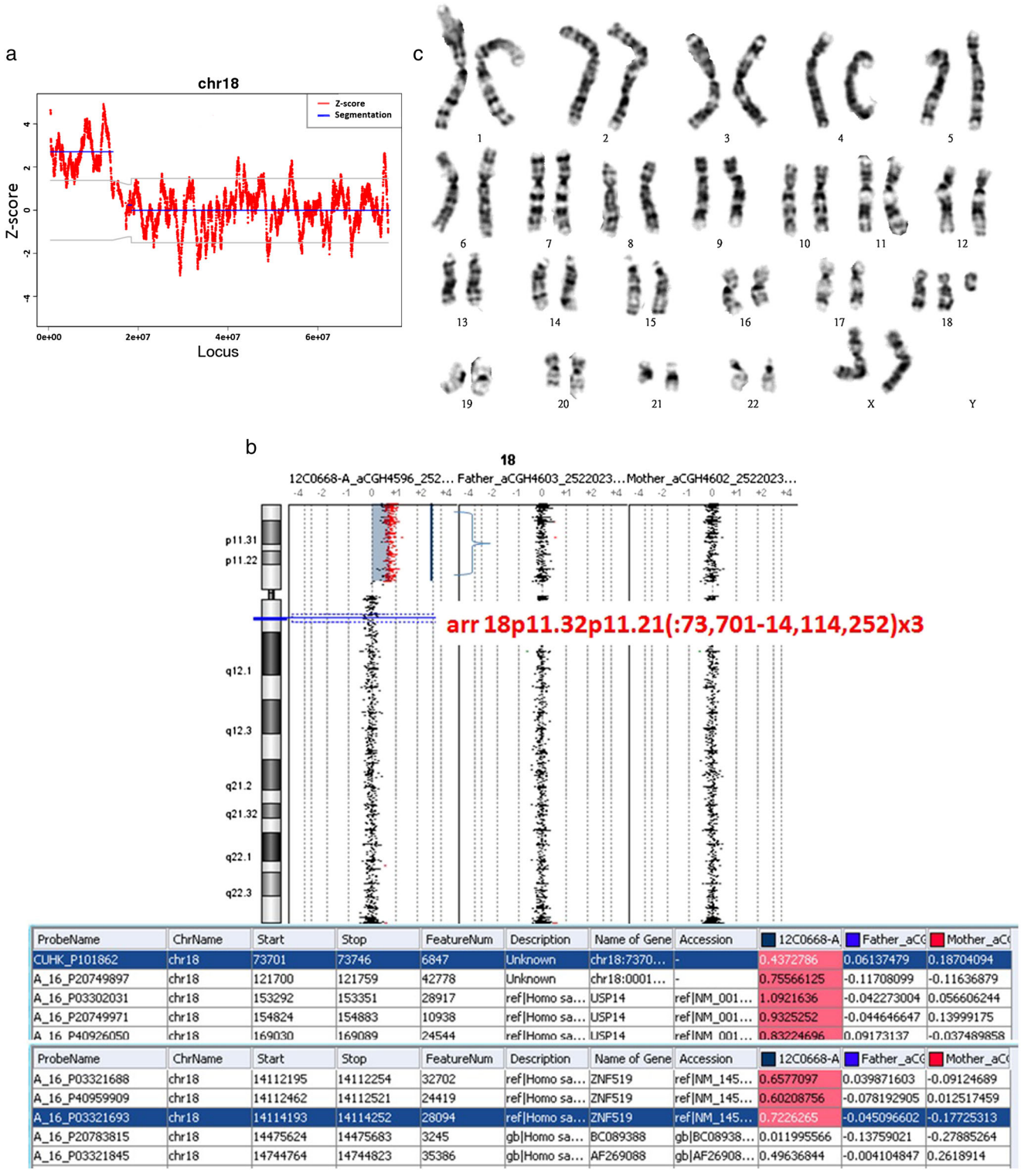
Case 1 was a 36-year-old woman with one previous miscarriage. She requested the NIPT as a primary screening test for fetal Down syndrome at 13^{+4} weeks. Pre-test sonogram was normal. NIPT report at 15^{+3} weeks showed normal numbers of chromosomes 21 and 13. The *t*-score for chromosome 18 was 2.03, for which the FCAPS analysis was performed. The FCAPS suggested that there was an approximately 13 Mb duplication involving chromosome 18p (chr18:483 517–14 400 897, corresponding to 18p11.32–p11.21) (Figure 1a). Sonogram at 15^{+3} weeks did not show any structural anomalies. After extensive counseling, the couple opted for amniocentesis and aCGH (to exclude possible additional deletions and duplications), which was performed at 16^{+5} weeks. aCGH showed a duplication of about 14 Mb, suggesting partial trisomy 18p (Figure 1b). Final karyotyping showed that it was a case of mosaic isochromosome tetrasomy 18p at a ratio of 7:23 (abnormal to normal cells) (Figure 1c). A repeat ultrasound scan did not show any structural abnormalities. The couple finally determined for pregnancy termination. The medical termination was uncomplicated. The *postmortem* examination showed no structural abnormalities.

