Gineco.eu [12] 208-210 [2016]
DOI: 10.18643/gieu.2016.208

Non-invasive prenatal testing for rare chromosomal anomalies

C. Coroleucă[1], C.A. Ionescu[1], L. Pleș[2], M. Dimitriu[1], M. Banacu[1], I. Popescu[1], L. Șerbănescu[3]

1. UMF "Carol Davila" Bucharest, Clinical Emergency Hospital "Sf. Pantelimon" Bucharest
2. UMF "Carol Davila" Bucharest, Clinical Emergency Hospital "Sf. Ioan" Bucharest
3. Faculty of Medicine, "Ovidius" University of Constanta, Romania

Correspondence: Dr. C.A. Ionescu e-mail: antoniuginec@yahoo.com

Abstract

After numerous clinical validation studies, non-invasive prenatal testing for fetal aneuploidy detection is now a clinical reality. While non-invasive prenatal testing was accepted due to the high accuracy for fetal trisomy (21, 18 and 13) detection, recent research showed that genome-wide analysis is able to detect other fetal and maternal (mosaic) aneuploidies. In the present paper, we discuss the clinical advantages and challenges of genome-wide circulating free fetal deoxyribonucleic acid (DNA) profiling and targeted detection of rare chromosomal anomalies. The use of cell-free DNA tests for sex chromosome aneuploidies and microdeletion syndromes screening cannot be currently recommended without restriction on the basis of the present data. Expanding non-invasive prenatal testing (NIPT)-based prenatal screening to include this anomalies not only raises ethical concerns related to information and counseling challenges but also risks reversing the important reduction in invasive testing achieved with implementation of NIPT for aneuploidy.
Keywords: *non-invasive prenatal testing, aneuploidy, microdeletion*

Introduction

Since 2011, non-invasive prenatal testing (NIPT) for fetal aneuploidy detection is now a clinical reality, only after numerous clinical validation studies applied massively parallel sequencing of maternal plasma cell-free deoxyribonucleic acid (cffDNA) using either whole-genome sequencing or targeted-sequencing methods (chromosome selective methods or single nucleotide polymorphism methods)[1-6].

The International Society for Prenatal Diagnosis, the American College of Obstetricians and Gynecologists and the Royal College of Obstetricians and Gynecologists have issued position statements primarily not only on the use of NIPT for trisomy 21 detection but also for the other common autosomal aneuploidies (trisomy 18 and 13)[3,5-11]. However, it is important to point that NIPT is not a diagnostic test for fetal aneuploidy, and therefore, a positive NIPT result requires an invasive test to confirm the findings. Furthermore, these societies considered to be insufficient evidence to support the use of NIPT for screening in the general population.

While the implementation of NIPT was accepted due to the high accuracy for fetal trisomy 21, 18 and 13 detection, several studies have showed that genome-wide analysis is able to detect other fetal and maternal (mosaic) aneuploidies[1,3]. In this review, we discuss the clinical advantages and challenges of genome-wide circulating free fetal DNA profiling and targeted detection of rare chromosomal anomalies.

We have selected 25 articles from the available literature published in the past 5 years regarding noninvasive prenatal testing, analyzing the three aneuploidies tested on a large scale (Down Syndrome, Edwards Syndrome and Patau Syndrome) and rare chromosomal anomalies.

From the total number of the selected articles, 16 are clinical studies[2,4,12-25] (i.e. 3 of them referring to procedural costs)[15,24,25], 2 meta-analysis[5,6] and 7 position papers and guidelines issued by relevant scientific societies[3,7-10,11,26].

The detection of fetal chromosomal anomalies by sequencing cffDNA from maternal plasma

Several groups have showed the ability to detect all fetal chromosomal aneuploidies, chromosomal structural anomalies, and submicroscopic copy number variations by sequencing cffDNA from maternal plasma[1,2,11,14,26]. Despite detecting other fetal aneuploidies which might aid in the interpretation of fetal development, clinical implementation of genome-wide NIPT analysis in the routine clinical setting remains limited[1,11,14,15].

