Advances in prenatal screening

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BACKGROUND With the current reduction in obstetric and gynaecological specialists, a pregnant woman's first antenatal visit to the obstetrician or hospital antenatal clinic now frequently occurs after 14 weeks of pregnancy. Therefore the onus for providing prenatal screening and diagnosis advice will increasingly fall to the general practitioner.
OBJECTIVE It is important that GPs are familiar with the current screening strategies pertinent to the most common chromosome abnormalities (principally Down syndrome) and to the most common genetic syndromes (such as cystic fibrosis).
DISCUSSION Currently, combined first trimester nuchal translucency assessment and biochemical screening is the most reliable method for prenatal Down syndrome screening. It provides approximately 90% detection in the hands of appropriately trained and accredited practitioners. Antenatal screening for cystic fibrosis is clearly of benefit in a high risk group. Screening all pregnant women requires considerable genetic counselling and laboratory resources and its routine use is still keenly debated in Australia.

General practitioners are usually the first point of contact in early pregnancy and increasingly they are involved in shared antenatal care. In Australia fewer obstetricians are providing pregnancy services and the demand for hospital antenatal clinics is rapidly expanding. There is a trend toward later first antenatal visits, often beyond the ideal gestation to give prenatal advice.

Maternal age in pregnancy is increasing. The median age is now 29.0 years and 16% of women are 35 years of age or over.[1] Therefore, the proportion of pregnant women undertaking some form of antenatal screening and diagnosis is increasing. These changes will see the onus to provide prenatal screening and diagnosis advice increasingly fall to GPs. It is vital that GPs are familiar with the current screening strategies in relation to the common chromosome and genetic syndromes.

Prenatal screening for chromosome abnormalities

Down syndrome (DS) is the most common chromosome abnormality with a live birth incidence of 1.2 per 1000 in Australia and is the most common known cause of severe intellectual disability.

Prenatal screening relates to the selection of 'at risk' individuals (designated by maternal age, ultrasound or biochemical tests) for definitive prenatal diagnostic testing (by chorionic villus sampling or amniocentesis). Screening is appropriate for pregnant women of ALL ages.[2]

Screening based on maternal age

Down syndrome screening programs were initially based on the recognition that an individual's risk increased with advancing maternal age. Offering prenatal definitive testing to women aged 35 years of age or above results in detection of approximately 30–45% of affected pregnancies. This low detection rate relates to the fact that approximately 60% of affected individuals are born to younger women and that there is low utilisation of invasive tests in older women (43% in New South Wales in 1996).[3] Consequently, several population based screening programs have been developed to improve antenatal detection (Table 1).

Table 1. Antenatal screening methods

Screening test	Gestation	Down syndrome detection rate (5% FPR)
First trimester serum biochemistry (free ßhCG and PAPP-A)	10–13	60
First trimester ultrasound Nuchal translucency (NT)	11–14	80[6]
NT plus FTS	11–13	90[8]
Second trimester biochemistry (hCG, oestridiol and alphafetoprotein)	14–18	60–70[5]
Second trimester ultrasound markers (eg. echogenic cardiac foci, pyelectasis, nuchal fold thickening)	18–20	20–30 (low sensitivity and high FPR)

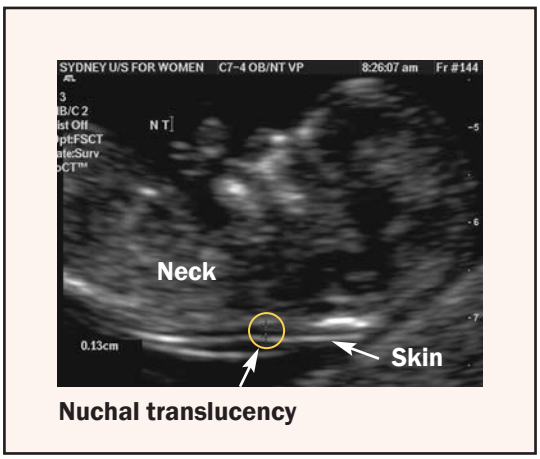

Figure 1. Ultrasound image showing measurement of nuchal translucency at 12 weeks gestation

Second trimester ultrasound screening

Second trimester ultrasound assessment may detect structural anomalies such as cardiac defects and duodenal atresia, or ultrasound 'markers' such as nuchal fold thickening, pyelectasis, echogenic cardiac foci, and others suggestive of DS. Individually, these markers have low sensitivity and a high false positive rate (FPR) in detecting aneuploidy, as they may be found in 10% of normal fetuses.

The trisomy 21 detection rate using second trimester ultrasound alone is approximately 40%. Algorithms combining maternal age with different ultrasound markers have improved the detection rates for trisomy 21 to between 60 and 75% for a FPR of 15%.[4] The accuracy of the screening test is highly operator dependent. The available data do not support the use of second trimester ultrasound screening as a primary screening test for DS.[2]

Second trimester serum screening

Down syndrome risk can be calculated by combining maternal age and the levels of human chorionic gonadotropin, oestriol and alphafetoprotein in maternal serum at 15–19 weeks of pregnancy. Women whose adjusted risk exceeds 1 in 250 are offered amniocentesis.