CASE 2

Case 2 was a 38-year-old parity 1 woman. She requested the NIPT as a primary screening test for fetal Down syndrome at 12^{+4} weeks. Pre-test sonogram did not detect any anomalies. The NIPT result was negative for T21 and T13. The *t*-score for chromosome 18 was low (−3.22). Routine FCAPS analyses showed that there was an approximately 6 Mb deletion in chr18q (chr18:21904494–28240116, corresponding to 18q11.2–q12.1) (Figure 2a). The copy number ratio of that segment was about 0.5, which was equivalent to the loss of one haploid dosage with a very low *t*-score of −11.9. Therefore, the origin of the deletion was highly likely to be maternal in origin. After removing this segment from basic analysis for aneuploidy, the *t*-score for chromosome 18 was normalized ($t = 0.19$). Therefore, the final report for this patient was low risk for T13, T18 or T21, but had high chance of carrying an 18q deletion of maternal origin. The couple after counseling agreed to maternal karyotyping and aCGH study of maternal white blood cell. aCGH showed an approximately 3.5 Mb deletion from chr18:22821811–26278943, corresponding to 18q11.2–18q12.1 (Figure 2b). This deletion is of unclear clinical significance. Two genes were involved in the region, cadherin 2 (*CDH2*) and carbohydrate sulfotransferase 9 (*CHST9*). Copy number change in *CHST9* has been shown to be associated with hematologic malignancies, although the functional significance is still uncertain at the moment.[5] The final karyotyping showed 46,XX with no obvious deletion at 18q11.2q12.1 at 500 band level (Figure 2c). The couple after counseling decided not to proceed with other prenatal diagnostic tests. The pregnancy was still ongoing.

CASE 3

Case 3 was a 35-year-old primigravida. She requested the NIPT as a primary screening test for fetal Down syndrome at 14^{+4} weeks. Pre-test sonogram did not detect any anomalies. The NIPT result was negative for T21, T18 or T13. At that time, analysis for deletion/duplication was not yet a routine. However, scan at 19^{+1} weeks of gestation showed unilateral cleft lip and palate, small cerebellum (at −2.6SD) and prominent cerebral ventricles (atrial width = 0.97 cm). After counseling, the couple opted for amniocentesis.



ProbeName	ChrName	Start	Stop	FeatureNum	Description	Name of Gene	Accession	12C0668-A	Father_aC	Mother_aC
CUHK_P101862	chr18	73701	73746	6847	Unknown	chr18:7370...	-	0.4372786	0.06137479	0.18704094
A_16_P20749897	chr18	121700	121759	42778	Unknown	chr18:0001...	-	0.75566125	-0.11708099	-0.11636879
A_16_P03302031	chr18	153292	153351	28917	ref|Homo sa...	USP14	ref|NM_001...	1.0921636	-0.042273004	0.056606244
A_16_P20749971	chr18	154824	154883	10938	ref|Homo sa...	USP14	ref|NM_001...	0.9325252	-0.044646647	0.13999175
A_16_P40926050	chr18	169030	169089	24544	ref|Homo sa...	USP14	ref|NM_001...	0.83224696	0.09173137	-0.037489858

