Lindquist Anthea (Orcid ID: 0000-0002-8662-5188)

Prenatal diagnostic testing and atypical chromosome abnormalities following combined first-trimester screening: implications for contingent models of non-invasive prenatal testing

Authors: Anthea Lindquist[1,4], Alice Poulton[1], Jane Halliday[1,3], Lisa Hui[1,2,4]

Affiliations

1. Public Health Genetics, Murdoch Children's Research Institute
2. Department of Obstetrics and Gynaecology, University of Melbourne
3. Department of Paediatrics, University of Melbourne
4. Mercy Perinatal, Mercy Hospital for Women

Corresponding Author:
Anthea Lindquist
Department of Obstetrics and Gynaecology
Mercy Hospital for Women
163 Studley Rd, Heidelberg VIC 3084
Email: aclin123@gmail.com

Running Head: Prenatal testing and atypical abnormalities

Keywords: non-invasive prenatal testing , combined first trimester screening , PaPP-A , anueploidy , NIPT , serum screening

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/uog.18979

ABSTRACT

Objectives: To perform a population-based analysis of a combined first trimester screening (CFTS) cohort for (i) changes in uptake of invasive prenatal diagnosis by CFTS risk, and (ii) prevalence and methods of ascertainment of atypical chromosome abnormalities.

Methods: Retrospective cohort study of state-wide prenatal datasets from Victoria, Australia. A three-step approach was undertaken: i) record-linkage between serum screening and diagnostic results; ii) comparison of rates of diagnostic testing by CFTS risk categories in a 2014-15 CFTS cohort with a historical cohort from 2002-04; (iii) detailed analysis of atypical abnormalities from 2014-15 by CFTS risk, individual serum analyte level and indications for diagnostic testing.

Results: In 2014-15, there were 100,418 CFTS results issued for 146,776 births (68.4%). The overall prevalence of atypical chromosome abnormalities in the entire CFTS cohort was 0.10%, highest in those with CFTS risk >1 in 10 (4.6%), or serum analyte levels <0.2 MoM (6.9% and 5.2% for PaPP-A and β-HCG respectively). Almost half (49.1%) of women with PaPP-A <0.2 MoM had a trisomy 21 risk of less than 1 in 100. The majority (55%) of atypical abnormalities occurred in women with CFTS risk below 1 in 300, and were most commonly ascertained via ultrasound abnormality (47.1%).

Conclusion: Concerns regarding missed diagnoses of atypical chromosome abnormalities where NIPT is offered after high-risk CFTS can be mitigated if diagnostic testing is offered to women with: trisomy 21 risk >1 in 100, serum PaPP-A or bHCG < 0.2 MoM, or ultrasound-detected abnormality. This has implications for contingent models of screening.

Funding: Mercy Perinatal Research Fellowship to AL; National Health and Medical Research Council Early Career Fellowship (1105603) to LH; and National Health and Medical Research Council Senior Research Fellowship (1021252) to JH.

INTRODUCTION

Until the recent introduction of non-invasive prenatal testing (NIPT), combined first-trimester serum screening (CFTS) was the dominant form of screening for trisomy 21 in many countries. As NIPT spread globally following its initial launch in China and USA in 2011, considerable debate has waged over the most appropriate method of integration into clinical practice and local population-based screening programs (1-3).

NIPT was initially promoted as a secondary screening test for pregnancies already at increased risk of trisomy 21 (T21) based on conventional screening tests or maternal age (4), but it is now considered an acceptable primary screening test for women of any background risk (5). There are ongoing concerns, however, that replacing CFTS with NIPT for trisomy 21 screening will lead to a decline in the 11-13 week- nuchal translucency (NT) ultrasound and subsequent missed opportunities for early detection of fetal structural malformations (6).

There are additional concerns that the use of NIPT as a secondary screening test, the so-called "contingent" model (7, 8) may lead to a reduction in the detection of atypical chromosome abnormalities previously identified through diagnostic testing after high-risk CFTS (9, 10). One population-based study has reported an increased risk of atypical chromosome abnormalities in women with low maternal serum markers (PaPP-A or β-HCG <0.2MoM), independent of CFTS risk (11). However, the corresponding CFTS results were not reported for the group with very low serum

analyte levels, making it unclear if they would have been offered diagnostic testing based on CFTS result alone. The role of ultrasound anomalies in the detection of atypical abnormalities was also not examined in that study.

