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Prenatal testing

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In the course of a generation, the information available to families regarding the health and future of their unborn children has been transformed. In the era of our parents and grandparents, even the number of babies to be born was a mystery. Following delivery of the first baby, careful checking for the appearance of another head or foot was routine. The arrival of the second twin made for a crowded bassinet in the back seat of the car on the way home, as well as hasty modifications to the cot, sleeping arrangements and siblings' expectations.

Through the 1970s and 1980s, the advent of prenatal ultrasound meant that the expected number of babies was no longer a surprise. Furthermore, being able to measure the size of a fetus provided greater certainty around exact gestational age and estimated date of birth. As ultrasound technology advanced, identification of structural abnormalities in the fetus, problems with heart and brain development, for example, became possible. Prior knowledge of birth defects would inform decisions for the future of the pregnancy, enable effective intervention where possible and optimise the time, place and mode of birth.

In tandem with advances in ultrasound and fetal imaging, the last four decades has seen the rapid evolution of prenatal genetic screening. Through the 1980s, screening for Down syndrome — the most common chromosome condition affect-

ing newborns — gradually entered antenatal care. Screening was initially undertaken by looking at the placental hormone profile in the mother's bloodstream in the second trimester ('triple test' or 'quadruple test'). Women who received a 'high-risk' result would be offered a diagnostic test from 15 weeks in the form of an amniocentesis, to confirm or exclude Down syndrome in their unborn baby. By the 1990s, screening methods improved using a combination of ultrasound and blood markers in first trimester ('combined first trimester screening'), allowing not only more accurate identification of pregnancies at increased risk, but also earlier diagnosis. First trimester diagnostic testing in the form of chorionic villous sampling became more widespread, allowing chromosomal and genetic testing of the baby to be known by 13 weeks, affording patients more reproductive choice and greater privacy in decision making.

In the last five years, the focus in prenatal screening has shifted to non-invasive prenatal screening (NIPS) after it was discovered that DNA originating from the placenta is released into the maternal bloodstream. Without the risks associated with sampling the fetal tissues directly, fetal DNA can be measured and analysed with a simple maternal blood test. While this screening test is highly accurate, errors do occur and confirmation with an invasive test (amniocentesis or chorionic villus sampling) is still required before a final diagnosis can be made (Table 1).

Our capacity to interrogate the fetal genome continues to increase exponentially. These exciting advances in genomic technology mean the yield of genetic information from prenatal genetic samples is much greater, and far more complex. Families may be given results reporting changes in their baby's genome of uncertain significance, where the long-term outlook

Table 1: Screening and diagnostic tests for chromosomal abnormalities in the fetus

	Screening tests			Diagnostic tests
	Combined first trimester screening	Second trimester serum screening	Noninvasive prenatal screening NIPS	Chorionic villus sampling (CVS), amniocentesis
Type of test	Blood test and ultrasound	Blood test	Blood test	Needle aspirate of placenta or amniotic fluid
Analytes	Nuchal transluency PaPP-A beta-HCG	Estriol, beta-HCG alphafetoprotein (inhibin)	Plasma cell-free DNA	Fetal cells
Timing of test	Blood 9–13 w Ultrasound 11–13 w	14–20 weeks	From 10 weeks	CVS 11–13 weeks Amnio ≥ 14 weeks
Conditions detected	Trisomy 21, 18 13; structural anomalies	Trisomy 21 and 18	Trisomy 21, 18, 13; sex chromosome conditions	Many chromosome conditions
Detection rate for trisomy 21	90%	75–80%	99%	99.99%
False positive rate for trisomy 21	3–5%	7–8%	<1%	<1%
Test failure rate	<1%	<1%	<1–5%	<1%

is not yet known. Sometimes families are provided with information regarding conditions they have not heard of, with implications they may not fully understand. The need for considered ethical debate regarding breadth of testing, and for high quality accessible genetic counselling has never been greater. Technology has advanced to the point where parents could soon know their baby's entire genome from a simple maternal blood test performed at 10 weeks' gestation, the time when our grandmothers were excitedly wondering if indeed they were pregnant, and if so, could they possibly be carrying more than one baby? Like them, we need to consider whether we wish to know such information in advance. Life could be forever changed once we do.

Fetal abnormalities: an overview

Every baby has a risk of having a genetic condition or a structural birth defect. Prenatal screening is offered to pregnant women to provide them with more information about the health of the unborn baby. It is a key ethical principle that such screening must be voluntary and only undertaken after an informed decision by the woman, in accordance with her personal values.

Screening for birth defects includes genetic screening for Down syndrome and other chromosomal disorders, as well as a careful check of the fetal anatomy with ultrasound, usually performed at 18–22 weeks gestation. Approximately 4% of fetuses will have a major birth defect involving the central nervous system, face, heart, kidney, gastro-intestinal or skeletal system. Minor birth defects such as skin tags are more common, but usually have no, or only minor cosmetic, implications. Many congenital defects have a genetic origin, either from chromosomal defects (approximately 10%), single gene disorders (approximately 5%) or multiple contributing pathways (approximately 25%).

