Noninvasive prenatal screening or advanced diagnostic testing: caveat emptor

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The past few years have seen extraordinary advances in prenatal genetic practice led by 2 major technological advances; next-generation sequencing of cell-free DNA in the maternal plasma to noninvasively identify fetal chromosome abnormalities, and microarray analysis of chorionic villus sampling and amniotic fluid samples, resulting in increased cytogenetic resolution.

Noninvasive prenatal screening of cell-free DNA has demonstrated sensitivity and specificity for trisomy 21 superior to all previous screening approaches with slightly lower performance for other common aneuploidies. These tests have rapidly captured an increasing market share, with substantial reductions in the number of chorionic villus sampling and amniocentesis performed suggesting that physicians and patients regard such screening approaches as an equivalent replacement for diagnostic testing. Simultaneously, many clinical programs have noted significant decreases in patient counseling.

In 2012 the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development funded a blinded comparison of karyotype with the emerging technology of array comparative genomic hybridization showing that in patients with a normal karyotype, 2.5% had a clinically relevant microdeletion or duplication identified. In pregnancies with an ultrasound-detected structural anomaly, 6% had an incremental finding, and of those with a normal scan, 1.6% had a copy number variant.

For patients of any age with a normal ultrasound and karyotype, the chance of a pathogenic copy number variant is greater than 1%, similar to the age-related risk of aneuploidy in the fetus of a 38 year old. This risk is 4-fold higher than the risk of trisomy 21 in a woman younger than 30 years and 5- to 10-fold higher than the present accepted risk of a diagnostic procedure. Based on this, we contend that every patient, regardless of her age, be educated about these risks and offered the opportunity to have a diagnostic procedure with array comparative genomic hybridization performed.

Key words: array comparative genomic hybridization, cell-free fetal DNA, chorionic villus sampling, fetal chromosome abnormalities, maternal serum combined screening, next-generation sequencing

Progress in science predictably leads to improvements in medical care. Historically, new laboratory tests and procedures go through 2 major phases.[1,2] First, there is a phase of development in which investigators have an idea leading to a testable hypothesis. This is followed by testing, refinement, patent applications, and publication of results. Once proof of concept is accepted and the product is ready for translation to care, creation of a market occurs based on demand for the new approach. This is followed by a phase of diffusion in which the new concept moves into the broader community.

Predictably, as the new concept is adopted by practicing physicians and their patients, there is a rapid expansion of utilization with variable levels of physician training and understanding, resulting in performance somewhat less than that initially projected. Eventually, with increasing community exposure, there is improvement in performance and better recognition of the appropriate integration into care.

Over the past several years, 2 important tools in screening and testing for fetal chromosomal abnormalities have developed on separate tracks: microarray analysis of chorionic villus sampling and amniotic fluid samples, resulting in increased cytogenetic resolution, and next-generation sequencing of cell-free DNA in the maternal plasma to noninvasively identify common fetal chromosome abnormalities (Table 1).

These technologies have had widely divergent pathways toward incorporation into prenatal practice. Before widespread introduction into care, fetal microarray analysis was vetted through blinded National Institutes of Health–funded trials performed by agnostic investigators. This approach was similar to that taken with other paradigm-changing prenatal diagnostic and therapeutic modalities such as chorionic villus sampling, first-trimester aneuploidy screening using biochemistry and nuchal translucency, antenatal steroids for fetal lung maturation, progesterone for the prevention of preterm birth, and many others.[3-5]

On the other hand, cell-free DNA screening has been a laboratory-developed test marketed by the developers to the practitioner and patient communities without multiple, independent studies and trials validating its performance prior to its introduction into the marketplace.

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TABLE 1
New laboratory methods for screening and diagnosis

Methods	Description
Array comparative genomic hybridization (also known as microarray)	Array comparative genomic hybridization is a molecular cytogenetic technique used for the determination of DNA copy number (deletions or duplications). Array comparative genomic hybridization can interrogate for the presence or absence of much smaller segments of DNA than can be seen by karyotype. There are enormous differences in the resolution of array comparative genomic hybridization methods varying from nearly whole chromosomes that are virtually indistinguishable from levels provided by fluorescent in situ hybridization to very high resolutions. Low resolution is used for fresh transfers in preimplantation diagnosis with in vitro fertilization and by some laboratories on amniocentesis and chorionic villus sampling specimens. High resolution is currently possible only on direct fetal material such as amniocentesis and chorionic villus sampling. The higher the resolution, the greater the finding of abnormalities but the greater the incidence of variants of uncertain significance segments. By lowering the resolution, the variants of uncertain significance segment percentage goes down, but so does the finding of abnormalities. Because the amount of DNA is much higher in chorionic villus sampling specimens than amniocentesis, chorionic villus sampling array comparative genomic hybridization usually has a faster result time than amniocentesis.
Next-generation sequencing (also called Next Gen) or massively parallel sequencing	These are similar approaches of modern sequencing techniques through which an entire genome can be sequenced within a day. Next-generation sequencing platforms sequence millions of small fragments of DNA simultaneously in parallel. Bioinformatics can then identify the origin of the fragments and map them to the reference human genome. Each of the DNA bases are sequenced multiple times so that variation in the genome can be detected.
Massively parallel sequencing	This uses a next-generation platform and bioinformatics to categorize the origin of fragments. By determining the number of fragments from specific chromosomes (or portions of chromosomes) and comparing this with the expected contribution, additional or missing chromosomal material can be diagnosed. For example, chromosome 21 normally contributes about 1.32% of all the DNA. If there is 2% in a sample, this suggests 3 copies of chromosome 21. This is one approach used in noninvasive prenatal screening, also called cell-free fetal DNA.
Selective sequencing and selective probes	In another approach to noninvasive prenatal screening, fragments from only selected chromosomes of interest (eg, 21, 18, 13) are sequenced or probed. These probes or sequencing for chromosome (eg, 21, 18 and 13) combined with the fraction of fetal DNA in the maternal circulation are used to screen for fetal aneuploidy. Noninvasive prenatal screening methods can all vary by the depth of discrimination detected and/or reported (ie, how small a fragment difference they can evaluate). All of these have high sensitivities for detecting trisomy 21 but lower performance for other trisomies (eg, trisomies 13 and 18 and sex chromosomes) and much lower performance for microdeletions and duplication syndromes (eg, DiGeorge, Prader-Willi, Angelman).
Single-nucleotide polymorphisms	These are single base pair differences in the genome and are the most common type of DNA variation in man.
Copy number variants	These reflect changes in sequences of DNA: duplications or deletions. These copy number variants can be clearly pathological, ambiguous, or benign.
Variants of uncertain significance	The percentage of ambiguous copy number variants are called variants of uncertain significance. These have decreased in percentage from about 2% several years ago to now less than 1% as with increasing experience, formerly variants of uncertain significance segments can now be classified as pathological or benign.

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This commentary represents the opinions of the 3 authors who have each been involved in prenatal diagnosis and screening for more than 30 years. It is intended to give our perspective on the relative advantages and disadvantage of the emerging technologies and to present potential pathways to clinical translation. Because many of the terms used may be new to many readers, we provide a short explanation of relevant ones (Table 1).

Improved diagnostic capability: chromosomal microarrays

The resolution of prenatally performed standard karyotypes is limited by the use of light microscopy and is typically quoted at about 7 million base pairs but in clinical, prenatal practice may be 10 million base pairs or higher. Molecular cytogenetic analyses such as array comparative genomic hybridization (array comparative genomic hybridization, or microarrays) does not require direct visualization of the chromosomes and has the ability to identify much