Although there have been numerous studies reporting the overall decline in invasive testing since the introduction of NIPT (12-15), there is a lack of population-based data on the relationship between numerical CFTS risk and uptake of diagnostic testing in the pre- and post NIPT eras. If clinicians are providing the recommended pre-test counselling on the limitations of NIPT (16), it is possible that women at the highest risk of atypical abnormalities (e.g. those with CFTS risks > 1 in 50) would continue to have high rates of diagnostic testing.

In view of this rapidly evolving prenatal screening environment and the debate regarding thresholds for contingent models of NIPT, we performed a population-based study to (i) analyse the uptake of diagnostic testing after CFTS according to numerical risk result, and (ii) analyse the prevalence and ascertainment of atypical chromosome abnormalities and pathogenic copy number variations (CNVs) by CFTS risk and individual serum marker MoMs.

METHODS

The Australian state of Victoria has approximately 73,000 births p.a., median maternal age 31.5 years, average fertility rate 1.7 births per woman, and average weekly disposable household income AUD 998 (http://www.abs.gov.au).

Voluntary screening for fetal chromosome and structural abnormalities in Victoria is universally available (17). Government rebates are available for CFTS, second trimester serum screening (STSS) and the mid-trimester morphology ultrasound with variable out-of-pocket cost to the patient (typically < AUD200). CFTS, in addition to ultrasound measurement of NT, includes serum measurement of Pregnancy-associated Plasma Protein A (PaPP-A) and free β-hCG. STSS is conducted between 14 and 20 weeks as a quadruple panel including alpha-feto protein, unconjugated

estriol, free β-hCG and dimeric inhibin A. Invasive testing is fully government-funded in the public sector to increased-risk women and partially-funded if performed in the private sector. There is no additional charge for chromosomal microarray in the public sector. NIPT is not currently government-funded and the total cost (approximately AUD 500) is borne by the patient.

2014-15 Data sources

This study linked state-wide records of prenatal screening with records of prenatal diagnostic tests from 1st January 2014 to 31st December 2015. Data on prenatal serum screening, including CFTS and STSS, were obtained from the Victorian Clinical Genetics Service. CFTS risks for T21, trisomy 18 (T18) and trisomy 13 (T13) were calculated with LMS Alpha program, version 8 (http://www.lmsalpha.com/).

Data on all Victorian women undergoing invasive prenatal diagnostic testing (amniocentesis or chorionic villus sampling) prior to 25 weeks gestation from January 2014 to December 2015 were obtained from the Victorian Prenatal Diagnosis Database (see Acknowledgements for contributors). The gestational age limit of 25 weeks was designated to capture invasive testing performed after routine first and second trimester screening (13). The screening and diagnostic datasets were probabilistically linked using LinkageWiz™, using family name, date of birth and postcode as individual identifiers. Potential data matches were manually examined and confirmed or rejected using the clerical review tool in LinkageWiz. For records without compete identifiers, manual linkage was performed in Excel, using a combination of name, maternal age, and dates of screening and diagnostic testing.

In our population, women with a screening result for T21 of 1 in 300 or higher are reported as “high risk” and are offered genetic counselling. Clinical pathways for high risk women include: secondary screening with NIPT at their own cost, invasive diagnostic testing with G-banded karyotype or chromosomal microarray (CMA), or no further testing. Women with a risk result less than 1 in 300 may also opt for NIPT or diagnostic testing, according to individual preference.

Analysis

Following data-linkage between the screening and diagnostic datasets, CFTS results were coded as (i) high risk (≥1 in 300 for T21, ≥1 in 175 for T18 and ≥1 in 100 for T13) according to standard clinical reporting practice, (ii) low risk, or (ii) unknown risk. Women with unknown risk were women who had undergone serum screening but had incorrect dates, or who did not have a nuchal translucency measurement supplied for risk calculation. These were excluded from the CFTS analysis, but included in the serum analyte analysis. STSS results were coded as high risk if the risk of T21 was ≥1 in 250, the risk of T18 ≥1 in 200 or the risk of neural tube defect was increased due to alpha-fetoprotein level >2.5MoM (17). Descriptive analysis was performed in STATA version 14.