ProbeName	ChrName	Start	Stop	FeatureNum	Description	Name of Gene	Accession	12C0668-A	Father_aC	Mother_aC
A_16_P03321688	chr18	14112195	14112254	32702	ref|Homo sa...	ZNF519	ref|NM_145...	0.6577097	0.039871603	-0.09124689
A_16_P40959909	chr18	14112462	14112521	24419	ref|Homo sa...	ZNF519	ref|NM_145...	0.60208756	-0.078192905	0.012517459
A_16_P03321693	chr18	14114193	14114252	28094	ref|Homo sa...	ZNF519	ref|NM_145...	0.7226265	-0.045096602	-0.17725313
A_16_P20783815	chr18	14475624	14475683	3245	gb|Homo sa...	BC089388	gb|BC08938...	0.011995566	-0.13759021	-0.27885264
A_16_P03321845	chr18	14744764	14744823	35386	gb|Homo sa...	AF269088	gb|AF26908...	0.49636844	-0.004104847	0.2618914

Figure 1 Study results of Case 1. (a) Result of FCAPS analysis of maternal plasma, demonstrating the suspicious duplication in chromosomal 18. (b) Result of aCGH study of DNA from amniocentesis, showing a duplication in chromosome 18: arr 18p11.32p11.21 (:73 701–14 114 252)x3. (c) Karyogram of those cells with isochromosomes

BGI-Shenzhen was requested to re-analyze the sequencing data by FCAPS. Within same day, the FCAPS analysis suggested that there was a 16 Mb duplication in chromosome 3 (Chr3:475 973–16 661 622, corresponding to 3p26.3–p24.3) (Figure 3a), although additional smaller deletions or duplications could not be excluded because of the relatively low fetal concentration at about 5%.

QF-PCR showed normal numbers of chromosomes 21, 18 and 13. The couple then requested aCGH, and the report was available at 20^{+0} weeks, showing a 19 Mb duplication in

