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Chorionic villus sampling in the cell-free DNA aneuploidy screening era: careful selection criteria can maximise the clinical utility of screening and invasive testing

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Chorionic villus sampling in the cell-free DNA aneuploidy screening era:

careful selection criteria can maximise the clinical utility of screening and invasive testing

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DISCLOSURE OF INTERESTS

DLN, LHK and RP-D are partners in a private ultrasound practice that provides both chorionic villus sampling and cell-free DNA screening. FN is employed by a commercial entity that provides cytogenetic testing and cell-free DNA testing. This manuscript has not been published or concurrently submitted for publication elsewhere.

WHAT IS ALREADY KNOWN ABOUT THIS TOPIC?

- The improved performance of cell-free DNA screening in the prenatal identification of common aneuploidies has led to a reduction in rates of invasive prenatal testing
- A proportion of potentially pathogenic atypical aneuploidies will not be identified by cfDNA screening

WHAT DOES THIS STUDY ADD?

- This large series of CVS procedures in a high-throughput tertiary centre provides further evidence of the impact of cfDNA screening in reducing CVS rates
- While the introduction of cfDNA screening has increased the diagnostic yield of each CVS procedure, there is a probable over-representation of T21 in these late first trimester samples
- However, the risk of not identifying a pathogenic chromosomal abnormality is low if cfDNA screening is offered in the absence of a structural fetal anomaly, increased nuchal translucency or relevant family history.

KEYWORDS:

Chorionic villus sampling

Cell-free DNA screening

Nuchal translucency

Prenatal diagnosis

Aneuploidy

Karyotype

Non-invasive prenatal testing

ABSTRACT

Objectives

To quantify the impact of cell-free DNA (cfDNA) screening on chorionic villus sampling (CVS) test indications and outcomes in a tertiary maternity service.

Methods

Retrospective cohort study of all CVS procedures performed for any indication on singleton pregnancies at The Royal Women's Hospital, Melbourne, and at Women's Ultrasound Melbourne, Australia, between August 2008 and February 2015. Karyotypes were classified according to pathogenicity and detectability by standard cell-free DNA screening panels.

Results

2051 CVS procedures, 25 373 twelve-week scans, and 2394 cfDNA tests were performed. The CVS rate per 12-week scan fell from 9.8% to 3.9% following introduction of cfDNA screening. The yield of pathogenic chromosomal anomalies per CVS increased from 12.9% to 25.2%, with 70% of pathogenic results now comprising T21, up from 52%. Sixteen (5.3%) of the pathogenic chromosomal abnormalities identified on CVS would not have been predicted by current cfDNA tests.

Conclusions

There is an evolving tension between improved screening performance for common aneuploidies offered by cfDNA testing, and the increasing diagnostic utility of molecular karyotyping. However, the risk of not identifying pathogenic chromosomal abnormalities is low if cfDNA screening is offered in the absence of a structural fetal anomaly, increased nuchal translucency or relevant family history.

INTRODUCTION

First trimester aneuploidy screening has been revolutionised by the clinical availability of cell-free DNA (cfDNA) testing in maternal serum.[1] For trisomy 21, this technology consistently achieves sensitivities and specificities of greater than 99%.[2,3] It is not surprising, therefore, that the uptake of this testing has been rapid,[4] given that its improved performance lowers patients' chances of having an invasive procedure, such as chorionic villus sampling (CVS) or amniocentesis.

Chorionic villus sampling is the 'gold standard' prenatal diagnostic test in the first trimester, and permits genetic testing of a pregnancy from as early as 10 weeks' gestation.[5] The test is technically demanding for the operator, with some studies suggesting that as many as 250 procedures are required before technical competence is achieved.[6] Although CVS may be performed for prenatal diagnosis of a heritable condition, paternity testing, or karyotypic assessment of an anomaly identified on a 12-week scan, the most common indication remains a high-risk result on aneuploidy screening. Sequential improvements in aneuploidy screening test performance have, however, reduced the number of such referrals over the last 15 years,[7,8] and have been associated with a desirable increase in the diagnostic yield of invasive testing.[9,10] Early experience elsewhere would suggest that cell-free DNA screening has only continued this trend,[11,12] with significant implications for the training of future

practitioners,[13] which was already difficult to deliver due to improvements in conventional aneuploidy screening.[14-16]

This study aimed to quantify the impact of cfDNA screening on CVS testing in a high-throughput tertiary maternity service located in a jurisdiction with a high uptake of aneuploidy screening. Further detail regarding the local context in which this study was performed is provided in Table 1. We hypothesised that the advent of cell-free DNA screening has had a demonstrable impact on:

- the number of CVS procedures performed
- the relative proportion of various indications for this procedure
- the diagnostic yield from each test, and
- the relative proportion of aneuploidies identified.

