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Recent advances of genomic testing in perinatal medicine

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Abstract

Rapid progress in genomic medicine in recent years has made it possible to diagnose subtle genetic abnormalities in a clinical setting on routine basis. This has allowed for detailed genotype-phenotype correlations and the identification of the genetic basis of many congenital anomalies. In addition to the availability of chromosomal microarray analysis, exome and whole genome sequencing on pre- and postnatal samples of cell free DNA has revolutionized the field of prenatal diagnosis. Incorporation of these technologies in perinatal pathology is bound to play a major role in coming years. In this communication, we briefly present the current experience with use of classical chromosome analysis, fluorescence *in situ* hybridization, and microarray testing, development of whole genome analysis by next generation sequencing technology, offer a detailed review of the history and current status of noninvasive prenatal testing using cell free DNA, and discuss the advents of these new genomic technologies in perinatal medicine.

G-banding chromosome analysis

For several decades, traditional G-banding chromosome analysis has been an integral part of clinical work up of many neonatal deaths, still births, pregnancy losses in the first and second trimester, as well as prenatal diagnosis using amniocentesis and chorionic villus sampling. Approximately 30% of miscarriages result from aneuploidy and at least 0.3% of newborns have numerical chromosome abnormalities, which can be detected by traditional karyotyping[1]. Classical chromosome analysis enables the detection of large genomic alterations (Fig 1A), including triploidy, aneusomy, balanced and unbalanced chromosomal rearrangements of at least 10–20 Mb in size, and mosaicism (Table 1). However, it requires

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sterile and viable tissue samples to establish cell culture. The need for cellular proliferation to obtain metaphase spreads, and the relatively low-resolution of G-banding are the limiting factors in assessment of fetal genomic abnormalities.

Fluorescence *in situ* hybridization (FISH) analysis

The limited resolution provided by the traditional karyotypes has led to widespread use of fluorescence *in situ* hybridization (FISH) analysis. Using this adjunct technique, clinically significant chromosomal aberrations can be detected in metaphase or non-dividing interphase cells (Figure 1B, C) with a resolution from 150 to 250 kb, depending on the probe size (Table 1). The use of specific DNA probes, which are complementary to the chromosomal region of interest, allows a rapid detection of a whole chromosome aneusomy, large and submicroscopic rearrangements, including microdeletions and duplications within known disease-associated regions of the genome. The major advantage of FISH is rapid quantitation and visualization of physical location of specific DNA sequences in individual cells. Most clinical cytogenetic laboratories use a set of commercially available FISH probes to identify genomic alterations in specific, targeted chromosomal regions, such as FISH panels for detection of trisomy 13, 18, 21 and monosomy X (Aneuvysion FISH, Fig 1B) or TUPLE (HIRA)/ARSA assay to test for deletions in the DiGeorge critical region on chromosome 22q11.2 (Fig 1C). Despite the fact that FISH improves our ability to study abnormal chromosome structure at a resolution that exceeds that of classical karyotype, FISH-based tests do not provide genome-wide analyses, but are limited to the targeted genomic regions of interest. Therefore, genome wide DNA copy number analysis using microarray technology is usually the first choice in many cases.

Array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) microarrays

The term “chromosomal abnormality” describes any rearrangement in chromosome number or structure and includes multiple categories such as: aneuploidy, aneusomy, deletions and duplications (involving terminal and interstitial segments), triplications, derivative chromosomes, and complex rearrangements (Fig 2, 3). About 1 baby in 150 in the United States and Europe is born with a chromosomal abnormality, and it has been estimated that they are detected in about 25% of all miscarriages and stillbirths, and 50–60% of first trimester miscarriages[1].

The introduction of high-resolution methods for whole genome analysis, such as aCGH microarrays, also known as chromosomal microarray analysis (CMA), has dramatically improved the sensitivity of detecting many genetic conditions that arise from DNA copy number alterations (genomic disorders)[2]. Genomic disorders are caused by chromosome rearrangements, which result in a gain or loss of dosage-sensitive gene(s), and are characterized as either recurrent or nonrecurrent aberrations. The recurrent rearrangements, which are commonly interstitial deletions and duplications, occur within chromosomal regions flanked by repetitive sequences, such as low copy repeats or segmental duplications. These architectural features mediate chromosomal rearrangements, resulting in hot spots for recurrent deletions and duplications[2]. Examples of common recurrent abnormalities include

Prader-Willi and DiGeorge syndrome. In contrast to recurrent rearrangements that have the same size and fixed breakpoints, nonrecurrent aberrations have varied sizes and breakpoints for each patient and are generated by different molecular mechanisms[2]. Chromosomal abnormalities are responsible for more than 300 human syndromes, (and the list continues to grow), and are more common than Mendelian single-gene disorders.