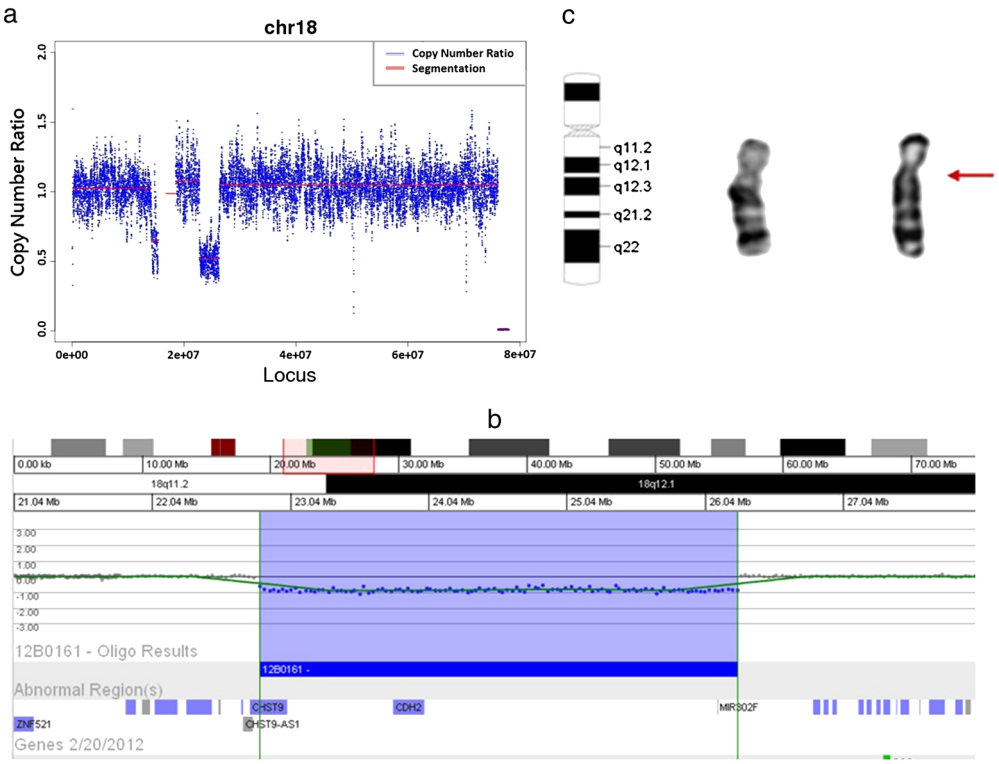


Figure 2 Study results of Case 2. (a) Result of FCAPS analysis of maternal plasma, demonstrating the suspicious deletion in proximal long arm of chromosomal 18. (b) Result from CGX-135K aCGH showing 3.46 MB copy loss at 18q11.2–18q12.1. Genes involved in the deletion region include cadherin 2 (*CDH2*), carbohydrate sulfotransferase 9 (*CHST9*) and a microRNA (*MIR302F*). (c) Idiogram of chromosome 18 and partial karyogram showing two chromosome 18 with no obvious deletion at 18q11.2–18q12.1 (arrow)

chromosome 3p and a 10 Mb deletion in chromosome 18q (Figure 3b). The karyotype confirmed an unbalanced translocation of 46,XY, der(18)t(3:18)(p24:2,q22) (Figure 3c), which was subsequently found to be inherited from the father who was a balanced translocation carrier. The couple finally decided to have pregnancy termination, which was uncomplicated. The couple declined *postmortem* examination, but cleft lip and palate was confirmed on external examination.

CASE 4

Case 4 was a 37-year-old primigravida. She presented for the NIPT because of positive first trimester combined screening test. Pre-NIPT ultrasound showed the absence of nasal bone but otherwise a normal singleton pregnancy with a nuchal translucency of 1.6 mm. After counseling, the couple changed their mind to have a chorionic villus sampling (CVS) and agreed to donate a blood sample for the NIPT as a quality assurance sample (for that, BGI-Shenzhen was not aware of the scan or other laboratory test results until the NIPT report was issued).

The CVS was performed at 14^{+0} weeks of gestation. QF-PCR was available at 14^{+1} weeks, showing normal copies of chromosomes 21, 18 and 13, but suspected mosaic XXY.

The NIPT result was available at 15^{+5} weeks. There was high suspicion of mosaic 47,XXY (*t*-score = 4.06), mosaic trisomy 21 (*t*-score = 3.32) and mosaic trisomy 7 (*t*-score = 6.32).

The full karyotype at 16^{+5} weeks showed mos 49,XXX,+7,+21 [24]/46,XY[6] (Figure 4). Although ultrasound examination showed a normal fetus with male phenotype, the patient subsequently requested an amniocentesis, which was performed at 16^{+6} weeks. Both QF-PCR and karyotype were normal (46,XY). This indicated the presence of confined placental mosaicism. The pregnancy was still ongoing.

CASE 5

Case 5 was a 44-year-old parity 1 woman with three previous spontaneous abortions. She had a regular monthly cycle with normal ovulation. The index pregnancy was conceived by *in vitro* fertilization. She was referred at 12^{+5} weeks of gestation for fetal Down syndrome screening. Although the Nuchal translucency was 3.2 mm with an adjusted risk of Down syndrome of 1:4, the patient declined the offer of diagnostic test but instead opted for the NIPT test.

The NIFTY report indicated that the fetus was not affected by trisomy 21, 18 or 13. The X chromosome concentration, however, was significantly lower than expected (*t*-score = −26). This finding could have been due to fetal 45X syndrome (Turner syndrome) but was considered to be unlikely for two reasons. Firstly, the *t*-score was not compatible with previous cases of fetal 45X syndrome detected in the laboratory, in which the average *t*-score was −5.70 (interquartile range (IQR): −6.23 to −4.50). Secondly, if the abnormal finding were due to fetal 45X syndrome, the estimated fetal DNA concentration in the maternal plasma would have been 55.4%, a level which has been rarely encountered in the laboratory. Therefore, the laboratory suggested that the most likely cause was maternal mosaicism.

