Joanne Kelley (Orcid ID: 0000-0003-2507-0155)
Lisa Hui (Orcid ID: 0000-0002-9720-3562)

Title: Increased nuchal translucency after low risk noninvasive prenatal testing: what should we tell the prospective parents?

Short running title: Increased NT after low risk NIPT

Manuscript word count: 4,452

No of tables: 5

No of figures: 3

Authors and affiliations

Joanne Kelley[1]

George McGillivray[1,2]

Simon Meagher[1,3,4]

Lisa Hui[1,4]

1. Department of Perinatal Medicine, Mercy Hospital for Women, Heidelberg VIC, Australia
2. Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville VIC, Australia
3. Monash Ultrasound for Women, Monash IVF Group, Melbourne VIC, Australia
4. Department of Obstetrics and Gynaecology, University of Melbourne, Parkville VIC Australia

Corresponding author:

Joanne Kelley
Department of Perinatal Medicine, Mercy Hospital for Women, 163 Studley Road, Heidelberg VIC 3084, Australia
Telephone: +61 38458 4346
E-mail: jkelley@mercy.com.au

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/pd.6024.

Conflict of interest statement: None to declare.

Funding statement: LH received salary support from a Medical Research Future Fund emerging leader fellowship.

Data availability statement: NA. No new data was created in this manuscript.

Ethics statement: Ethics approval not required as no new data was created in this manuscript.

Keywords

Nuchal translucency, NIPT, aneuploidy, chromosomes, prenatal screening, increased NT, cell-free DNA, Noonan's syndrome, fetal exome, RASopathy, fetal anomaly

What is already known about this topic?

- Noninvasive prenatal testing (NIPT) is a superior screen for the common autosomal trisomies 13, 18 and 21
- Increased nuchal translucency (NT) is associated with chromosomal, genetic and structural anomalies
- Options for advanced genomic testing are increasing

What does this paper add?

- Review of the definition "increased NT" in the context of changing practice and guidelines

- Synthesized data from available literature to provide updated clinical guidance on management of pregnancies with increased NT and low risk NIPT, incorporating psychosocial needs of parents

Abstract

Three decades ago, the observation that first trimester fetuses with excess fluid accumulation at the back of the neck were more likely to be aneuploid, gave rise to a new era of prenatal screening. The nuchal translucency (NT) measurement in combination with serum biomarkers and maternal age, resulted in the first trimester combined screening (FTCS) program. The introduction of noninvasive prenatal testing (NIPT) over the past decade has resulted in the option for parents to receive highly sensitive and specific screening information for common trisomy from as early as 10 weeks gestation, altering the traditional pathway FTCS pathway. The retention of the 11-13 week NT ultrasound remains important in the detection of structural anomalies, however the optimal management of pregnancies with a low risk NIPT result and an isolated increased NT measurement in an era of advanced genomic testing options is a new dilemma for clinicians. For parents, the prolonged period between the initial diagnosis in first trimester, and prognostic information at each successive stage of investigations up to 22-24 weeks, can be emotionally challenging. This article addresses the common questions from parents and clinicians as they navigate the uncertainty of having a fetus diagnosed with an increased NT after a low risk NIPT result and presents suggested approaches to management.

Introduction

Three decades ago, the critical observation that first trimester fetuses with excess fluid accumulation at the back of the neck were more likely to be aneuploid gave rise to a new era of prenatal screening.[1] The nuchal translucency (NT), as it became known, subsequently formed a key element of the first trimester combined screen (FTCS) in combination with serum biomarkers and maternal age. The FTCS quickly became the benchmark for prenatal screening for trisomy 21, 13 and 18, providing detection rates of 85% or more for pregnancies at 11-13 weeks gestation.[2] However, with the implementation of noninvasive prenatal testing (NIPT) over the past decade, FTCS has yielded some of its dominance, with 20% or more of women in some countries now choosing NIPT as their first line screening test.[3,4,5]

Although NIPT has the highest sensitivity and specificity of any screening test for the common autosomal aneuploidies,[6] professional societies continue to recommend and endorse the retention of the 11-13 week NT ultrasound as it provides the opportunity for accurate pregnancy dating, identification of multiples/chorionicity and early detection of fetal anomalies, .[7,8] In the current molecular era of NIPT, microarray and fetal exome sequencing, the debate has shifted from the value of retaining the 11-13 week ultrasound for early structural anomaly detection, to questions about the optimal management of pregnancies with a low risk NIPT result and an increased NT measurement.[9,10] This article addresses the common questions from parents as they navigate the uncertainty of having a fetus diagnosed with an increased NT after a low risk NIPT result, and presents the rationale for our approach to management.

Nuchal translucency measurement

1.What is the NT and why is it measured?

The NT is described as the subcutaneous fluid visualised behind the fetal neck on ultrasound in first trimester.[1] It is a powerful marker of aneuploidy when measured in fetuses with crown rump length (CRL) of 45-84 mm correlating with gestations of 11+0 to 13+6 weeks.[1] Distinctions between increased NT, enlarged jugular sacs, cystic hygroma (a septated increased NT), or hydrops in the first trimester can be unclear; however clinical management follows a similar approach regardless of the variations in appearance (Figure 1).[11] A heterogeneous range of conditions are associated with increased NT, reflecting varied underlying mechanisms including cardiac dysfunction, altered composition of the extracellular matrix, venous congestion in the head and neck, and failure of lymphatic drainage.[12]

Measuring the NT remains an important component of the 11-13 week scan in the context of a low risk NIPT result, as a marker for genetic conditions and fetal anomalies that would not be detected on NIPT (Table 1).[13,14] Prognostically, the overall risk of an adverse pregnancy outcome after normal conventional karyotype is proportional to the NT measurement (Table 2).[15]

Key counselling points: *The measurement of NT remains an important component of the 11-13 week ultrasound due to association with structural and genetic anomalies not detected by NIPT. There is an 80% risk of adverse pregnancy outcome when the NT measures > 6.5mm (Table 2).*

2.What is an abnormal NT measurement?

