Review Article

Genetics in prenatal diagnosis

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Abstract

The options for prenatal genetic testing have evolved rapidly in the past decade, and advances in sequencing technology now allow genetic diagnoses to be made down to the single-base-pair level, even before the birth of the child. This offers women the opportunity to obtain information regarding the foetus, thereby empowering them to make informed decisions about their pregnancy. As genetic testing becomes increasingly available to women, clinician knowledge and awareness of the options available to women is of great importance. Additionally, comprehensive pretest and posttest genetic counselling about the advantages, pitfalls and limitations of genetic testing should be provided to all women. This review article aims to cover the range of genetic tests currently available in prenatal screening and diagnosis, their current applications and limitations in clinical practice as well as what the future holds for prenatal genetics.

Keywords: Copy number variants, foetal anomaly, genetic testing, next-generation sequencing, prenatal diagnosis

INTRODUCTION

It was only 65 years ago that the number of human chromosomes was correctly determined to be 46 instead of 48, highlighting the rapidity with which our knowledge of the human genome has developed.[1] This crucial re-calibration provided the framework for the standardised reporting of various common chromosomal aneuploidies. It was the use of genetics in the paediatric population that paved the way for genetic diagnosis by connecting specific clinical phenotype(s) to chromosomal aberration(s). Thus, it was only a matter of time before genetic testing shifted to the earlier prenatal period by means of prenatal testing [Figure 1].[2-24]

One of the first mentions of prenatal diagnosis was the use of deviation of optical density at 450 nm (OD 450) of the amniotic fluid to predict foetal anaemia in rhesus isoimmunised pregnancies by Sir William Liley in 1961,[25] and the first attempt at prenatal genetic diagnosis dates back to the 1950s, where sex determination was done prenatally through identification of the Barr body.[8,26,27] Subsequent karyotyping was performed on cultured cells from amniotic fluid to detect imbalanced translocations and Down syndrome in the 1960s.[13,23,28]

In the 1970s, the uptake of invasive testing, namely amniocentesis, increased steadily.[29] It was initially performed for the indication of advanced maternal age and subsequently for high-risk results on aneuploidy screening tests or following the detection of foetal anomalies on ultrasound that were suggestive of chromosomal aneuploidy.

NON-INVASIVE PRENATAL DIAGNOSIS

Prenatal screening

The 'triple test' was first introduced in 1988; it involved serum measurements of maternal alpha-foetoprotein, human chorionic gonadotrophin (hCG) and unconjugated oestriol, which when taken together with maternal age, provided a detection rate for Down syndrome of 60%.[30] The subsequent addition of serum measurements of inhibin-A to this screening method, which became known as the quadruple test, improved the detection rate for Down syndrome to 70%.[31]

The combined first trimester screen was then introduced in 1997, in which serum measurements of pregnancy-associated

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Received: 30 Nov 2021 **Accepted:** 06 Oct 2022 **Published:** 19 Jan 2023

How to cite this article: Lim KM, Mahyuddin AP, Gosavi AT, Choolani M. Genetics in prenatal diagnosis. Singapore Med J 2023;64:27-36.

Access this article online

Quick Response Code:

Website: https://journals.lww.com/SMJ

DOI: 10.4103/singaporemedj.SMJ-2021-433

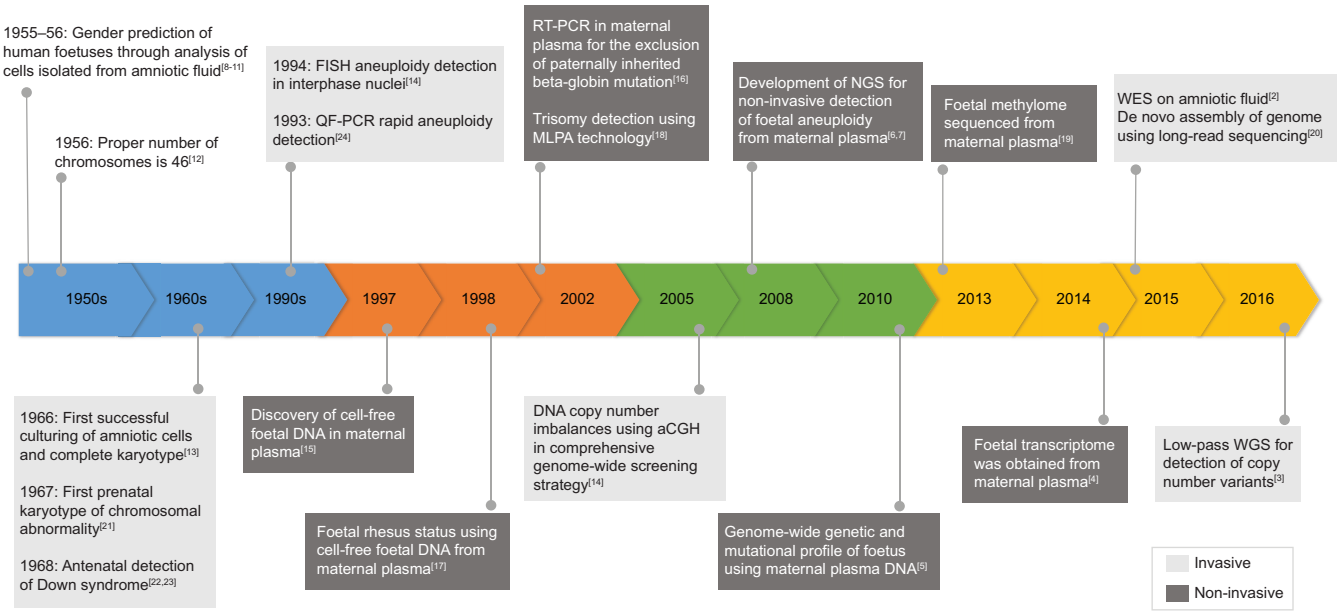

Figure 1: Diagram shows the shift from karyotype to non-invasive prenatal diagnosis and beyond. aCGH: array comparative genomic hybridisation, FISH: fluorescence *in situ* hybridisation, MLPA: multiplex ligation-dependent probe amplification, NGS: next-generation sequencing, QF-PCR: quantitative fluorescence polymerase chain reaction, RT-PCR: reverse transcription polymerase chain reaction, WES: whole exome sequencing, WGS: whole genome sequencing

plasma protein A (PAPP-A) and free beta-hCG were performed. Together with the sonographic measurement of foetal nuchal translucency between 11 and 14 weeks of pregnancy, the combined first trimester screen had a detection rate for Down syndrome of 85% to 90% and a false-positive rate of 5%.[32,33] In 2003, it was found that addition of the sonographic absence of the foetal nasal bone between 11 and 14 weeks of pregnancy yielded a detection rate of 90%, while reducing the false-positive rate to 0.5%.[34]

Ultrasound

Before the advent of ultrasound, birth outcomes were a matter of providence. The first reported use of a contact compound 2D ultrasound scanning machine was in 1958,[35] which simply aimed to obtain ultrasound images of the pregnancy and the foetus. Fast forward to 60 years later, it is impossible to conceive of practising obstetrics without the aid of one of the several types of ultrasound available today. The introduction of ultrasound into obstetrical care has resulted in the routine identification of foetal structural anomalies. Ultrasound performed in the first trimester has been shown to detect major foetal anomalies in 46% of low-risk or unselected populations and in up to 61% of high-risk populations.[36] Foetal structural anomalies are found in up to 3% of all pregnancies and in 1 in 300 women attending a third-trimester scan.[37]

