*Current Commentary*

# Current Status of Testing for Microdeletion Syndromes and Rare Autosomal Trisomies Using Cell-Free DNA Technology

*Yuval Yaron, MD, Jacques Jani, MD, Maximilian Schmid, MD, and Dick Oepkes, MD*

**Noninvasive prenatal testing using cell-free DNA in maternal blood for trisomy 21 was introduced in 2011. This technology has continuously evolved with the addition of screening for trisomy 18 and trisomy 13 followed by the inclusion of sex chromosome aneuploidies. Expanded noninvasive prenatal test panels have recently become available, which enable screening for microdeletion syndromes such as the 22q11.2 deletion (associated with the velocardiofacial syndrome) and others. However, the performance data for these microdeletion syndromes are derived from a small number of samples, mostly generated in vitro. Rigorous performance evaluation, as was done at least for trisomy 21 testing using cell-free DNA analysis, is difficult to perform given the rarity of each condition. In addition, detection rates may vary considerably depending on deletion size. Importantly, positive predictive values (PPVs), strongly influenced by the low prevalence, are expected to be significantly lower than 10% for most conditions. Thus, screening in an average-risk population is likely to have many more false-positives than affected cases detected. Conversely, testing in a high-risk population such as fetuses with cardiac anomalies may have higher PPVs, but a negative result needs to be considered carefully as a result of uncertain information about detection rates and a significant residual risk for other copy number variants and single gene disorders. This article integrates current knowledge on cell-free DNA testing for microdeletions with the aim to assist clinicians and policymakers in designing optimal programs for screening in pregnancy.**

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Analysis of cell-free DNA in maternal blood as a noninvasive method for the detection of trisomy 21 became available to pregnant women in October 2011. Since its introduction, the application of this technology has continuously evolved, first with the addition of screening for trisomy 18 and trisomy 13 followed by sex chromosome aneuploidies and recently by the introduction by some laboratories of testing for a small number of known microdeletions syndromes as well as a few rare trisomies. The expansion of cell-free DNA testing panels to include microdeletions may be driven by several distinct goals. First, it appears logical to use the information that can be obtained by cell-free DNA testing to the fullest to offer noninvasive assessment for as many conditions as possible. Second, the initial reason to develop noninvasive testing, the desire to eliminate the complications associated with invasive testing, would be best achieved if cell-free DNA analysis could approximate the capabilities of invasive methods, particularly with the use of chromosomal microarray. However, before concluding that testing for more conditions is inherently better, it is important to take a closer look at the implications of testing for these additional conditions using the currently available technology.

## ESTIMATED DISEASE PREVALENCE AND REPRESENTATION OF DELETIONS

The estimated prevalence of the conditions included in some of the microdeletion panels vary over

*From the Genetic Institute, Tel Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel; University Hospital Brugmann, Université Libre de Bruxelles, Brussels, Belgium; Medical University of Vienna, Vienna, Austria; and Leiden University Medical Centre, Leiden, The Netherlands.*

*Corresponding author: Yuval Yaron, MD, Prenatal Genetic Diagnosis Unit, Genetic Institute, Tel Aviv Sourasky Medical Center, 6 Weizmann Street, Tel Aviv 64239, Israel; e-mail: yuvaly@tlvmc.gov.il.*

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a wide range,[1–11] as described in Table 1. Most of these data is based on postnatal studies and thus does not account for any affected fetuses lost in utero. The increasing use of microarray technology on prenatal samples from invasive diagnostic procedures may provide better estimates of prevalence for these conditions.

## CURRENT LANDSCAPE IN PRENATAL MICRODELETION TESTING BY CELL-FREE DNA

Currently, several noninvasive prenatal test laboratories provide "expanded panels" that include testing for some microdeletion syndromes using cell-free DNA. Panels include the 22q11.2 deletion (associated with the velocardiofacial, or DiGeorge, syndrome), 1p36 deletion, 15q11.2-q13 deletions (associated with Prader-Willi and Angelman syndromes), terminal deletion of the 5p (Cri-du-Chat syndrome), and terminal deletion of 4p (Wolf Hirschhorn syndrome). Some companies offer testing for these conditions as an "opt-in" choice, some include all available microdeletions by default and requisition forms allow to "opt-out" of the entire expanded panel, whereas others offer an "expanded panel" and a "regular panel," which includes the 22q11.2 deletion by default.

As a result of the relative rarity of most microdeletion syndromes, an adequate clinical study to assess detection rates and positive predictive values (PPVs) may be difficult to perform. Alternative study designs using archived samples and artificial mixtures of abnormal DNA in maternal plasma have been used to provide at least an estimate of test accuracy. Detection rates quoted by companies range from 60% to greater than 99%.[12]

In this context, it is important to mention that performance is dependent on deletion size, with 3 Mb being the approximate lower limit for detection using the current methodology. However, approximately 15% of all cases of 22q11.2 deletions are smaller than that. Thus, quoting a detection rate greater than 97% may be misleading because this refers to performance for detection of deletions greater than 3 Mb only rather than all clinically relevant ones. False-positive rates, important in light of attempting to reduce invasive testing, were 0.8% for a single microdeletion in this study.[12] As a result of the relatively small number of patients studied and the resulting wide confidence intervals for these numbers, the actual false-positive rate for the entire panel could well exceed 1%.

