A systematic review evaluating non-invasive techniques to diagnose genetic disorders in a human fetus and the ethical implications of their use

Matthew Irwin
Sixth Year Medicine (Undergraduate)
University of New South Wales

Matthew Irwin is in his final year of Medicine at the University of New South Wales. His passion for rural healthcare has afforded him membership on advisory committees and opportunities to present at conferences including the World Congress of Anaesthesiologists. Upon accepting his NSW Rural Achiever Award, Matt pledged a life of servicing rural patients with otherwise limited resources.

Introduction: Genetic disorders are a significant cause of neonatal morbidity and mortality. [1] Diagnosing a genetic disorder currently involves invasive tissue sampling which carries an increased risk of miscarriage. The discovery of cell-free fetal DNA (cffDNA) in maternal plasma has enabled the development of non-invasive prenatal diagnostic tests (NIPD). [2,3] The scientific and ethical implications are examined. **Methods:** Medline, PubMed and Cochrane Library were searched for original research articles, review articles and meta-analyses focussed on screening and diagnosis of fetal genetic disorders. **Results:** 422 original research and review articles were assessed using processes in the Cochrane Handbook for Systematic Reviews of Interventions. [4] Using maternal plasma obtained during the second trimester, researchers were able to sequence the fetal genome with up to 98% accuracy. Clinicians reported the test will improve prenatal screening uptake, and reduce morbidity and mortality associated with genetic disorders. Ethicists argue it has implications for informed consent, rates of termination, reliability of future applications, inadvertent findings in clinical settings, commercial exploitation and inconsistent use of the technology internationally. **Conclusions:** Once NIPD tests utilising cffDNA are refined and costs reduced it is likely its implementation will affect both specialist genetic and routine antenatal services. However, given the complex set of ethical, legal and sociocultural issues raised by NIPD, professional education, public engagement, formal evaluation and the development of international standards are urgently needed. Health systems and policy makers must prepare to respond to cffDNA technology in a responsible and effective manner.

Introduction

Most pregnant women wish to be reassured that their unborn baby is healthy. [5] The aim of antenatal care is therefore to select screening and diagnostic tests that are accurate, safe and can be performed sufficiently early to allow parents to plan ahead or terminate the pregnancy in the event that fetal abnormality is diagnosed. [6] Genetic disorders are a significant cause (20%) of neonatal mortality. [1] At present, maternal serum screening, alone or in combination with ultrasound, is used to identify fetuses at risk of aneuploidy and other disorders. [7] Unfortunately, neither maternal serum screening nor ultrasound provide information on the genetic constitution of a fetus or allow a definitive diagnosis to be made. [8] For this, fetal cells must be invasively sampled from the placenta (chorionic villus tissue), amniotic fluid or fetal blood - all of which increase the risk of miscarriage. [9,10] This increased risk makes the decision to use invasive prenatal diagnosis difficult, particularly as there are still only very limited treatment options. [11] As a result, the medical community has sought to develop reliable and safe methods for achieving non-invasive prenatal diagnosis (NIPD), in addition to future treatment options. [12] Through NIPD, researchers hope to improve screening uptake, and reduce morbidity and mortality associated with genetic disorders. [1] Ethicists argue that NIPD transects existing distinctions between screening and diagnostic tests, and has implications for informed consent or choice. [12]

Methods

MEDLINE, PubMed and Cochrane Library were searched weekly between September 2012 and April 2013 for original research articles, review articles and meta-analyses focussed on screening and diagnosis of fetal genetic disorders. MeSH headings used were: Genetics, Medical, Genetics Testing and Fetus. Search terms used were: non-invasive, whole-genome and sequencing. Results were limited to human studies written in English between 1995 and 2013.

Results

The search resulted in 422 articles being identified; these were subsequently examined. The majority of publications were original research and review articles, although there was one meta-analysis by Alfirevic *et al.* (2003). [6] Many publications (217) were excluded for their limited scope or irrelevance.

Maternal serum screening and ultrasound are current methods of choice for screening pregnancies at risk of genetic disorders. [8,13] However, both methods rely on measuring epiphenomena rather than core pathology. Consequently, both tests have limited sensitivity and specificity and can only be used within a relatively narrow gestational period. [14] To achieve a definitive diagnosis chorionic villi sampling (CVS), amniocentesis or cordocentesis must be used. [6,8]

CVS is an invasive diagnostic procedure performed after 10 weeks gestation that is used for karyotyping when first trimester screening suggests a high risk of aneuploidy. [8] It is also used for fetal DNA analysis if the parents are known to be carriers of an identifiable gene mutation, such as cystic fibrosis or thalassaemia. [9] The procedure involves ultrasound-guided aspiration of trophoblastic tissue using either the trans-cervical or trans-abdominal routes. The tissue is then analysed with fluorescence *in situ* hybridisation polymerase chain reaction (FISH PCR). Like CVS, amniocentesis involves ultrasound-guided aspiration of amniotic fluid but is performed after 15 weeks gestation. [6] Cordocentesis involves direct sampling of fetal blood from the umbilical cord but is rarely performed and will not be discussed further in this article.

