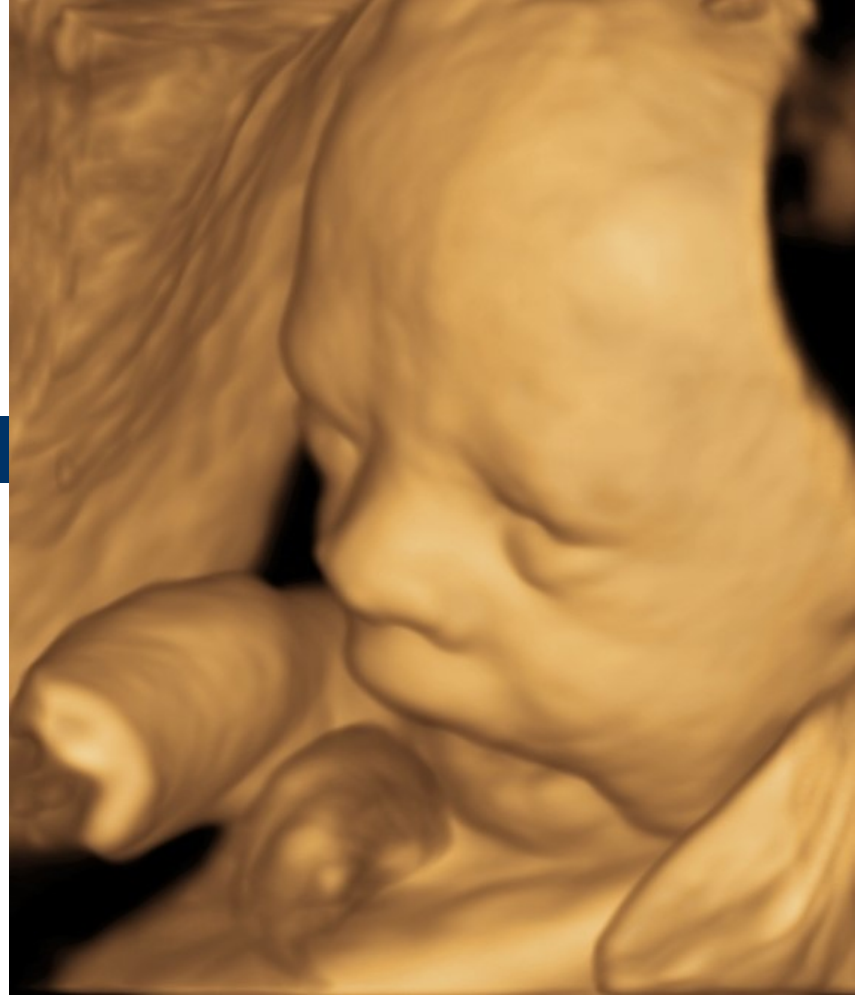
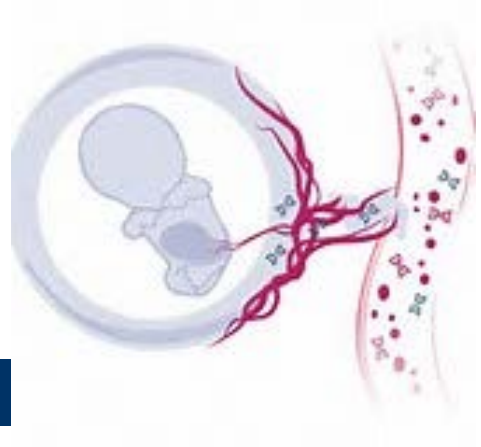


