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REVIEW

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Noninvasive screening by cell-free DNA for 22q11.2 deletion: Benefits, limitations, and challenges

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Abstract

Cell-free DNA (cfDNA) testing for fetal aneuploidy is one of the most important technical advances in prenatal care. Additional chromosome targets beyond common aneuploidies, including the 22q11.2 microdeletion, are now available because of this clinical testing technology. While there are numerous potential benefits, 22q11.2 microdeletion screening using cfDNA testing also presents significant limitations and pitfalls. Practitioners who are offering this test should provide comprehensive pretest and posttest prenatal counselling. The discussion should include the possibility of an absence of a result, as well as the risk of possible discordance between cfDNA screening results and the actual fetal genetic chromosomal constitution. The goal of this review is to provide an overview of the cfDNA testing technologies for 22q11.2 microdeletions screening, describe the current state of test validation and clinical experience, review “no results” and discordant findings based on differing technologies, and discuss management options.

1 | INTRODUCTION

Cell-free DNA (cfDNA) testing for fetal aneuploidy is one of the most important technical advances in prenatal care, and its uptake has increased rapidly. It is based on quantification or qualification of the cfDNA fragments circulating in the maternal plasma, which are derived from both the mother and conceptus, specifically from the apoptosis of the cytotrophoblast, the external layer of the placenta.[1-3] The maternal cfDNA contribution to the overall circulating fragments greatly exceeds the placental contribution, also known as the “fetal fraction.”

Additional chromosome targets beyond common aneuploidies, including screening for 22q11.2 deletion syndrome (22q11.2DS), are offered by some cfDNA testing providers. The screening for 22q11.2DS is proposed as an adjunct to common trisomy screening, either in isolation or in combination with other rarer microdeletions and trisomies. Practitioners who offer this test option should provide comprehensive pretest and posttest prenatal counselling. Key elements of this discussion should include the potential risk for no result and of possible discordance between cfDNA screening results and the actual fetal genetic chromosomal constitution, which may occur because of differing technologies.

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1.1 | 22q11.2DS and prenatal phenotype

The genetic defect underlying the 22q11.2DS is not a single chromosomal entity, but rather represents a group of different microdeletions all located in the 22q11.2 chromosome band. 22q11.2DS is a contiguous gene deletion syndrome inherited in an autosomal dominant manner. Only 10% of individuals will inherit the chromosomal defect from a parent and, of note, often the parent may have a mild phenotype that can go unrecognized, even by health care professionals. This is in keeping with the highly variable expressivity of this condition, even among identical twins, despite complete penetrance.[4-6] Studies have demonstrated enrichment of a maternal origin both for inherited and de novo deletions.[7,8]

The 22q11.2 region is particularly enriched with segmental duplications showing a high level of sequence homology, commonly referred to as “low copy repeats” (LCRs) (Figure 1). The presence of

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these LCRs (from A-H) predisposes this region to recombination errors due to a mechanism called "nonallelic homologous recombination" (NAHR). This mechanism is the underlying cause of the *de novo* cases. In most cases, with 22q11.2DS (85%), there is a typical three megabase microdeletion (=3 million bases, Mb) in size between the LCRs A and D that includes the TBX1 candidate gene. The remaining 15% of patients have "atypical" or "nested" 22q11.2 microdeletions (ranging in size from 1.5 to 0.7 Mb). The 22q11.2DS community has determined that the various syndromes associated with all different types of 22q11.2 deletions (from 0.7 to 3 Mb) should be grouped under the correct name of "22q11.2DS" to limit both clinical and scientific confusion. [6,9] A categorization of the 22q11.2 microdeletions has been attempted based on their size, location, and gene content.[10] The region can be broken down in to proximal, central, and distal deletions. Proximal deletions include A-B, A-D, A-E, and A-F (the majority of which are A-B and A-D deletions). These patients usually (≈90%) have a *de novo* deletion and are likely to have a more severe phenotype. B-D or C-D central deletions do not include the more proximal critical genes, and they are more likely to be inherited (75%). Distal deletions can be further categorized in to three types. Type 1 includes C-E, D-E, and D-F deletions. The phenotype includes the features of 22q11.2DS and, in addition, the risk for preterm birth (69%) prior to 37 weeks of gestation thereby requiring attention to potential complications related to preterm delivery. Most (62%) of these microdeletions are *de novo*. Type 2 includes E-F deletions and, because of the limited number of reported cases, clinical features are not well characterized. Type 3 includes any microdeletion involving SMARCB1 gene and are all *de novo*.[10]