In their prospective series of 1982 clinical cases, Lau et al.[14] detected 7 cases of full aneuploidy for chromosomes other than 21, 18, 13, X or Y. Follow-up testing and pregnancy outcome were provided for 5 of these and confined placental mosaicism (CPM) was confirmed in four of the five cases. Two pregnancies (including the case unconfirmed) were complicated by fetal growth restriction and delivered at 33-34 weeks gestation. In the two cases without any follow-up investigations, ultrasound was normal, the pregnancies were continued and no abnormalities were reported after birth.

The clinical utility of screening for all aneuploidies detected using NIPT, particularly in low-risk pregnancies, is controversial because this could lead to an increase in invasive procedures in women for whom this would

Received: *August 21, 2016*
Revised: *September 12, 2016*
Accepted: *October 22, 2016*

not normally be considered[1,2,11]. Some authors questions routine screening for sex chromosome aneuploidies (SCA) , given the variability in phenotype of, for example, monosomy X (Turners), XXY (Klinefelter), XYY and XXX individuals, with some only identified as adults due to fertility problems[1,11]. Moreover, the accuracy of SCA detection is lower in comparison with trisomy 21 detection (90.3-93.0% with false-positive rates of 0.14% to 0.23%)[11].

After NIPT implementation for common chromosome disorders and promising results for clinically significant copy number variations, one of the obvious extensions to the technology is testing for monogenic disorders[2]. The possibility of NIPT for single-gene disease has already been documented with digital polymerase chain reaction (PCR), enabling the detection of mutations associated with major blood disorders like β-thalassemia[12,19], sickle cell anemia[20], hemophilia[21] and the determination of RhD antigen genotypes[22].

The molecular diagnosis

Digital PCR remains technically challenging for most molecular diagnostic laboratories and thus this technology has not seen rapid translation into clinical practice. Currently only NIPT for RhD genotyping is clinically available, being highly accurate for determining the presence of an RhD-positive fetus[22].

Relative haplotype dosage analysis of the hemoglobin β gene combined with digital PCR was successfully applied to correctly genotype the established fetuses from pregnancies at risk for β-thalassemia[2]. Using next generation sequencing for HbE and the four most common β-thalassemia mutations found in South East Asia, Xiong et al.[12] obtained an overall sensitivity for detection of paternal mutations of 100% (95%, CI=92.4-100%) and a specificity of 92.1% (95%, CI= 79.2-97.3%).

Yan Xu et al.[13] showed in their study that noninvasive prenatal testing is efficient for Duchene muscular dystrophy, using a newly developed haplotype-based approach.

Clinical experience with this single-nucleotide polymorphism-based non-invasive screening test for 22q11.2 deletion syndrome indicates that these deletions have a frequency of approximately 1 in 1000 in the high risk population with access to NIPT, identifiable through this test. 22q11.2 deletion syndrome (i.e. DiGeorge or velocardiofacial syndrome) is the most common, with reported prevalence ranging from 1 in 2000 to 1 in 6000 live births. Use of this screening method requires the availability of counseling and other management resources for high-risk pregnancies, as the positive predictive value was just 18%[23].

Weigang et al.[2] used Sanger and whole-exome sequencing to identify familial ATPase, Cu^{++} transporting, β polypeptide gene mutations, responsible for the rare autosomal recessive disorder known as Wilson Disease. The study describes the development and validation of a novel assay termed circulating single-molecule amplification and resequencing technology (cSMART) for counting single allelic molecules in plasma. It showed that the suitability of cSMART for NIPT, with Wilson Disease as proof of concept.

Shan et al.[16] investigated the possibility of using targeted capture sequencing to detect fetal de novo pathogenic mutations responsible for skeletal dysplasia, which are a group of heterogeneous genetic diseases that affect the development of the chondro-osseous tissue. They have a prevalence of 2-5/10000 newborns. Three families whose fetuses were affected by skeletal dysplasia and two control families whose fetuses were affected by other single gene diseases were included in this study. Sixteen genes related to some common lethal skeletal dysplasia were selected for analysis. The causal mutations were specifically identified in the plasma and the results were identical to those obtained by sequencing amniotic fluid samples.