The report findings were explained to the patient, and she agreed to undergo maternal karyotyping that showed a

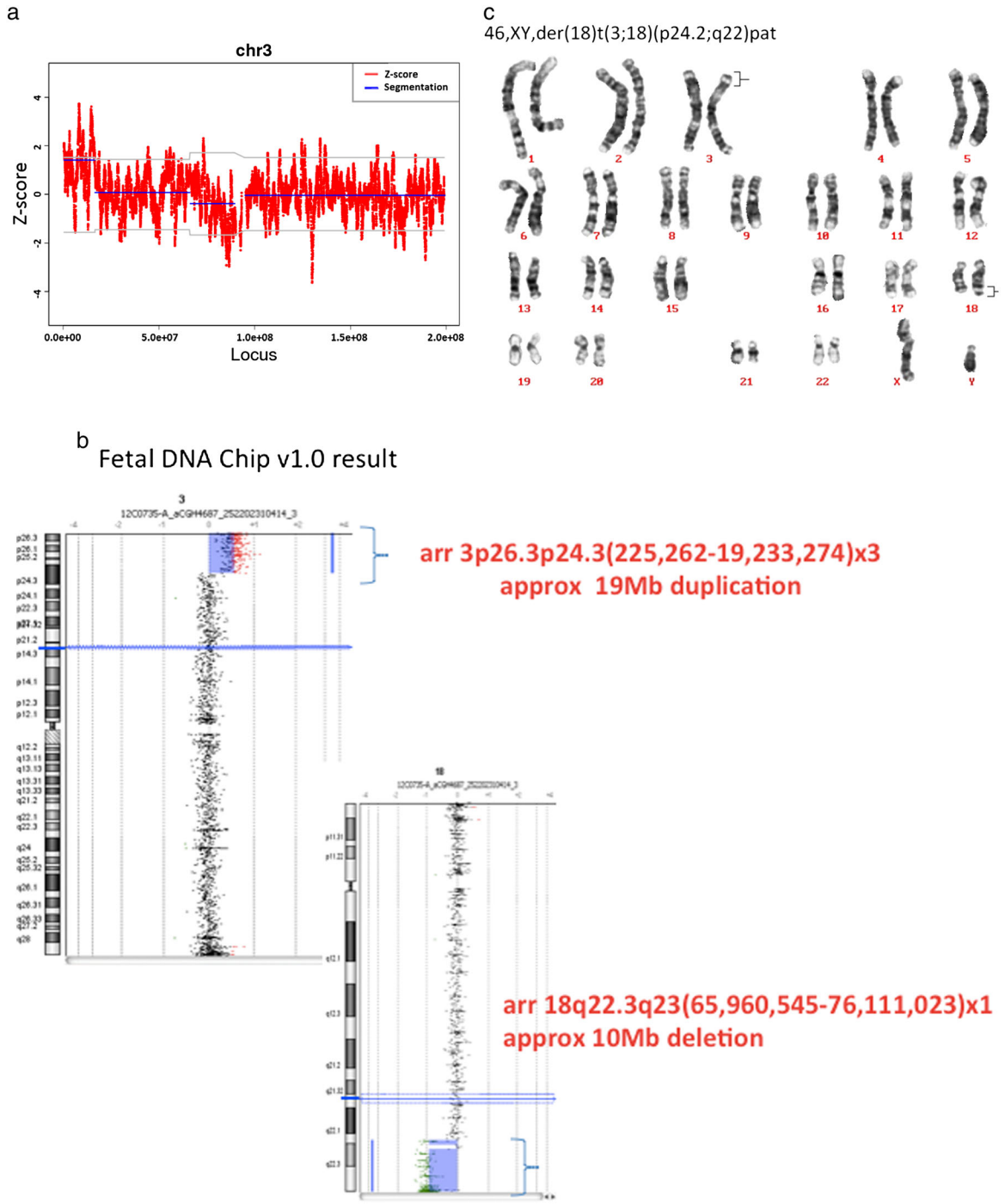


Figure 3 Study results of Case 3. (a) Result of FCAPS analysis of maternal plasma, showing a duplication in the short arm of chromosome 3. (b) Result of aCGH study of DNA from amniocentesis, confirming the duplication in chromosome 3 but an additional deletion in chromosome 18. (c) Result of final karyotyping confirming the aCGH result