Diagnostic karyotype results were explored according to CFTS risk for T21, PaPP-A MoM and free β-hCG MoM subcategories. Normal karyotype included 46XX, 46XY and balanced translocations. Major chromosome abnormalities included T21, T18, T13, other autosomal trisomies, triploidy, sex chromosome anomalies, pathogenic copy number variations (CNVs), unbalanced translocations, and level 3 mosaicism. Pathogenic CNVs included deletions or duplications in a region associated with an abnormal phenotype (18). Atypical abnormalities were defined as major abnormalities not detectable on standard five-chromosome NIPT, that is, excluding trisomies 21, 18 and 13, monosomy X, and sex chromosome trisomies. CNVs of uncertain or unknown significance were excluded.

Data for the historical comparison group, 2002-2004, were obtained from a prior published study from the Victorian Prenatal Diagnosis Database (19). No additional data analysis was performed on this cohort.

RESULTS

A combined total of 110,712 serum screening tests (CFTS n=103,319; STSS n=7,393) were performed among 146,776 births during the 2014-15 study period, representing a population uptake rate of 75.4%. This compared with a population

uptake of 48.1% of in 2002-04 (CFTS n = 41,663; STSS n = 19,072). STSS represented 6.7% of total serum screening tests in 2014-15, and was not included in the remainder of the analysis.

Of the women who had first trimester serum screening performed, 2.8% (2,901/103,319) did not have a CFTS risk reported due to missing NT data, or an US performed outside of the specified gestational age. These women were excluded from the CFTS analysis, but included in the analysis of serum analytes. Of the 100,418 women who had a CFTS result issued, 3.2% (3,199) had a high-risk result and 90.4% (90,787) a low-risk result for T21. Overall, 2.2% (2,226) of women who had CFTS underwent diagnostic testing.

Diagnostic testing by CFTS risk group: 2014-2015 vs 2002-2004

Among women with a CFTS result for T21 of $\geq$ 1 in 300, the rate of invasive diagnostic testing dropped from 74.1% in 2002-04 to 42.3% in 2014-15 (Figure 1). A decline of similar magnitude was also observed among the women with a T21 risk ≥1 in 50, from 89% to 59%. The number of major chromosome abnormalities was higher in 2014-15 compared with 2002-04, (406 vs 244 respectively), despite lower numbers of invasive diagnostic tests in 2014-15. This translated to a significantly higher diagnostic yield per invasive test respectively (18.2% vs 2.7%, χ^2=788.8, p<0.001) Table 1 summarizes the 406 chromosome abnormalities detected in the 2014-15 CFTS cohort.

Atypical chromosome abnormalities: 2014-15

Amongst the total 2014-15 CFTS cohort, the prevalence of major chromosome abnormalities was 0.4%; 25.1% of these were atypical chromosome abnormalities not detectable with NIPT (n=102). The risks of chromosome abnormality stratified by CFTS trisomy 21 risk are presented in tables 2 and 3. The prevalence of an atypical abnormality increased with CFTS risk, from 1.4% in women with T21 risks of $\geq$1 in 300 to 4.6% for women with risks of > 1 in 10 (Table 3). Over 40% (43/102) of atypical abnormalities were found amongst the low-risk CFTS group <1 in 1000. The

largest group of atypical abnormalities were pathogenic CNVs (n= 47), including 22 deletions or duplications of the 22q11.2 region.

The prevalence of atypical chromosome abnormalities among women with serum PaPP-A or free β-HCG levels <0.2MoM were 6.9% (20/291) and 5.2% (10/192) respectively (Table 4). We examined the corresponding individual CFTS risk results for women with serum markers <0.2MoM and found that 49.2% (58/118) of women with a PaPP-A result of <0.2MoM had CFTS risk for T21 below 1 in 100. Within this group, 39.7% (23/58) had an abnormal karyotype: T21 (n=4), T18 (n=7), T13 and 11 atypical abnormalities (triploidy (n=9), trisomy 16 and level III mosaicism).