Achondroplasia is the most common non-lethal skeletal dysplasia with an incidence of 5-15 per 100 000 live births. Chitty et al.[17] compared PCR and restriction enzyme digest (RED) of cell-free DNA with NGS assay in pregnancies at risk of achondroplasia and thanatophoric dysplasia. PCR-RED was performed in 72 cases and was correct in 88.6%, inconclusive in 7% with one false negative. NGS was performed in 47 cases and was accurate in 96.2% with no inconclusive results. For the study were selected fetuses at risk for achondroplasia or thanatophoric dysplasia, either because of sonographic findings or because of a relevant past family history. Orhant et al.[18] studied the *de novo* missense genetic mutation at nucleotide 1138 in fibroblast growth factor receptor 3 gene involved in >99% of achondroplasia cases. The study was based on two independent methods: digital-droplet PCR combined with mini-sequencing. Twenty-six women carrying fetuses at risk for achondroplasia were included and five were diagnosed as affected fetuses in maternal blood. The sensitivity and specificity of our test are 100% and 100% respectively.

The source of cffDNA is known to be placental in origin[1]. It is well documented from conventional cytogenetic examination of chorion villus sampling (CVS) tissue that CPM occurs in cases where placental tissue contains an abnormal cell line which is not present upon subsequent examination of amniocentesis or other fetal material. CPM is observed in around 1% of invasive tests[1,26] and is usually associated with normal fetal outcomes. It may be associated with intrauterine growth restriction, pregnancy loss or perinatal death[26]. Given that NIPT can result in false positives, positive results should be confirmed with invasive testing. Whether to perform CVS or amniocentesis is controversial. While CVS can be performed earlier than amniocentesis, it will also give false positive results in case of CPM. It is recommended to perform CVS and to examine all cell lines using either an uncultured sample by fluorescence in situ hybridization (FISH), as well as long-term culture of the sample. If all the results show aneuploidy, the results are reported to the patient. Otherwise, if the results are also mosaic, amniocentesis is recommended and analyzed by both FISH and karyotype[26].

Other causes of abnormal NIPT results can be twin pregnancies, maternal subclinical CNVs, maternal mosaicism and maternal tumors[1].

Conclusions

Microdeletion syndromes are candidate conditions for broader NIPT-screening scenarios that in the coming years may be considered also in settings where prenatal screening is a public health service. However, this requires more scientific evidence, as well as a thorough assessment of benefits and harms for those to whom the screening is offered. In particular an evaluation of the false positive rate is required as in some studies it has been reported to be as high as 3%. In addition, the limits of detection are unknown and small rearrangements may not be detected.

Increased demand for testing may have benefic economic implications for prenatal diagnostic services, but ethical issues remain, including directing resources to NIPT when used for information only and restricting access to safe tests if it is not cost-effective to develop NIPT for rare conditions.

The use of cfDNA tests for sex chromosome aneuploidies and microdeletion syndromes screening currently cannot be recommended without restriction on the basis of the present data. Expanding NIPT-based prenatal screening to include this anomalies not only raises ethical concerns related to information and counseling challenges but also risks reversing the important reduction in invasive testing achieved with implementation of NIPT for aneuploidy. ■

