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Opinion

Cell-free DNA testing for 22q11.2 deletion syndrome: appraising the viability, effectiveness and appropriateness of screening

In this issue of the Journal, Gross and colleagues present results from the first 6 months of clinical testing for 22q11.2 deletion syndrome (22q11.2 DS) using a single-nucleotide polymorphism (SNP)-based cell-free DNA (cfDNA) assay¹. In contrast to the numerous clinical validation studies published in rapid succession on cfDNA screening for Down syndrome, data on the new practice of microdeletion screening have been slow to appear, despite it now having been available commercially for over 2 years. This study is only the second large-scale report of cfDNA screening for 22q11.2 DS in clinical practice, and, as such, provides important information on the expansion of cfDNA beyond the common autosomal aneuploidies.

In this retrospective cohort study of 21 948 women referred for 22q11.2 DS screening, 5.3% were excluded due to their sample being unsuitable. Of the remaining 20 776 women, 92.1% had low-risk calls, 7.4% had results equivalent to the population risk ('risk unchanged') and 0.5% ($n = 97$) had high-risk calls for 22q11.2 DS.

Importantly, this study was not designed to clinically validate the sensitivity and negative predictive value of cfDNA screening for 22q11.2 DS. Rather, the study had the more modest objectives of assessing rates of follow-up diagnostic testing and clinical outcomes in the 95 cases with a suspected fetal deletion. Forty-eight of the 84 women known to have been provided information on invasive testing elected to have prenatal diagnosis (57% uptake). Results of prenatal and postnatal diagnostic testing were available for 61 of the high-risk cases, with 11 (18%) being confirmed on karyotyping or molecular testing as true positives. Eight of these 11 women had fetal ultrasound abnormalities detected prior to cfDNA screening.

As the authors acknowledge, one of the limitations of this study was the incomplete follow-up of women with high-risk results: data on confirmatory diagnostic testing were missing in 36% of cases and final pregnancy outcome was unavailable for 30.5%. Unfortunately, this precludes any precise assessment of the accuracy of SNP-based screening for 22q11.2 DS, or the drawing of firm conclusions about its effect on key outcomes such as newborn management and termination of pregnancy. The overall rate of termination of pregnancy for the total cohort is unknown; only three women were known to have terminated their pregnancy as a direct result of screening, but it is possible that more terminations occurred in the cases that did not participate in follow-up.

Another limitation of the study was the lack of outcome data in women with low-risk screening results. Therefore, the sensitivity and the negative predictive value of screening in clinical practice remain unconfirmed. Although clinicians were encouraged to report cases that were missed by screening, placing the onus on clinicians to report false-negative screening results for 22q11.2 DS is inadequate as many children with a mild phenotype will not be diagnosed in the newborn period (see Appendix). Furthermore, the relatively low prevalence of 22q11.2 DS means that a study even of this size (approximately 20 000 women) may still be insufficiently powered to produce a precise estimate of test sensitivity.

Positive predictive value

The positive predictive value (PPV) of a prenatal screening test is the probability that a woman with a positive test result truly has an affected fetus. This important measure is correlated positively with the background prevalence of the condition, as this alters the ratio of true and false positives ($PPV = \text{true positives} / (\text{true positives} + \text{false positives})$). In this study, the prevalence was approximately 1 in 1000, at least double the highest estimated prevalence in the general population, indicating a substantial degree of referral bias, which would be expected for a prenatal cohort. The reported overall PPV of 18% (11/61) is therefore higher than would be expected for an unselected population. Predictably, the PPV was highest in the subgroup with the classical ultrasound abnormalities associated with the syndrome (89%, 8/9).

The 'low-risk' subgroup in this study was defined narrowly as those women with known ultrasound findings that did not include any fetal structural anomalies associated with the syndrome. Women with missing ultrasound data were excluded from both the high- and low-risk groups for PPV calculation. Using this selective definition of low risk, the PPV was 5.1% (2/39). However, if the women with missing ultrasound data were to be included in the 'low-risk' group, then the PPV would drop to 3.8% (2/52). This contrasts with reported PPVs of 45.5–80.9% for cfDNA testing for Down syndrome in the general population^{2,3}.