Among women with a free β-HCG result of <0.2MoM, 75.0% (33/44) had a CFTS risk below 1 in 100 and 45.0% (18/44) of these women had a pregnancy with an abnormal karyotype. These included T18 (n=10) and 8 atypical abnormalities (triploidy (n=6), pathogenic CNV and trisomy 9).

The primary indications for testing among the 102 pregnancies with an atypical chromosome abnormality were: US abnormality (n = 38), T21 risk >1 in 100 (n=30), T21 risk 1 in 100-300 (n=5), T18 risk > 1in 150 (n=14), and high-risk NIPT (n=3) (Table S1). The remainder of the group had testing performed for other indications including advanced maternal age, family history of aneuploidy, or multiple indications. A substantial proportion of US-indicated tests were performed prior to 18 weeks, (44.7%; 17/38), representing the early detection of structural abnormalities prior to the routine mid-trimester morphology scan. There were 10 women who had an ultrasound abnormality nominated along with another indication for testing. When combined with the primary US indication group, ultrasound abnormality contributed to testing in 47% (n = 48) of the pregnancies with atypical abnormalities.

DISCUSSION

In this period of rapid change in prenatal screening, there has been increasing scrutiny of the contribution of conventional screening to aneuploidy detection over

that provided by standard five-chromosome NIPT. Our dataset is one of the few large population-based datasets able to report on the incremental value of individual serum markers and ultrasound abnormalities on the ascertainment of atypical abnormalities after CFTS. We confirmed an increase in risk of atypical chromosome abnormalities with increasing CFTS risk; 1.4% in those with a CFTS risk of ≥1 in 300 to 4.6% for those with a risk of >1 in 10. In concordance with a previously reported Danish national study, we observed that a substantial proportion of women with analyte levels <0.2 MoM were at increased risk of atypical abnormalities (6.9% for PaPP-A and 5.2% for free β-hCG) (12). Importantly, almost half of these women had a CFTS risk less than 1 in 100, suggesting that that individual serum markers should be independently considered in a decision pathway where NIPT is to be offered after CFTS. In our population, 42.2% of pregnancies with atypical chromosome abnormalities had a CFTS result <1 in 1000 and thus would not be offered diagnostic testing or NIPT within most contingent models.

Our results also show that ultrasound remains a major method of ascertainment of atypical chromosome abnormalities, contributing to the detection of almost one half of cases (47%). Furthermore, seventeen women had an atypical chromosome abnormality detected prior to 18 weeks gestation due to an US abnormality alone, highlighting the continued importance of high-quality first and second trimester ultrasound in the detection of chromosome abnormalities.

Based on our data, 90.2% of atypical abnormalities could be detected by offering diagnostic testing to women with (i) high-risk CFTS (>1 in 100 for T21), (ii) serum analytes <0.2 MoM, or (ii) US abnormality. The other tests that revealed atypical abnormalities were performed for advanced maternal age, which remains a relatively minor but enduring indication for invasive testing in our population. As there is no specific screening test for atypical abnormalities, the 90.2% detection rate using these suggested risk groups seems a reasonable approach, short of routinely offering diagnostic testing to all women, which has been proposed as a warranted approach by some opinion leaders (20).

Other developments in the expansion of NIPT beyond the common autosomal trisomies and sex chromosome abnormalities have the potential to further reduce missed diagnoses of atypical abnormalities, including microdeletions (21, 22), rare autosomal trisomies (23) and sub-chromosomal abnormalities (24). However, due to the paucity of robust clinical validity studies and the potential to inflate the screen-positive rate of NIPT, routine screening for these conditions with NIPT is not currently recommended by any professional society (Society for Maternal Fetal Medicine, American College of Obstetrics and Gynecology, American Congress of Human Genetics and Genomics, Royal Australian and New Zealand College of Obstetricians and Gynaecologists) (5, 25).