The benefit of CVS is that it can be performed at an earlier gestation, facilitating earlier diagnosis and providing the opportunity to terminate the pregnancy by suction curettage of the uterus. The benefits of amniocentesis include the lower background rate of miscarriage and the avoidance of isolated placental mosaicism occurring in 1% of samples. [8] The primary risk with CVS and amniocentesis is miscarriage. The level of risk is similar for the two tests (reported

risk ranges from 1% to 1 in 1600) and is operator dependent. [6,7] Researchers have attempted to reduce this risk by developing a NIPD that allows the direct analysis of fetal genetic materials. [2,12,14-22] Better screening tests will achieve a higher detection rate combined with a lower false positive rate, resulting in less invasive testing and fewer procedure-related miscarriages.

Much of the early work on NIPD focussed on the isolation of fetal nucleated cells that had entered into the maternal blood. [14] However, the concentrations of these cells were low, meaning the tests had low sensitivity and specificity. [15,22] Later methods were inspired by the presence of tumour-derived DNA in the plasma of cancer patients. [23,24] In 1997, Lo *et al.* (1997) observed an analogous phenomenon was present in pregnancy by identifying Y chromosomal DNA sequences in plasma of women carrying male foetuses. [25] Replication of this study has concluded that 10% of cell-free DNA (cffDNA) in a pregnant woman's plasma originates from the fetus she carries. [14,17,18,20] Since then, several groups have developed NIPD tests but most were only capable of detecting gross abnormalities such as aneuploidies, and were limited by small sample size and substandard accuracy. [17,18,22,26,27] In June 2012, Kitzman *et al.* (2012) reconstructed the whole-genome sequence of a human fetus using samples obtained relatively noninvasively during the second trimester, including paternal buccal DNA and maternal and cffDNA from the pregnant mother's plasma. [2] Predicting which genetic variants were passed from mother to fetus was achieved by resolving the mother's haplotypes - groups of genetic variants residing on the same chromosomes - and combining this result with shotgun genome sequencing of the father's DNA and deep sequencing of maternal plasma DNA. [19] Comparing the results of this method with cord blood taken at delivery found inheritance was predicted with 98.1% accuracy. The study sequenced only two fetuses at a cost of $50,000 each, and is yet to be reproduced. Researchers from Stanford University were able to sequence the fetal genome without a paternal saliva sample although this was less accurate than the method used by Kitzman *et al.* (2012). [18] This latter method forms the basis of commercially available NIPD tests being offered by laboratories. [28] In Australia, NIPD testing is currently limited to Trisomy 21, 18, 13 and abnormalities of sex chromosomes, is not eligible for a Medicare rebate and costs upwards of $1,250. [29] It is anticipated that analysing samples for NIPD locally will reduce the cost and drive demand. [30,31]

Discussion

Clinicians report that non-invasively diagnosing genetic disorders will reduce infant mortality and morbidity. [31] Ethicists argue the technology raises concerns for informed consent, rates of termination, reliability of future applications, inadvertent findings in clinical settings, commercial exploitation and inconsistent use of the technology internationally [12,32-36].

Informed Consent and Informed Choice

Ethicists believe NIPD testing transects existing distinctions between screening and diagnostic tests and has implications for informed consent and choice. [12] An example is screening for Down's syndrome, a common genetic disorder. Although a significant number of women may not already achieve informed choice for screening, at least a subsequent invasive diagnosis provides another opportunity for reflection as they consent to the procedure (CVS or amniocentesis). [34,35] Replacing this multi-step screening process with highly-predictive cffDNA testing may reduce opportunities for exercising informed choice. [12] In addition, despite the belief that introducing cffDNA testing will promote parental reproductive choice, it may indeed make proceeding with an affected pregnancy more difficult for two reasons: First, the decreased risks associated with cffDNA might lead women to feel 'pressured' into agreeing to the tests, or undergoing testing without informed consent, even if they potentially lead to outcomes with which they disagree. [33,36] Second, the lower risks might cause a shift in the extent to which society is supportive of those who chose to have disabled children. [10] In turn, worries over social disapprobation could prompt a loopback effect, where women feel more pressured to test and to terminate their pregnancies.