Structural abnormalities may be part of a specific syndrome, which means there is a collection of abnormalities in a characteristic pattern that are recognised as being genetically linked. This association between structural abnormalities and chromosome/genetic conditions is why an ultrasound-detected abnormality in the fetus is one of the most common reasons for prenatal testing with amniocentesis or chorionic villus sampling (CVS). Other causes of birth defects include fetal infection (e.g. Rubella), medical conditions affecting the mother (e.g. diabetes), exposure to toxic drugs (e.g. alcohol) or medicines (such as those used to treat epilepsy). However, in the majority of cases, the cause remains unknown.

Management of pregnancies where the fetus is known to have a fetal abnormality or serious medical condition involves detailed counselling regarding the implications of the diagnosis for the remainder of the pregnancy, labour and delivery, postnatal care and the long-term health implications. Some conditions may be amenable to treatment while the baby is still unborn, yet the mainstay for most will be postnatal surgery or continued medical follow-up and care. In some conditions where the outlook is lethal, palliative care will be offered to the newborn. In conditions where a fetus is diagnosed with a serious or life-threatening condition, some make the difficult decision of pregnancy termination.

Structural abnormalities in the fetus

Prevention of structural abnormalities in the fetus

Some women are at higher than average risk for fetal abnormalities. This includes women who have a family history of the condition, or who have had a previously affected child. Women with diabetes have an increased risk of malformations: their risk correlates directly with how well their sugar levels are controlled at the time of conception. Some medications are known to cause fetal damage, including blood thinners such as warfarin. Alcohol intake is well known to have harmful effects on fetal development. Obesity is also associated with a small but significant increase in risk for some structural abnormalities. Although this increase in risk is relatively small for the individual woman, this has an important impact at a population level, given that now half of all pregnant women are overweight or obese.

Prevention of structural abnormalities thus includes advice on optimising medications, diabetes control and weight prior to pregnancy, and avoiding alcohol intake from conception. Folic

acid (0.5 mg) should be prescribed to all women contemplating pregnancy to reduce the risk of spina bifida. For women at highest risk of a baby with spina bifida (those with a previously affected child, women with diabetes, women taking antiepileptic medication and obese women), high dose folate (5 mg daily) is recommended. Rubella and varicella are both potential infections associated with fetal damage, so vaccination prior to pregnancy is recommended. For other potential infections where no vaccine is available, personal hygiene measures (cytomegalovirus), dietary precautions (listeria, toxoplasmosis), and avoiding travel to high risk regions (Zika virus) are the mainstays of preventing fetal damage.

Detection of structural abnormalities in the fetus

The 'routine' midtrimester ultrasound is generally performed between 18 and 22 weeks' gestation. While many women and their partners look forward to this ultrasound as an enjoyable opportunity to 'see' and bond with their growing baby (and find out if they are having a boy or girl), there are important medical reasons for performing this scan. Measuring the size of fetal head, the abdominal circumference and the femur length provides greater certainty in estimating the gestational age and hence the due date of the fetus than menstrual dates alone. This is important for assessing fetal growth later in the pregnancy, and to avoid the risk of timing a planned birth too early or too late. The 18- to 22-week ultrasound also identifies multiple pregnancies, checks for an abnormally low placenta that may be unsafe for vaginal birth (placenta praevia), measures the length of the cervix (which may indicate an increased risk of preterm labour) and includes a detailed assessment of the fetal anatomy and growth.

A systematic search for structural abnormalities in the midtrimester scan will detect approximately 60% of major

anatomical defects (Grandjean et al., 1999) but may miss some minor abnormalities. The detection rate is limited by the nature of some abnormalities (which may not become apparent until later in pregnancy), the gestation at the time of the ultrasound, fetal position and number, and technical limitations including operator experience, ultrasound machine quality and maternal obesity which can limit visualisation. With improvements in technique, the next frontier is first trimester diagnosis of fetal structural abnormalities.

Most women will undergo a first-trimester ultrasound evaluation of the pregnancy. An ultrasound early in the first trimester is useful to confirm viability, reliably determine gestational age, establish the number of fetuses present and whether any, or all, of a multifetal pregnancy share a single placenta. By late in the first trimester, fetal anatomical structures are becoming apparent. The fluid-filled space at the back of the fetal neck (the 'nuchal translucency') can be measured when the crown-rump length of the fetus is between 48–84 mm (approximately 11–13 weeks), and this is useful as a screening test for trisomy 21 (see 'Screening tests for trisomy 21'), as well as for other structural defects, such as cardiac abnormalities.

A systematic evaluation of the biometry, intracranial structures, chest, abdomen and skeleton is generally performed at this ultrasound. In expert hands, it is suggested that a significant number of major structural abnormalities can be suspected, or diagnosed, by 13 weeks (Karim et al., 2016). Nevertheless, there are relatively few abnormalities that can be diagnosed with certainty in the first trimester; most fall into the category of 'may be seen' or 'may be suspected' early in pregnancy. While this can be helpful to triage early referral to an ultrasound specialist in later pregnancy, it can also create enormous anxiety for families during this time of diagnostic uncertainty.