Our historical comparison of invasive testing rates demonstrates a systematic shift away from invasive testing across all CFTS risk categories over the past decade, including the group with risks of >1 in 50 who traditionally had high rates of diagnostic testing. However, despite the decline in diagnostic testing, the diagnostic yield has increased markedly due to a number of factors. First, the incorporation of nasal bone into CFTS algorithm in 2011 reduces the false positive rate of CFTS (13). Second, the increasing use of NIPT as a secondary screening test after high-risk CFTS further reduces false positives. Finally, those women that have diagnostic testing are now likely to have fetal chromosome analysis with CMA. CMA made up 75% of all prenatal tests in Victoria in 2014-15 (13) and at least 50% of subspecialists now order CMA routinely for all diagnostic testing (26). The prevalence of pathogenic CNVs in our total diagnostic cohort of 2.0% (51/2211) is similar to the 2.2% reported by Vogel and colleagues in their population-based study on routine CMA after high-risk CFTS (27).

Our study was limited by the lack of access to NIPT data, including the numbers of women using NIPT, and the use of NIPT for primary or secondary screening. We could only speculate that the decline in diagnostic testing was in part, due to the introduction of NIPT. The changing pattern in uptake of diagnostic testing may also be due to the difference in the profile of women now accessing CFTS. In 2002-2004, CFTS was in its introduction phase with <50% population uptake and hence, a

selection bias in the women accessing CFTS may have existed (eg metropolitan residence, higher socioeconomic status, older). Now, with CFTS utilized by over 70% of the population, the preferences for diagnostic testing may have shifted with demographic characteristics, independent of other developments in prenatal screening such as NIPT.

We were also dependent on the information provided by the clinical referrer and the participating laboratories regarding the indications for testing, as we did not have direct access to individual ultrasound reports or hospital records. It is possible that relevant details were not captured with regard to the indications for testing, particularly those with ultrasound abnormalities or multiple indications.

Our analysis of the atypical chromosome abnormalities is limited by the absence of information on pregnancy outcomes. Access to data on pregnancy outcomes would have allowed us to determine pregnancy complications and birth outcomes for the various types of chromosome abnormalities, and ascertain rates of "missed" atypical abnormalities that were subsequently diagnosed at birth. We also confined ourselves to the cohort that had CFTS and did not include atypical abnormalities found in women who underwent other forms of screening, or no screening. This was to simplify the modelling for proposed screening pathways including universal CFTS.

CONCLUSION

This study demonstrates that the contemporary ascertainment of atypical chromosome abnormalities within a large CFTS cohort is predominantly via fetal structural abnormalities, and to a lesser extent, CFTS risk. A clinical pathway that offers diagnostic testing for women with CFTS >1 in 100, individual serum analytes <0.2MoM, or ultrasound abnormality would detect the vast majority (>90%) of atypical chromosome abnormalities not detected by NIPT in our population.

Acknowledgments

Contributors to the Victorian Prenatal Diagnosis Database

The Victorian Clinical Genetics Services (VCGS), Monash Medical Centre, and the private laboratories of Australian Clinical Laboratories and Melbourne Pathology form the contributors to the Victorian Prenatal Diagnosis Database. We gratefully acknowledge the following individuals within this collaboration for their cooperation and support: Lucy Gugasyan, Amanda Howden, Fiona Norris, Mark Pertile.

We are also grateful to Leonard Bonacquisto for contributing the serum screening data from VCGS.

Details of Ethics Approval

Human Research Ethics Committee (HREC) approval for the prenatal diagnosis data collection and associated research was obtained from the Royal Children's Hospital HREC on 17 Jan 2012 (Ref. No. 31135A) and Monash Health HREC on 18 Apr 2012 (Ref. No. 12063B).