Common postnatal phenotypic findings include growth and developmental delay, cardiac defects, cleft palate, recognizable facial features, learning disabilities, and immunodeficiency.[4-6,11,12] Regarding prenatal cases, to date, there is no data on the detection rate of 22q11.2DS by second trimester detailed ultrasound in an unselected average risk population. However, ultrasound abnormalities in fetuses with 22q11.2DS are quite common. [11-13] In a population of 272 fetuses with 22q11.2DS, the diagnosis was prompted by abnormal ultrasound findings in 86.8% of the cases.[8] Of note, at least one conotruncal and nonconotruncal cardiac defect was identified in 83.3% of this cohort. While cardiac defects rank as the most common prenatal ultrasound anomalies, it is worth bearing in mind that 22q11.2DS is a multiorgan syndrome, and other abnormalities, in addition to heart defects, may be detected.[10-12] Other ultrasound findings include thymic hypoplasia/agenesis (3.7%), craniofacial abnormalities (overt cleft palate/craniosynostosis; 5.9%), renal anomalies (9.2%), increased nuchal translucency/hygroma (7.4%), and polyhydramnios (9.2%). Except for cardiac abnormalities, the other anomalies are frequently found in association with other findings.[8] There are also case reports of 22q11.2DS fetuses with US scan showing congenital diaphragmatic, umbilical or inguinal hernia, tracheoesophageal fistula and oesophageal/laryngeal atresia, polydactyly, polymicrogyria, and dilation of the cavum septum pellucidum.[12,13] While beyond the scope of this review, the 3 and 3 T vessel ultrasound views have been well described and can help detect abnormalities in the outflow tracts.[14] While an effective technique to determine a thymic/thoracic ratio to screen for 22q11.2DS has been proposed, this measurement is helpful to evaluate a fetus with cardiac anomalies but should not be used as an independent screening modality.[15]

What is already known about this topic?

- Cell-free DNA (cfDNA) testing is a well-established technology for the evaluation of risk for fetal trisomies 21, 18, and 13. Despite a lack of comprehensive validation studies, cfDNA testing is already in use as a screening test for 22q11.2 deletion syndrome (22q11.2DS).
- Discordant 22q11.2 deletion findings as well as no definitive result can be encountered in routine practice. However, because of the paucity of clinical reports and the recent introduction of this test, practitioners may face challenges in management, including pretesting and posttesting counselling.

What does this study add?

This review provides an overview of (a) available cfDNA testing technologies for 22q11.2 microdeletion screening, (b) current test validation and clinical experience, (c) no results and discordant findings based on differing technologies, and (d) relevant management options.

1.2 | Prevalence of 22q11.2 deletions

The prevalence of 22q11.2 deletions in prenatal samples depends on the indication. Fetuses with cardiac heart defects (CHD) with or without extracardiac defects, have the strongest association with the presence of 22q11 deletions consistent with the known relationship between 22q11.2DS and congenital heart disease.[16]

However, of considerable interest is the case of an anatomically normal fetus with normal karyotype. Research has determined 22q11.2 deletions' prevalence in the "normal" first and second trimester population to be in the range of 1/800 to 1/1000 if all typical and atypical deletions are included (Table 1).[17,18]

However, although cases diagnosed in pregnancies at earlier gestational ages do not have any ultrasound abnormalities, they may have had abnormal second trimester ultrasound findings if the patients had elected to continue the pregnancies. Therefore, to understand the clinical utility of cfDNA-based screening for 22q11.2DS, it would be helpful to calculate the prevalence of 22q11.2DS in cases with a normal first and second trimester ultrasound investigation.

The prevalence is similar in miscarriages and products of conception.[19-21] This differs from the prevalence in an unselected cohort of neonates, which was found to be 1/3672 in a retrospective analysis of dried newborn bloodspots using microarray technology (CVS/AF vs neonates: OR 4.4821, 95% CI, 1.7370-11.5655; POCs vs neonates: OR 3.6356, 95% CI, 1.5370-8.5994).[22]

This drop in 22q11 deletion prevalence between the prenatal period and birth is likely secondary to elective termination of prenatal