Advances in ultrasound technology and use of 3D ultrasound can now delineate the exact location and extent of surface anomalies such as facial clefts and foetal neural tube defects, which aid better operative planning and prenatal counselling in the antenatal period. Multiplanar views on 3D ultrasound have increased the diagnostic accuracy of neural tube defects.[38] Improved prenatal detection of major foetal anomalies has a potential impact on the epidemiology of the condition. This was demonstrated in Denmark, where up to 89% of cases of spina bifida were diagnosed on ultrasound before 22 weeks, leading to a lower incidence of spina bifida secondary to women choosing to terminate their pregnancies, whereas in Sweden, there is lower acceptance of prenatal screening ultrasounds.[39]

Some anomalies such as cystic hygroma, omphalocoele, cardiac anomalies (i.e., atrioventricular septal defect and tetralogy of Fallot) and congenital diaphragmatic hernia have been shown to be strongly associated with aneuploidy and can be detected as early as 12 to 14 weeks. These conditions can easily be detected using standard G-banded karyotype, fluorescence *in situ* hybridisation (FISH) or quantitative fluorescence polymerase chain reaction (QF-PCR). However, if a structural anomaly is present, the preferred test would be chromosomal microarray analysis (CMA) because of the additional yield of pathogenic sub-chromosomal copy number variations (CNVs). There has been an observable reduction in diagnostic procedures involving high-risk serum screening tests globally because of the widespread adoption of aneuploidy screening, which involves non-invasive prenatal testing or screening (NIPT/NIPS). However, the rate of invasive testing following the detection of an ultrasound anomaly has remained steady.[40]

Cell-free foetal DNA from maternal blood

The discovery of cell-free foetal DNA in the maternal circulation and the subsequent advent of NIPS revolutionised the realm of aneuploidy screening.[15] The high sensitivity of 99% associated with common aneuploidies has resulted

in a significant decline in the number of invasive tests performed[29,41] because the high negative predictive value of the test often negates the need for a diagnostic test via amniocentesis or chorionic villus sampling (CVS), which carry miscarriage risks of 0.11% and 0.22%, respectively.[42]

Cell-based foetal DNA from maternal blood

Our group, and others, have shown that several different nucleated foetal cell types can be isolated from maternal blood and could potentially be used for cell-based non-invasive prenatal diagnosis (cbNIPD). These include haemopoietic progenitors,[43,44] foetal mesenchymal stem cells[45] and trophoblasts,[46-49] but most investigators agree that, by far, the best candidate nucleated foetal cell for cbNIPD is the foetal primitive erythroblast (i.e., the foetal nucleated red blood cell or FNRBC).[50] DNA derived from foetal cells recovered from the cellular fraction of maternal blood is 100% foetal in origin. It is essentially equivalent to the cells derived by amniocentesis and even has an advantage over CVS in that if FNRBCs are used, there is no risk of confined placental mosaicism (CPM).

FNRBCs retain all the advantages of nucleated foetal cells derived from maternal blood, but most importantly, they carry, and reflect, the true foetal genome. More recently, in 2017, He *et al*. used CD147[51-53] strategy, combined with epsilon-globin foetal cell identifier to develop a nanostructure microchip.[54] Then, in 2018, Wei *et al*. used the Percoll density gradient strategy combined with epsilon-globin to develop a microbead-based sedimentation method.[55] In the same year, Feng *et al*. used the CD147/epsilon-globin microchip to attempt chromosomal aneuploidy detection, but with limited success because of high false-positive rates.[56] The downstream analysis needed further refining, and therefore, in 2020, Cheng *et al*. attempted to use the Percoll/anti-CD147/epsilon-globin selection strategy for the simpler problem of foetal ABO genotyping.[57] This highlighted the challenges faced by the groups in taking this technology further downstream because of the inability to adequately analyse the FNRBC foetal genome.