The normal range of NT changes with gestational age in euploid fetuses.[12] When considered independently from the FTCS screening algorithm, an 'increased NT' triggering diagnostic evaluation has been variously defined as a fixed measurement of >=3.5mm[16], or a gestation-specific threshold of >= 99th centile.[17] Other authors suggest lower thresholds for offering genetic counselling and prenatal diagnosis such as 3.0mm[18,19] or the 95th centile.[10] The International Society for Ultrasound in Obstetrics and Gynecology (ISUOG) does not specify a definition of 'increased NT', but the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal Fetal Medicine (SMFM) now recommend thresholds of 3.0mm or the 99th centile, although these two definitions are not equivalent.[7,8]

Bardi et al recently calculated the Area Under the Curve (AUC) of NT measurement and found that the best NT threshold was 3.55mm for the prediction of congenital anomalies.[10] Similarly, the best cutoff for the prediction of adverse pregnancy outcome was NT of 3.6mm (AUC 0.779, for sensitivity of 70% and specificity of 75%). If a threshold below 3.5mm is to be used, we recommend a gestational-age controlled cutoff of 99th centile, or 1.9 multiples of the median (MoM) as this has similar detection rate for atypical chromosome abnormalities as a 3.0mm threshold, but with 31% fewer false positives.[20] This adjustment for gestational age is particularly relevant as women with low risk NIPT at 10-11 weeks may be advised to schedule their first trimester anatomy scan in the latter half of the 11-13+6 week window (ie 12+4 -13+6 weeks) in order to improve detection of structural defects.[21] This shift could have the effect of increasing the number of euploid fetuses that have an NT >3.0mm but below the 99th centile (Figure 2).

Key counselling points: *There is no clear international consensus on a definition of an increased NT after low risk NIPT, beyond the traditional fixed cutoff of 3.5mm. If a lower threshold is to be chosen, a gestational age-controlled threshold such as the 99th centile or 1.9 MoM may minimise false positive rates for atypical chromosome abnormalities.*

3. What about nuchal edema before 11 weeks gestation?

The increasing local practice of performing a "pre-NIPT" ultrasound at 10 weeks to exclude missed miscarriage, multiple pregnancy or incorrect dating prior to NIPT may lead to the detection of nuchal edema in fetuses with CRL < 45mm. This creates clinical uncertainty as there are no established protocols for management of an increased NT before 11 weeks. A recent retrospective study reporting outcomes in more than 100 fetuses with CRL of 28-45mm CRL and nuchal edema >2.2 mm (95th percentile for NT at 10 weeks) or hydrops showed that the risks of structural (4%) or chromosome abnormalities (19%) increased with NT size.[22] Of the 77/104 pregnancies that continued to a 11 to 13^{+6} weeks ultrasound, 82% had resolution of nuchal edema and these cases had fewer adverse outcomes (miscarriage, structural or chromosomal anomaly) than those who developed a NT ≥3.5 mm (10.9% vs 76.5%, respectively P < .001). Of the 76 women who received a low risk NIPT result, two received an atypical chromosome result from diagnostic testing. Both these fetuses had generalized edema, rather than isolated nuchal edema.

When there are concerning appearances before the timeframe for a formal NT assessment, our advice is to consider deferring NIPT and re-assess with ultrasound at 12-13 weeks. If an enlarged NT is still present or a structural abnormality is evident (including generalized edema) at 12-13 weeks, diagnostic testing should be offered. If the nuchal edema has resolved and no structural abnormalities are detected, then a choice of diagnostic testing or NIPT would be appropriate. More data from larger prospective studies are needed before we can confidently offer management recommendations based on the independent predictive value of nuchal edema at < 11 weeks.

Key counselling points: *While nuchal edema before 11 weeks may indicate an increased risk of miscarriage or fetal abnormality, many will resolve spontaneously. Further assessment at 12-13 weeks will assist in determining if a screening or a diagnostic approach is warranted.*

4.Did I do something to cause this?

Parents are often concerned that the finding of increased NT is due a behaviour in pregnancy, or sometimes other cultural or spiritual beliefs. Ideally this question is answered

by clinicians in a way that addresses both the psychosocial need for reassurance and practical elements of the question.

Key counselling points: *With few exceptions, identified causes of increased NT are unrelated to maternal behaviour or environmental exposures, and reassurance can usually be provided.*

Noninvasive prenatal testing

5.So what problems have been excluded by my low risk NIPT result?

What appears to be a straightforward question is becoming increasingly complicated given the emergence of numerous categories of NIPT with varying coverage of the genome. There are also variable detection rates within a particular assay depending on the chromosome, fetal fraction, platform and size of the genomic region of interest. Clinicians need to understand the specific NIPT product, its limitations (biological and statistical) and the concept of residual risk to answer this question for parents. In some settings, such as the Netherlands, where there is a single publicly funded provider with published data, this may be relatively straightforward.[23] However, in other settings such as Australia, there are a variety of providers, each with their own options. Most NIPT platforms in current clinical use in Australia utilize massively parallel sequencing, with single nucleotide polymorphism (SNP) based approaches less common. Options include:

- Targeted NIPT for the common autosomal trisomies: trisomy 21, 13 and 18, +/- sex chromosome aneuploidy (SCA) and fetal sex
- Genome-wide NIPT: autosomal trisomies of chromosomes 1-22, segmental chromosomal aneuploidy > 10Mb size for chromosomes 1-22, +/- SCA
- Targeted NIPT for the common autosomal trisomies with 22q11.2 microdeletion syndrome +/- other selected microdeletion syndromes
- Targeted NIPT plus triploidy +/- microdeletion(s) screening (SNP based option)

The confidence that a condition has been excluded by a low risk NIPT results depends on the negative predictive value (NPV). This is a function of the background prevalence of the condition and the sensitivity of NIPT for detecting that particular condition. The presence of a common autosomal aneuploidy in a pregnancy would be very unlikely after a low risk NIPT result (unless in mosaic form), particularly if the fetus appears structurally normal. However, confidence in the NPV for other conditions varies and is hampered by biological variation and limited published follow-up on cohorts with low risk NIPT results. In summary, we advise parents that a low risk NIPT cannot provide reassurance for conditions other that the common autosomal trisomies.

Key counselling points: *While the presence of a common autosomal aneuploidy is very unlikely after a low risk NIPT result, NIPT cannot exclude any condition with complete certainty. Most NIPT does not exclude microdeletions/duplications and mosaic forms of whole chromosome aneuploidy at all.*

Initial assessment

6. What should we do now?

a. Family and medical history

The value of taking a detailed pedigree or family history is two-fold; as an important tool for insight into family history of miscarriage, congenital anomaly, stillbirth, consanguinity, physical and intellectual disability, and as a means by establishing rapport with parents.[24] This exploration of both the genetic and psychosocial landscape may provide information regarding the potential for rare underlying monogenic causes and inheritance patterns of increased NT, and assist clinicians in establishing a working alliance with parents. A medical history may reveal potential risks factors for structural anomalies, such as pre-pregnancy diabetes mellitus, or teratogen exposure including congenital infection.

