Best Practice & Research Clinical Obstetrics and Gynaecology 42 (2017) 26–38

Contents lists available at ScienceDirect

Best Practice & Research Clinical Obstetrics and Gynaecology

journal homepage: www.elsevier.com/locate/bpobgyn

3

Noninvasive prenatal testing for fetal aneuploidy and single gene disorders

Hannah Skrzypek, MBBS (Hons), MRepMed, MRANZCOG, Maternal Fetal Medicine Fellow [a], Lisa Hui, MBBS, PhD, GradCertClinEpi, FRANZCOG, CMFM, DDU, Associate Professor Obstetrics and Gynaecology [a, b, c, d, *]

[a] *Department of Perinatal Medicine, Mercy Hospital for Women, 163 Studley Rd, Heidelberg, VIC 3084, Australia*
[b] *Translational Obstetrics Group, University of Melbourne, 163 Studley Rd, Heidelberg, VIC 3084, Australia*
[c] *Public Health Genetics Group, Murdoch Childrens Research Institute, Royal Children's Hospital, 50 Flemington Rd, Parkville, VIC 3052, Australia*
[d] *The University of Melbourne, Parkville, VIC 3010, Australia*

Keywords:
noninvasive prenatal testing
noninvasive prenatal screening
cell-free DNA
aneuploidy
prenatal screening

Our concept of nucleic acid biology has advanced dramatically over the past two decades, with a growing appreciation that cell-free DNA (cfDNA) fragments are present in all body fluids including plasma. In no other field has plasma DNA been as rapidly translated into clinical practice as in noninvasive prenatal testing (NIPT) for fetal chromosome abnormalities. NIPT is a screening test that requires confirmation with diagnostic testing, but other applications of cfDNA provide diagnostic information and do not require invasive testing. These applications are referred to as noninvasive prenatal diagnosis (NIPD) and include determination of fetal sex, blood group and some single gene disorders. As technology advances, noninvasive tests based on cell-free nucleic acids will continue to expand. This review will outline the technical and clinical aspects of NIPT and NIPD relevant to the daily practice of maternity carers.

* Corresponding author. Mercy Hospital for Women, 163 Studley Rd, Heidelberg, VIC 3084, Australia.
E-mail address: lisa.hui@unimelb.edu.au (L. Hui).

http://dx.doi.org/10.1016/j.bpobgyn.2017.02.007
1521-6934/

Introduction

Cell-free DNA (cfDNA) and RNA fragments are present in all body fluids in a dynamic state of constant turnover. CfDNA is comprised of small fragments of extracellular DNA that circulate freely in the bloodstream [1]. Technological progress in molecular genetics has enabled scientists to utilize this uniquely accessible genetic material for a whole new generation of blood tests that detect and monitor disease. In the field of obstetrics, this circulating DNA can be used for the noninvasive detection of fetal genetic abnormalities such as trisomy 21. In what has been a very rapid translation into clinical practice, noninvasive prenatal testing (NIPT) has revolutionised prenatal screening. Providers of prenatal care need to be familiar with the technical and clinical aspects of NIPT and these will be reviewed here.

Voluntary prenatal screening for fetal anomalies is a key component of antenatal care in developed countries and offers couples the option of obtaining more genetic information about their pregnancy. Although for some this information is not desired, for others, screening for common chromosome conditions such as Down syndrome (trisomy 21) provides an important opportunity to inform management. As diagnostic testing with amniocentesis or chorionic villus sampling (CVS) carries a cost and a procedural risk of miscarriage, screening tests are offered to better target diagnostic testing. Traditional screening approaches for trisomy 21 have considerable room for improvement, with detection rates of approximately 80–90%, for an average false positive rate of 5%. These performance characteristics translate to a substantial proportion of missed diagnoses and false positive "high risk" results leading to unnecessary invasive diagnostic testing [2].

Research efforts over the past two decades have therefore focused on developing a noninvasive yet highly accurate screening test that would increase detection and reduce false positive results. The combination of the discovery of cfDNA of fetal/placental origin in 1997 [3] and the rapid advances in DNA sequencing technology have combined to make this noninvasive approach a clinical reality in less than 15 years.

Biology of cfDNA

The majority of cfDNA in plasma is derived from haematopoietic cells that release fragments of DNA during cell turnover, but a large variety of solid organs also contribute to the circulating plasma pool [4]. In the non-pregnant state, the genomic profile (i.e. the proportional representation of each chromosome) in plasma cfDNA simply reflects the individual's karyotype and the size of each chromosome. During pregnancy however, cfDNA from the placenta is also released into the maternal plasma and this can be used to detect fetal chromosomal or genetic abnormalities (Figure 1).

The existence of cfDNA of fetal/placental origin in maternal plasma was first demonstrated through the detection of Y-chromosome specific sequences [3]. These DNA fragments, which originate from the outer cytotrophoblast, are released into the maternal circulation during apoptosis [5,6]. CfDNA arising from the placenta – so-called "cell-free fetal DNA" (cffDNA) – is detectable in maternal plasma from as early as 5 weeks gestation [7] and comprises 10–15% of the total plasma cfDNA [8]. CffDNA levels rise gradually with increasing gestation and are cleared quickly from the maternal circulation after delivery [9,10]. The circulating fetal DNA therefore represents the current pregnancy and does not persist from prior pregnancies.

Nomenclature

Prenatal testing based on analysis of cffDNA in the maternal plasma has been called *noninvasive prenatal testing* (NIPT) or *noninvasive prenatal screening* (NIPS) to distinguish it from traditional diagnostic methods of directly assessing the fetal karyotype using cells obtained via amniocentesis or CVS. Testing for fetal aneuploidy with cffDNA in maternal plasma is a *screening* test – it does not achieve diagnostic accuracy for aneuploidy and requires confirmation with pre or postnatal karyotyping.

Some applications of cffDNA can provide fetal diagnostic information and do not require subsequent confirmation with invasive testing. These applications are called *noninvasive prenatal diagnosis* (NIPD) and are currently primarily confined to the detection of paternally inherited conditions in the fetus (i.e. unique fetal DNA sequences that are not present in the woman's genotype), though research in the diagnosis of recessive conditions and de novo mutations continues to progress [11].