Trophoblast cells can be isolated non-invasively as early as 5 weeks of gestation,[58] and this cell type has recently gained popularity as one of the two cells of choice for cbNIPD.[59-66] But the yield of these cells from maternal blood is poor, and in one study, only half the cells isolated were of high quality and suitable for next-generation sequencing (NGS), whereas the rest were of poor quality, in S-phase or apoptotic.[67] Apoptotic trophoblast cells show loss of whole or segments of chromosomes, and produce noisy NGS signals, making clinical diagnosis difficult.[65] Again, the use of trophoblast cells will introduce the risk of CPM and false results. This CPM risk is not trivial; it is estimated to be up to 1.3% for CVS and ranges between 4.8% and 16.9% in potentially viable embryos.[68-71] Trophoblasts may take up to 4 weeks post-partum to clear from the maternal circulation and are unlikely to persist to the next pregnancy.[72] Furthermore, these cells allow for higher-resolution (~1 Mb) genome-wide CNV analysis.[67,73]

Prenatal diagnosis

Genetic conditions that can afflict an individual range from aberrations at the chromosomal level — that is, aneuploidy — to single-base-pair substitutions, deletions or duplications, each with wide-ranging deleterious effects on normal development in utero. Examples of chromosomal aberrations include the commonly screened for aneuploidies, trisomy 13, 18 and 21, which can present with multi-system abnormalities, some of which are potentially lethal. Submicroscopic deletions or duplications, otherwise known as copy number variants, although found in healthy individuals, are also an important known cause of intellectual disability and human disease.[74,75] At an even smaller resolution, substitution of a single base pair in the coding region of the genome can also result in diseases associated with high morbidity and mortality, such as sickle cell anaemia and cystic fibrosis. The following section discusses the different types of genetic tests available for prenatal diagnosis, and they are summarised in Table 1.

G-banded karyotyping

The gold standard for the investigation of a genetic cause for a foetal anomaly detected on ultrasound has traditionally been karyotyping. G-banded karyotype is performed on cultured cells obtained via amniocentesis or CVS and is able to detect genomic imbalances at a resolution of 5 Mb. Karyotyping is able to detect large deletions and duplications as well as chromosomal structural variants, such as inversions, balanced and unbalanced translocations and ring and marker chromosomes. Additionally, the analysis of at least 20 cells obtained from a cell culture for karyotype allows for the detection of mosaicism, which is defined as the presence of two or more cell lineages with different genotypes arising from a single zygote, in a single individual.[76]

However, because karyotyping relies on the collection of viable foetal tissue for culture and diagnosis, nonviable foetal tissue samples often lead to culture failure. In addition, cultured cells are prone to maternal decidual cell contamination, which could result in false diagnoses, and it may take up to 2 weeks to obtain a diagnosis; this is one of the main limitations of the test. To overcome this limitation, rapid aneuploidy tests are often used in conjunction with karyotyping to provide a quicker result, which is advantageous when dealing with parental anxiety following a possible foetal anomaly or genetic condition. The 2 rapid aneuploidy tests commonly used in clinical practice are FISH and QF-PCR.

Fluorescence in situ hybridisation

FISH was developed in the 1970s as a method of evaluating the presence or absence of a particular chromosomal segment by the labelling of specific DNA probes complementary to the genomic region of interest with fluorochromes, which are visible under a fluorescence microscope.[77] The

Table 1. Summary of genetic tests.

	Resolution	Coverage	Limitations	Turnaround time
G-banded karyotype	5–10 Mb	Monosomy, trisomy, structural variants, triploidy, mosaicism	Deletions/duplications smaller than 5 Mb, methylation defects, expansion repeat disorders, mitochondrial	10–14 days
Chromosomal microarray	100 Kb	Copy number variants, uniparental disomy, regions of homozygosity (SNP-array)	Deletions/duplications smaller than 100 Kb, methylation defects, expansion repeat disorders, mitochondrial, structural variants, balanced translocations	7–14 days
Whole exome sequencing	1 base pair	Single-base-pair substitutions/deletions/ duplications in the coding region	Methylation defects, expansion repeat disorders, mitochondrial, intronic variants, Robertsonian translocations	14 days
Whole genome sequencing	1 base pair	Single-base-pair substitutions/deletions/ duplications across entire genome	Methylation defects, expansion repeat disorders, mitochondrial, Robertsonian translocations	14 days