CHANGING FACE OF PRENATAL SCREENING

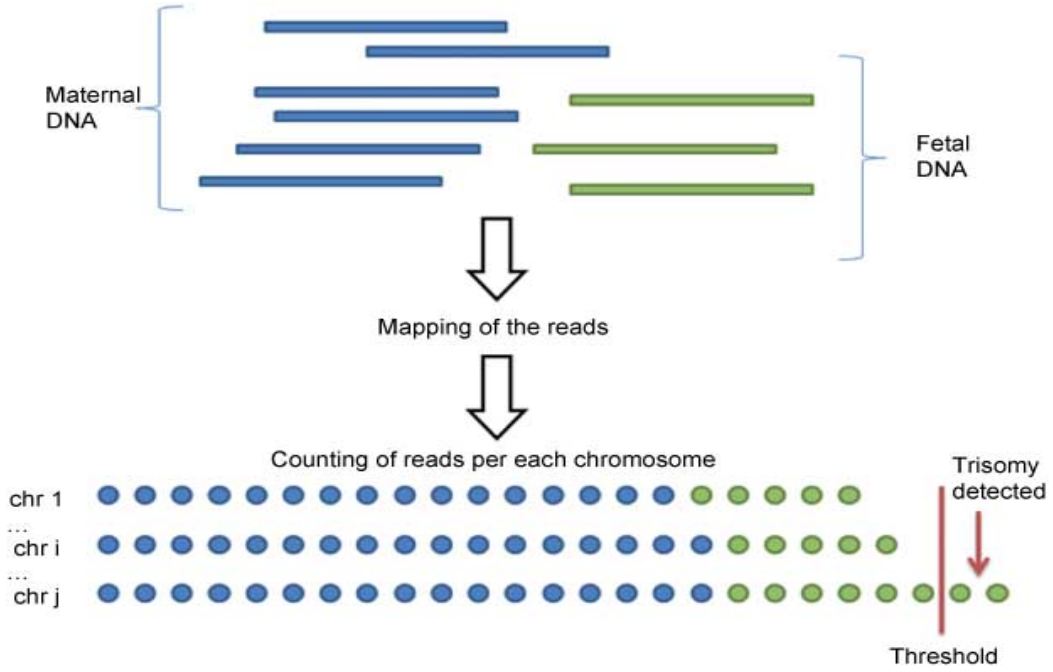
DR ADRIANA OLOG
MATERNAL FETAL MEDICINE

WHAT IS NIPT?



- Advanced screening test
- Provides risk calculation for the three most common trisomies and monosomy X
- Testing of the genome (placental) from maternal blood as a reflection of the fetal genome
- Short half life of 30 min, thus reflecting current pregnancy
- Complex technology detecting cfDNA (cell free DNA) of both maternal and fetal origin
- Fetal fraction normally about 10% after 10 weeks
- Critical fetal fraction 4% - minimum threshold of fetal DNA for reliable testing
- Non invasive, no risk to pregnancy

WHAT ARE THE PRINCIPLES OF NIPT?



- Fetal DNA is actually placental
- Fragments mapped and sequenced to a reference genome
- Fetal trisomy detected if higher than expected cfDNA fragments for a particular chromosome are counted
- Fetal fraction affected by
- Obesity
- Gestation
- Fetal aneuploidy

HOW ACCURATE IS NIPT?

FP rate of less than 1%

NIPT is currently the most sensitive screening test for trisomy 21

PPV of high risk CFTS for T21 is 2 – 6%

Sensitivities for T13 and monosomy X are similar to CFTS - NIPT may not be the best

Table 1. Cell-free DNA Test Performance Characteristics in Patients Who Receive an Interpretable Result*

			Age 25 years	Age 40 years
	Sensitivity (%)	Specificity (%)	PPV (%)	PPV (%)
Trisomy 21	99.3	99.8	33	87
Trisomy 18	97.4	99.8	13	68
Trisomy 13	91.6	99.9	9	57
Sex chromosome aneuploidy	91.0	99.6	--†	--

Abbreviation: PPV, positive predictive value.

VALIDATION IN AN AUSTRALIAN POPULATION

Table 1 Clinical indications for non-invasive prenatal testing (NIPT) in an Australian cohort of 1839 women

Indication for NIPT	n *(%)*
Advanced maternal age alone (≥ 35 years)	876 (47.7)
High-risk CFTS (≥ 1:300)	412 (22.4)
No specific risk factor	385 (20.9)
Prior aneuploidy in pregnancy or family history	49 (2.7)
Abnormal second-trimester ultrasound	38 (2.1)
Abnormal first-trimester ultrasound	26 (1.4)
Missed gestational-age window for CFTS	26 (1.4)
Unknown	14 (0.8)
High-risk second-trimester serum screening	9 (0.5)
Other	4 (0.2)

Table 2 Summary of clinical experience of non-invasive prenatal testing (NIPT) in an Australian study cohort of 1839 pregnant women

Clinical experience	n *(%)*
Overall invasive-testing rate	54/1839 (2.9)
Screen-positive rate of NIPT	45/1839 (2.4)
Invasive-testing rate after positive NIPT	35/45 (77.8)
PPV of NIPT*	29/35 (82.9)
Total false-positive rate of NIPT	6/1839 (0.3)
Invasive-testing rate after negative NIPT	15/1742 (0.9)
NIPT 'no result' on first blood draw	52/1839 (2.8)
Successful result after redraw	22/38 (57.9)
Not detected by NIPT	2/1794 (0.1)†
Total prevalence of aneuploidy (antenatal diagnosis only)	31/1839 (1.7)

*Confirmed with invasive test. †One failed assay and one atypical abnormality. PPV, positive predictive value.

WHO SHOULD HAVE NIPT?

- All women should be offered screening in pregnancy
- Pre-test counselling is crucial for all screening modalities and hence NIPT should not be performed without this
- NIPT has been validated as a screening modality for all risk women including high and low risk, clinical validation studies demonstrate similar sensitivity and specificity in both groups
- Varying PPV due to different background incidence in low and high risk populations
- Options as primary screening modality or as contingent screening for a high risk CFTS

WHAT ARE THE LIMITATIONS OF NIPT?