While fetal infections have been associated with nuchal edema, it is most strongly associated with fetal hydrops in second and third trimester.[25] In a study of 426 euploid pregnancies with increased NT at 10-14 weeks, six had maternal serology suggestive of recent maternal infection, but none were confirmed to have fetal infection.[26] Based on this study, we reserve serological testing for women with a specific clinical history that suggests an increased risk of cytomegalovirus, parvovirus or toxoplasmosis infection.

b. Specialist fetal ultrasound at 12-13 and 16 weeks

Structural anomalies are present in approximately 1 in 3 (30.7%) of euploid fetuses with NT >=3.5mm.[27] A detailed specialist obstetric ultrasound is recommended for all fetuses with an increased NT given the increased risk of a structural abnormality. The detection rate of

structural abnormalities with an expert 12-13 week ultrasound has improved considerably over the past decade, particularly with the use of transvaginal imaging, and it is now expected to detect at least 50% of anomalies seen in the general fetal population.[28,29]

The value of a 16 week ultrasound as both an assessment of further NT evolvement and other structural anomalies not seen at 13 weeks can provide parents with information that may prompt further investigation or provide reassurance.[13,30] In a recent study published by Le Lous et al, the 16 week scan detected the greatest proportion of associated structural abnormalities: the first trimester scan detected 31.3%, the 16 week scan 41.2% and the 22w scan detected the remaining 27.4%.[27] These authors showed that a normal 16 week scan was associated with favourable outcomes in 85% of pregnancies, and that those with a normal 20 week scan had almost 100% chance of a favourable outcome. Thus, an additional scan at 16 weeks is helpful for facilitating earlier diagnosis of major structural anomalies, or conversely, providing interim reassurance before the 20-22 weeks ultrasound.

If a lower NT threshold of >=95th centile is employed, the overall rate of associated structural abnormalities is lower, at about 1 in 10 (9.3%).[10] The majority of these structural anomalies are also detectable before 18 weeks, either at the time of the 11-13 weeks scan or at the time of tertiary referral (before 18 weeks).

Early diagnosis of a fetal anomaly provides parents with further prognostic information and provides a strong indication for diagnostic testing with chromosomal microarray (CMA).[31,32] The chance of additional clinically relevant finding on CMA after normal conventional karyotype is 7% in fetuses with increased NT and structural anomaly, compared with 4% in fetuses with an isolated increased NT.[16] However, where there is a clear diagnosis of a major structural anomaly, parents may feel they have sufficient information on which to base a decision about termination of pregnancy (TOP) without prenatal diagnostic testing. Parents should be supported appropriately and offered postnatal testing on products of conception.

c. Diagnostic testing with CMA

There are numerous studies examining the yield of CMA after normal karyotype for the isolated finding of increased NT.[16,19,33,34] A meta-analysis from 2015 demonstrated that 5% of fetuses with increased NT (predominantly defined in the included studies as >=3.5mm) had a clinically-significant copy number variant (CNV) detected after a normal karyotype.[16] In contrast, the yield of CMA after normal karyotype for a NT threshold of 3.0mm is similar to that for diagnostic testing in pregnancies without an ultrasound abnormality, that is, 0.37% (1 in 270).[19,33,34,35]

Key counselling points: *A medical history, detailed obstetric ultrasound and offer of diagnostic testing with CMA rather than karyotype should be part of the assessment of women with increased NT and low risk NIPT.*

Yield from prenatal diagnosis

7. What is the chance of a chromosome condition being detected on CMA after low risk NIPT?

Several recent studies have modelled the yield of CMA testing after low risk NIPT results for targeted NIPT including the SCA, summarized in Table 3. The most common conditions that would be missed by targeted NIPT include: triploidy, rare autosomal trisomies (often in mosaic form, including confined placental mosaicism), 22q11.2 deletion syndrome, other pathogenic CNVs, and mosaic monosomy X.[10,20,35] The risk of a clinically significant chromosome abnormality varies depending on the definition of increased NT, presence of associated structural abnormalities, as well as the coverage of the prior NIPT.

Overall, the frequency of a clinically significant condition being detected after prenatal diagnosis in fetuses with NT >=3.5mm or >=99th centile after low risk NIPT is 3.5-6.1%.[10,17,20] For those with NT 3.0-3.4mm or 95-99th centile, the risk is lower at 1.5-1.9%. However, when including all pregnancies with NT 3.0-3.4mm as the denominator, and not just the subgroup that undergoes prenatal diagnosis, the chance is lower at 0.37% (95%CI 0.05-1.35%).[20] However, this is still within a range where diagnostic testing is conventionally offered after screening and the option of diagnostic testing may be discussed with parents with this finding in pregnancy.

The presence of an associated ultrasound abnormality increases the risk of a chromosome abnormality. In a large population-based study with over 80,000 women, the risks of an atypical abnormality after low risk targeted NIPT (assessing chromosomes 21,13,18, X and Y)

increased from 3.1% to 11.0% in the presence of an additional structural anomaly. The most common pathogenic CNV detected was 22q11.2 deletion syndrome, consistent with this CNV ranking as the most common in other populations.[20]

The risk of a submicroscopic deletion/duplication after whole genome NIPT for fetuses with NT 3.0-3.4mm.

In a review synthesizing original data and previously published studies, Petersen et al calculated the residual risks in fetuses with NT 3.0-3.4mm and high risk combined first trimester result.[35] They reported that the risk of a CNV was 1 in 500 (0.2%) if a genome-wide 10-Mb resolution NIPT test had returned a low risk result.

Bardi et al reported the outcomes on 1007 fetuses with NT 95-99th centile after low risk whole genome NIPT and calculated a 0.9% risk of a submicroscopic CNV. This risk rose to 3% for those fetuses with NT >=99th centile.[10]

Key counselling points*: Increasing NT measurements are associated with higher chance of a pathogenic CNV, with the presence of additional ultrasound abnormalities further increasing this chance. A threshold that segregates those with a higher-than-background chance of an atypical chromosome condition is 3.5mm and/or > 99th centile[8]/ 1.9MoM[20]. Prenatal diagnosis and CMA provide important information to parents.*

Genomic testing

8. My microarray result was normal. Are there more genetic tests that I can have?

After a normal CMA in the presence of an increased NT, genetic counselling may turn to the option of testing for single gene conditions. About half of all single gene conditions associated with increased NT belong to the RASopathy family[10], and associated panels are the most common testing that is offered for increased NT after normal CMA.

RASopathy testing

Collectively known as RASopathies, this group of genetic conditions result from dysregulation of the *Ras/mitogen-activated protein kinase* (RAS-MAPK) signalling pathway. There are more than 20 genes in this pathway. The most common RASopathy syndrome, Noonan's syndrome, has a well-documented prenatal ultrasound phenotype including increased NT with or without cystic hygroma, hydrops, pleural effusion and cardiac abnormalities.[36,37] Diagnostic testing for RASopathies are commonly performed using next generation sequencing with a customised gene panel, or exome or genome sequencing with a targeted data analysis.