References

1. Brady P, Brison N, Van Den Bogaert K, de Ravel T, Peeters H, Van Esch H, Devriendt K, Legius E, Vermeesch JR. Clinical implementation of NIPT - technical and biological challenges. Version of Record Online, 2015, doi: 10.1111/cge.12598
2. Weigang L, Wei X, Guo R, Liu Q, Zheng Y, Chang J, Bai T, Li H, Zhang J, Song Z, Cram DS, Liang D, Wu L. Noninvasive prenatal testing for Wilson disease by use of circulating single-molecule amplification and resequencing technology (cSMART), Clinical Chemistry 2015, 61(1172), 181, doi: 10.1373/clinchem.2014.229328
3. Dondorp W, de Wert G, Bombard Y, Bianchi DW, Bergmann C, Borry P, Chitty LS, Fellmann F, Forzano F, Hall A, Henneman L, Howard HC, Lucassen A, Ormond K, Peterlin B, Radojkovic D, Rogowski W, Soller M, Tibben A, Tranebjærg L, van El CG, Cornel MC. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. Eur J Hum Genet 2015, 23(11), 1438-50, doi: 10.1038/ejhg.2015.57
4. Stokowski R, Wang E, White K, Batey A, Jacobsson B, Brar H, Balanarasimha M, Hollemon D, Sparks A, Nicolaides K, Musci TJ. Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies - Prenatal Diagnosis 2015, 35(12), 1243-6.
5. Taylor-Phillips S, Freeman K, Geppert J, Agbebiyi A, Uthman OA, Madan J, Clarke A, Quenby S, Clarke A. Accuracy of non-invasive prenatal testing using cell-free DNA for detection of Down, Edwards and Patau syndromes: a systematic review and meta-analysis. BMJ Open 2016, 6(1), e010002, doi: 10.1136/bmjopen-2015-010002.
6. Mackie FL, Hemming K, Allen S, Morris RK, Kilby MD. The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. BJOG 2016 May 31. doi: 10.1111/1471-0528.14050.
7. Benn P, Borell A, Chiu R. et al. Position statement from the aneuploidy screening committee on behalf of the board of the international society for prenatal diagnosis. Prenat Diagn 2013, 33(7), 622-9.
8. Gregg AR, Gross SJ, Best RG. et al. ACMG statement on noninvasive prenatal screening for fetal aneuploidy. Genet Med 2013, 15(5), 395-8.
9. Royal College of Obstetricians and Gynaecologists. Non-invasive prenatal testing for chromosomal abnormality using maternal plasma DNA. Scientific Impact Paper No 15, 2014, https://www.rcog.org.uk/en/guidelines-research-services/guidelines/sip15/
10. American College of Obstetricians and Gynecologists. Committee Opinion No. 545: Noninvasive prenatal testing for fetal aneuploidy. Obstet Gynecol 2012, 120(6), 1532-4.
11. Schmid M, Klaritsch P, Arzt W, Burkhardt T, Duba HC, Häusler M, Hafner E, Lang U, Pertl B, Speicher M, Steiner H, Tercanli S, Merz E, Heling KS, Eiben B. Cell-Free DNA testing for fetal chromosomal anomalies in clinical practice: Austrian-German-Swiss Recommendations for non-invasive prenatal tests (NIPT). Ultraschall in Med 2015, 36(05), 507-510, doi: 10.1055/s-0035-1553804
12. Xiong L, Barrett AN, Hua R, Tan TZ, Ho SSY, Chan JKY, Zhong M, Choolani M. Non-invasive prenatal diagnostic testing for β-thalassaemia using cell-free fetal DNA and next generation sequencing. Prenatal Diagnosis 2015, 35(3), 258-65.
13. Yan Xu MS, Xuchao Li MS, Hui-juan Ge ME, Bing Xiao MD, Yan-Yan Zhang MS, Xiao-Min Ying BS, Xiao-Yu Pan BE, Lei Wang MD, Wei-Wei Xie BM, Lin Ni BS, Sheng-Pei Chen BE, Wen-Ting Jiang MS, Ping Liu MM, Hui Ye BS, Ying Cao BS, Jing-Min Zhang MD, Yu Liu BS, Zu-Jing Yang MD, Ying-Wei Chen MD, Fang Chen MS, Hui Jiang MS, Xing Ji MS. Haplotype-based approach for noninvasive prenatal tests of Duchenne muscular dystrophy using cell-free fetal DNA in maternal plasma. Genetics in Medicine 2015, 17, 889-96 doi:10.1038/gim.2014.207