- Does not screen for all chromosomal anomalies and is very specific for T21/T18 and less so for T13 and the sex based aneuploidies
- Very high risk CFTS results with risk greater than 1:50, NIPT should not replace invasive testing as only 70% in this group have the common trisomies
- Not suitable for couples who request definitive testing, NIPT is not a diagnostic test
- Not suitable for parents who themselves carry a chromosomal anomaly, have had a bone marrow or organ transplant, twin pregnancy
- Not suitable in the context of fetal structural anomaly, cystic hygroma or an NT measurement greater than 3.5 mm
- Residual risk of chromosomal anomaly in the context of a normal NIPT following a high risk CFTS of 2 – 4%

WHEN IS NIPT PERFORMED?

- If the family request a high level advanced screening test
- The very high sensitivity and specificity of NIPT for T21 is unmatched by any other screening modality
- After 10 weeks to lessen risk of low fetal fraction
- No upper limit of gestation
- Fetal fraction influenced by maternal BMI so consider delaying in high BMI to avoid an inconclusive result
- Both high and low risk women should now be offered NIPT
- Should only be offered after appropriate pre-test counselling particularly in low risk women where the PPV is low

HOW DOES NIPT COMPARE TO OTHER SCREENING METHODS?

CFTS	NIPT
●Sensitivity 90%	Sensitivity 99.98%
●False positive 5%	False positive 1.2%
●False negatives 10%	False negatives 0.02-0.03%
●Uses priori risk, risk belongs	No priori risk
●to patient	
●Immediate calculation	Delay 5 working days
●1:300 cut off, difficult to	Easy interpretation >99.98%
●understand	vs <0.02%
●Pre/post test counselling	Pre/post test counselling

IS ANOTHER SCREENING METHOD NEEDED IF NIPT IS NORMAL?

- If NIPT is normal, performing a CFTS risk calculation should NOT occur
- Does not improve the sensitivity of NIPT for the common trisomies but increases the FP rate and hence the invasive testing rate
- Confusion in context of which “test to trust”
- Need for first trimester structural assessment remains, screening adjunct to NIPT
- Screening for prediction of placentally mediated pregnancy complications? Serum screening no longer performed as the are no longer required for contemporary trisomy screening. PAPP-A or BhCG evaluation in first trimester does not improve pregnancy outcome on their own and hence should not be performed as an indicator of placental function

SHOULD AN EARLY SCAN STILL BE OFFERED

- Dating
- Multiple pregnancy and chorionicity
- Viability
- Nuchal thickness measurement
- Correlation with structural anomalies and genetic syndromes
- Fetal structural assessment, increasing imaging resolution and understanding of early morphological assessment
- Assessment of other markers i.e. the IT
- Screening for maternal/fetal complications in pregnancy i.e. uterine arteries
- Maternal anatomy i.e. ovaries

THE IMPORTANCE OF AN EARLY STRUCTURAL ASSESSMENT

- Role of USS has not been well defined in the era of cfDNA
- Strongly advocated to occur as NIPT cannot replace early structural assessment
- Actually have very little in common but common false reassurance that “everything is ok” in the context of a low risk NIPT result
- Many structural anomalies will have a normal NIPT result eg MMC, and most neural tube defects, skeletal dysplasias
- Current recommendations are for tertiary level structural assessment including NT measurement, increases detection of fetal anomalies 9X in cases of normal NIPT
- Early detection allows more definitive testing, appropriate counselling and pregnancy termination with less maternal risk than at more advanced gestations

WHEN IS NIPT NOT SUITABLE?

- Early gestations
- Viability is not confirmed or gestation uncertain
- Structural concerns
- Cystic hygroma or NT measurement greater than 3.5 mm
- Concerns about other chromosomal anomalies or genetic syndromes i.e. family history
- Parents requesting definitive testing in the context of a high risk CFTS
- Parents with known genetic concerns e.g. balanced translocations

WHAT HAPPENS IF MY RESULT IS POSITIVE?

- Remember NIPT is a screening test with false positives and negatives.
- Abnormal result may be secondary to CPM, vanishing twin or maternal medical conditions.
- Hence result must be confirmed by invasive testing particularly if the result will be used for pregnancy management
- This may be pregnancy termination or continuation with specialised input, counselling and optimisation for birth
- Prompt referral recommended for discussion and further investigations
- Sex chromosome anomalies – commonly reported, exercise caution. Higher rate of placental mosaicism and biological variability for the X chromosome as well as higher risk of asymptomatic maternal variations (low level XO/XX increases with advancing gestation). Usually phenotypically mild, detailed post test counselling indicated
- Maternal malignancy may lead to false positives, always consider if positive across several conditions

WHAT HAPPENS IF I HAVE AN INCONCLUSIVE RESULT?

