Chapter 8
Novel Non-invasive Prenatal Diagnosis as Related to Congenital Adrenal Hyperplasia

Joe Leigh Simpson and Farideh Bischoff

Prenatal treatment of female fetuses having congenital adrenal hyperplasia (CAH) requires diagnosis of gender for genotype prior to genital differentiation. This approach currently involves chorionic villus sampling (CVS). In experienced hands CVS carries minimal risk of pregnancy loss, comparable to that associated with amniocentesis. Although offering considerable advantage by first trimester diagnosis, CVS is typically performed no earlier than 9-week gestation (7-week embryonic age) and usually not until 10–12 weeks. The procedure can be performed as early as 6–7 weeks, but it is technically difficult at that gestational age and associated with limb reduction defects. A preferable approach is definitive *non-invasive* diagnosis of fetal disorders. This could be achievable by analysis of either intact fetal cells or cell-free fetal DNA in maternal blood.

Intact Fetal Cells

Intact embryonic or fetal cells exist but are rare in maternal blood (1 fetal cell per cc or $10^7/10^8$ maternal cells); thus, one must enrich a maternal blood sample for the efficient analysis of rare fetal cells. Recovery can be accompanied through the use of beads to which antibodies are attached or by cell capture devices (i.e., microfluid-based system). These devices capture cells by attachment chemistry and fluid dynamics. One such device is CEE^{TM} (Biocept, Inc.), a hollow device (75 μm $\times$ 12 mm $\times$ 30 mm) that contains randomly distributed vertical posts. Microfluid-based cell capture devices can be coated with capture antibody (i.e., glycophorin-A (Gly-A)) to enrich for the fetal cell population. FISH (21-specific; X- and Y-centromeric probes) can then be performed within the device using standard fluorescent microscopy.

J.L. Simpson (✉)
Herbert Wertheim College of Medicine, Florida International University, Miami, FL, USA
e-mail: joe.simpson@fiu.edu

M.I. New, J.L. Simpson (eds.), *Hormonal and Genetic Basis of Sexual Differentiation Disorders and Hot Topics in Endocrinology*, Advances in Experimental Medicine and Biology 707, DOI 10.1007/978-1-4419-8002-1_8,

In pregnancies with a male fetus, fetal red blood cell detection was achieved in the majority of pregnancies, as validated by a Y interphase signal concomitant to independent confirmation by PCR of a Y sequence. Accuracy is essentially 100% in informative cases. Confirming fetal/embryonic cell origin in pregnancies with a female fetus requires an independent marker to distinguish fetal XX from maternal XX cells. However, difficulty arises in preserving optimal integrity for chromosome-specific FISH probes in the presence of independent markers, for which reason far fewer pregnancies with a female fetus are informative. Thus, intact fetal red blood cells in blood are considered to have limited value at present for definitive non-invasive prenatal diagnosis.

Cell-Free Fetal DNA

An alternative for definitive non-invasive prenatal diagnosis involves cell-free fetal DNA (cffDNA) in maternal blood. At least 5% of cell-free DNA in first trimester maternal blood is derived from the fetus; cffDNA can be recovered from plasma or maternal whole blood, through controlled lysis of cells (targeting weaker, apoptotic, fetal cells) and size fractionation. Cell-free fetal DNA for prenatal diagnosis is applicable earlier in pregnancy (6-week gestation) than CVS and is without risk of pregnancy loss. The strategy employed involves excluding a mutant paternal allele whose transmission is necessary for the fetus to be affected, even if substantial admixture exists with fetal and maternal blood. Cytogenetic indications include detecting fetal trisomy, being pursued by analyzing chromosome-specific fetal versus parental polymorphisms or by massive parallel genomic sequencing. Specific single gene indications include the following: (a) Determining fetal sex in couples at risk for X-linked recessive traits; (b) detecting a mutation transmitted from a father who has a DNA sequence that the mother lacks (e.g., Marfan syndrome or achondroplasia), as discussed above; (c) determining Rh(D) fetal status in an Rh-negative (dd) mother whose partner is heterozygous (Dd); and now (d) performing linkage analysis to detect 21-hydroxylase deficiency, linkage analysis required given existence of pseudogenes.

In conclusion, prenatal diagnosis has evolved from invasive diagnostic tests to risk-free definitive non-invasive diagnosis (cffDNA), while management can be initiated earlier in pregnancy.