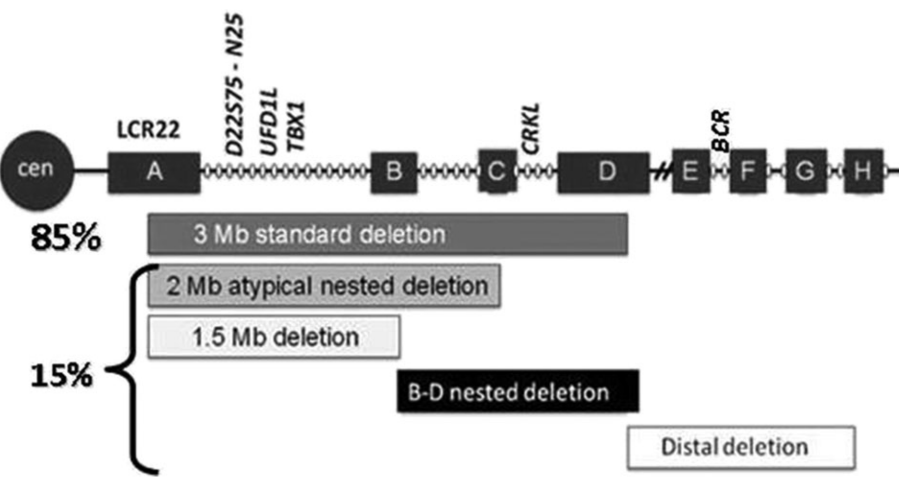

FIGURE 1 Schematic structure of the 22q11.2 chromosomal region. Eighty-five percent of 22q11.2DS patients have the 3 Mb typical deletion; the remaining 15% have other nested atypical deletions. Modified from GeneReviews, Donna M McDonald-McGinn, MS, CGC, Beverly S Emanuel, PhD, and Elaine H Zackai, MD, FACMG

TABLE 1 Calculated prevalence of 22q11.2 microdeletions in products of conception, prenatal samples, and newborns

	Study	22q11.2 Typical Deletions	22q11.2 Atypical Deletions	22q11.2 typical + Atypical Deletions
POCs and miscarriages	Maisenbacher et al, 2016[19]			17/17838
	Rosenfeld et al, 2015[20]—personal communication	2/515		
	Levy et al, 2014[21]—personal communication	1/1861		
	Total	3/2376		20/20 214
	Prevalence (95% CI)	1/792 (1/2328-1/270)		1/1011 (1/1561-1/655)
CVS/AF (anatomically normal fetuses)	Grati et al, 2015[17]	6/5953		
	Wapner et al, 2012[18]	3/3067	4/3067	
	Total	9/9020	4/3067	13/9020
	Prevalence (95% CI)	1/1002 (1/1905-1/528)	1/767 (1/1971-1/299)	1/694 (1/1187-1/406)
Neonates (term)	Sparso et al, 2016[22]			7/25703
	Total			7/25703
	Prevalence (95% CI)			1/3672 (1/7580-1/1179)

Abbreviations: AF, amniotic fluid; CI, confidence interval; CVS, chorionic villi sample; POCs, product of conceptions.

diagnosed cases (due to increased ultrasound scan resolution and hence improved detection of subtle cardiac and multiorgan structural defects at later gestational age) and a natural selection against fetuses with 22q11.2 deletion syndrome, particularly fetuses with the most severe phenotypes.

1.3 | Prenatal diagnosis—The gold standard

The "gold standard" method for detecting both typical and atypical 22q11.2 microdeletions remains chromosomal microarray (CMA), performed on invasively obtained prenatal samples (chorionic villi, CV; amniotic fluid, AF).[4] Depending on design and resolution, CMA can pick up known chromosomal deletions, which are described above. Rare sequence variants of TBX1 gene causing 22q11.2DS cannot be detected by this technology.[23,24] There are some targeted assays, such as Prenatal BACs-on-Beads (PNBoBs), Fluorescence In Situ Hybridization (FISH), and Multiple Ligation-dependent Probe Amplification (MLPA) that can be used for prenatal diagnosis, although with some limitations. Standard FISH analysis with clone D22S75-N25 and PNBoBs can detect the subgroup of microdeletions that include the smallest overlapping A-B region thereby covering the detection of 85% to 95% of 22q11.2 patients.[4] These tests can be applied in familial cases where the microdeletion has already been characterized and confirmed as detectable with these technologies.

Offering CMA testing for prenatal diagnosis in the setting of fetal anomalies is considered standard of care in many countries[25,26] (https://www.sigu.net/show/attivita/5/1/LINEE%20GUIDA; http://www.bsgm.org.uk/media/956141/g144_useofcmapregnancy_jun15.pdf). CMA technology can detect pathogenic copy number variants (CNVs) in approximately an additional 6% of abnormal fetuses with a normal standard karyotype and 9% in those with multiple anomalies.[18,27]

CMA's detection rate is particularly high because of the significant association of 22q11.2DS and cardiac anomalies, with pathogenic chromosomal abnormalities detected in up to 21% of such cases.[28-32] As a result, cfDNA screening would be of reduced clinical utility for pregnancies with ultrasound anomalies because the definitive diagnostic test would be recommended based on the presence of abnormal sonographic findings, regardless of cfDNA screening results.[33] CfDNA screening for 22q11.2DS may be of value in non anomalous

foetuses where there are no other "clues" suggestive of a potentially significant chromosomal anomaly. However, studies addressing clinical utility in this average risk population are still pending, and clear long-term benefits have not yet been demonstrated.