SNP: single nucleotide polymorphism

method is useful in the detection of an aberrant number of copies of a particular chromosome as well as in identifying translocations. Three types of probes for FISH are used in clinical practice: gene-specific probes, centromeric probes and whole-chromosome probes, which can detect a range of genetic abnormalities depending on the type of probe used. FISH does not have to be performed on actively dividing cells, and hence cell culture is not required, resulting in a turnaround time of 24 to 48 hours. However, despite having a higher resolution compared to karyotype of 100 to 200 kb,[78] FISH is not an efficient method for interrogating the entire genome for imbalances; the genomic sequence of interest must be known beforehand, based on clinical suspicion. FISH can be used for the rapid detection of the common aneuploidies trisomy 13, 18 and 21, sex chromosome aneuploidies and specific microdeletion syndromes such as DiGeorge, Prader Willi and Angelman syndrome.

Quantitative fluorescence polymerase chain reaction

QF-PCR is another rapid aneuploidy test that relies on the detection and amplification of specific genomic sequences called short tandem repeats (STRs) on chromosomes. STRs are found throughout the genome in healthy individuals, and the number of repeats is polymorphic, resulting in each individual having a relatively unique number of STRs on each chromosome. Using PCR amplification of specific STR markers, QF-PCR results in the quick identification of the number of chromosome copies in a DNA sample. Two separate peaks in a 1:1 ratio would suggest that an individual has inherited 2 copies of that chromosome — 1 peak representing each inherited allele. For an individual who has inherited three copies of that chromosome, there would be 3 individual peaks in a 1:1:1 ratio, or 2 peaks in a 2:1 ratio.[79] Occasionally, STR markers will be non-informative if both inherited alleles have the exact same number of repeats, but this is overcome by the use of multiple STR markers on a single chromosome. QF-PCR is a cost-effective method of aneuploidy screening,[80] has a very high detection rate for aneuploidies of chromosomes 13, 18, 21, X and Y of up to 98.6% to 100%[81,82] and a zero false-positive rate,[83] leading to its widespread clinical use in prenatal diagnosis of the common aneuploidies. However, QF-PCR does not provide information about structural variants, and follow-up with conventional cytogenetic analysis is still recommended to rule out inherited Robertsonian translocations, which has implications for subsequent pregnancies, particularly in the cases of trisomy 13 and 21.[83] The second main limitation of karyotype lies in its inability to detect submicroscopic deletions or duplications that are less than 5 Mb in size.

Chromosomal microarray

In the past decade, CMA has increasingly become the test of choice and may even replace the karyotype when investigating a foetus with multiple foetal anomalies. CMA has the ability to detect copy number variants at resolutions as small as 50 kb. In a landmark paper in 2012, it was shown that CMA was able to detect clinically relevant deletions or duplications in approximately 1 in 60 of structurally normal pregnancies (test indications for advanced maternal age or positive screening results) and in 1 in 17 pregnancies with a structural anomaly.[84] Since then, CMA has been shown to provide an incremental diagnostic yield over karyotype in foetuses with congenital anomalies in all major organ systems.[85-87] Additionally, the incremental diagnostic yield is as high as 9% when there are multiple foetal anomalies. A systematic review of 17 studies in 2015 showed that CMA provides a pooled incremental yield of 5% in foetuses with a nuchal translucency of more than 3.5 mm and a normal karyotype, which is increased to 7% when other anomalies are detected in addition to a thickened nuchal translucency. The increased diagnostic yield that CMA provides has led to recommendations from various governing bodies that CMA be performed as the first-line genetic test when a foetus is found to have a thickened nuchal translucency of more than 3.5 mm or has one or more major structural abnormalities.[88-90]

An added benefit of CMA is that it can be performed on nonviable tissue, unlike karyotyping. This is advantageous when investigating for a genetic aetiology for stillbirths, of which up to 13% have been shown to be a result of chromosomal abnormalities.[91] In an analysis of 532 stillbirths, CMA was able to provide a result in 87.4% of cases, compared to 70.5% with karyotype analysis.[92] CMA was also able to detect a genetic abnormality in more antepartum stillbirths (8.8%

vs 6.5%, $P = 0.02$) and stillborn foetuses with congenital abnormalities when compared with karyotype analysis (29.9% vs 19.4%, $P = 0.008$).

