CEOG Clinical and Experimental Obstetrics & Gynecology

Non-invasive prenatal detection for copy number variation

Y.H. Liu[1], L. Gao[2], Y. He[1], W.W. Xie[3], F. Xiong[4,5], H.J. Jiang[3,6], F. Chen[3], J.H. Qu[3], H.H. Huang[3], W.F. Xu[1], Y.Y. Lin[1], R.G. Xie[1], J.W. Lou[1], H. Jiang[3]

[1]Department of Prenatal Diagnosis Center, Dongguan Maternal and Child Health Hospital, Dongguan
[2]Department of clinical Laboratory, Henan Province People's Hospital, Zhengzhou; [3]BGI-Shenzhen, Shenzhen
[4]Department of Medical Genetics, School of Basic Medical Sciences, Southern Medical University, Guangzhou
[5]Key laboratory of Reproduction and Genetics of Guangdong Higher Education Institutes, Guangzhou Medical University, Guangzhou
[6]School of Biological Science and Medical Engineering, Southeast University, Nanjing (China)

Summary

Aim: To assess the technology of non-invasive prenatal testing (NIPT) and the robust mathematical model fetal copy-number analysis through maternal plasma sequencing (FCAPS) detecting large fetal deletions or duplications. *Materials and Methods:* Peripheral venous blood were taken from three pregnant women with high risk, and maternal plasma DNA were extracted and detected by NIPT and FCAPS. The results were validated through Array-CGH or karyotyping with amniotic fluid or umbilical cord blood obtained from the patients. *Results:* One out of three cases was positive by NIPT, but all were found with abnormalities by FCAPS. The results were further confirmed using array-CGH or karyotyping. *Discussion:* This study provides novel insights into noninvasive prenatal diagnosis using low-coverage maternal plasma sequencing to detect large fetal deletions or duplications, as well as correlations between fetal genotypes and phenotypes.

Key words: noninvasive prenatal testing, copy number variation, cell-free fetal DNA

Introduction

Chromosomal abnormalities such as aneuploidies and chromosomal deletions or duplications are the main causes of birth defects. More recent advances in genomics and genomic technologies have resulted in the development of a non-invasive prenatal testing (NIPT) using cell-free fetal DNA sequences isolated from a maternal blood sample; this has been used for fetal aneuploidies, particularly for trisomy 21, 18, and 13. Using next generation sequencing platforms, millions of amplified genetic fragments can be sequenced in parallel (massively parallel sequencing) and have achieved sensitivities and specificities as high as 99% [1]. However, sequencing-based non-invasive technologies focus on the numerical chromosome changes, and chromosomal structural abnormalities are still at the theoretical stage. The present authors' previous study proposed a robust mathematical model fetal copy-number analysis through maternal plasma sequencing (FCAPS) that could accurately detect fetal chromosome abnormality by maternal plasma DNA sequencing [2]. Here the authors report three cases by non-invasive prenatal detection of deletion/duplication using maternal plasma DNA sequencing. This study provides novel insights into non-invasive prenatal diagnosis using low-coverage maternal plasma sequencing to detect large fetal deletions or duplications, as well as correlations between fetal genotypes and phenotypes.

Materials and Methods

All samples were selected from the outpatient of the Dongguan Maternal and Child Health Hospital. Peripheral venous blood (5 ml) were taken from the pregnant women, and maternal plasma DNA were extracted and used for library construction and single-end sequencing followed a published protocol [1, 3]. For the data, sequencing was further analyzed by bioinformatics. All the materials and procedures obtained the approval from Institutional Ethics Committee of Dongguan Maternal and Child Health Hospital.

The FCAPS pipeline is a robust mathematical for the detection of large deletions and duplications. Overall, the authors divided the reference genome into about 300,000 sliding windows that shared the same number of reads rather than a fixed length. The number of reads in each window was calculated and the relative reads number (RRN) was defined as the ratio between the reads number of each window and the average reads number of all windows. Least-squares estimation was used for each window through all 140 negative control samples to obtain a significant linear relationship between the GC content and the RRN. Based on the linear relationship, the authors performed GC correction for a corrected relative reads number (CRN). Finally, a binary segmentation algorithm and dynamic threshold strategy were used to localize the breakpoint of fetal deletions or duplications and to determine the variation in chromosomal abnormality types.

Peripheral blood, amniotic fluid or umbilical cord blood were sampled, metaphase chromosomes from Phytohaemagglutinin (PHA)-stimulated blood lymphocytes of the above-mentioned samples were analyzed by standard GTG-banding procedures and karyotyping was performed using the cytovision system. Metaphases were captured with a cytovision digital imaging system.