1.4 | Current technologies behind cfDNA testing for fetal 22q11.2DS risk assessment

Targeted and genome-wide cfDNA tests have both been proposed as a screening tests for the detection of panels of microdeletions and microduplications, in particular for 22q11.2DS due to this syndrome's prevalence and clinical importance (Table 2).[34-42]

1.4.1 | Targeted technologies

The general strategy of targeted technologies is to create assays with molecular probes capturing/hybridizing and selectively amplifying only those circulating cfDNA fragments belonging to targeted chromosomes/loci. Then, only the subset of amplified fragments is analysed for risk assessment with different platforms (next generation sequencing [NGS] or microarray) at a high analytic depth. All targeted technologies are designed to interrogate the proximal deletions (A-B, A-C, A-D, A-E, or A-F), which are found in most patients with the characteristic phenotype. Therefore, depending on the type of test design, a minimal residual risk for the remaining uncovered atypical microdeletions should be considered.

Analytic validation of test performance has been reported on artificial samples and real plasma samples for three targeted technologies: single nucleotide polymorphisms-based (SNP-based), digital analysis of selected regions (DANSR), and targeted capture enrichment assay (TCEA) technologies (Table 3).[34-37] Results from experiments with artificial samples created by spiking plasma/genomic samples to simulate cfDNA should be considered with caution as these samples may not reflect the analytical performance of real plasma samples.

SNP-based technology analyses polymorphic loci over the A-D region.[34] Therefore, both the common and nested deletions can potentially be detected in this chromosomal segment. Sensitivity and false positive rate (FPR) with SNP-based technology for the larger 3 Mb A-D deletion were reported as 43/43 (100%) and 0/65 (0%), respectively, using artificial mixtures and 2/3 (75%) and 3/332 (0.9%) with real plasma samples.[34] Recently, a second analytical validation with an enhanced reflex protocol of high risk cases detected with the standard protocol demonstrated a sensitivity and specificity for the 3 Mb deletion of 90% (95% CI, 55.5-99.8) and 99.74% (95% CI, 98.6%-99.99%), respectively.[35] The enhanced protocol included an increased number of SNPs in the 22q11.2 A-D region, sequencing at a higher depth of read (~14 x 10^6 reads/sample), and an increased positive confidence call threshold. However, for 99 unaffected cases with a lower fetal fraction of 2.8% to 6.5%, only the paternal alleles could be interrogated, leaving open the possibility of a false negative call based on the inability to determine whether the fetus has a maternally derived de novo 22q11.2 deletion. In addition, samples reported as "risk unchanged" or "no call" (4.6%) were excluded from specificity calculations. Thus, the projected positive predictive value (PPV) in the general population based on the above performance characteristics is 19.6%.

In a retrospective review of over 80 000 patients, the PPV in the general population was 15.7% for 22q11.2DS with the original protocol.[38,39] Applying a post hoc analysis, using a revised protocol with a higher confidence threshold for reporting a case as high-risk for a microdeletion and reflex sequencing of high-risk cases (≥6 million reads/sample), the estimated PPV for the general cohort was 44.2% (Table 3). Most true positives occurred in the context of an abnormal prenatal ultrasound as 80.6% of screen positive cases had major ultrasound anomalies and, therefore, this study does not represent an unselected population. In cases without US findings at the time of blood sampling or, if present, were detected only after the cfDNA test, the PPV was 6.4% providing further support that PPVs may be inflated because of inclusion of cases with the high risk ultrasound findings,

TABLE 2 Summary of cfDNA testing technologies' characteristics for 22q11.2DS

Type of Test	Type of Technology	Covered 22q11.2 Region	Principle	Pitfalls with Detection
Targeted	SNP-based[34,35] DANSR[36] TCEA[37]	A-D A-C	Capture/hybridization of circulating cfDNA fragments belonging from 22q11.2 region only by complementary molecular probes. Only a restricted subset of amplified fragments is analysed for risk assessment (by NGS or microarray) with high analysis depth	Minimum fetal fraction is required; nested/atypical CNVs may not be detected depending on the type of probes design
Genomewide	MPSS[40,41]	A-H (whole chromosome 22)	Random sequencing by NGS of up to 25 million maternal and fetal circulating cfDNA fragments belonging to all chromosomes, including whole chromosome 22. 22q11.2DS is analysed by counting the sequenced fragments derived from the A-H region through a bioinformatic post hoc process by estrapolating only the selected fragments of interest and filtering out the remaining ones	Minimum fetal fraction is required; detection rate of 22q11.2 deletionsis negatively affected with decreasing sequencing depth, fetal fraction and imbalance size

Abbreviations: cfDNA, cell-free DNA; CNVs, copy number variants; DANRS, digital analysis of selected regions; MPSS, massively parallel shotgun sequencing; SNP-based, single nucleotide polymorphisms-based; TCEA, targeted capture enrichment assay.