Bardi et reported the overall rate of a single gene condition in fetuses with NT > 99th centile (including those with structural abnormalities) to be 3.3%, of which Noonan's syndrome made up almost half (1.4%)(Table 4).[10] When there are multiple ultrasound features associated with RASopathies, an overall yield of 10-14% has been reported after a normal CMA.[36-40] In the most recent study, Scott et all found that the overall diagnostic yield for RASopathy testing after a normal CMA was 14% (50/352), but was much lower for isolated

NT (1/90) compared with NT with other typical ultrasound abnormalities (16%, 27/167) (Table 4).[39] These findings are consistent with the large study on prenatal RASopathy testing by Stuurman et al, which recommended that RASopathy testing only be offered for very large isolated NTs (>=5.0mm), or if at least one other typical ultrasound feature was present.[36]

Exome and whole genome sequencing

The final step in the prenatal diagnostic journey of increased NT could be exome or whole genome sequencing, with analysis of many genes linked to single gene conditions presenting before or after birth. One of the earliest fetal exome studies that included 5 fetuses with isolated increased NT did not return any positive diagnoses.[41] Since then, larger cohorts of fetuses with isolated and non-isolated increased NT have been studied with sequencing after normal microarray (Table 5).[42-48] Yang et al performed trio exome sequencing on 73 fetuses with isolated first trimester increased NT >=3.5mm and normal CMA.[45] Three of four cases with pathogenic variants developed structural anomalies on ultrasound at mid pregnancy, leading to pregnancy termination. When considering only those fetuses with isolated first trimester increased NT, normal CMA and with no ultrasound abnormalities in second trimester, the diagnostic yield of exome sequencing in that study was only 1.4% (1/70). Sparks et al performed exome sequencing on 127 second trimester fetuses with nonimmune hydrops, defined as the presence of fetal ascites, pleural or pericardial effusions, skin edema, cystic hygroma, increased NT, or a combination of these conditions.[44] The median gestation at diagnosis was 20 weeks (range 13.4 to 24.6). Among the 29 cases with increased NT or cystic hygroma (either isolated or concurrent with other anomalies), 31% (9/29) had a diagnostic variant. However, among the cases with isolated increased NT or cystic hygroma, the diagnostic yield was 7% (1/15). Not surprisingly, the presence of hydrops, effusions or other lymphatic system anomalies increased the yield of pathogenic variants up to 24-34%.[42,44] When combining all the apparently isolated NT > 3.5mm from these studies, the weighted average diagnostic yield was 3.7%, including RASopathy conditions. (Table 5).

When to offer fetal exome or RASopathy panel can be impacted by the competing elements of awaiting further ultrasound phenotypic information to guide appropriate testing, and parental desire for comprehensive and timely results. Genomic testing is expensive, requires careful pre- and post-test counselling, specific gene selection and variant interpretation in the context of the fetal phenotype. The cost to the patient, service and ultimately the public healthcare sector are important considerations that will vary according to the local context.

Key counselling points: *Single gene testing with a RASopathy panel is expected to have a yield of ~1% and exome sequencing ~ 3-4% for fetuses with isolated increased NT and normal CMA. Comprehensive pre- and post-test counselling, that includes setting realistic expectations of further testing are important considerations. Progressive fetal phenotype can better assist test choice, gene selection and variant interpretation.*

Managing uncertainty and supporting decision-making

9. When will I know if my baby is healthy? I am having trouble coping.

Receiving news about a possible or confirmed fetal anomaly is acutely distressing for parents.[49-51] Parents benefit greatly from timely access to information, support and multidisciplinary care when faced with an uncertain prognosis in their pregnancy.[49,52] Information provision of itself is not sufficient for parents to feel supported during the often months-long journey of uncertainty during the pregnancy. Information needs to be integrated and contextualized for parents, with attention to parent responses, questions and concerns.[53,54] Genetic counsellors can assist prospective parents to manage uncertainty by providing specific clinical information and anticipatory guidance around potential outcomes of further testing, as well as identifying areas of control and supporting coping mechanisms for the couple.[50,51,55] Supportive decision-making should be non-directive in terms of the decision itself, but directive in terms of guiding parents through the diagnostic journey.[49] Some parents may benefit from referral to specialist perinatal mental health services, particularly in context of previous mental health issues or persistent distress.[52]

Key counselling points*: Acknowledging the emotional impact of diagnostic uncertainty and providing guidance and support is a key component of care.*

10. What should I tell my family and friends? Can I have a termination of pregnancy?

Parents often have difficulty sharing uncertain prenatal information with family and friends, increasing the potential for social alienation, increased grief reactions and emotional

distress.[56] These feelings can be compounded by expectations of negative judgement a couple elects to have a termination of pregnancy.[57,58] Exploring sources of support with parents, offering simple written and verbal explanation example, and revising knowledge at subsequent information points can assist parents with this process.

Women and their partners may wish to discuss TOP early in the journey, particularly if the NT is large (Table 2). A woman's decision about TOP is dependent on many factors, including risk perception, tolerance for uncertainty, spiritual beliefs, personal and family experience of grief or disability, and many other components of complex decision-making. Parents may change their views regarding TOP when faced with a poor obstetric outcome or diagnosis of a genetic condition, and exploration of choices, appreciating context and providing non-judgemental support is essential. Access to safe and timely termination of pregnancy varies greatly according to location and healthcare setting. Clinicians caring for parents should have an awareness of the laws, accessibility, and barriers that could impact a woman's decision-making, and raise these potential issues with patients while counselling. Many couples benefit from the multidisciplinary nature of fetal medicine teams, with access to support from pastoral care, social workers, and other mental health care professionals.

Key counselling points: *Parents benefit from simple verbal and written resources to understand and explain the finding of increased NT in pregnancy. Clinicians have an obligation to be aware of services and facilitate referral for TOP as an essential component of patient care.*

Final assessment

11. My 20 week scan was normal and so was my CMA. What is the chance my baby will be born with a problem?

After a normal 20 week ultrasound showing resolution of nuchal edema and normal CMA, parents should be reassured that the chances of having a child with a significant health issue are probably not greater than the background risk.[59,30] A recently published prospective study compared the neurodevelopmental outcomes of 203 children who had isolated increased NT≥95th centile with a control group with normal NT measurements.[60] Using objective psychomotor testing at 2 years corrected age, the investigators found that the mean developmental quotient of the increased NT group was within the normal range, although it was lower than the control group. These reassuring findings also applied for those who had an isolated NT ≥ 3.5mm.[60]

While the majority of major cardiac defects are detectable by 18-22 weeks, a small proportion may not be recognisable until the third trimester or after birth.[20,29,61] Increased NT or hydrops are common features of serious skeletal dysplasias presenting in early pregnancy, however evaluation of long bones in the second or third trimester may prompt further investigation for other skeletal dysplasias.[62] Other structural defects, such as pulmonary or gastrointestinal anomalies, are less likely to be detected on ultrasound, even in the third trimester.[10,63] A 24 week fetal echocardiogram may be considered, depending on adequacy of prior fetal heart assessment at 20 weeks, parental concern and family history. The balance between comprehensive investigation and normalizing a pregnancy for parents is an important consideration in the counselling process.