This lower PPV is one of the reasons why cfDNA screening for microdeletions has not been met with the same enthusiasm as has that for autosomal trisomies^{4,5}. Ongoing protocol refinements, such as deeper sequencing of samples with high-risk calls, may improve the PPV in

Table 1 Comparison of clinical studies on 22q11.2 deletion syndrome (DS) screening with cell-free DNA

	Gross (2016) ¹	Helgeson (2015) ⁷	Taneja (2015) ⁸
Method	SNP	MPS	MPS
Total with 22q11.2 DS result	20 776	175 393	13 268
Total high-risk calls (SPR) (<i>n</i> (%))	97 (0.5)	32 (0.02)	10 (0.08)
Maternal deletion suspected	2§	20**	1‡‡
Prenatal diagnostic testing	49¶	6	NA
Postnatal diagnostic testing	13	8	NA
True positives (TP), fetal*	11	14	4
False positives†	50	0	5
No fetal diagnostic test result available	36	18††	NA
Reported false negatives	0	3	0
Fetal prevalence (TP/10000 women screened)	5.3	0.8	3
PPV (for fetal deletions only) (% (<i>n</i>))‡	11 (11/97)	44 (14/32)	40 (4/10)

Only first author of each study is given. Data are given as *n*, unless stated otherwise. Definitions of TP and PPV varied between included studies and differ from those used in this table. *Confirmed in fetus/newborn/products of conception with cytogenetic/molecular testing (including confirmed fetal cases with detected maternal deletions). Cases of detected maternal deletions *without* confirmation in fetus not included as TPs. †Detected maternal deletions not counted as FPs. ‡PPV (%) = TP/SPR; cases without fetal diagnostic outcomes assumed FPs. §One confirmed unaffected fetus, one not tested. ¶One case of suspected maternal deletion in which amniocentesis was also performed; fetus was unaffected. **Nine of these women were reported as having affected fetuses, but only two were confirmed with diagnostic testing and included as TPs in this table. ††Eight had no cytogenetic testing; nine had only maternal testing for suspected deletion; one was lost to follow-up. ‡‡Confirmed maternal deletion; fetal testing declined. MPS, random massively parallel sequencing; NA, data not available; PPV, positive predictive value; SNP, single-nucleotide polymorphism; SPR, screen-positive result.

future⁶. Regardless, it is true that expanding the scope of prenatal screening to include microdeletion syndromes will have some cumulative effect on false-positive results, thereby reducing the gains in specificity made by cfDNA screening for Down syndrome.

Other studies on 22q11.2 DS screening

In contrast to the numerous studies on the use of cfDNA for aneuploidy screening, there are very few published data on 22q11.2 DS screening in clinical practice. Only one other commercial provider has published its experience with microdeletion screening⁷, while another presented data at the most recent meeting of the International Society for Prenatal Diagnosis⁸ (Table 1). It is disappointing that, despite a combined total of more than 200 000 women undergoing microdeletion screening in these three studies, we still lack the basic performance metrics of sensitivity and negative predictive value for 22q11.2 DS in clinical practice. The striking variation in results suggests significant heterogeneity among the patient populations and/or assay performance, as well as differences in case definitions and methods of calculating PPV. Helgeson *et al.* included suspected/confirmed maternal deletions and fetal cases with 'clinical' (but not cytogenetic/molecular) confirmation as true positives, whereas Gross *et al.* did not. However, Gross *et al.* excluded cases with suspected/confirmed maternal deletions from all PPV calculations. To enable comparison, Table 1 presents newly calculated lower bound PPVs for each study, defined as the percentage of all screen-positive results that resulted in a cytogenetic/molecular diagnosis of an affected fetus/newborn (with cases without fetal diagnostic outcomes considered as FPs).

Nevertheless, drawing any firm conclusions from these data is impossible, because data on population prevalence,

clinical referral details, ultrasound findings, cytogenetic confirmation and pregnancy outcomes are incomplete. None of these studies performed systematic follow-up of women with a low-risk result, no doubt because of feasibility and cost barriers. One novel approach used in some clinical studies of Down-syndrome screening has been to provide women with an 'insurance policy' to create financial incentives for reporting false-negative results⁹. However, as mentioned above, detection of 22q11.2 DS at birth remains a problem because of the well-recognized delay in diagnosis in children without major structural defects¹⁰.

Criteria for screening for a health condition

The classic criteria for a health condition to warrant population screening are that it must: (i) be common; (ii) have a severe phenotype; and (iii) have a consistent natural history. Prenatal screening for 22q11.2 DS has been controversial because it only partially fulfills these criteria. The syndrome is less common than Down syndrome and has a variable phenotype and natural history, ranging from critically ill newborns to healthy, asymptomatic adult carriers.

The screening test itself must also show excellent performance characteristics in the general population in order to be considered acceptable for widespread use. Due to the rarity of microdeletions and the absence of any population screening, validation studies are much more difficult to perform for 22q11.2 DS than for Down-syndrome screening. Preclinical technical validation studies may require artificial plasma mixtures to supplement the scarce clinical samples from pregnant women¹¹. Furthermore, the sensitivity of 22q11.2 DS screening is limited by the presence of genetic variants. The SNP-based method used in this study is designed to detect the typical 3-MB deletion responsible for approximately 87% of cases, thereby limiting its

sensitivity and negative predictive value. The maximum detection rate of cfDNA is even lower for other microdeletion syndromes for which additional genetic mechanisms for the conditions exist, such as uniparental disomy.