METHODS

This is a retrospective cohort study of CVS procedures performed or supervised by a group of seven specialist sonologists in public practice at the Royal Women's Hospital, Parkville, Victoria, Australia, and in private practice at Women's Ultrasound Melbourne, Australia, during the 5 year period from August 2008 to July 2013 (pre cfDNA screening), and then August 2013 to February 2015 (18 months following the clinical availability of cfDNA

screening). All singleton pregnancies undergoing CVS between 11 to 14 weeks' gestation were eligible for inclusion. Multiple pregnancies were excluded.

Data were collected from the electronic ViewPoint ultrasound picture archiving and communication system (GE Healthcare, Connecticut, USA) at both the Royal Women's Hospital and Women's Ultrasound Melbourne. Additional outcome data were obtained from electronic clinical information systems at the Royal Women's Hospital.

The following data were collected for each procedure: indication, limited maternal demographics, gestational age (determined by the crown-rump length at the time of the procedure), results of the 12-week scan (including measurement of the nuchal translucency [NT] and the presence of any anomalies), procedure-related data (including number of needle insertions, immediate complications, need for repeat sampling), genetic result, and pregnancy outcome. It is standard practice at the centres involved in this study for the NT to be measured in all patients undergoing CVS, either at the time of the procedure, or at a scan immediately preceding it. It is also standard practice for a 12-week scan to be offered to all patients undergoing cfDNA screening as the primary aneuploidy screening test, and for patients whose fetuses demonstrate a nuchal translucency of > 3.5 mm to be offered invasive testing, regardless of the results of conventional combined screening or cfDNA testing.

A risk for trisomy 21 was assigned to each pregnancy, using the best-performing screening test that had been performed (cfDNA, combined first trimester screening, nuchal translucency and maternal age, or maternal age alone if the NT had not been recorded), regardless of the indication for the CVS. Most genetic tests were performed at the Victorian Clinical Genetics Service, the centralised cytogenetic testing provider for the state of Victoria, with a small proportion (<5%) undertaken at private pathology laboratories. Abnormal chromosome results were categorised as trisomy 21, 13 or 18, sex chromosome aneuploidy, or 'other' (including polyploidy, translocations, and microduplications and deletions), and were further dichotomised as pathogenic or benign (conservatively including those anomalies at low or uncertain risk of an abnormal phenotype). Karyotyping was performed on all CVS samples, even those procured to assess for heritable single gene defects. Molecular karyotyping became routinely available in 2012, and is now effectively replacing G-banded karyotyping for CVS samples, regardless of the indication for the test.

Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Melbourne.[17] Analysis of variance (ANOVA) tests were used to analyse the mean value of baseline characteristics in the pre- and post-cfDNA screening cohorts.

As a retrospective, anonymised, chart-based audit project, this study posed no risk to patients, and met the criteria established for quality assurance activities outlined in the NHMRC guideline *Ethical Considerations in Quality Assurance and Evaluation Activities*.[18]

Correspondence confirming this was received from the local institutional Human Research Ethics Committee.

RESULTS

A total of 2051 CVS procedures, 25 373 twelve-week scans, and 2394 cfDNA tests was performed during the overall study period, as depicted in 6-month increments in Figure 1. The populations before and after the introduction of cfDNA screening were not statistically different with respect to mean maternal age (35.84 vs. 35.49 years, p = 0.241) or gestation at which the CVS was performed (12.58 vs. 12.67 weeks, p = 0.069). Prior to the incorporation of the presence of the fetal nasal bone in combined first trimester aneuploidy screening algorithms (in 2010), 12% of 12-week scans proceeded to CVS. After this time, but before cfDNA, there was an 8.9% CVS rate per 12-week scan, which dropped to 3.9% after August 2013 – a 56% reduction.

<Figure 1>

Table 2 outlines the number and proportion of indications for CVS procedures prior to and following cfDNA test availability. There were 23 high-risk cfDNA results, which comprised 8% of indications for CVS post August 2013. Of these, 21 (91%) had chromosomal anomalies, all of which confirmed the abnormality suspected on cfDNA testing. There was one false

positive each for trisomy 13 and 45,X (Turner syndrome). Thirteen of the 23 (57%) had nuchal translucencies > 3.5 mm, and would have been offered invasive testing based on this finding alone. A total of 299 nuchal translucencies in the entire cohort were > 3.5 mm, of which 150 (50%) had a pathogenically abnormal karyotype. The introduction of cfDNA screening did not result in a change in the annualised rate of CVS procedures performed for fetal anomalies (9 per annum pre-cfDNA, 8.67 per annum post).

<Table 2>

Molecular karyotyping became routinely available in 2012. In the entire cohort, molecular karyotyping was performed on 12% of CVS samples, but in 74% of samples obtained in the last six months of the study.

Figure 2 shows the relative proportions of aneuploidies identified by CVS prior to and following cfDNA test availability. The overall yield of karyotypic anomalies per CVS has increased from 14.8% to 28.4%, with the yield of pathogenic anomalies increasing from 12.9% to 25.2% per CVS test. A similar proportion of chromosomal anomalies was pathogenic in the two periods: 87.4% prior to cfDNA screening, and 88.8% thereafter, although the proportion of pathogenic CVS results comprising T21 has increased from 52% to 70%.