Revised manuscript accepted for publication September 29, 2016

The anatomy of the postnatal fetus was determined by a professional anatomist. The local medical research ethics committee reviewed the study, and informed consent was obtained from the participant or the proband's parents.

The authors obtained amniotic fluid or umbilical cord blood from the pregnant women, and peripheral blood samples from the couples after written informed consent.Genomic DNA was isolated from samples and detected by Array-CGH according to standard procedures. The detective data were analyzed by DECIPHER and OMIM data [4].

Results

Case 1 was a 24-year-old woman with gravida 1 and para 0 presented for a routine pregnancy test at 23 gestational weeks. The heart beat rate/minute gives much more information that there has been no sign of abortion during the early pregnancy. Sonography screening at 23 gestational weeks showed that the fetus possibly had polycystic kidney and bilateral ventricular asymmetry (Figure 1).

Anamnestic evaluation indicated that the parents were not consanguineous and had no family history of genetic diseases or history of virus infections, fever, and any other problems during pregnancy. After genetic counseling, the NIPT was performed at 24 gestational weeks with informed written consent.

About 6.48 million single-end reads were obtained for the test sample, corresponding to 226.7 Mb of sequence data, of which 84.76% mapped uniquely to a human genome reference (NCBI Build 36, hg18). The NIPT showed a low risk of fetal trisomies 21, 18, and 13, but a high risk for monosomy 18. The authors used a practical method Fetal Copy-number Analysis through Maternal Plasma Sequencing (FCAPS) to detect monosomy 18. They observed a 21.34 Mb deletion at the end of the long arm of chromosome 18 (53,333,583-74,673,666) and a 14.35 Mb duplication of chromosome 18 (38,987,507–53,333,582) (Figure 1A). The abnormalities covered 47.8% of chromosome 18 with the 18qter.

To validate the prenatal test result, umbilical cord blood was sampled at 31 gestational weeks. Karyotype analysis revealed that the fetus had 46,XX,del (18)(q21.3q23) (Figure 1B). Analysis of the parental chromosomes showed a normal karyotype, so the fetal chromosome 18 deletion was de novo. After genetic counseling, the parents decided to terminate the pregnancy at 34 gestational weeks. However, the fetal duplication of chromosomal 18 from massively parallel sequencing (MPS) was not seen by the karyotype analysis and the low-coverage whole-genome sequencing with fetal tissue DNA. The authors conducted low-coverage whole-genome sequencing from parental blood samples and found no duplications or deletions. The result of the fetal duplication on chromosome 18 was a false positive signal without a clear cause. Anatomical analysis showed the fetus to present an enlarged liver, left centerline, polycystic kidney, overlapping fingers, low-set ears, and single transverse palmar creases on the left hand (Figure 1C).

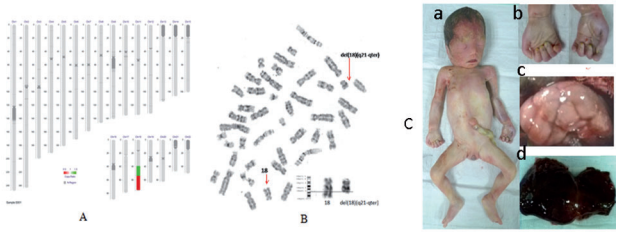

Figure 1. — The karyotpye and phenotype analysis on the fetus with 46,XX,del (18)(q21.31q23)
(A) Digital karyotyping using non-invasive maternal plasma sequencing. Gray, no deletions or duplications; dark gray, genomic N-regions; Red, deletion; green, duplication. This sample shows a 21.34 Mb deletion from 18q21.31 to 18q23. (B) G-banded fetal karyotype showing deletion of chromosome 18 from 18q21.31 to 18q23. (C) Fetal phenotype with chromosome 18q abnormality. (a) Integral phenotype of the fetus: eyes closed, short neck, and low-set ears. (b) Overlapping fingers and single transverse palmar creases on the left hand. (c) Slightly swollen kidney similar to polycystic kidney. (d) Slightly swollen liver volume and center line of the left.