14. Lau TK, Cheung SW, Lo PS. et al. Non-invasive prenatal testing for fetal chromosomal abnormalities by low-coverage whole-genome sequencing of maternal plasma DNA: review of 1982 consecutive cases in a single center. Ultrasound Obstet Gynecol 2014, 43 (3), 254-64.
15. Benn P, Curnow KJ, Chapman S, Michalopoulos SN, Hornberger J, Rabinowitz M. - An Economic Analysis of Cell-Free DNA Non-Invasive Prenatal Testing in the US General Pregnancy Population - PLoS One. 2015, 10(7), e0132313. doi: 10.1371/journal.pone.0132313
16. Shan D , Yuan Y, Wang Y, Chen C, Gao C, Yu S, Liu Y, Song WA, Zhu H, Yang L, Deng H, Su Y, Yi X. Non-invasive prenatal diagnosis of lethal skeletal dysplasia by targeted capture sequencing of maternal plasma. Published: July 19, 2016, http://dx.doi.org/10.1371/journal.pone.0159355
17. Chitty LS, Mason S, Barrett AN, McKay F, Lench N, Daley R, Jenkins LA. Non-invasive prenatal diagnosis of achondroplasia and thanatophoric dysplasia: next-generation sequencing allows for a safer, more accurate, and comprehensive approach. Prenat Diagn 2015, 35(7), 656-62, doi: 10.1002/pd.4583
18. Orhant L, Anselem O, Fradin M, Becker PH, Beugnet C, Deburgrave N, Tafuri G, Letourneur F, Goffinet F, Allach El Khattabi L. et al. Droplet digital PCR combined with minisequencing, a new approach to analyze fetal DNA from maternal blood: application to the non-invasive prenatal diagnosis of achondroplasia - Version of Record online: 7 APR 2016, doi: 10.1002/pd.4790
19. Lun FM, Tsui NB, Chan KC, Leung TY, Lau TK, Charoenkwan P. et al. Noninvasive prenatal diagnosis of monogenic diseases by digital size selection and relative mutation dosage on DNA in maternal plasma. Proc Natl Acad Sci U S A 2008, 105, 19920-5.
20. Barrett AN, McDonnell TC, Chan KC, Chitty LS. Digital PCR analysis of maternal plasma for noninvasive detection of sickle cell anemia. Clin Chem 2012, 58, 1026-32.
21. Tsui NB, Kadir RA, Chan KC, Chi C, Mellars G, Tuddenham EG. et al. Noninvasive prenatal diagnosis of hemophilia by microfluidics digital PCR analysis of maternal plasma DNA. Blood 2011, 117, 3684-91.
22. Tsui NB, Hyland CA, Gardener GJ, Danon D, Fisk NM. et al. Noninvasive fetal RHD genotyping by microfluidics digital PCR using maternal plasma from two alloimmunized women with the variant RHD(IVS3+1G>A) allele. Prenat Diagn 2013, 33, 1214-6.
23. Gross SJ, Stosic M, McDonald-McGinn DM, Bassett AS, Norvez A, Dhamankar R, Kobara K, Kirkizlar E, Zimmermann B, Wayham N, Babiarz JE, Ryan A, Jinnett KN, Demko Z, Benn P. Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome. Ultrasound Obstet Gynecol 2016, 47(2), 177-83.
24. Verhoef TI, Hill M, Drury S, Mason S, Jenkins L, Morris S, Chitty LS. Non-invasive prenatal diagnosis (NIPD) for single gene disorders: cost analysis of NIPD and invasive testing pathways 2016, 36(7), 636-42.
25. Yin A, Peng CF, Zhao X, Caughey BA, Yang JX, Liu J, Huang WW, Liu C, Luo DH, Liu H, Chen Y, Wu J, Hou R, Zhang M, Ai M, Zheng L, Xue RQ, Mai MQ, Guo FF, Qi Y, Wang D, Krawczyk M, Zhang D, Wang Y, Huang Q, Karin M, Zhangegi K. Noninvasive detection of fetal subchromosomal abnormalities by semiconductor sequencing of maternal plasma DNA. Proc Natl Acad Sci U S A 2015, 112(47), 14670-5, doi: 10.1073/pnas.1518151112
26. Mardy A, Wapner RJ. Confined placental mosaicism and its impact on confirmation of NIPT results. American Journal of Medical Genetics Part C: Seminars in Medical Genetics Special Issue: Laird G. Jackson, M.D. Celebratory Festschrift 2016, 172(2) 118-22.