The most common structural abnormalities are those involving the heart and the major blood vessels. Some of these can be life threatening, and their detection is crucial to ensure optimal care of the baby before, during and after birth. Some are relatively minor, but can be a 'signpost' to look carefully for the presence of associated structural or genetic abnormalities. Prenatal testing with amniocentesis or CVS are usually offered if a heart abnormality is found, as many are associated with an increased risk of a chromosome abnormality.

A common abnormality of the central nervous system is a neural tube defect, such as spina bifida. The incidence of children being born with neural tube defects has dramatically decreased since routine screening and folic acid supplementation in pregnancy has commenced. More recently, open fetal surgery has been shown to improve the outcome of children with spina bifida (Adzick et al., 2011). Fetal surgery involves opening the uterus and operating on the spina bifida at around 26 weeks in pregnancy to reduce the risk of damage to the spinal cord during pregnancy. Nevertheless, fetal surgery such as this carries risk to the mother and her future pregnancies, and this needs to be weighed carefully against any potential fetal benefit. In Australia, this procedure has only been performed in Brisbane to date. Given the small number of cases that would meet the criteria for fetal surgery annually, it seems unlikely that more than one surgical centre would be required for a country like Australia with a relatively small population.

The detection of other abnormalities during pregnancy with prenatal ultrasound has clear benefits for the fetus, the baby and the family. An example of fetal benefit is found in twin-to-twin transfusion syndrome. In this condition, identical twins sharing a placenta have an imbalance of blood flow between them, creating a life-threatening situation for both. Minimally

invasive fetal surgery for this condition involves placing a telescope in the uterus and obliterating all placental vessels running between the twins using laser. This procedure has an established place, improving survival and reducing disability of twins with this condition. Infants with either gastroschisis or exomphalos (where the front wall of the abdomen has not formed correctly) will require immediate admission to intensive care for stabilisation prior to surgery in the hours immediately following birth. For these families, prenatal diagnosis facilitates appropriate surveillance during pregnancy, transfer to a hospital with expert neonatal facilities and preliminary discussions with the paediatric surgical team. Even for other conditions where postnatal surgery is not immediately indicated (e.g. in cleft lip and palate), families benefit from knowing the approach to feeding, surgery and the likely cosmetic result by liaising with the multidisciplinary cleft team prior to birth.

Genetic abnormalities in the fetus

Screening for aneuploidy

Normally, the genetic material (or DNA) contained in our cells is packaged into 46 chromosomes, arranged in 23 pairs. The first 22 pairs are called autosomes (numbered 1 to 22). The final pair (the X and Y chromosomes) are called the sex chromosomes because they determine if a fetus develops as a male or female. A normal chromosome count will be reported as 46,XX- female, or 46,XY- male. *Aneuploidy* is the term given where there is an extra or missing copy of a chromosome in every cell. Aneuploidy can affect the autosomes or the sex chromosomes. Many aneuploidies are lethal in early pregnancy, and aneuploidy is the leading contributor to early miscarriage. Down syndrome (also known as trisomy 21) is caused by a

complete or partial third copy of chromosome 21 (Figure 1). Trisomy 21, which underlies Down syndrome, is the most common chromosomal abnormality among livebirths. The chromosome report is 47,XX+21, or 47,XY+21, indicating the presence of an extra whole chromosome (47 instead of 46), and which chromosome is affected (+21).

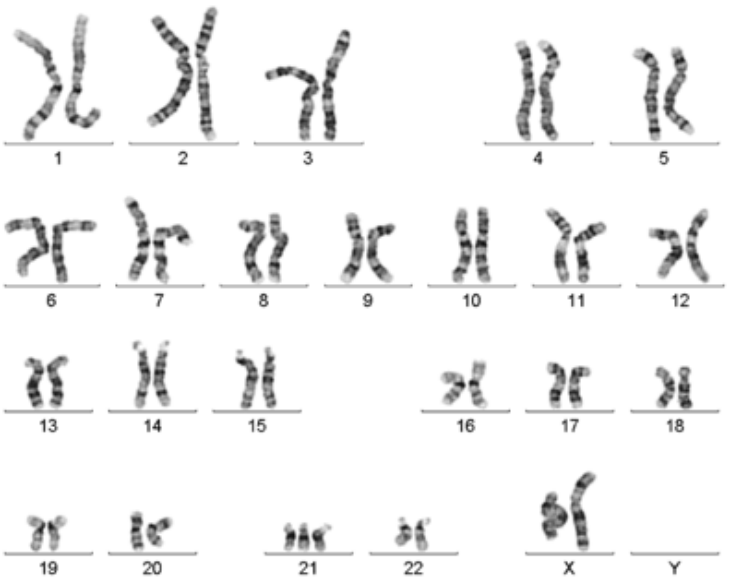

Figure 1: G-banded karyotype from a female fetus with trisomy 21 — note three copies of chromosome 21 arrowed.
Image courtesy of the Victorian Clinical Genetics Service.