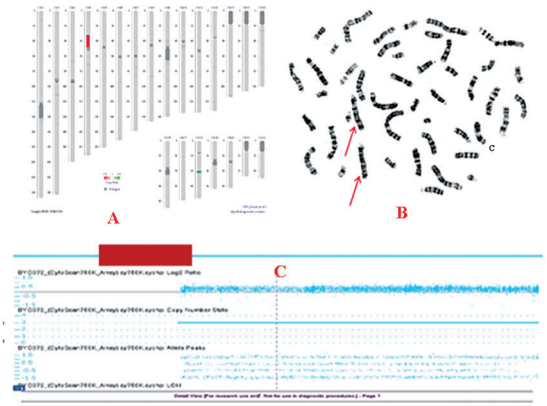

Figure 2. — The results of NIPT and FCAPS, Karyotype and ArrayCGH
A.Digital karyotyping using non-invasive maternal plasma sequencing. Gray, no deletions or duplications; dark gray, genomic N-regions; Red, deletion; green, duplication. This sample shows a 21.34 Mb deletion from 8Mb deletion in chromosome 4p15.1.B. karyotyping indicates that two 4 chromosomes are normal.C. Digital karyotyping using Array-CGH. Red represents deletion; This sample shows an approximately 10Mb deletion from 4p15.1.

Case 2 was a 37-year-old woman with gravida 1 and para 0. At 22 weeks of gestation for prenatal Down's screening

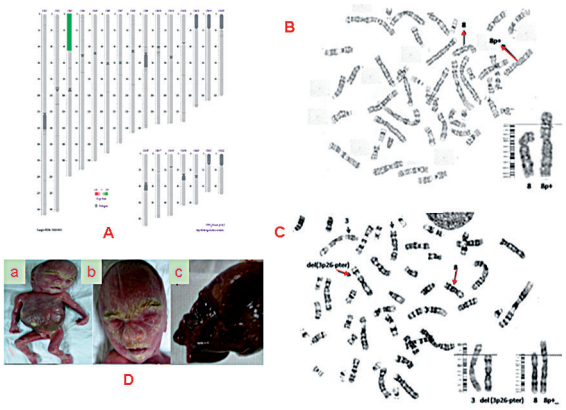

Figure 3. — The digital karyotyping of NIPT and FCAPS, karyotype, and anatomy
A) Digital karyotyping using non-invasive maternal plasma sequencing. Gray, no deletions or duplications; dark gray, genomic N-regions; Red, deletion; green, duplication. This sample shows an approximately 34.82Mb duplication in chromosome 3q26.1-29;B. G-banded fetal karyotype showing duplication of chromosome 8 pter. (C) Maternal karyotyping showed a karyotype of 46,xx,t(3;8)(3q26;8p23). D) The fetal shows low-set ears (a) and orbital hypertelorism (b). Anatomy shows left ventricular septal defect (c).

was at higher risk, the sonography screening showed that the fetus possibly had finger deformity and single umbilical artery (Figure 2). After counseling, the couple opted for NIPT. About 5.49 million single-end reads were obtained for the test sample, corresponding to192.17 Mb of sequence data, of which 84.40% mapped uniquely to a human genome reference (NCBI Build 36, hg18). The NIPT result was negative for T21, T18, and T13. Considering the sonography abnormal, for case 2 the FCAPS analysis was performed. The result showed that there was an approximately 8Mb deletion in chromosome 4p (chr4: 27923389-35991754, corresponding to 4p15.1) (Figure 2A). The couple after counseling agreed to amniocentesis for karyotyping and aCGH study. The result of karyotyping was normal (Figure 2B), but aCGH showed an approximately 10Mb deletion from chr4: 263998823-40512576, corresponding to 4p15.1 (Figure 2C). The couple finally determined for pregnancy termination at 28 gestational weeks.

Case 3 was a 24-year-old woman with three previous miscarriages. She had three unexplained abortions at the third trimester. At six gestational weeks, she was hospitalized because of repeated vaginal bleeding for two days. At 16 weeks, maternal serum screening test of Down syndrome was performed and the result showed a high risk of T21(1/110). Cervical cerclage was performed due to cervical incompetence at 18 gestational weeks. At 26 gestational weeks, sonography screening showed that fetal possibly had ventricular septal defect and placenta thickening. After counseling, the couple opted for NIPT. About 6.77 million single-end reads were obtained for the test sample, corresponding to 236.82Mb of sequence data, of which 84.06% mapped uniquely to a human genome reference (NCBI Build 36, hg18). The NIPT result was negative for T21, T18, and T13. Considering the sonography abnormal, for case 3 the FCAPS analysis was performed. The result showed that there was an approximately 34.82Mb duplication in chromosome 3q (chr3: 164567125-199384518, corresponding to 3q26.1-29) (Figure 3A). With written informed consent, the pregnant woman requested an amniocentesis at 24 weeks. The result of karyotyping is 46, XY, dup(8pter) (Figure 3B). The report findings were explained to the patient, and she agreed to undergo maternal karyotyping that showed a karyotype of 46,xx,t(3;8)(3q26;8p23) (Figure 3C). The couple finally opted for pregnancy termination at 28 weeks. The fetal birth weight was 850 grams, a stillbirth with body length of 32 cm. The postmortem examination showed left ventricular septal defect. Other abnormal included low-set ears and orbital hypertelorism (Figure 3D).