Termination of pregnancy (TOP)

In Australia, there is broad agreement that TOP is ethically and legally permissible in some circumstances. [11,33,37] However, the laws are notoriously unclear, outdated and inconsistent between states and territories. [38,39] In many jurisdictions it is legally defensible for a clinician to perform a TOP at any gestation if they can justify the harms of continuing with the pregnancy outweigh the risks of termination. [40] For this reason, access to TOP is very much dependent on the clinician, which may be problematic if cffDNA testing becomes more widespread and moves outside the existing setting of medical genetics, where high standards of relevant ethical practice and the professional duty of non-directive counselling are firmly entrenched. [12]

Accuracy and reliability of NIPD

Despite improved accuracy by utilising fetal nucleic acids, the sensitivity and specificity of even the most accurate method is still less than 100%. [2] Maintaining an acceptably high sensitivity and specificity will also be a challenge, as researchers discover an ever-increasing number of sequences associated with pre-existing diseases. [12] To do this will require careful monitoring within different applications. [33] Without it, the personal, sociocultural, legal and ethical ramifications of false positives and negatives may be devastating. For example, additional invasive testing may be undertaken, healthy fetuses may be terminated, and children may suffer psychologically should they discover their parents would have terminated them if they had known of their diagnosis. [34]

Inadvertent findings in clinical settings

The Kitzman *et al.* method requires paternal buccal DNA to sequence the fetal genome and may therefore inadvertently disclose misattributed paternity. [41] However, so too may the Stanford University method that forms the basis of commercially available NIPD but that does not require paternal buccal DNA. [18] In a trial of 18 subjects, researchers using the latter method were able to predict 70% of the paternally inherited haplotypes in the fetus with 94–97% accuracy. [18] Of course, the correlation of these findings to the clinical setting would likely still require paternal buccal DNA to confirm paternity. The potential for inadvertent disclosure of misattributed paternity would be a particular concern if cffDNA testing were ever incorporated into routine antenatal screening as a greater number of women who may not have been adequately forewarned would be exposed to the risks such information may bring.

Commercial and international uses

The likely increase in the accessibility of NIPD using cffDNA tests made available via the internet has major implications, particularly for fetal sex selection. [12] In China [42] and India, [43] population skewing has already been observed as a result of unlawful sex selection practices favouring male children. Some ethicists believe cffDNA could significantly aggravate or extend this problem. [44] The development of cffDNA technology within the commercial sector is also a concern as some companies choose only to sell the service rather than invest in research and development eg: babygendermentor.com. The provision of testing direct-to-consumers raises a complex set of issues relating to the role of 'gatekeepers' in prenatal testing and access to non-clinical applications of the technology. [33] In addition, it may even impact upon the provision made through Medicare for ongoing care, including diagnostic confirmation, interventional procedures (such as TOP) and medical advice. [5] Having commercial players involved may result in elements of professional practice, including informed consent and counselling, being difficult to enforce considering international legislative and regulatory boundaries. [12] The cultural context is also highly relevant to how consumers access cffDNA testing. For example, its use in countries where access to safe TOP is limited or absent is

ethically questionable and could cause significant social and medical problems. [45]

Conclusion

The utilisation of cffDNA for safe and reliable NIPD has opened the way for accurate sequencing of the fetal genome and the ability to diagnose an ever-increasing number of genetic anomalies and their clinical disorders. Once methods such as those by Kitzman *et al.* and researchers at Stanford University are refined and costs reduced it is likely the implementation of cffDNA testing will affect both specialist genetic and routine antenatal services, improve screening uptake, and reduce morbidity and mortality associated with genetic disorders. As a result of the pace of development, there is concern that cffDNA testing transects existing distinctions between screening and diagnostic tests, having implications for informed consent, termination rates and commercial. Given the complex set of ethical, legal and sociocultural issues raised by NIPD, both professional education and public engagement are urgently needed. Formal evaluation of each test should be required to determine its clinical accuracy, and laboratory standards should be developed alongside national best practice guidelines to ensure that cffDNA testing is only offered within agreed and well-supported pathways that take account of the aforementioned issues. This development has the potential to deliver tangible improvements in antenatal care within the next 5-10 years, and health systems and policy makers around the globe must now prepare to respond to further developments in cffDNA technology in a responsible, effective and timely manner.

Conflict of interest

None declared.

Correspondence

M Irwin: matt@irwinmd.com.au

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