The Australian experience shows variation in effectiveness of biochemical screening. Some states (South Australia and Victoria) have attempted to make this a population based screening program but overall utilisation in Australia is only approximately 30%.

The New South Wales Reference Laboratory reported a 59% detection rate for trisomy 21 using a triple serum marker test.[5] The FPR of screening is 5.3–5.5%. While this is clearly superior to invasive testing on maternal age grounds alone, the detection of other chromosome abnormalities is generally poor as is the detection of trisomy 21 in younger women.

First trimester ultrasound screening

Over the past decade first trimester nuchal translucency (NT) assessment by ultrasound at 11–14 weeks of pregnancy has emerged as an alternate strategy to second trimester biochemical and ultrasound screening for aneuploidy (Figure 1). Combining maternal age and the thickness of the NT measurement can derive DS risk.[6] If the risk exceeds 1 in 300, karyotyping is offered either by chorionic villus sampling (CVS) or, later, by amniocentesis. One advantage of this approach is that screening in the first trimester allows earlier surgical methods of pregnancy termination in the event of fetal anomaly.

The largest published study reported detection rates of 82% for trisomy 21 and 80% for other chromosome abnormalities (FPR 7.7%) in over 96 000 women.[6] Critical to the success of this strategy is a formal program for continuous audit and structured sonographer training. There is now a national training, accreditation and audit program funded by the Commonwealth Department of Health and Aging to ensure standardisation of

image acquisition across the 100 centres in Australia offering this form of screening. In 2001, over 20% of pregnant Australian women had NT screening.

Approximately 4% of euploid fetuses with enlarged NT have a major structural anomaly (principally cardiac defects).[7] Thus, careful fetal morphology assessment at 18–20 weeks of pregnancy is important in this group. First trimester ultrasound has the additional benefit of providing accurate pregnancy dating, exclusion of unrecognised early pregnancy failure, accurate chorionicity assessment in twins and detection of many severe morphological fetal anomalies (such as anencephaly).

Combination of first trimester ultrasound and serum screening

First trimester serum screening based on the placental proteins free beta-hCG and PAPP-A is capable of identifying approximately 60% of DS fetuses. Accurate gestation information is critical to the accuracy of serum testing. Free beta-hCG tends to be elevated in DS and PAPP-A is decreased. The combination of NT and serum screening has yielded detection of approximately 90% DS fetuses with a FPR of 5%.[8,9]

Algorithms have recently been derived to assess the DS risk in twin pregnancies[10] and the risk for trisomies 13 and 18[11,12] in conjunction with NT assessment. Prospective studies are awaited to assess their value in clinical practice. Preliminary information that low levels of both PAPP-A and free beta-hCG may be associated with pregnancy complications such as miscarriage, pregnancy induced hypertension, growth restriction and diabetes[13] has yet to be confirmed in large prospective studies. First trimester serum screening does not provide neural tube defect information.

Future possibilities

Fetal nasal bone

A link between the absence of the fetal nasal bone on first trimester ultrasound and DS has been described in one study in a high risk population.[14] The nasal bone was absent in 75% of DS fetuses and in only 0.5% of normal fetuses. Similar figures have been reported from small, low risk studies.[15,16]

Nasal bone absence is thought to be independent of maternal age and other screening parameters,[14] although this is questioned in one recent study.[17] If the association is shown in low risk populations, DS detection could improve to over 90% through a combination of nasal bone assessment, maternal age, NT and serum screening for a 1% FPR. This would dramatically reduce the number of invasive tests required in any given population of screened pregnant women.

New horizons – fetal DNA extraction, multiplex fluorescent PCR

Exciting advances in molecular technology have enabled extraction of free fetal DNA from the maternal plasma as early as 10–14 days after conception. Conventional polymerase chain reaction (PCR) techniques are currently being used in preliminary studies to identify the SR-Y gene and the rhesus D genes.[18] The reliability of PCR and maternal cell contamination may limit the clinical application of this technique to a screening, rather than a diagnostic role in determination of chromosomal and genetic anomalies.

A newer technique called multiplex fluorescent PCR may revolutionise antenatal screening. This is substantially more sensitive than conventional PCR. This technique has wide ranging applications including fetal gender identification, single gene defect diagnosis (such as cystic fibrosis), DNA 'fingerprinting' and paternity. The potential exists for 90% detection of the common trisomies, including DS, with less than 1% FPR. Invasive testing would still be required for diagnosis.

Prenatal screening for cystic fibrosis

Cystic fibrosis (CF) is the most common inherited condition in caucasians. The disorder is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene located on chromosome 7. One in 25 caucasian Australians is a carrier and one in every 2500 babies born in Australia is affected. As it is recessively inherited, one in four children born to parents who are both carriers will be affected.