TABLE 3 Validations of cfDNA tests for 22q11.2 deletions

Type of Test	Study	Technology	Covered Region	Study Type	Sample Type	Performances, %	Comments
Targeted	Wapner et al, 2015[34]	SNP-based	A-D	Analytical validation	43 artificial samples with 22q11.2DS	Analytic detection: 100 (91.8-100)	Validation based on 3 Mb deletion
					65 artificial samples w/out 22q11.2DS	Specificity: 100 (94.4-100)	
				Clinical verification	3 plasma samples with 22q11.2DS	DR: 66.7 (20.8-93.9)	
					332 plasma samples w/out 22q11.2DS	Specificity: 99.1 (97.4-99.7)	
	Ravi et al, 2018[35]	SNP-based enhanced (v2)[a]		Analytical validation	10 plasma samples with 22q11.2DS	DR: 90% (55.5-99.8)	- Performances referred to 3 Mb deletion;—for 99 unaffected cases with a fetal fraction of 2.8%-6.5%, the sample was evaluated only for the presence or absence of the paternally inherited haplotype; —samples reported as "risk unchanged" or "no call" (4.6%) were excluded from specificity calculations;—estimated PPV (with a prevalence of 1/1442 of A-D deletion in the general population) is estimated PPV is 19.6%
					409 plasma samples w/out 22q11.2DS	Specificity: 99.74% (98.6%-99.99%)	
	Martin et al, 2017[39] (including Gross et al, 2015[38])	SNP-based		Retrospective, registry-based clinical study	Plasma samples from a screening cohort of the general population	General population: FPR: 0.3 (0.2-0.4) PPV: 15.7 (8.5-54.4) Low risk population - PPV: 6.4 High risk population—PPV: 82.6	6.85% and 3.75% of the samples received a no result or a result of "risk unchanged," respectively; the study is not aimed to calculate the DR and NPV
		SNP-based enhanced (v1)[b]				General population (projected performances): FPR: 0.07 PPV: 44.2 Low risk population—PPV: 18.5 High risk population—PPV: 100	
	Schmid et al, 2017[36]	Digital Analysis of Selected Regions (DANSR)		Analytical validation	122 artificial samples with 22q11.2DS	Analytic detection: 75.4 (67.1-82.2)	Deletion sizes tested: 1.96-3.25 Mb; simulated fetal fraction levels from 4% to 33%
					1614 plasma samples from presumed unaffected pregnancies	Specificity: 99.5 (99.0-99.7)	
				Clinical verification	7 plasma samples with 22q11.2DS	DR: 71.4 (35.9-91.8)	Assessment of 22q11.2 del performed with FISH or microarray
					210 plasma samples w/out 22q11.2DS	Specificity: 100 (98.2-100)	
	Neofytou et al, 2017[37]		A-C	Analytical validation	3 artificial samples with 22q11.2DS	Analytic detection: 66.7 (20.8-93.9)	Analytic validation based on 3 Mb deletion

(Continues)

TABLE 3 (Continued)

Type of Test	Study	Technology	Covered Region	Study Type	Sample Type	Performances, %	Comments
		Targeted capture enrichment assay (TCEA)			50 artificial samples w/out 22q11.2DS	Specificity: 100 (92.9-100)	
Genome-wide	Helgeson et al, 2015[40]	Massively Parallel Shotgun Sequencing (MPSS)	A-H (whole chr 22)	Retrospective, registry-based clinical study	Plasma samples from a screening cohort of a high risk population	High risk population: PPV: 96.9-100 (82.0-100)	No result rate for 22q11.2DS not reported; false negatives of 2.2 Mb in size

Abbreviations: cfDNA, cell-free DNA; FISH, Fluorescence In Situ Hybridization; SNP-based, single nucleotide polymorphisms-based.

[a]V2 of enhanced protocol includes increased #SNPs in 22q11.2 A-D region, increased positive confidence call threshold, and reflex sequencing at a higher depth of read (~14 million reads/sample) of high-risk cases.

[b]V1 of enhanced protocol includes increased positive confidence call threshold and standard depth of read of greater than or equal to 3.2 million reads/sample and reflex sequencing at a higher depth of read (≥6 million reads/sample) of high-risk cases.

such as cardiac anomalies. Calculation of false negative rate and negative predictive value was beyond the scope of this registry-based study because screen negative cases did not undergo microarray testing. This study was directed at the larger 3 MB deletion, although there is the possibility, as previously mentioned, that nested atypical deletions in the A-D region may be detected. However, performance characteristics may be lower if one takes into account all 22q11.2DS cases, including the non-3 MB deletions.