Trisomy 21 is the leading cause of intellectual disability associated with a recognisable chromosomal abnormality. Children with trisomy 21 have some common physical characteristics (short stature, flattened nasal bridge, protruding tongue, short neck) and intellectual disability is universal, usually in the mild (IQ 50–70) to moderate (IQ 30–50) range. Up to 50% of newborns will have congenital heart disease and other structural abnormalities that may be identified prenatally,

such as intestinal blockages requiring surgery. Medical problems may include an underactive thyroid gland, hearing and eye abnormalities and an increased risk of some malignancies. Behavioural and psychiatric problems are more common, including autism and early onset dementia.

Trisomy 21 affects approximately 1 in 700 pregnancies, although this depends on the maternal age of the population being studied. In most cases of trisomy 21, the extra copy of chromosome 21 has come from the egg, an event that becomes more common with increasing maternal age (Figure 2). This is why miscarriage (due to aneuploidy), and livebirth with trisomy 21, are both more common in older women. Because trisomy 21 is common, and has important medical implications, screening for trisomy 21 is considered an important component of antenatal care. Not all families wish to avail themselves of screening, but all should be offered. In the event that screening returns a high-risk result, confirmation of the diagnosis is made

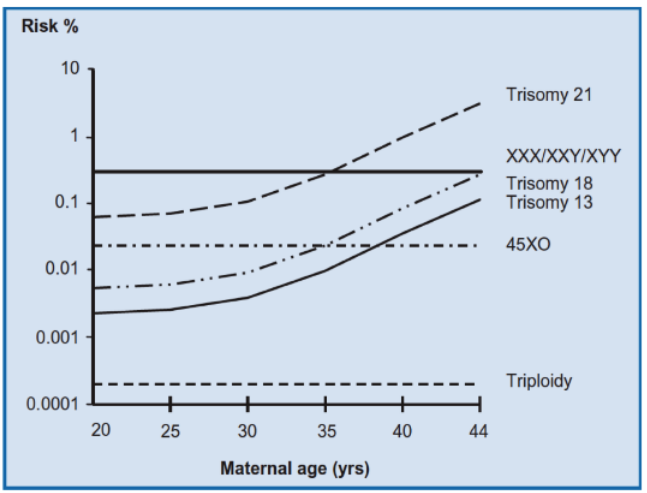

Figure 2: Maternal age-related risk of fetal chromosome conditions (%).
Reproduced with permission from The 11–13+6 weeks scan, Kypros H. Nicolaides, Fetal Medicine Foundation, London, 2004.

with either an chorionic villus sampling or amniocentesis, directly accessing genetic material from the fetus.

Historically, screening with maternal age alone was the first attempt to screen pregnancies for trisomy 21. All women aged 35 or 37 years of age and over (this differed in different jurisdictions) at the time of delivery were offered an amniocentesis to determine whether their fetus was affected. This screening was recognised as being highly inefficient, since a large number of women were classified as high risk, or 'screen positive', requiring a diagnostic procedure, yet this only detected about 30% of fetuses with trisomy 21 in the population. While the individual risk for older women is much higher (about 1 in 100 chance of a term infant with trisomy 21 for a 40-year-old woman; see Figure 2), these women represent a relatively small proportion of the pregnant population.

For this reason, attention has turned away from identifying 'high-risk women' to identifying features of the 'high-risk pregnancy,' utilising both ultrasound and biochemistry markers so that screening is universally applicable. These tests are summarised in Table 1. Many of these ultrasound and biochemical features are also associated with trisomy 13 and trisomy 18, and so a risk can also be given for these conditions. These chromosomal abnormalities are the next most likely (after trisomy 21) to result in livebirth, although are almost always lethal. The challenge with all risk-based screening programs is ensuring sufficient health literacy to understand the numerical risks being presented. Faced with a risk of 1:60 or 1:600, many women are unsure which represented the higher risk. Visual probability aids can assist by providing a graphical representation of risk, and there are publically available decision aids that can be used to assist with counselling (https://www.mcri.edu.au/prenatal-screening).

Screening tests for trisomy 21

(1) Second trimester maternal serum screening ('triple test' or 'quadruple test')

There are hormone markers derived from either the placenta or fetus that can be measured in the maternal blood. Through the 1980s it was noted that the profile of these markers was often different in pregnancies affected by trisomy 21. Second-trimester maternal serum screening involves taking a blood sample between 14 and 20 weeks' gestation, and recording maternal age as well as other relevant variables. The risk associated with maternal age alone is then adjusted according to the hormone profile pattern and a new risk, specific to this pregnancy, is generated. When screening incorporates all four blood markers, the detection rate for Down syndrome in well dated pregnancies is approximately 75% using a cut-off risk of 1:250 or higher where the false positive rate is 5% (Cuckle & Maymon, 2016). This means that 5% of women will be screen positive, having an adjusted individual risk result between 1:2 (highest possible 'screen positive' risk) and 1:250 (lowest possible 'screen positive' risk). Almost all of these results will turn out to be false positives, that is, women will be found to have an unaffected fetus at the time of diagnostic testing. In the last three decades since the advent of screening with midtrimester maternal serum screening, considerable effort has been made to maximise the detection rate, but minimise the false positive rate. This is because every false positive generates anxiety for women and their families. It mostly results in performing a diagnostic procedure (amniocentesis) that carries a small risk of miscarrying a much wanted pregnancy.