REFERENCES

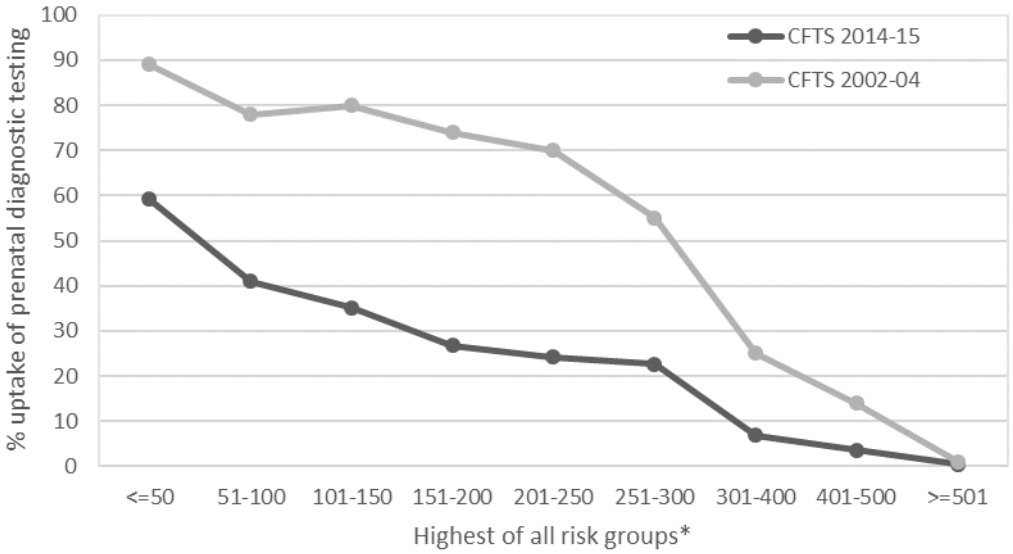
1. Oepkes D, Page-Christiaens GC, Bax CJ, Bekker MN, Bilardo CM, Boon EM, et al. Trial by Dutch laboratories for evaluation of non-invasive prenatal testing. Part I-clinical impact. Prenat Diagn. 2016;36(12):1083-90.
2. Dickinson JE. Noninvasive prenatal testing: known knowns and known unknowns. Aust N Z J Obstet Gynaecol. 2014;54(5):397-9.
3. Borrell A, Stergiotou I. Cell-free DNA testing: inadequate implementation of an outstanding technique. Ultrasound Obstet Gynecol. 2015;45(5):508-11.
4. Diagnosis ISfP. Prenatal detection of Down Syndrome using Massively Parallel Sequencing (MPS): a rapid response statement from a committee on behalf of the Board of the Internataional Society for Prenatal Diagnosis. International Society for Prenatal Diagnosis; 2011 24/10/2011.
5. Gregg AR, Skotko BG, Benkendorf JL, Monaghan KG, Bajaj K, Best RG, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med. 2016;18(10):1056-65.
6. Sonek JD, Cuckle HS. What will be the role of first-trimester ultrasound if cell-free DNA screening for aneuploidy becomes routine? Ultrasound Obstet Gynecol. 2014;44(6):621-30.

7. Gil MM, Giunta G, Macalli EA, Poon LC, Nicolaides KH. UK NHS pilot study on cell-free DNA testing in screening for fetal trisomies: factors affecting uptake. Ultrasound Obstet Gynecol. 2015;45(1):67-73.
8. Morris S, Karlsen S, Chung N, Hill M, Chitty LS. Model-based analysis of costs and outcomes of non-invasive prenatal testing for Down's syndrome using cell free fetal DNA in the UK National Health Service. PLoS One. 2014;9(4):e93559.
9. Maxwell S, Dickinson JE, Murch A, O'Leary P. The potential impact of NIPT as a second-tier screen on the outcomes of high-risk pregnancies with rare chromosomal abnormalities. Aust N Z J Obstet Gynaecol. 2015;55(5):420-6.
10. Susman MR, Amor DJ, Muggli E, Jaques AM, Halliday J. Using population-based data to predict the impact of introducing noninvasive prenatal diagnosis for Down syndrome. Genet Med. 2010;12(5):298-303.
11. Petersen OB, Vogel I, Ekelund C, Hyett J, Tabor A, Danish Fetal Medicine Study G, et al. Potential diagnostic consequences of applying non-invasive prenatal testing: population-based study from a country with existing first-trimester screening. Ultrasound Obstet Gynecol. 2014;43(3):265-71.
12. Larion S, Warsof SL, Romary L, Mlynarczyk M, Peleg D, Abuhamad AZ. Uptake of noninvasive prenatal testing at a large academic referral center. Am J Obstet Gynecol. 2014;211(6):651 e1-7.
13. Hui L, Hutchinson B, Poulton A, Halliday J. Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy. Genet Med. 2017.
14. Robson SJ, Hui L. National decline in invasive prenatal diagnostic procedures in association with uptake of combined first trimester and cell-free DNA aneuploidy screening. Aust N Z J Obstet Gynaecol. 2015;55(5):507-10.
15. Warsof SL, Larion S, Abuhamad AZ. Overview of the impact of noninvasive prenatal testing on diagnostic procedures. Prenat Diagn. 2015;35(10):972-9.
16. Sachs A, Blanchard L, Buchanan A, Norwitz E, Bianchi DW. Recommended pre-test counseling points for noninvasive prenatal testing using cell-free DNA: a 2015 perspective. Prenat Diagn. 2015;35(10):968-71.
17. Hui L, Muggli EE, Halliday JL. Population-based trends in prenatal screening and diagnosis for aneuploidy: a retrospective analysis of 38 years of state-wide data. BJOG. 2016;123(1):90-7.
18. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med. 2012;367(23):2175-84.
19. Jaques AM, Collins VR, Muggli EE, Amor DJ, Francis I, Sheffield LJ, et al. Uptake of prenatal diagnostic testing and the effectiveness of prenatal screening for Down syndrome. Prenat Diagn. 2010;30(6):522-30.