The use of CMA has also been investigated in growth-restricted foetuses, where a genetic aetiology is known to be responsible for up to 20% of cases.[93] In a systematic review of 10 studies where CMA was performed for growth-restricted foetuses with a normal karyotype, results revealed a 4% incremental yield over karyotyping in foetuses without congenital anomalies and a 10% incremental yield when congenital anomalies were present in addition to growth restriction.[94]

The 2 main types of CMA techniques are array comparative genomic hybridisation and single nucleotide polymorphism (SNP) array.[95] The former relies on the quantification of genomic DNA in a patient sample using DNA probes that detect sequences across the entire genome. Patient DNA and DNA from a reference genome are labelled with fluorescent dyes of different colours and are mixed together in equal quantities. The signal emitted at each probe is interpreted digitally by the CMA platform, which is represented as a log2 ratio. When the quantity of genomic data in the patient sample is the same as that in the reference sample, the log2 ratio is zero, indicating that 2 copies of DNA material were detected at that locus. A loss (deletion) or gain (duplication) of genomic material is represented by a negative or positive log2 ratio, respectively. SNP-based arrays rely on the use of SNPs, which are distributed throughout the human genome. The fluorescent signal intensity obtained from a patient sample is compared to that from a reference sample to determine gains and losses in genomic material. Additionally, allelic data obtained from SNP arrays allow the detection of regions of homozygosity as well as uniparental disomy, where both alleles of a particular chromosome have been inherited from the same parent. This is of clinical significance in certain chromosomes that carry imprinted genes, resulting in disease phenotypes. SNP arrays also have the ability to detect triploidy, which can only be determined from allelic data.[95] However, because both CMA methods rely on the quantity of genomic data as compared to a reference sample, the technique is unable to detect balanced translocations where there is no overall net gain or loss of genomic material. Additionally, the resolution of CMA relies on probe density, which may not be evenly distributed across the genome and is platform dependent. Hence, cryptic deletions and duplications (usually <50 kb) smaller than the distance between probes may be missed. CMA also has limited ability in detecting low-level mosaicism (<20%) compared to conventional cytogenetics.[96] Also, genetic conditions that are caused by mutations in the mitochondrial genome or repeat expansion disorders such as fragile X syndrome cannot be detected on CMA. However, with the current evidence of improved diagnostic yield with CMA in the prenatal setting as well as the availability and affordability of testing, CMA is likely to become the first-line test in the investigation of foetuses with structural abnormalities.

Next-generation sequencing

NGS refers to DNA sequencing technology that has evolved beyond the founding Sanger sequencing method described in 1975.[97] High-throughput methods now allow rapid and simultaneous sequencing of large volumes of DNA, making the interrogation of the entire human genome down to single-base-pair resolution possible. There are several approaches to the use of NGS in prenatal diagnosis, and in increasing order of cost and turnaround time, they are as follows: (1) targeted panel sequencing, (2) whole exome sequencing (WES), and (3) whole genome sequencing (WGS).