(2) Screening with first trimester nuchal translucency

In the 1980s, it was observed that fetuses with trisomy 21 are more likely to have an increased fluid space behind the neck

(called the 'nuchal translucency'), which can be measured using ultrasound between 11–13 weeks. The trisomy 21 detection rate using nuchal translucency measurement alone is approximately 75%, using a risk cutoff of 1:300 or higher, and a false positive rate of 5%.[4] This test has comparable accuracy to second-trimester maternal serum screening, but screening and diagnostic testing is performed earlier in pregnancy. For families who would consider termination of an affected pregnancy, earlier screening and diagnosis offers greater privacy surrounding decision making for the future of the pregnancy. Termination of pregnancy is also more straightforward, and so easier to access earlier in gestation.

(3) Screening with first trimester nuchal translucency plus biochemical markers

First-trimester combined screening refers to measuring two substances in the maternal blood in the first trimester, specifically pregnancy-associated plasma protein A (PAPP-A) and hCG, and then combining these results with the nuchal translucency measurement. With first-trimester combined screening, patients have a blood test performed at 9–12 weeks, followed by an ultrasound at 11–13 weeks. The adjusted risk reflects the additional information obtained by both the maternal serum screening and nuchal translucency results when applied to the background maternal age risk. Using the dual modalities of ultrasound and maternal serum screening results in an improved detection rate. The detection rate using first-trimester combined screening is approximately 90% at a cutoff of 1:300 and a false positive rate of 5%.[4]

(4) Non-invasive prenatal screening

An exciting new development in the area of prenatal genetic diagnosis is non-invasive prenatal screening (NIPS). This has

revolutionised screening for trisomy 21, and has the potential to completely change the paradigm of prenatal genetic testing.

Background to NIPS

DNA is our 'genetic template'. It contains all the instructions for our development and survival and this unique genetic template is faithfully replicated and contained in the nucleus of almost every cell in our body. When cells break down, DNA fragments contained in the nucleus are released into our blood stream, called cell free DNA (cfDNA). Plasma contains cfDNA that has origins from every organ of our body. In pregnant women, cfDNA released from the placenta is also found in the blood (Figure 3). Because the placenta is growing so rapidly in early pregnancy, cells are constantly dividing and breaking

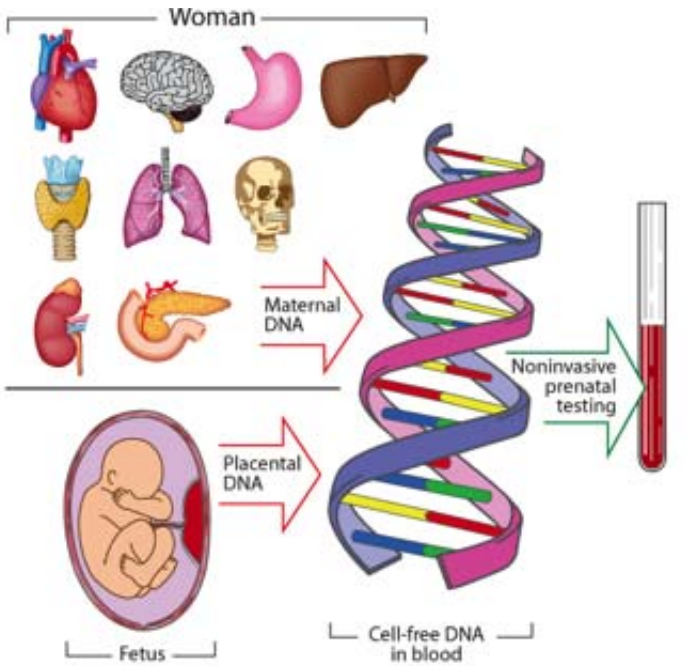

Figure 3: Noninvasive prenatal testing.
Image courtesy of Mercy Perinatal (https://mercyperinatal.com/toolkit).

down. From as early as 5 weeks' gestation, cfDNA of placental origin is detectable in the maternal blood stream, and by 8 weeks it comprises 10–15% of the total cfDNA pool. The circulating pool of fetal cfDNA clears quickly after delivery, and so testing at 10 weeks only represents cfDNA from the current pregnancy (Skrzypek & Hui, 2017).

The test

Non-invasive prenatal screening (NIPS) involves an analysis of the maternal cfDNA at approximately 10 weeks. Using a simple maternal blood test, the cfDNA is identified and these fragments undergo millions of 'reads' of their genetic sequence. The cfDNA fragments can then be assigned to a chromosome of origin. In a normal pregnancy, there should be equal representation of DNA sequences from each chromosome. If there is an over-representation of DNA originating from chromosome 21, it is assumed this has come from a placenta, and therefore fetus, that has an extra copy of chromosome 21, that is, trisomy 21. NIPS is the most advanced screening test not only for trisomy 21 (with a detection rate of over 99%), but also for trisomies 13 and 18, where detection rates exceed 97%. The false positive rate for NIPS is well under 1% (Cuckle & Maymon, 2016). Accordingly, the American College of Medical Genetics and Genomics recommends advising all pregnant women that NIPS is the most sensitive screening option for trisomies 21, 13 and 18 (Cuckle & Maymon, 2016), and it has rapidly transitioned into clinical practice. Despite its excellent screening performance, it should be noted that an invasive test is always required to confirm the diagnosis.