karyotype of mos 45,X[3]/46,XX[27]. As a result, the fetal X chromosome dosage could not be estimated non-invasively. Because the patient decided to continue with her pregnancy even if the fetus was affected by Turner syndrome, she opted not to have an invasive test. A routine morphology scan at 20 weeks of gestation was normal, and the patient delivered a phenotypically normal baby at 38 weeks of gestation. Fetal blood was taken for karyotyping at birth, which confirmed that the fetus was not affected by either a pure or mosaic Turner syndrome.

COMMENTS

These unusual cases showed that information generated from whole genome sequencing approach can be used for the identification of additional chromosomal copy number abnormalities, including non-aneuploidy structural chromosomal deletions, duplications and mosaicism. With further improvement in bioinformatics, it is likely that NIPT by maternal plasma sequencing (MPS) will enable the detection of deletions and duplications down to 5 Mb, a resolution similar to that of conventional prenatal cytogenetics.

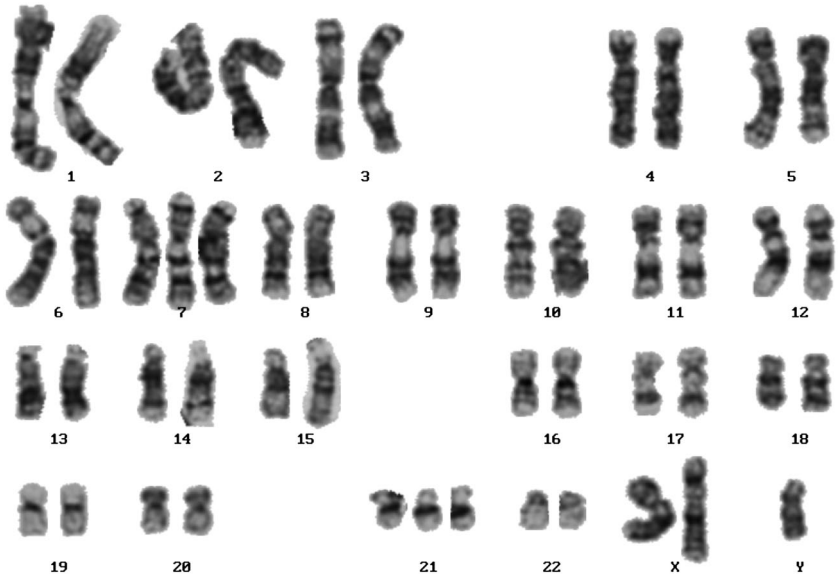


Figure 4 Karyogram of chorionic villi from Case 4, showing one cell with triple trisomies

Although it is too early to conclude whether it would be cost-effective to do so, such feasibility brings NIPT closer to conventional karyotyping on fetal samples collected through invasive tests. The major advantage of our approach is that the analyses for additional chromosomal structural changes were based on sequencing data already obtained from routine NIPT for common fetal aneuploidies. There was no requirement for extra sequencing depth, unlike previous reports that the detection of deletion/duplication required about 50-fold more sequencing data.[6,7] Therefore, this additional analysis has negligible implication on the total cost and reporting time.

All early studies on the use of MPS of maternal plasma for the NIPT of fetal Down syndrome involved the sequencing of all DNA fragments throughout the whole genome, but analyses were limited to the chromosomes of interest. The majority of information from sequencing was therefore wasted. Recent studies have proposed the use of a selective approach by target sequencing of only the part of human genome of interest, so as to minimize the cost.[8,9] If this targeted approach were used, it is likely that the abnormalities in most of the cases reported here would have been missed. With the rapid development and advances in technology, the differences in cost between our MPS approach and the targeted approach would become minimal.