Key counselling points: *Parents should be reassured of a likely good pregnancy outcome when second trimester investigations are normal. Clinical examination of the baby prior to discharge from hospital should inform the need for postnatal follow-up.*

Conclusion

Parents are now faced with a myriad of choices around screening and testing options in pregnancy. The experience of receiving a low risk result on NIPT and a subsequent 12-13 week ultrasound with an increased NT is a contemporary dilemma, made more complex by the emerging options for single gene testing. The psychological journey for parents is made even more challenging by the prolonged period between initial diagnosis in first trimester, and prognostic information at each successive stage of investigations up to 22-24 weeks (Figure 3). The balance between comprehensive investigation and normalizing a pregnancy for parents is an important consideration throughout management. As ever, the integration of new genomic testing into prenatal care is associated with ethical, counselling and healthcare resource utilization challenges. Despite the continuous advances in technology, supporting parents with appropriate information and supporting decision-making remains a constant principle to guide practice in the genomic era.

Acknowledgements.

We thank Mr Leonard Bonacquisto (Victorian Clinical Genetics Services) for providing the data for Figure 2, and to the multidisciplinary fetal medicine team, Department of Perinatal Medicine, Mercy Hospital for Women, Melbourne, for comments on the manuscript.

References

1. Nicolaides KH, Azar G, Snijders RJ et al. Fetal nuchal oedema: associated malformations and chromosomal defects. *Fetal Diagn Ther* 1992; 7:123-131
2. Snijders RJ, Noble P, Sebire N et al. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998; 352:343-346
3. Hui L and Bianchi DW. Noninvasive prenatal testing for aneuploidy – the vanguard of genomic medicine. *Annual Review of Medicine* 2017; 68:459–72.
4. Gadsbøll K, Petersen OB, Gatinois V, et al; The NIPT-map Study Group. Current use of noninvasive prenatal testing in Europe, Australia and the USA: A graphical presentation. *Acta Obstet Gynecol Scand*. 2020; 99: 722– 730.
5. Lindquist A, Hui L, Poulton A, et al. State-wide utilization and performance of traditional and cell-free DNA-based prenatal testing pathways: the Victorian Perinatal Record Linkage (PeRL) study. *Ultrasound Obstet Gynecol*. 2020;56(2):215-224.
6. Gil MM, Accurti V, Santacruz B, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol* 2019;53(6):734-742.
7. Salomon LJ, Alfirevic Z, Audibert F, et al. ISUOG updated consensus statement on the impact of cfDNA aneuploidy testing on screening policies and prenatal ultrasound practice. *Ultrasound Obstet Gynecol*. 2017;49(6):815-816.
8. American College of Obstetricians and Gynecologists Practice Bulletin No. 226. Screening for fetal chromosome abnormalities 2020. Downloaded from

https://www.smfm.org/publications/328-practice-bulletin-226-screening-for-chromosomal-abnormalities Sept 5, 2020.

9. Reiff ES, Little SE, Dobson L, et al. What is the role of the 11- to 14-week ultrasound in women with negative cell-free DNA screening for aneuploidy? *Prenat Diagn.* 2016;36(3):260-5.
10. Bardi F, Bosschieter P, Verheij J, et al. Is there still a role for nuchal translucency measurement in the changing paradigm of first trimester screening? *Prenat Diagn.* 2020;40(2):197-205.
11. Molina FS, Avgidou K, Kagan KO, et al. Cystic hygromas, nuchal edema, and nuchal translucency at 11-14 weeks of gestation. *Obstet Gynecol.* 2006;107(3):678-83.
12. Nicolaides KH. The 11-13 weeks scan. Fetal Medicine Foundation, London 2004.
13. Souka AP, Von Kaisenberg CS, Hyett JA, et al. Increased nuchal translucency with normal karyotype. *Am J Obstet Gynecol.* 2005;192(4):1005-21. Erratum in: *Am J Obstet Gynecol.* 2005;192(6):2096.
14. Yagel S. Integration of nuchal translucency screening into the first-trimester fetal anatomy scan: the time has come. *Ultrasound Obstet Gynecol.* 2021;57(1):29-31.
15. Ayräs O, Tikkanen M, Eronen M, et al. Increased nuchal translucency and pregnancy outcome: a retrospective study of 1063 consecutive singleton pregnancies in a single referral institution. *Prenat Diagn.* 2013;33(9):856-62. Erratum in: *Prenat Diagn.* 2016;36(2):199.
16. Grande M, Jansen FA, Blumenfeld YJ, et al. Genomic microarray in fetuses with increased nuchal translucency and normal karyotype: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol.* 2015;46(6):650-8.
17. Miranda J, Paz Y, Miño F, et al. Should cell-free DNA testing be used in pregnancy with increased fetal nuchal translucency? *Ultrasound Obstet Gynecol.* 2020;55(5):645-651.
18. Berger VK, Norton ME, Sparks TN, et al. The utility of nuchal translucency ultrasound in identifying rare chromosomal abnormalities not detectable by cell-free DNA screening. *Prenat Diagn.* 2020;40(2):185-190.
19. Su L, Huang H, An G, et al. Clinical application of chromosomal microarray analysis in fetuses with increased nuchal translucency and normal karyotype. *Mol Genet Genomic Med*. 2019;7(8):e811.

20. Hui L, Pynaker C, Bonacquisto L, et al. Re-examining the optimal nuchal translucency cutoff for diagnostic testing in the cell-free DNA and microarray era: results from the Victorian Perinatal Record Linkage study. *Am J Obstet Gynecol.* 2021;3:S0002-9378(21)00537-8.
21. Rossi AC, Prefumo F. Accuracy of ultrasonography at 11-14 weeks of gestation for detection of fetal structural anomalies: a systematic review. *Obstet Gynecol* 2013;122:1160-1167.
22. Ramkrishna J, Menezes M, Humnabadkar K, et al. Outcomes following the detection of fetal edema in early pregnancy prior to non-invasive prenatal testing. *Prenat Diagn.* 2021;41(2):241-247.
23. van der Meij KRM, Sistermans EA, Macville MVE, et al for the Dutch NIPT Consortium. TRIDENT-2: National Implementation of Genome-wide Non-invasive Prenatal Testing as a First-Tier Screening Test in the Netherlands. *Am J Hum Genet.* 2019;105(6):1091-1101.
24. Bennett RL. The family medical history as a tool in preconception consultation. *J Community Genet.* 2012;3(3):175-83.
25. Sileo FG, Kulkarni A, Branescu I, et al. Non-immune fetal hydrops: etiology and outcome according to gestational age at diagnosis. *Ultrasound Obstet Gynecol.* 2020;56(3):416-421.
26. Sebire NJ, Bianco D, Snijders RJ, et al. Increased fetal nuchal translucency thickness at 10-14 weeks: is screening for maternal-fetal infection necessary? *Br J Obstet Gynaecol.* 1997;104(2):212-5.
27. Le Lous M, Bouhanna P, Colmant C, et al. The performance of an intermediate 16th-week ultrasound scan for the follow-up of euploid fetuses with increased nuchal translucency. *Prenat Diagn.* 2016; 36(2):148-53.
28. Grande M, Arigita M, Borobio V, et al. First-trimester detection of structural abnormalities and the role of aneuploidy markers*. Ultrasound Obstet Gynecol.* 2012;39(2):157-63.
29. Syngelaki A, Hammami A, Bower S, et al. Diagnosis of fetal non-chromosomal abnormalities on routine ultrasound examination at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol.* 2019;54(4):468-476.