In targeted panel sequencing, selected genes responsible for a group of monogenic disorders are sequenced based on the observed phenotype and clinical suspicion. A targeted testing approach potentially allows for greater sequencing depth and reduced cost and turnaround time of testing compared to WES but may result in a lower diagnostic yield, depending on the type of abnormality and gene panel available. A review of 127 cases of non-immune hydrops foetalis showed that WES provided a diagnostic yield of 29% compared to 18% on the largest targeted hydrops foetalis gene panel available at the time.[98] Conversely, targeted gene panels appear to have a high diagnostic yield in cases of anomalies of a particular organ system, such as skeletal dysplasias.[99] Hence, the application of gene panels should be individualised to the foetal anomaly detected. Additionally, this approach relies heavily on the accurate identification and diagnosis of the phenotype observed on antenatal ultrasound, which may not be as easily defined compared to the postnatal setting.

WES involves selective sequencing of the coding regions of the human genome, which constitutes 1% to 2% of all genomic DNA. Up to 85% of disease-causing mutations are found within the exome, making it a worthwhile testing strategy with a relatively high diagnostic yield. Initial studies showed that WES was able to provide a definitive genetic diagnosis in 21% of 24 cases with ultrasound abnormalities with a normal CMA result.[100] Following this, the PAGE cohort study showed that WES identified a diagnostic genetic variant in 8.5% of 610 foetuses undergoing invasive testing for thickened nuchal translucency or structural anomaly with a normal karyotype and CMA result.[101] Diagnostic rates from subsequent studies varied widely depending on the cases selected for WES,[85,102] with rates up to 80% in very carefully selected cases.[103] Since 2015, numerous studies have been performed that look at the added advantage that WES has over karyotype and CMA in the detection of various foetal anomalies. The additional yield that WES provides appears to vary greatly according to the type of anomaly detected, with the highest yield in foetuses with multiple anomalies of up to 23%.[104] Whether WES is the genetic test of choice when investigating an isolated thickened nuchal translucency remains to be determined. In foetuses with isolated thickened nuchal translucency above the 99th centile, WES has

been shown in a systematic review to provide an additional diagnosis over CMA in 4% of cases.[105] In view of the higher cost of WES compared to CMA and the time-sensitive nature of prenatal diagnosis, current evidence suggests that proper triage of which cases might benefit most from WES using a stepwise approach is necessary rather than the widespread application of WES to all cases of foetal anomalies.

The limitations of WES include the inability to detect intronic variants, certain structural variants involving intronic regions and expansion repeat disorders. Additionally, despite being able to provide an additional diagnostic yield above CMA, WES is limited in its accurate detection of copy number variants. This is because of the nature of the sequencing method, which involves short-read sequences in which the accurate assessment of repetitive elements in the genome is technically difficult, as well as PCR-dependent issues such as GC content bias.[106] WGS, which refers to sequencing of both the coding and non-coding regions of the human genome, has the potential to overcome the limitations of WES. Sequencing read depth refers to the number of copies of each region represented in the pool of fragments, and because of the error rate of NGS, a depth of 30× is considered adequate for diagnostic WGS.[107] At this depth, WGS is able to detect variants at a single-base-pair resolution across the genome. However, this is at the expense of increased cost and turnaround time, limiting its practical use in routine prenatal diagnosis. Additionally, the detection of variants of unknown significance and secondary findings are increased with WES and WGS. This may in turn increase parental anxiety and have possible consequences on the purchasing of insurance for the individuals tested, highlighting the importance of adequate pretest counselling.

EMERGING SEQUENCING TECHNOLOGIES

Low-pass whole genome sequencing

Low-pass WGS was first described as a feasible NGS method for detecting copy number variants over conventional CMA in 2016 by Dong *et al*.[3] At a read depth of 0.25×, this method was able to accurately detect all copy number variants detected by conventional CMA in prenatal and postnatal samples as well as abortuses. The technique was also able to detect mosaicism at lower levels of 25% compared with CMA. Additionally, diagnostic rates in demised foetuses were higher with this technique compared to CMA. Subsequent studies demonstrated the ability of low-pass WGS to accurately detect pathogenic CNVs at a higher coverage and resolution of the genome while requiring less DNA input compared to CMA.[108,109] Balanced translocations and inversions as well as their breakpoints are also able to be identified with paired end sequencing in low-pass WGS — a known limitation of CMA and WES.[110] The optimised low-pass WGS protocol was able to achieve a turnaround time and cost that was either comparable to or 50% less than those for current CMA tests,[111] heralding the likely replacement of CMA altogether by low-pass WGS.