<Figure 2>

Fifty-eight (17%) of all chromosomal anomalies in the entire cohort (including all indications) would not have been predicted by current standard cfDNA testing panels (assuming perfect test performance) that cover chromosomes 13, 18, 21 and the sex chromosomes. Of these, 34 (9.9%) occurred in patients with a background risk of a structural fetal anomaly (i.e. high risk on screening, past history of aneuploidy, risk of non-chromosomal heritable conditions), whereas 24 (7%) were identified in patients whose fetuses already had structural concerns evident or were at increased risk of developing these (carriers of balanced translocations).

However, as outlined in tables 3 and 4, only sixteen of these 58 anomalies (27.6%) were deemed pathogenic. Of these pathogenic anomalies, five had a nuchal translucency < 3.5 mm (the institutional threshold for offering a CVS) and no sonographic anomalies: in the absence of another indication for invasive testing, these karyotypic anomalies may have been missed even if cell-free DNA aneuploidy screening is combined with a 12-week scan. That said, only *one* of these pathogenic anomalies (CPM for T16 with subsequent FGR) was identified following high-risk combined first trimester screening: the other four were unbalanced translocations identified by CVS because of known parental carriage of the balanced form.

<Table 3>

<Table 4>

Twenty-seven of the chromosomal anomalies not detectable by cfDNA screening (47%) were identified following high-risk conventional combined first trimester screening. Of these 27, five were deemed pathogenic, and four of these led to termination of pregnancy. Further detail about each of these 27 cases is presented in Table 5.

<Table 5>

Ten CVS procedures were unsuccessful in obtaining a result: seven samples were insufficient, while three were a consequence of laboratory technical problems. Two were successful on a second attempt, the remainder underwent later amniocentesis. All insufficient samples were anticipated.

DISCUSSION

Certain aspects of the context in which this study was performed merit consideration in the interpretation of its results. The increase in NT scans performed over the course of the study reflects the population growth and stable birthrate in Melbourne, Australia, and a consequent increase in maternity care provision, with the Royal Women's Hospital delivering

6563 babies in the first year of the study, increasing to 7579 in the last. In response to this, capacity and throughput has increased in both ultrasound practices involved in this study. Although it has grown, the population has not changed in any other material way.

Even prior to the introduction of cfDNA screening, the diagnostic yield of pathogenic chromosomal anomalies from CVS procedures in this study was relatively high – 12.9% – when compared to rates in other centres. Rather than a high threshold for invasive testing, this diagnostic yield likely reflects the tertiary referral role of the practices in this study, which results in a patient population with higher pre-test probabilities for karyotypic anomalies. It may also reflect the high uptake of conventional combined first trimester aneuploidy screening in this population,[19] which in the setting of the present study has achieved a detection rate for T21 of 91.8% for a false positive rate of 4.5%.[20]

This study demonstrates a clear shift in the relative proportions of aneuploidies identified in CVS samples following the availability of cfDNA screening, with trisomy 21 now comprising over two-thirds of pathogenically abnormal karyotypes. This is likely to be an over-representation of the true proportion of Down syndrome relative to other aneuploidies in the late first trimester,[21] and reflects the performance characteristics of cfDNA testing for this aneuploidy. Coupled with the overall decline in CVS rates, this suggests fewer atypical aneuploidies are being identified at this early gestation, and unless a structural anomaly or fetal growth restriction prompts a later amniocentesis, such chromosomal defects may not

be identified until after birth. Consequently, the almost-twofold increase in the diagnostic yield per CVS procedure afforded by cfDNA screening, while desirable in principle, may have come at the cost of the earlier identification of atypical, potentially pathogenic karyotypic abnormalities.

There is thus an evolving tension between the improved sensitivity and specificity of cfDNA testing for 'typical' aneuploidies, such as trisomy 21, and the increasing awareness of the pathogenicity of microdeletions and duplications identifiable using molecular karyotyping techniques on CVS or amniocentesis samples. In time, it may be possible for cfDNA testing to encompass not only molecular karyotyping[22] and assessment of single gene defects,[23] but fetal whole-genome sequencing as well.[24] At present, however, full molecular karyotyping is only available in the context of diagnostic testing, which has been shown to be safer than previously thought.[25-28] Some authors have suggested that adopting cfDNA testing as a second-tier investigation following a high-risk combined first trimester screen would miss only a very small proportion of chromosomal anomalies,[29] whereas others indicate that up to 16.9% of aneuploidy would be missed, even when diagnostic testing is limited to G-banded karyotyping.[30]