30. Bilardo CM, Timmerman E, Pajkrt E, van Maarle M. Increased nuchal translucency in euploid fetuses - what should we be telling the parents? *Prenat Diagn.* 2010;30(2):93-102.
31. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med.* 2012;367(23):2175-84.
32. Robson SC, Chitty LS, Morris S, et al. Evaluation of Array Comparative genomic Hybridisation in prenatal diagnosis of fetal anomalies: a multicentre cohort study with cost analysis and assessment of patient, health professional and commissioner preferences for array comparative genomic hybridisation. Southampton (UK): NIHR Journals Library; 2017.PMID: 28182369.
33. Maya I, Yacobson S, Kahana S, et al. Cut-off value of nuchal translucency as indication for chromosomal microarray analysis. *Ultrasound Obstet Gynecol*. 2017;50(3):332-335.
34. Yang X, Li R, Fu F, et al. Submicroscopic chromosomal abnormalities in fetuses with increased nuchal translucency and normal karyotype. *J Matern Fetal Neonatal Med*. 2017;30(2):194-198.
35. Petersen OB, Smith E, Van Opstal D, et al. Nuchal translucency of 3.0-3.4 mm an indication for NIPT or microarray? Cohort analysis and literature review. *Acta Obstet Gynecol Scand.* 2020;99(6):765-774.
36. Stuurman KE, Joosten M, van der Burgt I, et al. Prenatal ultrasound findings of rasopathies in a cohort of 424 fetuses: update on genetic testing in the NGS era. *J Med Genet.* 2019;56(10):654-661.
37. Croonen EA, Nillesen WM, Stuurman KE, et al. Prenatal diagnostic testing of the Noonan syndrome genes in fetuses with abnormal ultrasound findings. *Eur J Hum Genet.* 2013;21(9):936-42.
38. Ali MM, Chasen ST, Norton ME. Testing for Noonan syndrome after increased nuchal translucency. *Prenat Diagn.* 2017;37(8):750-753.
39. Scott A, Di Giosaffatte N, Pinna V, et al. When to test fetuses for RASopathies? Proposition from a systematic analysis of 352 multicenter cases and a postnatal cohort. *Genet Med.* 2021;10. Epub ahead of print. PMID: 33568805.
40. Sinajon P, Chitayat D, Roifman M, et al. Microarray and RASopathy-disorder testing in fetuses with increased nuchal translucency. *Ultrasound Obstet Gynecol.* 2020;55(3):383-390

41. Drury S, Williams H, Trump N, et al. Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities. *Prenat Diagn.* 2015;35(10):1010-7.
42. Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. *Lancet.* 2019;393(10173):758-767.
43. Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal Assessment of Genomes and Exomes Consortium. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet.* 2019;393(10173):747-757.
44. Sparks TN, Lianoglou BR, Adami RR, et al. Exome Sequencing for Prenatal Diagnosis in Nonimmune Hydrops Fetalis. *N Engl J Med.* 2020 Oct 29;383(18):1746-1756.
45. Yang X, Huang LY, Pan M, et al. Exome sequencing improves genetic diagnosis of fetal increased nuchal translucency. *Prenat Diagn.* 2020;40(11):1426-1431.
46. Xue S, Yan H, Chen J, et al. Genetic Examination for Fetuses with Increased Fetal Nuchal Translucency by Genomic Technology. *Cytogenet Genome Res.* 2020;160(2):57-62.
47. Choy KW, Wang H, Shi M, et al. Prenatal Diagnosis of Fetuses With Increased Nuchal Translucency by Genome Sequencing Analysis. *Front Genet.* 2019;16(10):761.
48. Daum H, Meiner V, Elpeleg O, Harel T. Fetal exome sequencing: yield and limitations in a tertiary referral center. *Ultrasound Obstet Gynecol.* 2019;53(1):80-86.
49. Hodgson J, Pitt P, Metcalfe S, et al. Experiences of prenatal diagnosis and decision-making about termination of pregnancy: A qualitative study. *Aust N Z J Obstet Gynaecol.* 2016;56(6):605-613.
50. Hodgson J, McClaren BJ. Parental experiences after prenatal diagnosis of fetal abnormality. *Semin Fetal Neonatal Med.* 2018;23(2):150-154.
51. Werner-Lin A, Barg FK, Kellom KS, et al. Couple's Narratives of Communion and Isolation Following Abnormal Prenatal Microarray Testing Results. *Qual Health Res.* 2016;26(14):1975-1987.
52. Dempsey AG, Chavis L, Willis T, et al. Addressing Perinatal Mental Health Risk within a Fetal Care Center. *J Clin Psychol Med Settings.* 2021; 28(1):125-136.
53. Austin J, Semaka A, Hadjipavlou G. Conceptualizing genetic counseling as psychotherapy in the era of genomic medicine. *J Genet Couns.* 2014;23(6):903-9.
54. Kessler S. Psychological Aspects of Genetic Counseling. IX. Teaching and Counseling. *J Genet Couns.* 1997;6(3):287-95.

55. Lou S, Petersen OB, Lomborg K, Vogel I. How do geneticists and prospective parents interpret and negotiate an uncertain prenatal genetic result? An analysis of clinical interactions. *J Genet Couns.* 2020;29(6):1221-1233.
56. Howard ED. Family-centered care in the context of fetal abnormality. *J Perinat Neonatal Nurs.* 2006;20(3):237-42.
57. France EF, Hunt K, Ziebland S, Wyke S. What parents say about disclosing the end of their pregnancy due to fetal abnormality. *JAMA.* 2013; 321: 550–552.
58. Giurgescu C, Penckofer S, Maurer MC, Bryant FB. Impact of uncertainty, social support, and prenatal coping on the psychological well-being of high-risk pregnant women. *Nursing Research.* 2006; 55: 356–365.
59. Souka AP, Krampl E, Bakalis S, et al. Outcome of pregnancy in chromosomally normal fetuses with increased nuchal translucency in the first trimester. *Ultrasound Obstet Gynecol.* 2001;18(1):9-17.
60. Buffin R, Fichez, A, Decullier E, et al. Neurodevelopmental outcome at 2 years of corrected age in fetuses with increased nuchal translucency thickness and normal karyotype compared with matched controls. *Ultrasound Obstet Gynecol.* 2021;57(5):790-797.
61. Minnella GP, Crupano FM, Syngelaki A, et al. Diagnosis of major heart defects by routine first-trimester ultrasound examination: association with increased nuchal translucency, tricuspid regurgitation and abnormal flow in ductus venosus. *Ultrasound Obstet Gynecol.* 2020;55(5):637-644.
62. Khalil A, Pajkrt E, Chitty LS. Early prenatal diagnosis of skeletal anomalies. *Prenat Diagn.* 2011;31(1):115-24.
63. Baer RJ, Norton ME, Shaw GM et al. Risk of selected structural abnormalities in infants after increased nuchal translucency measurement. *Am J Obstet Gynecol.* 2014;211(6):675.e1-19.