In the future, it seems likely that it will be possible to make a non-invasive diagnosis for many genetic conditions (Skrzypek, & Hui, 2017). Already, NIPS has expanded to include condi-

tions that are not part of traditional population screening programs, such as microdeletion syndromes (e.g. 22q11.2 deletion syndrome) and sex chromosome conditions (such as Kleinfelter syndrome or 47,XXY). As the depth of information available to parents regarding their unborn baby increases, so does the imperative for broad societal engagement with the ethics of prenatal testing. For example, NIPS can reliably determine fetal gender in early pregnancy, but concerns regarding use of this technology for sex selection have meant that gender reporting is banned in some countries.

Diagnostic tests for aneuploidy

Among women who screen high risk for aneuploidy, the available diagnostic tests are chorionic villus sampling (CVS), and amniocentesis. These are ultrasound-guided procedures done as an outpatient and do not require anaesthetic. Amniocentesis and chorionic villous sampling access the fetal genetic material directly, and are the only way that aneuploidy — or any other genetic disorder affecting the fetus — can be confirmed or definitively ruled out.

Chorionic villus sampling is an ultrasound-guided procedure where a needle is passed into the placental tissue and placental (chorionic) villi are aspirated (Figure 4). The procedure is most commonly performed between 11–14 weeks, and is commonly performed after abnormal first trimester screening. The risk of miscarriage following CVS is approximately 1 in 500. Amniocentesis is an ultrasound guided procedure performed after 15 weeks' gestation where approximately 10–15 ml of amniotic fluid is aspirated (Figure 5) from the amniotic sac. This reaccumulates over the next 48 hours. The miscarriage risk of amniocentesis is lower (approximately 1 in 1000), but this needs to be weighed against a later diagnosis.

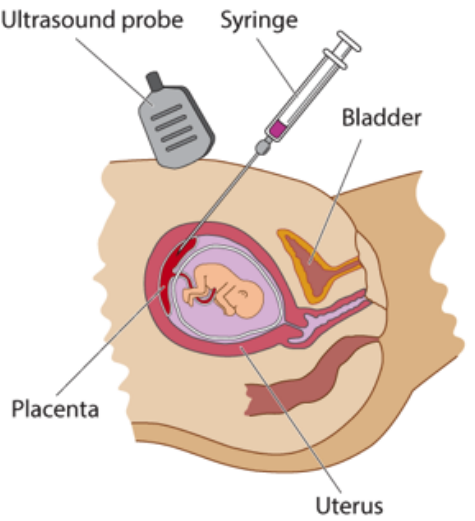

Figure 4: Chorionic villous sampling.
Image courtesy of Mercy Perinatal (https://mercyperinatal.com/toolkit).

Diagnostic tests: what can be known?

The amount of information that can be obtained from sampling this genetic material is far greater than just confirming the presence — or absence — of a whole chromosome. The initial result from the sample is called the Fluorescent In Situ Hybridisation (FISH) result. This is a test where fluorescent-labelled probes are attached to regions on individual chromosomes, thus identifying a missing or extra copy of that chromosome. The result is available in 48 hours, and so FISH is a useful test to promptly exclude the major chromosomal abnormalities, trisomy 21, 18, 13, and sex chromosome abnormalities. Standard karyotyping (Figure 1) involves examination of all chromosomes under a microscope and will diagnose disorders of chromosome number (aneuploidy), and abnormalities in the structure of chromosomes, such as missing pieces (deletions), extra pieces (duplications) and parts that have come off one

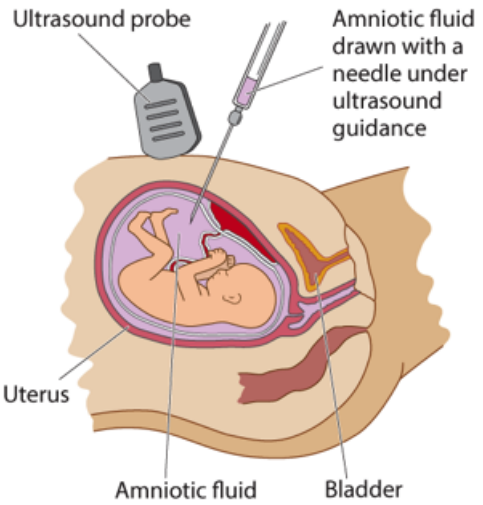

Figure 5: Amniocentesis.
Image courtesy of Mercy Perinatal (https://mercyperinatal.com/toolkit).

chromosome and attached itself to another (translocations). The result from a banded karyotype takes approximately two weeks.