Studies that have utilised molecular karyotyping indicate that standard cfDNA testing alone would not identify up to 23.4% of potentially clinically significant karyotypic abnormalities that would otherwise have been identified by combined first trimester screening and

invasive testing.[31] Incorporating a nuchal translucency threshold into the decision algorithm for invasive testing will reduce this proportion, with a recent cohort study demonstrating that when a CVS is prompted by *either* an abnormal cfDNA result *or* a nuchal translucency of 3 mm or greater, only 5.2% of significant abnormalities would not be identified.[32] As noted earlier, our institutional policy is to offer a CVS when the NT is > 3.5 mm. In this study, for those abnormal CVS results that would not have been detected by cfDNA testing, applying *either* this lower nuchal translucency threshold of ≥ 3 mm *or* the local standard of 3.5 mm would have failed to identify only five pathogenic fetal karyotypic anomalies overall, representing 1.7% of all pathogenic CVS results in this series. Four of these CVS procedures were performed on account of parental carriage of a balanced translocation, and only one following a high risk result on combined first trimester screening (cf. Table 4).

Among the five pathogenic chromosomal anomalies in this cohort identified following combined first trimester screening but deemed not detectable by cfDNA testing (cf. Table 5), only one (CPM for T16 with subsequent FGR) had a nuchal translucency of < 3 mm. This suggests that the risk of *not* identifying a pathogenic chromosomal abnormality is low if cfDNA aneuploidy screening is offered in the absence of a structural fetal anomaly, increased nuchal translucency or relevant family history, and highlights the ongoing clinical relevance of the 12-week NT scan. However, had molecular karyotyping been performed on the entire cohort, rather than just 12% thereof, the percentage of karyotypic anomalies not able to be identified by cfDNA testing would likely have been higher, in keeping with the previously

demonstrated substantial increase in diagnostic yield afforded by microarray-based karyotyping in prenatal diagnosis.[33]

Strengths and Limitations

The primary strength of this study is the size of its cohort, and the level of detail that could be obtained regarding individual cases. This is in contrast to population-level studies of trends in invasive prenatal testing,[11] which generally lack specific data about the indication for procedures or pregnancy outcomes. In particular, given that this study spans the period prior to and following the availability of cfDNA aneuploidy screening, it permits an appraisal of the impact of this new technology on the performance and outcomes of CVS procedures in the hands of experienced operators. Additionally, molecular karyotyping was available for almost half of the study period (from 2012 onwards), affording an assessment of its additional diagnostic yield above that of G-banded karyotyping alone.

This study possesses some limitations that must be considered in the interpretation of its results. It assumes that all changes in the rate of and indications for CVS procedures following August 2013 were a consequence of the introduction of cell-free DNA testing, although other unidentified factors may have had an impact. The magnitude of any such impact, however, is likely to have been small, and the stable number of 12-week scans in the

latter part of the study suggests against other factors being responsible for the decline in CVS numbers.

CONCLUSION

This study demonstrates a reduction in CVS rates following the introduction of cfDNA aneuploidy screening, with a consequent increase in the diagnostic yield per procedure, potentially at the expense of early detection of atypical aneuploidies. However, it was found that the risk of missing a pathogenic chromosomal abnormality is low if cfDNA aneuploidy screening is offered in the absence of a structural fetal anomaly, increased nuchal translucency or relevant family history. It is recognised that a small proportion of potentially pathogenic karyotypic abnormalities will be missed by any 'non-invasive' screening program, leading some authors to suggest that the potential yield from universal prenatal molecular karyotyping is such that *all* pregnant women should be offered invasive testing for this purpose.[34] At the very least, the potential risks and benefits of all available prenatal screening and diagnostic strategies must be discussed in detail with pregnant women by caregivers who are themselves well informed about this rapidly evolving field. Such nuanced and time-consuming counselling is perhaps at odds with the direct-to-consumer marketing approach taken by many cfDNA testing providers,[35] whose product does not even require a clinician's referral in many jurisdictions. As always, enthusiasm for a new technology needs

to be tempered by a rational assessment of its risks and benefits,[36] both for individual patients and for the community as a whole.

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Study data were collected and managed using REDCap electronic data capture tools hosted at The University of Melbourne.[17] REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

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FIGURE CAPTION LIST

Figure 1: Absolute numbers of CVS procedures, 12 week scans and cfDNA tests performed over the study period

Figure 2: Relative proportions of all aneuploidies (benign and pathogenic) identified in CVS samples pre- and post-cfDNA testing availability