Table 1. **Conditions associated with increased NT after low risk targeted NIPT[#]**

Associations	Most common example
Structural anomalies	Cardiac
Microdeletions (pathogenic submicroscopic copy number variants)	22q11.2 deletion syndrome*
Single gene disorders	Noonan syndrome
Other chromosomal	Triploidy*

[#] Assuming targeted NIPT has excluded trisomy 21, 13, 18 and sex chromosome aneuploidy

*Some NIPT assays screen for these conditions

Table 2. Risk of abnormal pregnancy outcome after NT > 3.5mm and normal karyotype (adapted from Ayras et al 2013)

NT (mm)	n	Chance of liveborn without congenital anomaly	Risk of abnormal outcome
3.5-4.4mm	157	141 (90%)	1 in 10
4.5-5.4mm	38	30 (79%)	2 in 10
5.5-6.4mm	11	5 (45%)	5 in 10
≥6.5mm	17	3 (18%)	8 in 10
Total	**223**	**149 (67%)**	**1 in 3**

Table 3. Modelled yield of chromosomal microarray in pregnancies with increased NT after low risk targeted NIPT for trisomy 21/13/18 and sex chromosome aneuploidy

First author, year	Increased NT criteria	No. of pregnancies with CMA	Yield of CMA N (%)
Bardi 2020	>=99th centile	1007	45 (4.5)*
Hui 2021^	>=3.5mm	329	20 (6.1)
	>= 1.9 MoM	399	22 (5.5)
Miranda 2020	>99th centile	226	13 (3.5)
Bardi 2020	95th-99th centile	894	13 (1.5)$^{\$}$
Petersen 2020	3.0-3.4mm and high risk FTC	522 (pooled cohort)	10 (1.9)$^{\#\#}$
Hui 2021$^{\wedge}$	3.0-3.4mm	129	2 (1.6%)

CMA, chromosomal microarray; FTC, first trimester combined screening; NIPT, noninvasive prenatal testing; NT, nuchal translucency

*15 autosomal trisomies and triploidy plus 30 CNVs (calculated from tables 2 and 4)

$^{\$}$ 3 autosomal trisomies, 2 triploidy and 8 CNVs (calculated from tables 2 and 4)

$^{\wedge}$ Includes results of cases with postnatal testing on products of conception

$^{\#\#}$ Result calculated by excluding 9 susceptibility CNVs from the 19 total 'undetectable' abnormalities in pooled cohort in table 1 in Petersen et al.

Table 4. Yield of RASopathy testing in fetuses with increased NT and normal chromosomal microarray

First author, year	Criteria for RASopathy testing	No. of tested fetuses	Yield of RASopathy panel (%)
Isolated increased NT			
Bardi 2020	NT >= 99th centile	1007	14 (1.4)
Scott 2021	Isolated increased NT	90	1 (1)
Mixed cohorts of isolated and non-isolated increased NT			
Scott 2021	Increased NT, polyhydramnios, hydrops, effusions, congenital heart disease, and/or renal anomalies	352	50 (14)
Sinajon 2020	NT >=3.5mm	103	3 (3)*

*all 3 positive cases had additional ultrasound findings

Table 5: Yield of fetal exome sequencing for isolated increased NT > 3.5 mm after normal microarray

First author, year	Definition of increased NT	No. with isolated increased NT	Diagnostic yield n (%)
Lord 2019	>=4.0mm	93	3 (3.2)
Petrovski 2019	>= 3.5mm	30	1 (3.3)*
Yang 2020	>= 3.5mm	70	1 (1.4)
Sparks 2020	>= 3.5mm	15	1 (7)
Choy 2019	>=3.5mm	34	3 (8.8)
Total pooled yield		**242**	**9 (3.7%)**

*Inferred from Table 1 in Petrosvki et al 2019

Figure legends

Figure 1A. A normal NT measurement in a fetus at 13 weeks 0 days gestation. 1B. An increased NT in an 11 week fetus. 1C. Cystic hygroma (nuchal edema with septations) at 11 weeks.

Images courtesy of A/Prof Simon Meagher, University of Melbourne.

Figure 2. Distribution of nuchal translucency measurements among 81,244 singleton pregnancies in Victoria, 2015-16.

Data courtesy of Leonard Bonacquisto, Victorian Clinical Genetics Services.
Software: Alpha Version 8.0.16281.67, Logical Medical Systems Ltd, London, United Kingdom
CRL, crown rump length; NT, nuchal translucency, MoM, multiples of the median

Figure 3. Example of diagnostic pathway for increased NT and low risk NIPT.

Figure 1A. A normal NT measurement in a fetus at 13 weeks 0 days gestation. 1B. An increased NT in an 11 week fetus. 1C. Cystic hygroma (nuchal edema with septations) at 11 weeks.

Images courtesy of A/Prof Simon Meagher, University of Melbourne.