Our cases showed that NIPT by MPS is a highly sensitive technology. It detected abnormalities in the maternal genome (Cases 2 and 5), in the placenta (Case 4) or the fetus (Cases 1 and 3). It detected mosaicism at a level that was not detected by QF-PCR (Case 4). It is comprehensive and covered the whole genome, providing a more accurate answer than QF-PCR (Case 4). Our data showed that a positive NIPT can be due to anomalies solely of maternal or placenta origin, which led to two important implications. Firstly, it is expected that more 'false positives' of NIPT due to abnormalities of maternal/placental origin will be encountered with more widespread use of NIPT because confined placental mosaicism is probably much commoner than what is usually believed, occurring in at least 4.8% of the term placenta.[10] This raises a fundamental question of whether amniocentesis is a more appropriate and reliable follow-up diagnostic test than CVS in cases of positive NIPT, especially if there is absence of sonographic features in the fetus suggestive of trisomy. Second implication is that a positive NIPT test with normal amniocentesis result might not represent a technical 'false positive' but a true finding not affecting the fetus but the placenta or the mother. Because of these potential 'problems', all abnormal findings revealed by MPS should be interpreted with caution and preferably by those who have extensive experience in this area.

Case 3 demonstrated that even if routine in-depth analysis of MPS data was not performed, the data could be re-analyzed when necessary, with ultrafast reporting time. This has the potential of enabling pregnant couple to make timely decision when fetal anomalies are detected at the mid-trimester. In this case, only the duplication but not the deletion was detected. This was because the fetal concentration was only 5%, at which 40× more sequencing data were required to detect the 10 M deletion. However, it is foreseeable that this could be overcome by better bioinformatics methodologies in the future.

Our cases also demonstrated the inherent limitation of molecular tests. In Case 1, although both NIPT and aCGH study suggested the presence of trisomy 18p without ambiguity, the final karyotype result showed that it was a case of mosaic tetrasomy. This was because results of molecular genetic tests are a reflection of 'averaged' genetic dosage of a sample and average genetic material within a cell. This usually would not carry any significance because in most individuals, the chromosomal constitutions of all cells are the same. However, this occasionally could potentially lead to misleading results as in this case. The tetrasomy led to doubling of the dosage, whereas mosaicism reduced the average dosage, resulting in an overall dosage mimicking a trisomy status. Given this potential limitation, results of molecular tests must be interpreted with caution again, especially when uncommon abnormalities are detected, or in the absence of detectable fetal anomalies. Formal karyotyping is mandatory in these circumstances.

Counseling for unexpected findings, especially with rare conditions, can be difficult. For example, case I was initially suspected to be trisomy 18p but ultimately was found to be mosaic tetrasomy 18p. Most publications on trisomy 18p started with a similar statement such as 'Most of the patients have either an apparently normal phenotype or only minor anomalies, and may or may not have mental retardation'.[11,12] However, a more detailed analysis of reported cases showed that mental retardation was present in eight of 14 (57.1%) reported cases of pure trisomy 18p without associated abnormalities of other chromosomes.[13] The phenotype of tetrasomy 18p is more distinct, including physical and growth abnormalities, and developmental delay and cognitive impairment are universally present.[14] On the other hand, only a few cases of mosaic tetrasomy 18p have been reported, and therefore, no conclusion concerning phenotype can be drawn.[15,16] Similar situation happens when unexpected findings are detected by amniocentesis or chorionic villus sampling. It is therefore important in the pre-test counseling to discuss with the couple whether they would like to be informed of such additional findings.

Case 5 is of particular relevance when NIPT is only limited to the detection of common trisomies. It showed that maternal mosaicism for aneuploidy could result in a 'false positive' NIPT result. Although the X chromosome was involved in this case, this phenomenon of maternal mosaicism could occur in other chromosomal aneuploidies including trisomy 21. A previous study has suggested that at the present sequencing depth, fetal trisomy 21 can be detected by NIPT of maternal plasma when the fetal DNA concentration is above 3.9%.[17] This means that if the pregnant woman has a low-level mosaic trisomy 21 of 4%, the NIPT result will be 'falsely' positive. At such low-level mosaicism, it is very likely that the affected pregnant woman will have normal or mild phenotype that would escape clinical detection.[18]

In conclusion, NIPT is a new technology. It is a very sensitive test for fetal common aneuploidies and other chromosomal abnormalities not confined to the fetus. It is expected that this new information will enhance our understanding of many previously undetected chromosomal aberrations; although at this initial stage, this additional information must be interpreted with caution

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Non-invasive prenatal testing (NIPT) by maternal plasma DNA sequencing is highly sensitive and specific in detecting fetal aneuploidies.