DANSR assay probe design analyses circulating fragments that map within the A-D region.[36] Therefore, both the common and nested deletions in this chromosome region can be covered. The analytical sensitivity using simulated pregnancies and real plasma samples with deletions ranging in size from 1.96 to 3.25 Mb in size was 75.2% (95% CI, 67.1%-81.8%); the specificity calculated using a clinically normal population was 99.6% (95% CI, 99.1%-99.8%). (Table 3) Different fetal fraction levels (from 4% to 33%) were simulated in the analytical validation. The smallest deletion size detected was 1.96 Mb, and deletions were detected across the entire range of fetal fractions.

TCEA assay probe design analyses circulating fragments belonging from a region of 2.44 Mb in size located between LCR-A and LCR-C, therefore, proximal A-D, A-C, and A-B deletions can be covered.[37] A proof of concept study using three artificial affected and 50 artificial unaffected pregnancies was carried out to test the analytical sensitivity and specificity of the assay. All samples were correctly classified (Table 3).

1.4.2 | Genome-wide technology

Retrospective descriptions of commercial testing using nontargeted technologies based on genome-wide massively parallel shotgun sequencing (MPSS) methodologies indicate that with this counting approach, detection of microdeletion syndromes, including 22q11.2DS, is technically possible.[40-42] Screening for the entire A-H region is feasible because MPSS is based on the random (shotgun) sequencing by NGS of up to 25 million maternal and fetal circulating cfDNA fragments belonging to all chromosomes, including the whole chromosome 22. Consequently, 22q11.2DS is analysed by counting the sequenced fragments derived from the A-H region through a bioinformatics post hoc process by extrapolating only the selected fragments of interest and filtering out the remaining ones. Therefore, unlike targeted technologies, MPSS does not allow for the selective increase of the depth of analysis over a specific narrowed chromosome region. As a consequence, performance is negatively affected with decreasing size of microdeletions, fetal fraction, and sequencing coverage.[43,44] A simulation study with a shallow MPSS achieving a 0.2x coverage showed an average sensitivity for 3 Mb deletions of approximately 60%.[43] In agreement with this projection, Helgeson et al[40] reported that all false negative samples with this coverage had a fetal 22q11.2 deletion of an average size of 2.2 Mb. In addition, the incidence of fetal deletions was three-fold lower than with SNP-based targeted technology[39] (0.01%, 24/80 449 versus 0.03%, 21/175 393), strongly suggesting under-ascertainment by MPSS. Yatsenko and colleagues (2015)[45] described a case of maternal and fetal atypical central B-D microdeletion reported as "high risk for 22q11.2 deletion" without the specification of its size and boundaries.

This is an example of ambiguity in test reporting with MPSS that may impact pregnancy management. The lack of definition of microdeletion boundaries with MPSS may be related to the high sequence homology of cfDNA fragments derived from the 22q11.2 region interfering with the accurate alignment of the obtained reads, the reduced sequencing depth and number of informative sequences.

PPV using MPSS has been reported to be above 80% in high risk fetuses with known cardiac defects at the time of blood sampling.[40] Like the targeted technologies, data on PPV of MPSS in the low risk population are very limited for several reasons, not least of which are the absence of thorough, prospective confirmatory array studies, and follow-up for both negative and positive results.

In conclusion, PPVs may vary by technology (genome-wide MPSS-based and targeted SNP-based technologies).[46-48] In the published studies, the specific clinical context and indications for cfDNA screening are unknown in the majority of cases. In addition, the total number of women who underwent cfDNA screening is not readily available. Even a key factor such as the minimum required FF% to reliably detect 22q11.2 deletions in clinical samples with different technologies has yet to be determined. Prospective blinded clinical studies with molecular verification of all screen positive and negative cases are ongoing and will hopefully provide answers to these open questions. In summary, robust data on test performance are still lacking for all technologies and more research is required.

1.5 | “No result” with different cfDNA testing technologies and possible management options

“No result” rate for full chromosome aneuploidies attributable to low FF have been reported varying from 0.1% to 6.1% depending on technology and test version; reasons related to low assay quality metrics account for a no result rate ranging from 0.1% to 2.8%.[49]

Presently, data regarding “no calls” or “risk unchanged” for 22q11.2 deletions on clinical samples have only been reported for SNP technology.[39] In Martin et al[39] 6.85% (5511/80 449) of samples were excluded for reasons such as test failures and cancellations, while 3.75% of reports were “risk unchanged.” From a practical, clinical point of view, the patient will be left with the initial general population risk.