20. Evans MI, Wapner RJ, Berkowitz RL. Noninvasive prenatal screening or advanced diagnostic testing: caveat emptor. Am J Obstet Gynecol. 2016;215(3):298-305.
21. Helgeson J, Wardrop J, Boomer T, Almasri E, Paxton WB, Saldivar JS, et al. Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. Prenat Diagn. 2015;35(10):999-1004.
22. Gross SJ, Stosic M, McDonald-McGinn DM, Bassett AS, Norvez A, Dhamankar R, et al. Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome. Ultrasound Obstet Gynecol. 2016;47(2):177-83.
23. Pertile MD, Halks-Miller M, Flowers N, Barbacioru C, Kinnings SL, Vavrek D, et al. Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease. Sci Transl Med. 2017;9(405).
24. Lefkowitz RB, Tynan JA, Liu T, Wu Y, Mazloom AR, Almasri E, et al. Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants. Am J Obstet Gynecol. 2016;215(2):227 e1- e16.
25. Gynaecologists RAaNZCoOa. Prenatal screening and diagnosis of chromosomal and genetic conditions in the fetus in pregnancy. Royal Australian and New Zealand College of Obstetricians and Gynaecologists; 2016.
26. Hui L, The S, McCarthy EA, Walker SP. Emerging issues in invasive prenatal diagnosis: Safety and competency in the post-NIPT era. Aust N Z J Obstet Gynaecol. 2015;55(6):541-6.
27. Vogel I, Petersen OB, Christensen R, Hyett J, Lou S, Vestergaard EM. Chromosomal microarray as a primary diagnostic genomic tool for pregnancies defined as being at increased risk within a population based combined first-trimester screening program. Ultrasound Obstet Gynecol. 2017.

FIGURE LEGENDS

Figure 1. Rates of diagnostic testing by combined first trimester screening (CFTS) result in 2002-2004 and 2014-2015



*includes a high-risk result across any of T21, T18 or T13

Table 1. Abnormal fetal karyotype results in the 2014-15 combined first trimester screening cohort*

Abnormal karyotype	N (% of total abnormalities)
Total major abnormality	304 (74.9%)
T21	215
T18	45
T13	15
Sex chromosome aneuploidy	29
Total atypical abnormalities	102 (25.1%)
Other autosomal trisomy	3 (T16 and T9)
Triploidy	20
Pathogenic CNV*	47
Mosaicism	30
Other	2
TOTAL	406

*Total prenatal diagnostic tests =2,226

Table 2. Chromosomal abnormalities stratified by risk of trisomy 21 on combined first-trimester screening (CFTS) amongst the total study population (146,776 births)