- Incidence of a no test or inconclusive result is 1 – 4%
- Conditions which increase maternal cell turnover can decrease the fetal fraction and lead to no test
- Obesity - increased cell turnover in adipose tissue and higher volume of distribution, 30% over 120 kg, consider CFTS or redraw in these women
- Autoimmune disease such as SLE particularly if flare or recent disease activity

- Those women who have a failed test or inconclusive result are at increased risk of fetal aneuploidy due to a low fetal fraction

HOW DO I ACCESS NIPT?

- NIPT is a service available to all pregnant women following pre-test counselling
- As now offered to low risk women, available as a test for GPs and obstetricians
- Tertiary and MFM input is always available
- Generation QML
- Harmony S&N

SHOULD I OFFER TESTING FOR MICRODELETIONS?

- Rapid expansion of scope of NIPT with attempt to include microdeletion panel
- Rare sub chromosomal abnormalities, small less than 5 Mb hence harder to detect
- Lack of knowledge of true incidence in the general population due to lack of screening but approximately 1:1000 of all structurally normal pregnancies
- Clinical validation studies are limited, data is slow and not promising, sensitivity and specificity unknown
- PPV for 22q11.2 deletion syndrome was 3.8% in recent study looking at an unselected population
- Wide phenotypic variability, ethical considerations as up to 30% of 22 q11.2 mutations inherited from either parent who may be mildly or not affected
- **Currently, microdeletion panel should NOT be performed until deeper sequencing improves detection rates and clinical validation studies become available**

EXTENDED MICRODELETION PANEL

Syndrome	Prevalence (in Caucasian population)	Phenotype	Expected ultrasound findings
22q11 deletion syndrome	1 in 4,000	**Highly variable** - no features to cleft lip/palate, congenital heart defects, hypoparathyroidism, T-cell immunodeficiency, dysmorphic features, intellectual/developmental delay, increased risk of psychiatric disorders – or minimally affected	Nil to congenital cardiac defects, cleft lip or palate.
1p36 deletion syndrome	1 in 5,000 -1 in 10,000	**Variable but all have intellectual delay** – dysmorphic features, seizures, vision problems, hearing loss, short stature, neurological anomalies, facial clefts, congenital heart defects, renal anomalies.	Facial clefts, cardiac defects.
Prader-Willi syndrome	1 in 10,000 - 1 in 30,000	**Relatively consistent** – Severe neonatal hypotonia, feeding difficulties, small genitalia (both males and females). Child onset obesity, developmental delay, short stature, dysmorphic features, behavioural problems and hyperphagia (excessive eating and food obsession).	Nil
Angelman syndrome	1 in 15,000	**Relatively consistent** – Ataxia, severe intellectual disability, speech impairment, seizures, hyperactivity and behavioural issues, autism spectrum disorder, apparently 'happy' affect.	Nil
5p deletion syndrome (Cri du chat)	1 in 15,000 to 1 in 50,000	**Relatively consistent** – severe intellectual and developmental delay, dysmorphic features, hypotonia, microcephaly.	Diagnosis after soft markers on ultrasound reported but rare.[3]

SHOULD I OFFER SCREENING IN FIRST TRIMESTER FOR MATERNAL MEDICAL CONDITIONS?

- Uses risk factors, MAP, uterine artery Doppler assessments, PIGF and PAPP-A, detection rates of over 90% but with FP of 10 % for early onset disease
- Very poor prediction for late onset pre-eclampsia, growth restriction and stillbirth even in well controlled clinical trials
- Wide discrepancies in validation studies, lack of robust clinical application evidence in an Australian population
- Globally have low PPV in both low and high risk pregnancy, low background incidence 0.5%
- Complex, trained assessment, expensive

SCREENING FOR MATERNAL MEDICAL CONDITIONS

- Currently predictive tests for placental mediated adverse pregnancy outcomes are not endorsed by major bodies such as sMFM, ACOG, RANZCOG and SOMANZ
- Aspirin and calcium have a modest but consistent effect on reduction of rates but is a relatively safe and cheap treatment option
- Treat everyone?
- Biochemistry less often collected with the move towards NIPT
- Only potential benefit is more intensive pregnancy management in high risk women

CONCLUSION

- NIPT is an advanced screening test with the best screening capacity for T21
- Should be offered to all pregnant women, with detailed pre-test counselling highlighting its limitations
- Should not replace first trimester structural assessment and NT measurement
- Extended microdeletion panel screening is not currently endorsed
- First trimester screening for maternal complications in pregnancy shows promising detection rates but currently not endorsed in the Australian population
- Recommendation for risk factor screening at this stage and low threshold consideration for LDA