There are no professional guidelines at this time on how to manage “no results” or “risk unchanged” for 22q11.2DS and whether ultrasound scans (beyond those that would normally be carried out) are indicated for a level of a priori risk of, at most, approximately 1/1000. Growth restriction and intrauterine growth restriction (IUGR) have been reported in cases of 22q11.2DS with central and distal deletions.[10] Future studies are necessary to investigate if, similar to T13 and T18 pregnancies[50], 22q11.2DS pregnancies associated with IUGR and small placentas also have reduced fetal fraction leading to a no result report.

1.6 | Discordant cfDNA results for 22q11.2DS

22q11.2DS discordant results may have the same underlying biological origins as those reported for common trisomies.[51]

1.6.1 | Insufficient fetal fraction

Low fetal fraction can be a source of false negative results if the threshold is too low in relation to the microdeletion size and the analysis depth. Therefore, there are three options the laboratories can use in this situation to minimize the false negative rate: (a) adjust the minimum fetal fraction threshold and/or (b) the analytic depth and/or (c) the minimum size of genomic imbalance.[35,37,39,41,43,44] Increasing depth of sequencing across the entire genome will improve detection rates for specific microdeletions when using MPSS technology.[43] Using additional probes to capture more fragments from the region of interest may improve performance of targeted methodologies at low fetal fractions.[35] In addition, similar to MPSS, increasing sequencing depth is an option when NGS-based targeted analysis is used.[35,39] Of note, all these strategies will likely come at significantly increased cost per sample.

1.6.2 | Feto-placental mosaicism

The conceptus' cfDNA circulating in the maternal plasma is mainly derived from the apoptosis of the placental cytotrophoblast.[1-3] In approximately 1% of the pregnancies, the genetic constitution of the cytotrophoblast does not match that of the fetus because of the presence of a feto-placental mosaicism.[52] Several cases of discordant cfDNA testing results for full chromosome aneuploidies attributed to feto-placental mosaicism have been published.[51,53] However, only a few case reports have been described regarding feto-placental mosaicism involving microdeletions/duplications.[54-56] Interestingly, Bunnell and colleagues (2017)[54] described the placental follow-up of a false positive case by SNP-based method because of the presence of a 22q11.2 microdeletion confined to the cytotrophoblast in a mosaic form and not confirmed in either the maternal or fetal genomes.

The size of this biological phenomenon is essentially unknown, and certainly, more research is needed on this topic. Collection of placental samples following a 22q11.2 false positive cfDNA test or following the late detection of 22q11.2DS in screen negative cases is strongly encouraged.

1.6.3 | Maternal 22q11 deletion

In some cases, the high-risk result is caused by the presence of the 22q11.2 deletion in the maternal instead of the fetal genotype.[39-41] Table 4 summarizes maternal and fetal 22q11.2 deletions detected in three clinical studies.

Cases with maternal deletions might be identified in assays that look at relative copy number in the context of a fetal fraction measurement.[39,40] However, none of the available screening tests can reliably discriminate a maternal from a fetal copy number change with confidence. Until a robust and validated cfDNA testing analysis is available, CMA of the mother remains a potential explanation for a false-positive cfDNA test result.[57]

Most of what we know regarding the pattern of inheritance of 22q11.2DS syndrome has been primarily derived from cases diagnoses postnatally and from fetuses diagnosed with this syndrome based on ultrasound findings. Scant data have been published regarding

TABLE 4 Maternal and fetal 22q11.2 deletions reported in three clinical studies

Study	Type of cfDNA Testing Technology	Type of Prenatal Population	Number of Study Cases	Number of Positive 22q11.2 Deletion Test Results	Number of No Follow-up	Number of Positive Cases with Molecular Test	Number of False Positive Cases	Number of Confirmed 22q11.2 Deletions	Number of Maternal 22q11.2 Deletions	Number of Fetal 22q11.2 Deletions, %	Number of Maternal + Fetal 22q11.2 Deletions
Helgeson et al, 2015[40]	MPSS	High-risk	175 393	32	9	23		23	2	9	12
Martin et al, 2017[39] (including Gross et al, 2015[38])	SNP-based	General (high+low risk)	80 449	289[a]	130	159	129	30	6	24	
Pescia et al, 2016[41]	MPSS	High-risk	6388	2	1	1		1			1

Abbreviations: cfDNA, cell-free DNA; MPSS, massively parallel shotgun sequencing; SNP-based, single nucleotide polymorphisms-based.

[a]Including maternal deletions.

the proportion of inherited and de novo deletions in anatomically normal versus anatomically abnormal fetuses. The national institute of child health and human development (NICHD) clinical trial reported a 22q11.2DS in three and eight cases of the low and high risk populations, respectively; all of them were de novo in origin.[18] More studies are certainly needed on this topic to improve pretest and posttest counselling.