Discussion

Fluorescence *in situ* hybridization (FISH), microsatellite analysis, and high-resolution array comparative genomic hybridization (aCGH) are the standard approaches for identifying chromosomal abnormalities. However, none of these methods can be used for non-invasive prenatal detection. MPS is undergoing dramatic decreases in cost and increases in throughput and offers new possibilities for disease-related research and clinical practice [5, 6]. Several studies have used MPS technology for non-invasive prenatal testing for fetal aneuploidies for low-coverage whole-genome sequencing of maternal plasma DNA and have achieved sensitivities and specificities as high as 99% [1]. Unlike previous studies for deletions and duplications, the present authors identified fetal chromosome deletion / duplication by MPS from maternal plasma. This technique could prevent fetal loss and parental anxiety caused by amniocentesis or chorionic villus sampling [7]. Although case 1 showed the result of the fetal duplication on chromosome 18 was a false positive signal without a clear cause, the authors accurately identified the remaining cases of chromosomal deletions/ duplication using less than seven million sequencing reads. Wide clinical application of MPS for detecting large chromosomal deletions or duplications still faces challenges such as the need for increased data-processing capacity, improved genome-wide sequencing coverage, and genetic counseling about novel deletions and duplications. We still lack a comprehensive evaluation of the performance of the sequencing approach for detecting chromosomal deletions and duplications. Therefore, at this time, results from sequencing should be confirmed by invasive procedures [8], but alternative diagnosis solutions,

such as FCAPS, should be applied before final prenatal diagnosis results are given.

In conclusion, the introduction of MPS of maternal plasma for prenatal testing could dramatically improve the efficiency for detecting large, partial chromosomal deletions and duplications. The present study provided novel insights into non-invasive prenatal diagnosis using low-coverage maternal plasma sequencing to detect large fetal deletions or duplications.

Acknowledgements

The authors thank the patients' families for their cooperation. This study was supported by the Key Project of Science and Technology of Dongguan (2011105102012, 2012105102003, and 2013108101021) and also funded by the Shenzhen Birth Defect Screening Project Lab (JZF No. [2011] 861), and approved by Municipal Commission for Development and Reform and Key Laboratory Project in Shenzhen (CXB201108250096A) and Key Laboratory of Cooperation Project in Guangdong Province (2011A060906007), and Science and technology program in Guangzhou (201300000095). Guangdong Province Science and Technology Plan Project (20130318C).

References

[1] Lau T.K., Chen F., Pan X., Pooh R.K., Jiang F., Li Y.*, et al.:* "Noninvasive prenatal diagnosis of common fetal chromosomal aneuploidies by maternal plasma DNA sequencing". *J. Matern. Fetal Neonatal Med.*, 2012, *25*, 1370.

[2] Peters D., Chu T., Yatsenko S.A., Hendrix N., Hogge W.A., Surti U., Bunce K.*, et al.:* "Noninvasive prenatal diagnosis of a fetal microdeletion syndrome". *N. Engl. J. Med.*, 2011, *365*, 1847.

[3] Jiang F., Ren J., Chen F., Zhou Y., Xie J., Dan S.*, et al.:* "Noninvasive Fetal Trisomy (NIFTY) test: an advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies". *BMC Med. Genomics*, 2012, *5*, 57.

[4] Hung C.C., Lin C.H., Lin S.Y., Shin J.C., Lee C.N., Su Y.N.: "Prenatal diagnosis of a fetus with a de novo trisomy 12p by array-comparative genomic hybridization (array-CGH)". *Gene*, 2012, *495*, 178.

[5] Bell C.J., Dinwiddie D.L., Miller N.A., Hateley S.L., Ganusova E.E., Mudge J.*, et al.* Carrier testing for severe childhood recessive diseases by next-generation sequencing. *Sci Transl Med*., 2011, *3*, 65ra4.

[6] Moorthie S., Mattocks C.J., Wright C.F.: "Review of massively parallel DNA sequencing technologies". *Hugo J*., 2011, *5*, 1.

[7] Simpson J.L., Bischoff F.: "Cell-free fetal DNA in maternal blood: evolving clinical applications". *JAMA*, 2004, *291*, 1135.

[8] Miller D.T., Adam M.P., Aradhya S., Biesecker L.G., Brothman A.R., Carter N.P.*, et al.:* "Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies". *Am. J. Hum. Genet.,* 2010, *86*, 749.

Corresponding Author:
YANHUI LIU, M.D.
Department of Prenatal Diagnosis Center
Dongguan Maternal and Child Health Hospital
Dongguan (China)
e-mail: liuliang7121500@163.com