Cystic fibrosis affects the exocrine glands resulting in thick mucus production in the lungs and the gut, leading to recurrent respiratory infections and poor absorption of nutrients. There is no cure for CF and treatment involves daily physical therapy and medication. Life expectancy varies depending on the severity of the illness. Some people will not survive past teenage years and others may live to approximately 50 years of age.

Table 2. Risk of CF carriage with a positive family history

Family history	Risk of being a carrier
Sibling affected	2 in 3
Sibling is a carrier	1 in 2
Niece/nephew affected	1 in 3
Aunt/uncle affected	1 in 3
First cousin affected	1 in 4

Table 3. Risk calculations for caucasians with no family history of cystic fibrosis, screening only for the delta F508 mutation

Test results for each parent	Risk of being a carrier	Risk of having a CF affected child	
Before screening	1/25 for each	1/25 x 1/25 x 1/4	1 in 2500
1 not detected 1 not screened	1/100 1/25	1/100 x 1/25 x 1/4	1 in 10 000
2 not detected	1/100 for each	1/100 x 1/100 x 1/4	1 in 40 000
1 heterozygous carrier 1 not screened	1 1/25	1 x 1/25 x 1/4	1 in 100
1 heterozygous carrier 1 not detected	1 1/100	1/100 x 1 x 1/4	1 in 400
2 heterozygous carriers	1 for each	1 x 1 x 1/4	1 in 4

Infertility in CF affected men is common due to congenital absence of the vas deferens.

Newborn screening

Neonatal screening has been the traditional method to detect unexpected CF cases. This has the supposed advantage of commencing an early monitoring and treatment program rather than making the diagnosis when the child fails to thrive and may already be severely compromised. However, a recent Cochrane review of newborn CF screening was unable to identify evidence suggesting benefit from this method of screening.[19]

Antenatal screening

Given the nature of the condition, antenatal screening and diagnosis seems logical as it provides parents with choice about pregnancy outcome. The American College of Obstetricians and Gynecologists recently recommended making CF screening information available to all couples regardless of their carrier risk. They also recommend that physicians should specifically offer screening to the higher risk groups.[20] The Human Genetics Society of Australia, the RACGP and the RANZCOG have made no such recommendations regarding CF screening. Generally antenatal screening in Australia is targeted to those with a family history of CF, rather than being routinely offered.

There is significant debate over sequential versus couple screening.[21,22] The former involves screening all pregnant women and then screening the partner in the 1 in 25 cases where the woman is found to be a carrier. Screening both parents simultaneously is more cost effectiveness as only 1 in 676 couples need counselling and further investigation.[23]

Definitive diagnosis of the potentially affected fetus is usually by CVS. However, amniocentesis, or rarely fetal blood sampling, can also achieve a definitive result.

Pretest counselling

There are ethical issues regarding DNA collection, testing and storage. Before embarking on carrier testing it is important the couple receives appropriate counselling. This would include:

- the risk of being a carrier (Table 2)
- the limitations of CF testing (not all mutations are screened for)
- the possible test outcomes (Table 3), and
- the option of prenatal testing (by CVS or amniocentesis) if both parents are carriers.

Screening is performed by buccal smear and there is currently no Medicare rebate.

Which genetic mutation to screen for

Since the CFTR gene was sequenced in 1989, nearly 1000 different mutations have been identified. The delta F508 mutation accounts for 75% of all caucasian carriers. However, it is present in only 28% of carriers of Ashkenazic Jewish descent. If there is a family history of CF with a known mutation, then screening can be targeted to that mutation. If the mutation is unknown, or there is no family history of CF, then screening is tailored to the ethnic background of the couple. Screening for multiple mutations increases the carrier detec-

tion rate (to 82% testing the eight most common mutations, and to 90% if the most common 31 mutations are tested). If there is no specific family history of CF and the couple is caucasian and unrelated, screening for delta F508 is sufficient.

Preimplantation genetic diagnosis

Advances in reproductive and molecular technologies have enabled couples at high risk for a CF affected child to select only embryos that do not carry the mutation to be implanted. A single cell is extracted from an eight cell embryo. DNA is then extracted from the cell and amplified by PCR until sufficient product is available to test for the CF mutation. The risk of misdiagnosis is estimated to be 0.05%.[24]

Conclusion

With the changes in demographics and provision of maternity services currently seen in Australia, GPs will increasingly shoulder the burden of antenatal screening advice. It is important that those who are involved in antenatal care are familiar with the current screening strategies for the common chromosomal and genetic abnormalities. Ongoing education in this area is vital as new techniques rapidly emerge and evolve.

SUMMARY OF IMPORTANT POINTS

- First trimester combined nuchal translucency and serum screening currently detects approximately 90% of Down syndrome fetuses. Education, training and audit are vital to its success.
- Preconceptual or prenatal couple screening for cystic fibrosis is recommended for those at high risk. Routine antenatal screening has not yet found acceptance in Australia.
- In the next decade molecular techniques applied to fetal DNA are likely to be the primary mode of screening.

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