## POSITIVE PREDICTIVE VALUE IN AN AVERAGE-RISK POPULATION

Unlike the common aneuploidies, microdeletions are not known to increase in frequency with maternal age. In addition, with the possible exception of 22q11.2 deletions and monosomy 1p36, the microdeletions on the currently offered cell-free DNA panels are unlikely to have fetal abnormalities that are visible on routine prenatal ultrasonography. Offering these microdeletion panels to all women undergoing cell-free DNA testing is essentially offering microdeletion screening to an average-risk population.

A useful way to consider the value of testing for microdeletions using cell-free DNA is by looking at the PPV. The PPV is the proportion of positive test results that represents "true-positives." This value is influenced by test performance and prevalence. Thus, for the most common 22q11.2 microdeletion, assuming a detection rate of 97.8%, a specificity of 99.24%, and a prevalence of one per 4,000 to one per 2,000, the PPV would be 3.2–6.2%, respectively.[12] For conditions with a lower prevalence, the PPVs will be even lower (Fig. 1). Thus, in a general screening population, most screen-positive cases will be false-positive.

## NEGATIVE PREDICTIVE VALUE IN AN AVERAGE-RISK POPULATION

Negative predictive value is the proportion of negative test results, which represent "true-negatives." In an average-risk population, the negative predictive

**Table 1. Population Estimates of Disease Prevalence and Representation of Detectable Deletions**

| Syndrome | Chromosome Location | Estimated Prevalence | Deletion Size (Mb) | % With 3 Mb or Greater Deletion |
|---|---|---|---|---|
| DiGeorge or velocardiofacial | 22q11.2 | 1:2,000[1]<br>1:4,000[2] | 3 (common deletion)[3] | 85 |
| Monosomy 1p36 | 1p36 | 1:5,000<br>1:10,000[4] | 1.5–10.5[5,6] | 85 |
| Angelman or Prader-Willi | 15q11.2-q13 | 1:20,000 | 5 and 6 (common deletions)[7,8] | 70[7,8] |
| Cri-du-Chat | 5p15 | 1:50,000[9] | 10–30[10,11] | 99[10,11] |

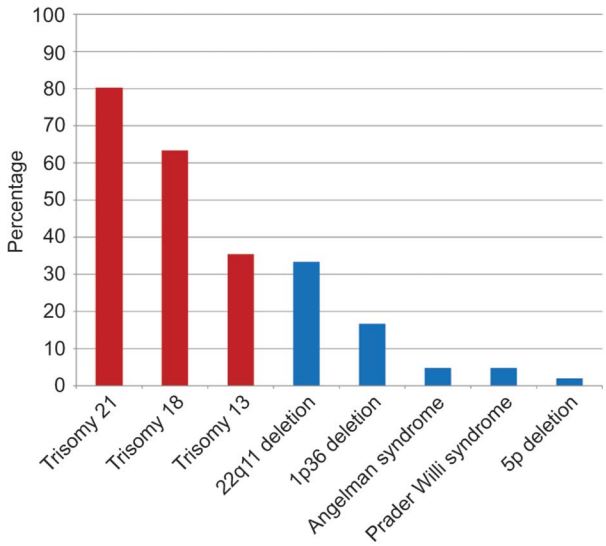


**Fig. 1.** Theoretical positive predictive values (PPVs) of cell-free DNA testing. The prevalence of known trisomies (*red*) and microdeletions (*blue*) was used to predict the expected PPVs for a test with a sensitivity of 100% and a false-positive rate of 0.1%. Theoretical PPVs are based on the following prevalence for each condition in a pregnant women aged 35 years at the time of screening: trisomy 21, 1 in 249; trisomy 18, 1 in 580; trisomy 13, 1 in 1,826[21]; 22q11 deletion, 1 in 2,000[1]; 1p36 deletion, 1 in 5,000[4]; Angelman syndrome, 1 in 20,000[7]; Prader-Willi syndrome, 1 in 20,000[8]; 5p deletion, 1 in 50,000.[9]

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value for the specific microdeletions on these panels remains high (greater than 99%) regardless of the detection rates and false-positive rate used. This is more the result of the relative rarity of the microdeletion syndromes rather than the test performance itself. Moreover, and highly relevant to the average-risk pregnant woman seeking reassurance, the deletions on these panels represent a relatively small proportion of all possible submicroscopic changes. One large prenatal microarray series demonstrated that clinically relevant fetal microdeletions or microduplications were present in 1.7% of samples whose clinical indications were advanced maternal age or positive serum screening results.[13] Closer examination of the microarray data reveals that, depending on the clinical indication, the deletions on the microdeletion panels represent approximately 6–11% of the clinically relevant deletions and duplications found.[13,14] Given a 1.7% a priori risk of any clinically significant microdeletion or microduplication being present in the fetus,[13] a negative microdeletion screening panel in an average-risk patient will, within the current range of reported detection rates and false-positive rate, only modestly reduce the risk to 1.6%. Therefore, cell-free DNA testing currently does not come close to invasive testing for clinically significant microdeletions using microarray.