CFTS Trisomy 21 risk group	Total pregnancies	PNDx n (%)	Abnormal karyotype (% of PNDx)	Atypical abnormalities* not detectable with NIPT n (% of PNDx)	Prevalence of atypical abnormalities as % of total pregnancies
>1 in 10	345	245 (71.0)	155 (63.3)	16 (10.3)	4.6
1 in 10 – 1 in 19	152	94 (61.8)	19 (20.2)	4 (4.2)	2.6
1 in 20 – 1 in 49	373	198 (53.1)	38 (19.2)	7 (3.5)	1.9
1 in 50 – 1 in 99	464	188 (40.5)	32 (17.0)	11 (5.9)	2.4
1 in 100 – 1 in 199	909	298 (32.7)	25 (8.4)	3 (1.0)	0.3
1 in 200 – 1 in 299	883	219 (24.8)	15 (6.8)	3 (1.4)	0.3
≥1 in 300 risk	3,199	1,257 (39.3)	286 (22.8)	45 (3.6)	1.4
1 in 300 – 1 in 999	6,505	233 (3.6)	29 (12.4)	15 (6.4)	0.2
≤1 in 1000	90,787	736 (0.8)	93 (12.6)	43 (5.8)	0.05
TOTAL	*100,418*	*2,226 (2.2)*	*406 (18.2)*	*102 (4.6)*	*0.1*

*atypical abnormalities: excluding T21, T18, T13, and sex chromosome aneuploidy

Table 3. Cumulative prevalence of abnormalities by trisomy 21 risk on combined first trimester screening

CFTS cumulative risk group	Prevalence of atypical abnormality (%)	% of all atypical chromosomal abnormalities in CFTS cohort (n=102)	Prevalence of total abnormalities by CFTS risk (%)	% of all total chromosomal abnormalities in CFTS cohort (n=406)
CFTS >1 in 10	16/345 (4.6)	15.7	155/345 (44.9)	38.1
CFTS >1 in 50	27/870 (3.1)	26.5	212/870 (24.4)	52.2
CFTS >1 in 100	38/1,334 (2.8)	37.3	246/1,334 (18.4)	60.6
CFTS ≥1 in 300	45/3,199 (1.4)	44.1	286/3,199 (8.9)	70.4
CFTS >1 in 1000	59/9,631 (0.61)	57.8	315/9,704 (3.2)	77.5
Total CFTS group	102/100,418 (0.10)		406/100,418 (0.40)	

Table 4. Chromosomal abnormalities stratified by pregnancy-associated plasma protein-A (PaPP-A) multiples of the median (MoM) and by free β-human chorionic gonadotropin (β-hCG) MoM amongst the study population (146,776 births)

Risk group	Total pregnancies*	PNDx performed (% total pregnancies)	Total Abnormal karyotype (% of PNDx)	Atypical karyotype (% of PNDx)	Prevalence of atypical karyotype (% of total pregnancies)
PaPP-A MoM					
<0.2	291	118 (40.5)	61 (51.7)	20 (16.9)	6.9
0.2 – 0.39	4,114	419 (10.2)	129 (30.8)	20 (4.8)	0.5
0.4 – 0.99	45,041	1,001 (2.2)	150 (15.0)	34 (3.4)	0.08
1.0 – 1.99	38,225	457 (1.2)	24 (5.3)	17 (3.7)	0.04
>=2.0	11,380	124 (1.1)	15 (12.1)	9 (7.3)	0.08
TOTAL	*99,051*	*2,119 (2.1)*	*379 (17.9)*	*100 (4.7)*	*0.1*
Free β-hCG MoM					
<0.2	192	41 (20.9)	25 (61.0)	10 (24.4)	5.2
0.2 – 0.99	40,143	622 (1.5)	109 (17.5)	29 (4.7)	0.07
1.0 – 1.99	53,041	1,117 (2.1)	204 (18.3)	49 (4.4)	0.09
2.0 – 3.99	8,899	353 (55.3)	55 (15.6)	11 (3.1)	0.1
4.0 – 4.99	623	59 (9.2)	11 (18.6)	3 (5.1)	0.5
>=5.0	421	37 (8.6)	6 (16.2)	1 (2.7)	0.2
TOTAL	*103,319*	*2,229 (2.4)*	*410 (18.4)*	*103 (4.6)*	*0.1*

*Total number of pregnancies includes women who had serum testing but no US and therefore no CFTS result