WHAT DOES THIS STUDY ADD?

- We report five cases of secondary findings of abnormal chromosome copy number when performing NIPT by maternal plasma sequencing for fetal aneuploidies.

REFERENCES

1. Dan S, Wang W, Ren J. Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11 105 pregnancies with mixed risk factors. Prenat Diagn 2012;1–8.
2. Lau TK, Chan MK, Lo PS. Clinical utility of noninvasive fetal trisomy (NIFTY) test – early experience. J Matern Fetal Neonatal Med 2012;25:1856–9.
3. Chen S, Lau TK, Zhang C. A method for non-invasive detection of fetal large deletions/duplications by low coverage massively parallel sequencing. Prenat Diagn Submitted
4. Leung TY, Vogel I, Lau TK. Identification of submicroscopic chromosomal aberrations in fetuses with increased nuchal translucency and apparently normal karyotype. Ultrasound Obstet Gynecol 2011;38:314–9.
5. Zhao X, Wu Q, Fu X. Examination of copy number variations of CHST9 in multiple types of hematologic malignancies. Cancer Genet Cytogenet 2010;203(2):176–9.
6. Peters D, Chu T, Yatsenko SA. Noninvasive prenatal diagnosis of a fetal microdeletion syndrome. N Engl J Med 2011;365:1847–8.
7. Jensen TJ, Dzakula Z, Deciu C. Detection of microdeletion 22q11.2 in a fetus by next-generation sequencing of maternal plasma. Clin Chem 2012;58:1148–51.
8. Ashoor G, Syngelaki A, Wagner M. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012;206:322.e1-5
9. Sparks AB, Struble CA, Wang ET. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012;206:319.e1-9.
10. Artan S, Başaran N, Hassa H, *et al.* Confined placental mosaicism in term placentae: analysis of 125 cases. Prenat Diagn 1995;15:1135–42.
11. Li S, Tuck-Muller CM, Martínez JE. Prenatal detection of de novo duplication of the short arm of chromosome 18 confirmed by fluorescence in situ hybridization (FISH). Am J Med Genet 1998;80:487–90.
12. Marical H, Le Bris MJ, Douet-Guilbert N. 18p trisomy: a case of direct 18p duplication characterized by molecular cytogenetic analysis. Am J Med Genet A 2007;143A:2192–5.
13. Mabboux P, Brisset S, Aboura A. Pure and complete trisomy 18p due to a supernumerary marker chromosome associated with moderate mental retardation. Am J Med Genet A 2007;143:727–33.
14. Sebold C, Roeder E, Zimmerman M. Tetrasomy 18p: report of the molecular and clinical findings of 43 individuals. Am J Med Genet A 2010;152A:2164–72.
15. Göcke H, Muradow I, Zerres K, Hansmann M. Mosaicism of isochromosome 18p. Cytogenetic and morphological findings in a male fetus at 21 weeks. Prenat Diagn 1986;6:151–7.
16. Pinto MR, Silva ML, Ribeiro MC, Pina R. Prenatal diagnosis of mosaicism for tetrasomy 18p: cytogenetic, fish and morphological findings. Prenat Diagn 1998;18:1095–7.
17. Fan HC, Quake SR. Sensitivity of noninvasive prenatal detection of fetal aneuploidy from maternal plasma using shotgun sequencing is limited only by counting statistics. PLoS One 2010;5:e10439.
18. Papavassiliou P, York TP, Gursoy N. The phenotype of persons having mosaicism for trisomy 21/Down syndrome reflects the percentage of trisomic cells present in different tissues. Am J Med Genet A 2009;149A:573–83.