Microarray (or molecular karyotype) examines the fetal genome in much greater detail than the conventional karyotype and has become the new standard in clinical testing for chromosome disorders. Microarray analysis can detect chromosomal deletions or duplications that are 100 times smaller than those identified on conventional karyotype. These small changes may be associated with significant health consequences for the baby. Nevertheless, microarray analysis also carries potential for findings of uncertain significance. This can create enormous anxiety for families when they are provided with additional information from a test, the significance of which is unknown. Although families will mostly request that these 'unknown variants' are reported, it is important that

adequate pre and post test information and/or counselling is available.

Screening for inherited genetic conditions during pregnancy

The vast majority of chromosome conditions such as Down syndrome are sporadic and are not inherited from the parents. In contrast, other genetic disorders can be inherited by the fetus from apparently healthy parents who are carriers of a faulty gene. Testing of the couple to determine the risk of an affected fetus is called reproductive 'carrier screening' (see Chapter 7). If testing of the parents indicates that the fetus is at risk of a serious genetic disease, then prenatal testing with CVS or amniocentesis can be performed, or alternatively, testing of the newborn at birth can be performed. In the past, carrier testing was only offered in the setting of affected family members, or for couples from high- risk ethnic groups. Now, however, carrier screening of couples with no prior family history is available for the more common inherited genetic diseases such as cystic fibrosis, spinal muscular atrophy, or fragile X syndrome. 'Expanded carrier screening' is an even more recent phenomenon where a healthy person with no family history of genetic disease may choose to be tested for many recessive diseases (over 100) with a single blood or cheek swab test.

Cystic fibrosis

Cystic fibrosis is the most common autosomal recessive disorders among Caucasians, and involves serious health risks to the child such as chronic lung disease, difficulty absorbing nutrients from the gut and reduced life expectancy. Autosomal recessive conditions are those where both parents are healthy, but carry one copy of a faulty gene, and one copy of the healthy gene. The offspring needs to inherit the faulty gene from both

parents to develop the disease, and so each pregnancy becomes like a game of '2-up': there is a 1-in-4 chance that a child of carrier parents will inherit the disorder ('2 heads') but a 3-in-4 chance the child will be unaffected ('two tails' or 'one head: one tail' or 'one tail: one head'). The carrier frequency for cystic fibrosis among Caucasian populations is approximately 1 in 25. This means the birth prevalence for cystic fibrosis is 1 in 2500, that is, 1/25 (the chance of the mother being a carrier) X 1/25 (the chance of the father being a carrier) X 1/4 (the chance of the fetus inheriting the faulty gene from both parents).

All newborns are screened for cystic fibrosis, but some families wish to know whether they are at risk prior to birth. Antenatal screening involves performing a cheek swab or blood test to check for the most common gene faults responsible for cystic fibrosis. Depending on the test methodology most carriers will be identified with this test. Where both parents are found to be carriers, diagnostic testing may be performed to assess whether the fetus is affected (1 in 4) or unaffected (3 in 4).

Thalassaemia

Thalassaemia is also an autosomal recessive condition affecting the production of haemoglobin, and is more common in some ethnic groups (African, South East Asian and Mediterranean individuals). Thalassaemia minor is the carrier state, but an infant affected with thalassaemia major will have a lifelong requirement for blood transfusions. This creates further medical complications related to iron overload, and people with thalassaemia major have a shortened life expectancy. Women found to have thalassaemia minor should be offered partner screening. If the partner is also found to have thalassaemia minor, they have a 1-in-4 risk of thalassaemia major in the offspring. Again, diagnostic testing with chorionic villus sampling or amniocentesis can be offered for this condition.

Other genetic conditions

With advancing technology, the opportunity to screen for more genetic disorders in the fetus has become available. There are some genetic conditions that are more common among particular ethnic groups. One such condition is Tay Sachs disease among Ashkenazi Jews. In these groups, a targeted screening panel can be offered. Fragile X syndrome is the second most common cause of severe learning difficulty and occurs in about 1 in 6000 births. Despite its frequency and severity, population screening is not widespread. Both male and female offspring are affected, but males generally have a more severe form resulting in moderate to severe disability. Affected females have a less severe disability, although the exact outcome can be difficult to predict. In at-risk families, fragile X syndrome is often transmitted from a 'premutation' carrier mother to her sons. Screening women to identify those at risk is feasible and could detect virtually all at-risk families.

Nevertheless, population screening has not been taken up as enthusiastically for fragile X syndrome as for trisomy 21: only Israel has a public health screening program. In part, this is because of the difficulty in counselling families about the likely outcome among affected females. Further, unlike trisomy 21 screening, the implications of fragile X syndrome screening are widespread, extending beyond this pregnancy in this couple. Fragile X syndrome carriers are at risk of premature ovarian failure and a late onset neurodegenerative disease called fragile X tremor ataxia syndrome. Screening therefore requires careful counselling: 'Would you want to know?' Moreover, the pregnant woman's sisters and other family members may also carry the genetic change. Pregnancy screening for fragile X syndrome can therefore open a 'Pandora's box' for the entire family. It is therefore imperative that any public health screen-

ing program is adequately resourced for the 'cascade screening' that may follow.