Other pitfalls have been reported with microdeletion detection using random massively parallel sequencing. A recent case report highlighted the problems of predicting phenotype when precise genomic coordinates for the deletion are not available from cfDNA¹². In this case, the woman and her child both carried a small atypical deletion that did not include the DiGeorge critical region, but this was only ascertained after birth, reportedly causing the patient considerable anxiety and expense.

Screening for 22q11.2 DS also has more implications for unexpected parental diagnoses than does Down-syndrome screening. Up to 28% of 22q11.2 deletions may be inherited from a mildly affected parent¹³. In this present study, only two maternal carriers were suspected (one confirmed with genetic testing, and the other suspected on clinical findings). In another recent study that used a random whole-genome sequencing method, 20 of 32 high-risk calls involved a suspected maternal component⁷. These unexpected maternal findings clearly pose ethical implications if widespread screening in the low-risk population is to be considered seriously.

Controversy regarding prenatal screening for 22q11.2 DS also involves the unknown impact on clinical outcome, including uptake of diagnostic testing and termination of pregnancy. Prenatal screening provides an opportunity for termination of pregnancy, but, with the variable and frequently mild phenotype, this is ethically more complex compared with current screening for the common autosomal trisomies.

Current standard of care for high-risk fetuses and newborns

Diagnostic testing for 22q11.2 DS is currently recommended for all newborns with a conotruncal cardiac anomaly (which occurs in approximately 75% of affected individuals), or neonatal hypocalcemia secondary to idiopathic hypoparathyroidism¹⁴. In the prenatal setting, the indications for diagnostic testing for 22q11.2 DS include: previous history of an affected child; known parental carrier (50% risk of affected fetus); and presence of a fetal conotruncal cardiac lesion (especially tetralogy of Fallot, interrupted aortic arch Type B or truncus arteriosus). The authors of this study rightly emphasize that diagnostic testing with chromosomal microarray, rather than cfDNA screening, remains the test of choice for high-risk patients. Eight of the 11 true-positive cases in this study could thus have been detected by prenatal ultrasound and diagnostic testing alone.

However, some women will decline diagnostic testing, despite the presence of a suspicious fetal structural abnormality, or will wish to have an additional risk assessment before deciding whether to undergo invasive prenatal testing. It is this small group of women who may benefit from cfDNA screening before delivery. These

women may find value in 22q11.2 screening for personal utility (knowledge as an end in itself), rather than clinical utility (informing decision-making). However, there are also potential adverse effects of screening without prenatal diagnostic follow-up, such as increased maternal anxiety and obstetric intervention after a high-risk call. It is also unknown whether those women who received a 'risk unchanged' (7.4%) or 'no call' (5.3%) screening result felt that their needs had been met.

Screening for 22q11.2 DS in low-risk populations

Most high-income countries have newborn screening programs that will identify a proportion of the structural manifestations of 22q11.2 DS, such as congenital cardiac malformations and cleft palate. Supporters of universal prenatal screening for 22q11.2 DS argue that prenatal diagnosis may improve long-term developmental outcomes by preventing newborn hypocalcemia and seizures. These complications have been linked to an increased risk of intellectual disability in a small retrospective case-control study¹⁵. Instituting prenatal or newborn screening to improve long-term intellectual outcome is an attractive proposal, but it has not been examined prospectively in any study.

Another argument for population-based screening is that it can enable provision of anticipatory care for developmental delay and prevent the distressing 'diagnostic odyssey' experienced by some families. Bales and colleagues¹⁶ reported that parents of children with 22q11.2 DS believe that early diagnosis could improve outcomes by facilitating access to services, ensuring more accurate medical advice and improving anticipation of health problems. However, these surveyed parents also identified some possible disadvantages of screening, especially for children with a mild phenotype. These included medicalization of a child's life from birth, and potential negative effects on parent-child bonding and parenting behavior.

Lessons from a newborn screening application

In 2011, an application was made to the United States Secretary's Advisory Committee on Heritable Disorders in Newborns and Children to have 22q11.2 DS incorporated into the recommended universal newborn screening panel¹⁷. This application, based on a novel polymerase chain reaction (PCR)-based assay that could potentially facilitate rapid diagnosis on a large scale, did not progress beyond the Nomination and Prioritization workgroup, because there were insufficient data available to refer it for a systematic evidence-based review. The reasons for the failure of this application are highly relevant to the current debate on prenatal screening. The stated reasons for the rejection of the newborn screening application were:

- The performance characteristics of the proposed screening test had not been assessed in a population-based study.