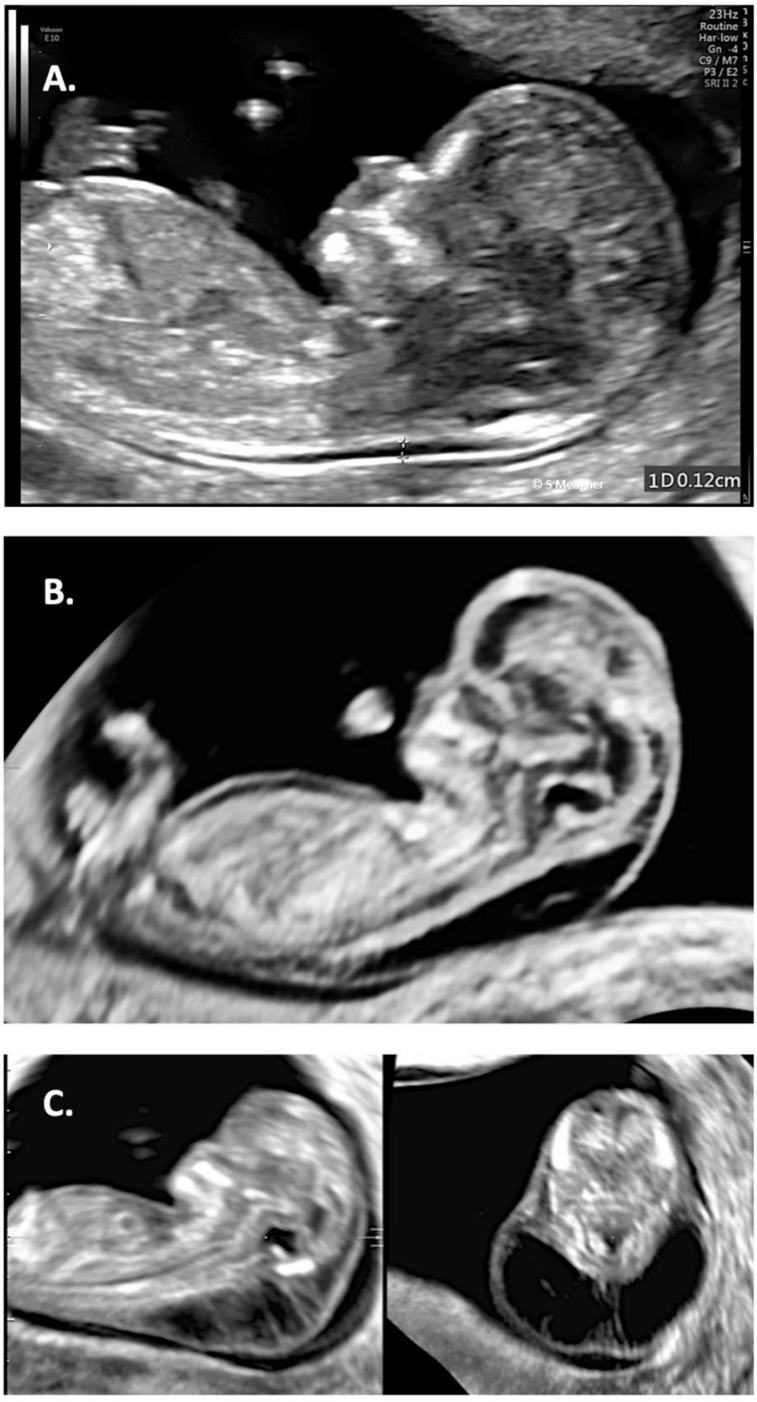
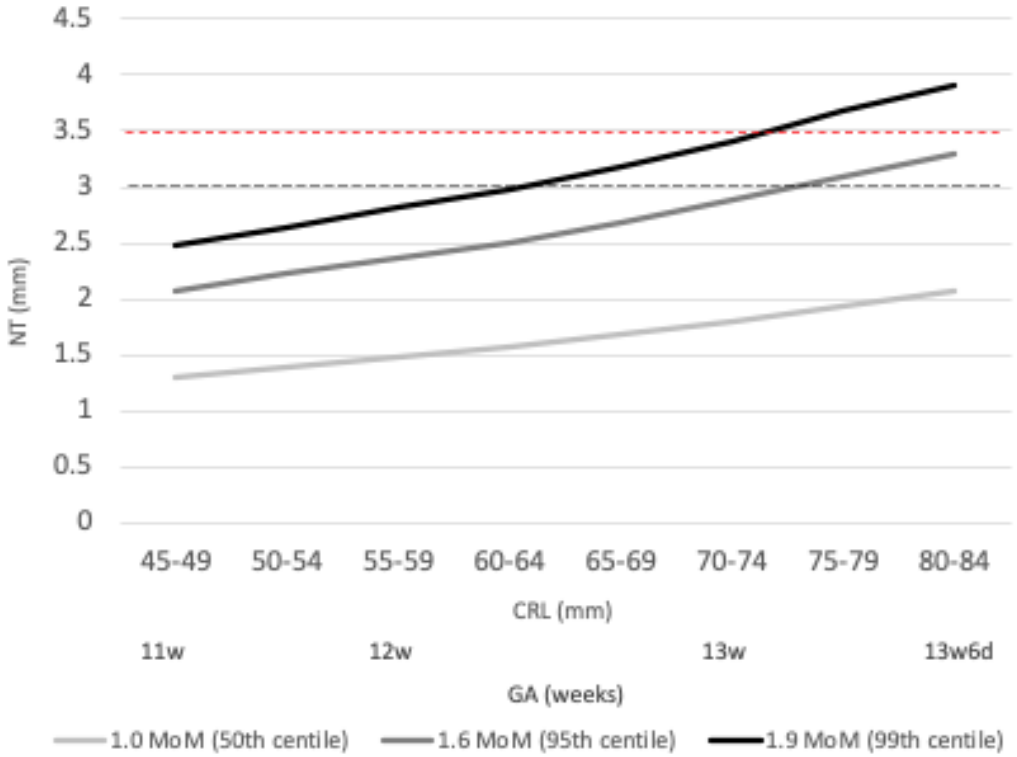


Figure 2. Distribution of nuchal translucency measurements among 81,244 singleton pregnancies in Victoria, 2015-16



CRL, crown rump length; NT, nuchal translucency, MoM, multiples of the median

Data courtesy of Leonard Bonacquisto, Victorian Clinical Genetics Services.
Software: Alpha Version 8.0.16281.67, Logical Medical Systems Ltd, London, United Kingdom

Figure 3. Example of diagnostic pathway for increased NT and low risk NIPT

Diagnostic pathway	Key counselling points
Increased NT after low risk NIPT	• Increased chance of atypical chromosome condition, single gene condition, fetal structural anomaly, perinatal loss • Overall risk of adverse outcome for NT ≥ 3.5mm is 1 in 3* (See Table 2)
First trimester tertiary scan	• 31% risk of structural anomaly with NT ≥ 3.5mm* (ref. 28)
CVS (or amniocentesis)	• Chromosomal microarray recommended, 11% chance of an atypical chromosome abnormality if a structural anomaly present vs 3% chance if no structural anomaly present (ref. 20) • CVS may be preferable to amniocentesis, as it provides more DNA that can then be stored for subsequent genetic testing if required
16w scan	• 85% favourable outcome if normal ultrasound at 16 weeks (ref. 28) • Consider testing for RASopathy syndromes if NT at 11-13 weeks was > 5.0mm and no alternative diagnosis by 16 weeks, or if other ultrasound features present (15% yield)
20w scan	• Reassuring if normal, 98% normal outcome (ref. 10) • Consider exome sequencing if multiple anomalies (24% yield) • Exome yield of ~3-4% for isolated NT > 3.5mm (including RASopathies) • Consider 24 week fetal echocardiogram
Newborn examination	• Reassuring if normal newborn examination, no long term follow up usually recommended

*Data on outcomes after normal karyotype used as proxy for outcomes after low risk NIPT