Fragile X syndrome screening highlights some of the challenges of prenatal genetic testing. Families often wish to 'know everything' about their unborn fetus. Accordingly, commercial companies now target women planning a pregnancy with direct to consumer marketing of genetic screening panels for over 100 conditions. Access to testing is not difficult, as no medical referral is required, and only a saliva or blood sample with payment is required. The attraction is clear: to know that 'everything is fine' before embarking on a pregnancy. However, up to one quarter of people are found to be carriers for at least one recessive condition following testing with an 'extended' panel of genes (Lazarin et al., 2013). This result would then trigger testing of the partner and genetic counselling. The challenges are: (1) ensuring adequate pretest counselling, to ensure women understand what they are being tested for, and to confirm their wish to know their carrier status; and (2) adequate counselling resources to manage the post-test fallout when abnormal results are received.

Screen before or during pregnancy?

Couples at risk of transmitting inherited conditions to their fetus may be identified by screening in pregnancy. There are established screening pathways for families known to be at high risk, and population screening is offered to identify carriers of common and important conditions such as cystic fibrosis and thalassaemia. Screening prior to pregnancy allows couples more choice with reproductive planning (Figure 6). Pre-pregnancy screening has a chequered history. In Cyprus, for example, population screening of young adults for thalassaemia was introduced in the 1970s to reduce the public health

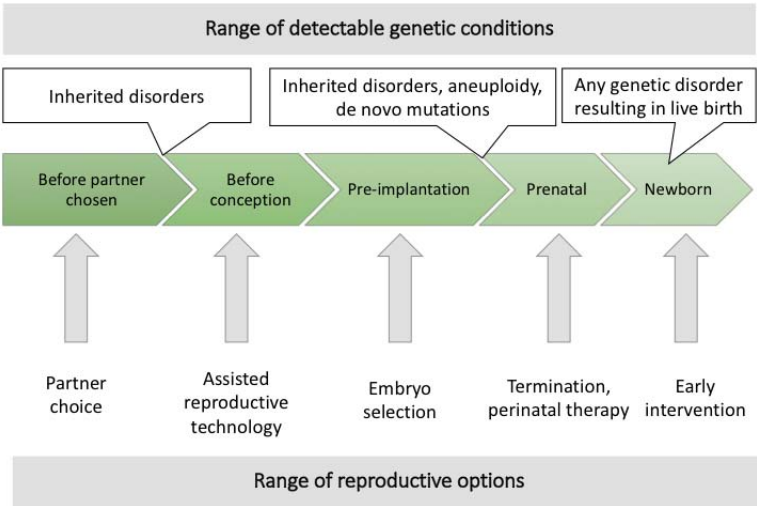

Figure 6: Timing considerations in relation to reproductive screening.

burden and financial costs associated with this disease. The cost of treating thalassaemia would be greater than the entire health budget without this program being instituted. This compulsory carrier screening and counselling was actively supported by the Orthodox Church of Cyprus, a necessary partner since the church was needed to sanction and bless a marriage. While two carriers are free to marry, in practice they rarely do so. The impact on population health was immediate and dramatic. From being a country with one of the highest rates of thalassaemia, the birth rate of affected infants fell to almost zero. While such directive programs fall outside contemporary bioethical norms, pre-pregnancy identification of high-risk couples also enables them greater reproductive planning choice. It is possible to consider assisted reproductive options such as pre-implantation genetic diagnosis or use of donor egg or sperm to minimise the risk of an affected child.

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) refers to the process where an embryo created using in vitro fertilisation (IVF) technology can be tested, and confirmed to be healthy, prior to being placed back in the uterus. PGD is most commonly offered to couples known to be at risk of transmitting a serious heritable disorder to their offspring. PGD for sex-selection for social reasons such as 'family balancing' is not permitted in Australia but can be offered to families at risk of transmitting sex-linked serious medical conditions where the genetic basis is not known. Rarely, families may request PGD to identify a child that would be a compatible stem cell cord-blood donor, or bone-marrow donor, for a sibling affected with a life threatening disease. In general, the aim of PGD is to ensure a healthy pregnancy unaffected by the genetic disorder in question, and obviate the need for prenatal testing and termination of an affected pregnancy. The benefits of PGD need to be weighed against the disadvantage that IVF is always required, even for couples who are not infertile. IVF is costly and brings some additional risks, particularly multiple pregnancy. The chance of a misdiagnosis is very small with PGD, but confirmatory testing by invasive testing such as CVS or amniocentesis is nevertheless offered in all pregnancies.

Conclusion

The application of genetic screening and fetal ultrasound in obstetrics has revolutionised pregnancy care. The opportunity to look into the health of the next generation has never been greater as screening and diagnostic testing have become more sophisticated. For clinicians and patients alike, it is a constant struggle to keep abreast of the latest technological developments that are being rapidly transitioned into clinical care. The

success of such programs will depend on not just adequate counselling of pregnant women, but broad societal engagement with the ethics of screening and the value we place on 'knowing all that we can'. Such discussion will be informed by a bioethical framework including respect for maternal autonomy, but also respecting the principle of beneficience — doing more good than harm — and justice, the use of health resources wisely and equitably to do the most good we can do.

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