- The impact of testing on diagnosis was unclear without population-based data.
- No published studies were available to show the benefits or effectiveness of early treatment or diagnosis in mild cases.
- No pilot studies of proposed screening test algorithm and treatment protocols were available to provide the data necessary to inform an evidence-based review.

All these points are entirely applicable to the current case for prenatal screening. Moreover, the potential harms of screening are greater in the prenatal period, as confirmation of a high-risk screening result requires an invasive test (with a small attendant risk of miscarriage), the postnatal phenotype is difficult to predict, and termination of an unaffected pregnancy may occur if post-test genetic counseling and access to diagnostic testing are inadequate.

The future of prenatal screening: evolving concepts of utility

From a public health perspective, which emphasizes health improvements at the population level, there is clearly insufficient evidence to support routine prenatal screening for 22q11.2 DS.

From a clinical perspective, which emphasizes the use of prenatal screening to inform diagnostic testing and therapeutic choice, the available data show some limited utility. If clinical utility is measured by the uptake of prenatal diagnostic confirmation, then 22q11.2 DS screening in this population was useful in approximately half of the total cohort. This compares with diagnostic uptake rates of 32–100% for suspected autosomal trisomies in other clinical reports of cfDNA screening^{18–20}. However, when it is considered that the majority of affected fetuses in this study had typical ultrasound abnormalities that would have prompted an offer of genomic testing, then the clinical utility of cfDNA screening for 22q11.2 DS appears less convincing. As the authors of this study conclude, appropriate resources for genetic counseling and high-risk pregnancy management must be available if 22q11.2 DS screening is offered.

The genomic era is broadening our concepts of utility in genetic screening. The concept of ‘personal utility’ emphasizes the value of genomic information *per se*, regardless of its clinical use or health outcomes²¹. If we view ‘willingness to pay’ for microdeletion screening as a monetary metric for personal utility, then consumer demand appears to be substantial. This assumes that women who elected to have microdeletion screening added to their ‘routine’ cfDNA panel all received appropriate pretest counseling. Local context, including attitudes towards people with disability, health resources and acceptability of termination of pregnancy, will also have an important influence on how women view microdeletion screening. If women are choosing prenatal screening ‘for information only’, then advances in methods of newborn screening for 22q11.2 DS, such as digital droplet PCR, may diminish

the interest in prenatal screening²². Before we completely accept personal utility as sufficient justification for offering microdeletion screening to women, we need to understand more about women’s experience of this test, with a focus on the perceived benefits and disadvantages. Developing metrics of personal utility for genomic information will become increasingly important as the scope of prenatal screening expands²³.

Clinicians recognize the enormous benefits provided by the rapid translation of cfDNA technology into clinical practice and are excited by the future prospects for perinatal care. However, it is evident that we have entered an era in which the scope of prenatal screening is no longer directed by traditional public health policy processes. Many of us now work in an environment in which an expanding ‘menu’ of tests from the commercial sector exists alongside traditional population-based prenatal screening programs. Currently, no professional society actively endorses routine cfDNA screening for 22q11.2 DS, and some advise explicitly against it²⁴. While the study of Gross *et al.* is an important addition to the literature, it is unlikely that the clinical experience of 22q11.2 DS screening reported here will dramatically shift the current consensus. As ever, adherence to the principles of screening, informed decision-making and critical appraisal of the published evidence should continue to guide local clinical practice.

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APPENDIX

Background on cfDNA screening for 22q11.2 deletion syndrome

The first technical reports of successful fetal microdeletion detection using cfDNA from maternal plasma were published in 2011²⁵ and 2012²⁶, suggesting exciting new opportunities for non-invasive prenatal screening. 22q11.2 DS, also known as DiGeorge syndrome or velocardiofacial syndrome, is the second most common genetic syndrome after trisomy 21. Its most significant severe health consequences include conotruncal cardiac anomalies, velopharyngeal insufficiency, thymic and immune dysfunction, hypocalcemia and seizures. It has an extremely wide phenotypic spectrum, with over 180 associated clinical features. Approximately one third of children have normal development (or only mild speech delay), and two thirds have some degree of developmental delay, ranging from mild to severe learning difficulties¹³. Individuals are also at increased risk of mental health disorders in adult life, such as psychosis and bipolar disorder²⁷.

Due to the variable and frequently mild phenotype, at least one third of affected individuals are not diagnosed until childhood or adulthood²⁸. Children with cardiac defects are more likely to be diagnosed before birth or in the first postnatal year (mean age at diagnosis, 0.6 years) compared with children without cardiac defects (mean age at diagnosis, 6.9 years)¹⁰. The true newborn prevalence of 22q11.2 DS is therefore uncertain, as no country performs population screening for this condition. Population estimates vary greatly from 1 in 2000 births to 1 in 7600, the conventionally quoted figure being 1 in 4000^{29–33}.