TABLE 1: The context in which this study was performed

Australian health system	• Three funding sources: public funding (from either the Federal or State government, depending on the service), private health insurance (only contributes to private inpatient care episodes), and patient co-payment (out of pocket)[37] • Patients electing to receive maternity care in the public system pay no out-of-pocket costs for basic investigations and all antenatal/intrapartum/postnatal care
Epidemiology of aneuploidy screening in the state of Victoria	• High uptake of aneuploidy screening across the state (greater than 80%)[19] • High rate of invasive procedures for 'screen positive' women, and high rates of termination for aneuploidy
Second trimester maternal serum screening test	• Performed between 14 and 20 weeks' gestation • Serum testing only: inhibin-A, free beta-hCG, alpha feto-protein, unconjugated oestriol • The only aneuploidy screening option in Victoria that is fully funded by the state • Reports risks for T21, T18 and NTDs • Detection rate for T21 in this population of 72.7%, for a false positive rate of 7.8%[20]
Combined first trimester screening test	• Comprises serum testing of PAPP-A and free beta-hCG between 9 and 13+6 weeks' gestation, and a fetal nuchal translucency scan between 11+1 and 13+6 weeks • Cost of both the serum test and the ultrasound only partially covered by public funding, resulting in an out-of-pocket cost for all patients • Reports risks for T21, T18 and T13 • Detection rate for T21 in this population of 91.8% for a false positive rate of 4.5%[20]
Cell-free DNA screening	• Available since August 2013, five providers, all test chromosomes 13, 18 and 21 ± sex chromosomes

	• Initially all tests performed in overseas laboratories, but available locally since 2015 • All costs are charged to the patient directly ($AUD 450 – 600), and patients can self-refer for this test • No agreed policy on its use: initially, 63.8% of cfDNA tests in Australia were performed as first-tier screening tests,[38] increasing to > 80% currently.

TABLE 2: Relative proportions of indications for CVS tests pre and post-cfDNA testing availability

	Pre-cfDNA (Aug 2008 - Jul 2013) *n* (%)	**Post-cfDNA (Aug 2013 - Feb 2015) *n* (%)**
High risk on screening or by maternal age alone	1229 (69.5)	160 (56.7)
Known risk of heritable condition	218 (12.3)	26 (9.2)
Combination of fetal anomaly and high risk on screening	157 (8.9)	37 (13.1)
Previous pregnancy with aneuploidy	81 (4.6)	13 (4.6)
Major fetal anomaly	45 (2.5)	13 (4.6)
Other	39 (2.2)	10 (3.6)
High risk on cfDNA screening	0 (0)	23 (8.2)
Total	**1769**	**282**

TABLE 3: Results of CVS testing before and after introduction of cfDNA screening

	Pre-cfDNA era (Aug 2008 - Jul 2013) *n* (%)			**Post-cfDNA era (Aug 2013 - Feb 2015) *n* (%)**		
Total number of CVS procedures	1769			282		
Total number of karyotypically normal results	1499 (84.7)			200 (70.9)		
Total number of karyotypic anomalies	262 (14.8)			80 (28.4)		
Total number of pathogenic karyotypic anomalies	229 (12.9)			71 (25.2)		
Total number of failed procedures	8 (0.4)			2 (0.7)		
	N	**% of total CVS procedures**	**% of pathogenic results**	***n***	**% of total CVS procedures**	**% of pathogenic results**
Number of **pathogenic** karyotypic anomalies not detectable by cfDNA	11	0.62	4.8	5	1.8	7
By indication: High risk on screening or maternal age alone	*3*	*0.17*	*1.3*	*2*	*0.7*	*2.8*
*Known risk of heritable condition (chromosomal)**	*6*	*0.34*	*2.6*	*1*	*0.4*	*0.4*
Known risk of heritable condition (other)	*0*	*0*	*0*	*0*	*0*	*0*
Combination of fetal anomaly & high risk on screening	*0*	*0*	*0*	*1*	*0.4*	*1.4*
Major fetal anomaly	*2*	*0.1*	*0.9*	*1†*	*0.4*	*1.4*

* Includes translocations and microduplications / deletions.
† This patient had cfDNA screening, which returned a low-risk result. The 12-week scan had demonstrated an NT of 3.77 mm and multiple abnormalities. The CVS returned a result of 47,XY+9 (trisomy 9), and the patient elected to terminate the pregnancy.

TABLE 4: Spectrum of abnormal CVS results not detectable by cfDNA screening across the entire cohort (*n* = 58)

Indication	Benign	Pathogenic
High risk on screening or by	22	5

maternal age alone **(see table 5 for further details on these cases)**	CPM* involving various chromosomes not associated with adverse pregnancy outcomes (9 cases) ‡‡ Microduplication on chromosome X of maternal origin (2 cases) Inversion of maternal origin Microduplication on chromosome 8 of maternal origin Balanced translocation chromosomes 14 and 17 of maternal origin †† Microduplications on chromosomes 9 and 11 of paternal origin Microduplications on chromosome 22 of paternal origin †† Microduplications on chromosome 5 of paternal origin Microduplication on chromosome 15 of maternal origin †† Microduplication on chromosome 1 of maternal origin †† Microduplication on chromosome 16 of maternal origin Pericentric Y inversion †† CPM for translocation chromosomes 4 and 12 – FGR with IUFD‡ at 21 weeks ††	CPM for trisomy 16 – high risk for FGR†,†† Trisomy 2 (confirmed on amnio) – ToP§ Trisomy 9 (confirmed on amnio) – ToP Trisomy 4 (confirmed on amnio) – ToP 22q microdeletion – ToP
Known risk of heritable	8	7