1.6.4 | Vanishing twin

Confined placental chimerism is a well-known phenomenon, where a vanishing twin and its trophoblastic remnants will continue to shed cytotrophoblasts and cfDNA into the maternal circulation beyond the time of demise.[58] Although a case of cfDNA test discordance has not yet been reported, given the 22q11.2 microdeletion prevalence in miscarriages and POC of one in 1000 (Table 1), a false positive cfDNA result could in theory be caused by the presence of a 22q11.2 deletion in the reabsorbed twin.

1.6.5 | Absence of heterozygosity

Absence of heterozygosity (AOH) for a chromosomal region might be interpreted by the SNP-based algorithm as a deletion. This may occur when the fetus apparently shows a single set of alleles in the region of interest and both maternal and paternal allele sequences are identical, which can happen either by chance or when the parents are consanguineous and inherit the sequence in question from a common ancestor.[59] This reason was hypothesized as the underlying mechanism for two false positive cases for microdeletions by SNP-based technology showing large regions of AOH.[60]

2 | CONCLUSIONS

There are numerous potential advantages to detecting 22q11.2 microdeletions in prenatal period[4,5,12,57], including ensuring that the delivery team and neonatal services are on alert. Knowing this genetic information can potentially prevent life-threatening complications, such as depressed calcium levels in the newborn.[61]

However, cfDNA screening for 22q11.2 microdeletions requires further validation before it is used routinely in practice. Currently, there are no published prospective studies where microarray confirmation was obtained for all screen positive and negative cases. As a result, adequate performance characteristics are not available. However, at least two prospective studies analysing all cases with microarray are ongoing and will provide these relevant data. The key to counselling is two-fold: (a) make patients aware that a screening test is not diagnostic and (b) fully explain that validated PPV and NPV values are not yet available. For those who desire to know whether their fetus has the deletion, invasive testing remains the only option.

In addition, there are no formal clinical utility studies that demonstrate a positive, beneficial impact on longer term outcomes. Of note, there have been publications that describe newborn screening for 22q11.2 microdeletions. The ability to detect 22q11.2 DS shortly after birth could further impact any discussion related to the clinical utility of prenatal screening, if the reasoning behind prenatal

screening is to prevent missed diagnoses in the newborn intensive care unit.[62] Given the lack of robust clinical validation and utility studies, pretest counselling must make clear that a screen negative result does not mean that there is no deletion present.

Other important counselling considerations include the recommendation that a positive prenatal 22q11.2 deletion screen report requires confirmatory invasive testing using CMA.[57] Pretest counselling should also emphasize that invasive prenatal diagnostic testing with CMA is the standard of care for all cases in which a fetal anomaly has been detected. A positive non-invasive prenatal testing (NIPT) result in these circumstances may require confirmation by invasive testing, and a negative result should also lead to invasive testing because, as discussed, the sensitivity of NIPT is not 100%.

In addition, the meiotic non-allelic homologous ricombination (NAHR) products can not only be deleted but also can appear with a reciprocal duplication of 22q11.2 region as well.[4,5] Therefore, providers also need to be aware that 22q11.2 reciprocal duplications can be detected by cfDNA testing and, if the technology does not filter out this information, posttest counselling might be challenging because of the extremely variable phenotype associated with this result.[63] A full discussion on 22q11.2 duplications is beyond the scope of this review, but it is worth noting that while the phenotype tends to be milder, counselling can be challenging because of the variable phenotype (ranging from normal to significant findings such as autism spectrum disorder and birth defects).[63,64]

Finally, professional organizations diverge when it comes to the use of cfDNA assessment for microdeletions/duplications. American college of medical genetics (ACMG) recognizes women should be informed about availability of the test, provided detection rate, specificity, PPV, and NPV of each microdeletion screened, when available.[65] International society for prenatal diagnosis and therapy (ISPD) and international society of ultrasound in obstetrics & gynecology (ISUOG) statements provide an overview of the issues related to additional chromosome targets.[66,67] The american college of obstetricians and gynecologists (ACOG) and the society for maternal-fetal medicine (SMFM) have recently reiterated their position and are very clear that routine cfDNA testing is not advised until further clinical validity studies in average risk populations are available.[68] The Austrian-German-Swiss Recommendations and Italian Society of Human Genetics similarly state that cfDNA test for microdeletions cannot be recommended as a routine prenatal test based on currently available data (https://www.sigu.net/show/attivita/5/1/NIPT).[69]

DISCLOSURES

F. R. G. is a full-time employee of TOMA laboratory without ownership shares. She is an advisory board member for Roche and consultant for Menarini Biomarkers. TOMA laboratory performs cfDNA test using DANSR technology. S. J. G. is CEO of The ObG Project, medical director of Sema4, and consultant for Genoox. Sema4 performs cfDNA test using SNP-based and genome-wide MPSS-based technologies.

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