## PREDICTIVE VALUE IN A HIGH-RISK POPULATION

Studies of pregnancies with a fetal cardiac abnormality diagnosed prenatally have identified 22q11.2 deletions in 5–10% of cases, depending on the defect seen.[15,16] Given the higher prevalence of 22q11.2 deletion in this population, the PPV of cell-free DNA testing for this condition will increase proportionally. If one recalls that the lower limit of size of a microdeletion detectable by current technology is 3 MB, the increase in PPV of 22q11.2 deletion for high-risk populations comes at a dire cost because approximately 15% of 22q11.2 deletions are only 0.75 Mb in size and will not be detected. Moreover, such cardiac anomalies may be the result of a different chromosomal copy number variant or other genetic etiology.[17,18] A negative cell-free DNA test leaves many genetic anomalies unrecognized before birth. Thus, it is obvious that cell-free DNA testing should not be an acceptable alternative to invasive testing in patients with prenatal-detected cardiac abnormalities.

## RARE AUTOSOMAL TRISOMIES

Laboratories are also including screening for triploidy, trisomy 9, trisomy 16, and trisomy 22, each of which has the potential to add an additional 0.1% or more to the false-positive rate (personal communication). Reported false-positive rates vary by laboratory; however, the addition of these expanded panels to fetal aneuploidy screening may push the cumulative false-positive rate closer to 2%, or even higher. Moreover, any screening test with false-positive results for lethal anomalies that are very hard to miss on ultrasound examination, such as triploidy and trisomy 13, could be regarded as unjustified. This also may be argued for trisomy 16 and 22, in which most embryos or fetuses are spontaneously aborted in the first trimester (unless mosaic). Screening is particularly beneficial for non-lethal diseases leading to a life with serious handicaps for which parents may choose termination or for which beneficial treatments or other useful perinatal management options exist. Therefore, even before cell-free DNA testing, the inclusion of trisomies 18 and 13 in first-trimester screening algorithms has been a matter of controversy.

## CELL-FREE DNA TESTING FOR MICRODELETIONS: HOW TO PROCEED

The best way to evaluate the overall benefit compared with harm of new screening tests is to carefully

analyze the effects of implementing such tests on the overall performance and unwanted side effects of the complete screening program.[19] Programs can be adapted by adding new tests and keeping the existing ones, by replacing old by new tests, or variations such as changing existing cutoff levels for calling certain steps in the screening process "positive."[20] Some of these evaluations can be done by modeling; however, clinical reality might be quite different from expectations, in particular uptake is difficult to predict. The least we should do, as clinicians and organizations promoting changing current screening protocols, is to critically analyze the effect of the innovations and to be willing to redesign our programs when our expectations are not met. It is up to those offering expanded panels including microdeletion syndromes and rare trisomies to provide up-to-date performance data including PPVs and negative predictive values, and false-positive and -negative rates. These data need to come from comprehensive clinical validation studies in the population to be tested rather than in vitro studies or analysis of a limited amount of preselected samples. Such studies ideally should be prospective, blinded, and have outcome data on all participants. For very rare conditions, this may not be possible. Therefore, the true sensitivity and specificity will remain uncertain. It is obvious that for transparency for the patient and conservation of health resources, the "opt-out" option must be clear for the ordering clinician and the patient for microdeletion testing.

Finally, it is clear that health policy needs to be primarily based on good evidence, but also involves much broader political as well as socioeconomic consideration. The conversation on which conditions deserve prenatal screening and what standards to accept in doing so cannot be left to commercial companies alone.

## DISCUSSION

Cell-free DNA testing is an exciting new technology that has already changed the prenatal testing paradigm for common fetal aneuploidies. The potential to expand from assessment for traditional screening targets to genome-wide detection of copy number variants appears to be of interest to clinicians. One step in this process has been the introduction of expanded panels that include a small list of rare microdeletion syndromes and additional aneuploidies. Integration of these expanded panels into clinical practice will require comprehensive published clinical validation studies and a demonstration of PPV and clinical utility in the population to be tested. However, current evidence regarding performance is based on scant data, of which very little was obtained from actual pregnancies with fetuses affected with the conditions tested. Furthermore, false-positive rates are cumulative. Thus, as the number of target conditions increases, the cumulative false-positive rate will increase. In exchange for the additional false-positives, the expanded panels may not add significantly to the overall detection of clinically relevant microdeletion syndromes in the general population. These facts should be conveyed to the patients during pretest counseling so that well-informed choices can be made.

To conclude, cell-free DNA testing for microdeletion syndromes and rare autosomal trisomies is currently unsupported by sufficient clinical evidence. Routine testing for these conditions should await comprehensive clinical validation studies and a demonstration of PPV and clinical utility in the population to be tested.

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