chromosomal condition	Balanced Robertsonian translocation chromosomes 13 and 14 (2 cases) †† Balanced Robertsonian translocation chromosomes 14 and 15 (2 cases) †† Balanced translocation chromosomes 10 and 14 of maternal origin †† Balanced translocation chromosomes 1 and 7 of maternal origin †† Balanced translocation chromosomes 4 and 10 of paternal origin †† Balanced translocation chromosomes 6 and 20 of maternal origin ††	Unbalanced translocation chromosomes 16 and 18 of paternal origin – ToP (2 cases) ¶, †† Microdeletion on chromosome 1 associated with fetal thrombocytopenia absent radius syndrome – ToP Xq28 microduplication – ToP †† Unbalanced translocation chromosomes 11 and 18 of maternal origin – ToP ¶ 3q26 duplication – ToP †† Xp11 microdeletion (Norrie disease) – ToP
Known risk of other heritable condition (e.g. single gene disorder)	2	0
	Microdeletions chromosome 19, de novo †† CPM for trisomy 2, normal amnio ††	
Combination of fetal	4	1

anomaly and high risk on screening	Microduplications of paternal origin (2 cases) Inversion of maternal origin CPM for marker chromosome, normal amnio	Trisomy 4 – ToP
Previous pregnancy with aneuploidy	4	0
	Balanced pericentric inversion chromosome 7 †† Microduplication of paternal origin †† CPM for mosaic microdeletion on chromosome 10, normal amnio †† Marker chromosome of maternal origin ††	
Major fetal anomaly	2	3
	Translocations of paternal and maternal origin; fetus with suspected megacystis and NT of 4 mm, ongoing pregnancy Variant chromosome 15; fetus with skeletal dysplasia – ToP**	Translocation chromosome 15; fetus with congenital diaphragmatic hernia – ToP** Complex rearrangement involving 9p duplication and deletion; fetus with renal anomaly – ToP** Trisomy 9; fetus with multiple anomalies – ToP

* CPM = confined placental mosaicism
† FGR = fetal growth restriction
‡ IUFD = intra-uterine fetal death
§ ToP = termination of pregnancy
¶ the size of the translocated segment of chromosome 18 in these cases rendered detection by cfDNA screening unlikely
** the fetal abnormality contributed to the decision for ToP in these instances
†† structurally normal fetus with a NT of < 3.5 mm on the 12-week scan
‡‡ two of these cases had NTs > 3.5 mm in otherwise structurally normal fetuses at 12 weeks, the remainder had normal 12-week scans

TABLE 5: Abnormal CVS results identified following high-risk combined first trimester screening that would not predictably be identified by cfDNA screening alone (*n* = 27)

Maternal Age (years)	Gestational Age (weeks)	cFTS* Trisomy 21 Risk (1:*x*)	Nuchal Translucency (mm)	Karyotype	Pathogenic	Pregnancy Outcome Following CVS
36	12.71	2	4.26	arr 8p23.2(3,734,897-5,962,953)x3 mat	No	Ongoing pregnancy
36	13.14	3	7.4	46,XY,inv(10)(p11.2q21.2)(mat)	No, incidental finding	Ongoing pregnancy
33	12.29	4	7.1	47,XY,+4	**Yes** (confirmed on amniocentesis)	Termination
38	12.29	20	2.4	46,XY,t(14:17)(q24.2;q21.31)mat	No, but relevant for future pregnancies[¶]	Ongoing pregnancy
40	12.43	22	1.3	47,XX,+22	No (Normal amniocentesis, CPM[†])	Ongoing pregnancy
36	12.29	22	1.7	47,XX,+4	No (Normal amniocentesis, CPM)	Ongoing pregnancy
38	13	24	3.8	48,XX,+mar+mar	No (Normal amniocentesis, CPM)	Ongoing pregnancy
25	12.29	27	2	47,XY,+12[17]/46,XY[13]	No (Normal amniocentesis, CPM)	Ongoing pregnancy

Maternal Age (years)	Gestational Age (weeks)	cFTS* Trisomy 21 Risk (1:*x*)	Nuchal Translucency (mm)	Karyotype	Pathogenic	Pregnancy Outcome Following CVS
37	13	32	3.4	arr[hg19] 12q24.21(116,229,013-116,547,287)x3 pat, Xp22.31(8,639,988-8,918,776)x2 mat	Uncertain: a male fetus with a maternally inherited duplication needs a healthy male relative with the same duplication to confirm this as benign; not confirmed for this pregnancy.	Ongoing pregnancy
35	12.43	34	3.71	47,XY,+2[4]	No (Normal amniocentesis, CPM)	Ongoing pregnancy
27	12.14	35	3.7	22q11.2(TUPLE1x1) dn	**Yes**	Termination
28	12.71	44	5.5	arr[hg19] 9p24.2(2,688,521-3,156,013)x3 pat 11q24.2(125,251,763-125,487,570)x3 pat	No	Ongoing pregnancy
34	12.57	46	1.62	47,XY+16	**Yes**, despite CPM being confirmed on amniocentesis, as placental insufficiency and growth restriction likely	Ongoing pregnancy