Long-read sequencing

Current NGS techniques rely on the massively parallel sequencing of short sequence reads of about 150 to 300 base pairs followed by its alignment to a reference genome to determine genetic variants down to a single-base-pair level.[112] Although it is able to detect a significant number of genomic variants, short-read sequencing has several limitations, such as the limited detection of structural variants (>50 bp),[20,113,114] the accurate detection of expansion repeat disorders such as fragile X syndrome and Huntington's disease[115] and differentiating pseudogenes from the actual gene of interest. Pseudogenes are sequences that have high sequence homology to known functional genes but do not produce functional proteins and can lead to impaired variant detection and false-positive results.[116,117] Additionally, short-read sequencing also relies on the need for PCR amplification, carrying with it the inherent challenges of sequencing regions of the genome with high GC content, with a significant proportion of the human genome that is GC-rich being inaccessible to such PCR-based sequencing methods.[118] Long-read sequencing is the latest advancement in the field of genetics and holds promise in overcoming the aforementioned limitations of current NGS techniques.[119,120] Long-read sequencing technologies involve the generation of sequences of up to >1 Mb in length followed by de novo assembly instead of alignment to a reference genome.[20,121] This has been demonstrated in several studies to have an improved ability to detect structural variants[122-124] and expansion repeat disorders[125,126] and discriminating pseudogenes from actual genes of interest.[127] Additionally, long-read sequencing has the ability to assign genetic variants to the maternally and paternally inherited chromosome, known as variant phasing. This is of particular importance when investigating compound heterozygosity in recessively inherited Mendelian disorders, to determine if variants are in cis or trans, which has implications for likelihood of pathogenicity and inheritance patterns. This advantage of long-read sequencing has been demonstrated in the field of pre-implantation genetic diagnosis in determining the parental origin of mutations to inform the risk of recurrence in subsequent pregnancies.[128,129] Long-read sequencing has yet to be widely applied in prenatal diagnosis. A recent study demonstrated the clinical utility of long-read sequencing in the determination of the pathogenicity of copy number variants of unknown significance in the *DMD* gene in the prenatal setting without the need for familial segregation, which often takes a significant amount of time. This testing approach led to the provision of timely genetic counselling and prompt pregnancy management, highlighting the important potential of long-read sequencing for future use in prenatal diagnosis where the time window for diagnosis is limited.[130]

CONCLUSION

As the complexity of options for prenatal genetic testing continues to expand, a commensurate increase in the quality

and quantity of genetic counselling services must also follow, given the paramount importance of adequate pretest and posttest counselling. Pretest counselling should cover the scope of the genetic tests performed as well as the possible results and their implications. Issues that should be covered include the cost, turnaround time, limitations of the test, variants of unknown significance, secondary findings, the potential implications for insurance coverage and the possibility of revealing non-paternity. Posttest counselling is of equal importance in explaining the results of the test and whether it provides a clinical diagnosis, as well as the options for the current pregnancy and future pregnancies. Therefore, referral to a medical geneticist for appropriate counselling is an essential component of the patient journey when considering prenatal genetic testing.

This article summarises the current practices as well as recent advances in the field of prenatal genetic testing, which will continue to evolve. The use of genetic testing should be carefully considered and individualised, with adequate and updated genetic counselling, to empower families to make informed choices regarding their pregnancy.

Financial support and sponsorship

This work was supported by National Medical Research Council, Clinician Scientist Award to Senior Investigator, Choolani M (NMRC/CSA/059/2014).

Conflicts of interest

Choolani M is a member of the SMJ Editorial Board, and was thus not involved in the peer review and publication decisions of this article.

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