Maternal Age (years)	Gestational Age (weeks)	cFTS* Trisomy 21 Risk (1:*x*)	Nuchal Translucency (mm)	Karyotype	Pathogenic	Pregnancy Outcome Following CVS
40	13.14	65	1.5	arr[hg19] 22q11.22q11.23(22,997,928-23,650,871)x3 pat	Susceptibility locus or benign CNV[‡,¶]	Ongoing pregnancy
36	13	68	3.75	arr[hg19] 5p15.33(820,030-1,314,303)x3 pat	No	Ongoing pregnancy
32	13.14	180	2.5	arr[hg19] 15q11.2(22,754,322-23,140,114)x1 mat	Variable penetrance[¶]	Ongoing pregnancy
31	13	181	1	46,XX,t(4;12)(q25;q15)dn	No: CPM (normal fetal microarray), although severe FGR[§] secondary to gene disruption around the breakpoints could not be excluded	Fetal death at 21 weeks: growth restricted but structurally normal fetus
42	12.29	194	1.5	47,XX,+2 [3]	No (Normal amniocentesis, CPM)	Ongoing pregnancy
32	13.86	197	1.3	47,XX,+5 [11]/46,XX [19]	No (Normal amniocentesis, CPM)	Ongoing pregnancy
36	11.14	200	1.15	47,XY,+5[21]/48,XY,+5+8[5]/46,XY [4]	No (Normal amniocentesis, CPM)	Ongoing pregnancy
38	12.71	209	1.52	47,XY,+8	No (Normal amniocentesis, CPM)	Ongoing pregnancy

Maternal Age (years)	Gestational Age (weeks)	cFTS* Trisomy 21 Risk (1:*x*)	Nuchal Translucency (mm)	Karyotype	Pathogenic	Pregnancy Outcome Following CVS
28	12.57	209	3	arr[hg19] 1p32.3(54,831,001-55,365,830)x3 mat	No	Ongoing pregnancy
33	12.29	229	3.11	Pericentric Y inversion 46,X,inv(Y)(p11q11)	No: benign variant	Ongoing pregnancy
45	12.14	268	2.4	arr[hg19] Xq28(154,215,554-154,302,678)x2 mat	Male fetus, significance unclear¶	Ongoing pregnancy
25	12.86	40 (T18)	3.7	arr[hg19] 16p13.11(14,929,070-16,520,463)x3 mat	Susceptibility locus, variable penetrance¶	Ongoing pregnancy
36	12.86	2 (T18)	3.6	47,XY,+2	**Yes** (confirmed on amniocentesis)	Termination
37	12.71	6 (T18)	3.9	47,XY,+9	**Yes** (confirmed on amniocentesis)	Termination

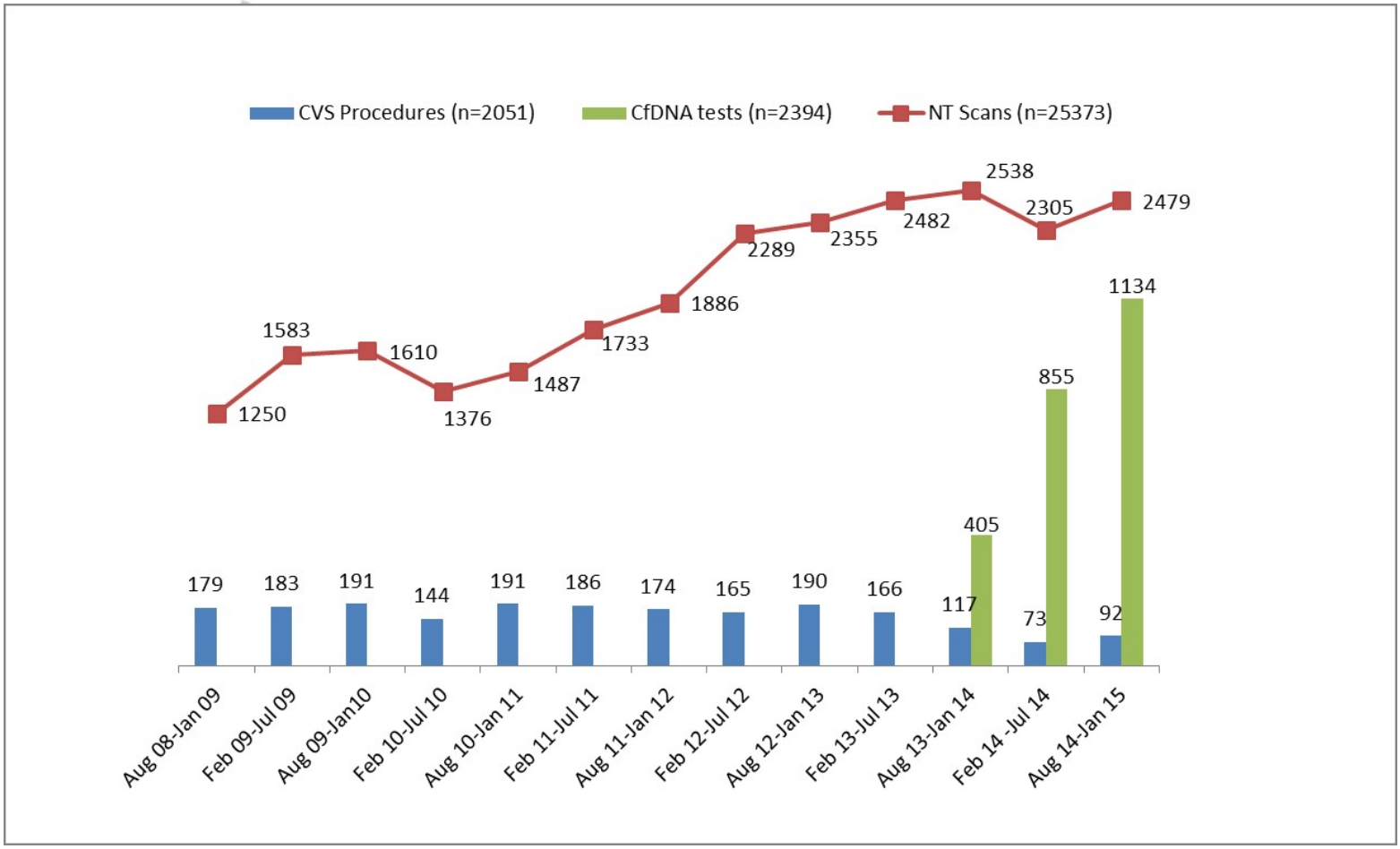
* cFTS = combined first trimester screening

† CPM = confined placental mosaicism

‡ CNV = copy number variant

§ FGR = fetal growth restriction

¶ the phenotype of the parent of origin was reported as normal



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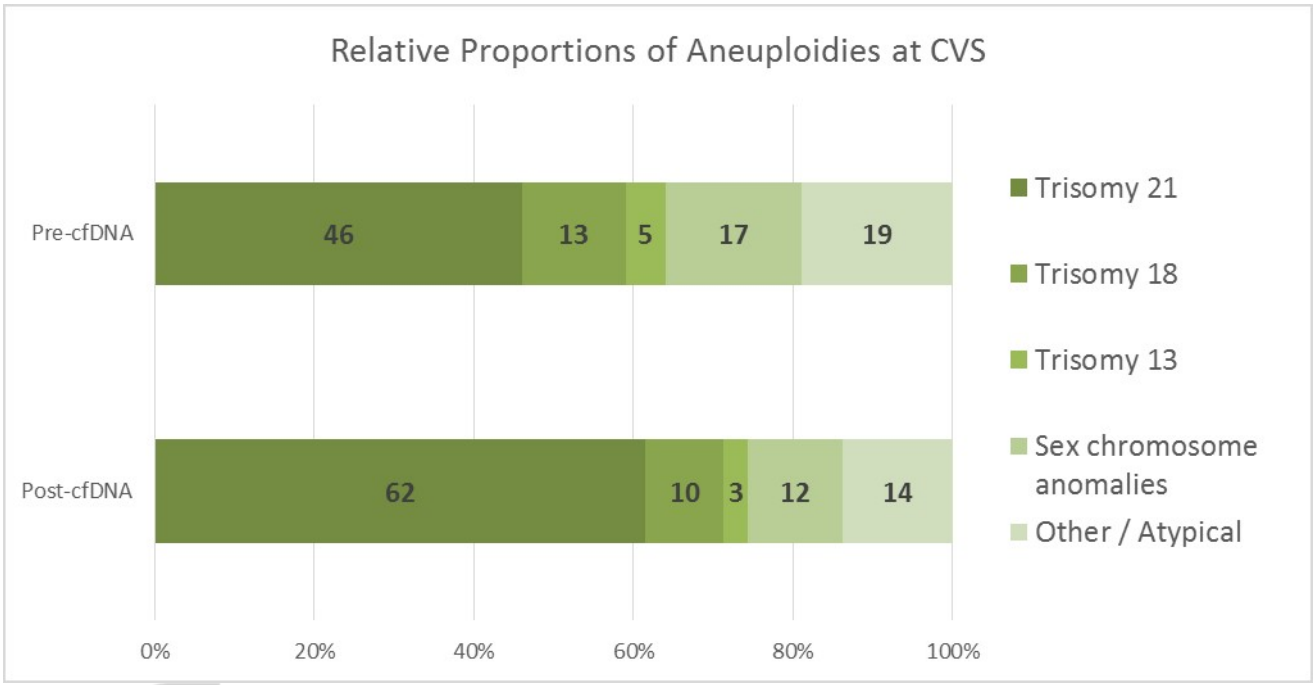

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