Microarray technology involves assessment of patient's DNA by thousands of DNA probes that have been previously selected from the human genome to generate a high-resolution molecular karyotype. To date, multiple microarray platforms are utilized for clinical diagnosis[3–13]. These whole genome microarrays contain from 60,000 to 400,000 oligonucleotide probes with particularly dense coverage within clinically relevant genes as well as probes spanning the rest of the genome. Whole genome CGH probes detect DNA copy number changes; whereas SNP microarrays detect single nucleotide polymorphisms, and CGH+SNP microarrays combine both types of oligonucleotide probes. High-resolution X chromosome specific microarrays that contain predominantly X chromosome derived probes are also available for clinical use.

Microarray analysis reliably detects DNA losses (deletions) and gains (duplications and triplications) as small as 10–100 kb in size, which gives at least 25-fold better resolution than classical chromosome analysis (Table 1). Whole genome microarray analysis has a substantially higher yield than G-banded karyotype, and, thus, has been recommended as a first tier diagnostic test in individuals with congenital anomalies, neurodevelopmental and intellectual disabilities, and autism spectrum disorders[4,5]. In the pediatric population the diagnostic yield is ~12–19%, and in neonates with multiple congenital anomalies it reaches up to 30% of cases, in comparison to 1–3% of cases positive by conventional karyotype[5,6]. The diagnostic value of chromosomal microarray for the analyses of stillborn pregnancies and fetal structural anomalies has been demonstrated in multiple centers[7–10]. These studies have shown that in prenatal diagnosis, microarray provides additional clinically relevant information in 1.7–2.5% of referred cases due to advanced maternal age or positive maternal serum screening, and up to 6.0% of cases with fetal structural anomaly on ultrasound[8–13]. Microarray analysis has multiple advantages over the other cytogenetic methods. It can be performed on direct (uncultured) specimens and, therefore, is more likely to provide a genetic diagnosis in pregnancy losses and stillbirths, where 10–30% of samples are not viable and will not grow in culture, or may fail due to other reasons such as maternal cell contamination. Microarray analysis detects copy number alterations through the entire genome at high resolution and can be completed within 3–5 days. In addition, the SNP and CGH+SNP combo microarrays can detect triploidy and copy number neutral chromosome abnormalities, such as long stretches of homozygosity that occur due to consanguinity, uniparental isodisomy (UPD)[3], or complete molar pregnancy (Fig 4).

Non-Invasive Prenatal Testing: the Existing Standard

The current standard of care for non-invasive prenatal screening for fetal genetic disease in low-risk expectant mothers involves serum-based first- and second-trimester screens, in which concentrations of specific protein markers associated with fetal malformations are determined in isolation or in combination with ultrasonography. Despite their widespread

use, these methods do not provide desirable levels of accuracy. For example, first-trimester screening via the measurement of pregnancy-associated plasma protein A and free [beta]-subunit of human chorionic gonadotropin combined with ultrasound measurement of nuchal translucency has a detection rate for trisomy 21 of 82–87% between 10 and 13 weeks of gestation, with a false-positive rate of 5% [14]. In addition to the fact that up to 18% of true positives are missed by these approaches, this relatively high false positive rate means that approximately 5% of expectant mothers will unnecessarily undergo either amniocentesis or chorionic villus samples (CVS). This is significant because these procedures have a risk of fetal loss that has been reported to be somewhere between 0.1 and 1.0%[15–18] and, not surprisingly, are a considerable source of parental stress and anxiety[19–21].

Non-Invasive Prenatal Testing: History and Background

In recent decades, there has been intense interest in the development of risk-free non-invasive alternatives to invasive methods of prenatal diagnosis. Initially, these efforts were focused on the isolation of nucleated fetal cells from maternal blood[22,23]. The goal of such approaches was to perform direct karyotyping on cells of fetal origin, but with recent progress in molecular analysis there is the possibility that such cells could be routinely subjected to whole genome amplification and copy number analysis. Unfortunately, however, fetal cell isolation did not translate well into clinical practice due to a lack of robust methods for the recovery of these rare cells. For example, a multi-centered trial using nucleated fetal cells reported a sensitivity of 41.4% for the detection of male fetal cells in maternal blood and a false-positive rate of 11.1%, results that are clearly unsatisfactory[24]. More recently, a number of reports have emerged in which novel cell isolation approaches have been used and these have re-ignited interest in this field[25–27]. Despite this resurgence of interest in fetal cell isolation, the current non-invasive testing relies on circulating cell-free fetal nucleic acids for the detection of fetal chromosome anomalies.

Circulating Cell-Free Fetal Nucleic Acids

It was shown in 1948 that circulating nucleic acids are present in plasma and serum[23] and further demonstrated in the 1970s that levels of circulating cell-free DNA were negatively associated with cancer survival[28]. In 1997, Dennis Lo and colleagues demonstrated that male fetal DNA is detectable by PCR in maternal plasma and serum[29]. The same group further described fundamentally important features of cell free fetal nucleic acid including the percentage of fetal genome equivalents relative to their maternal counterparts, the absolute number of these genome equivalents and the approximate rate at which they are cleared[31,31].

Developments in the non-invasive analysis of fetal nucleic acids have transformed prenatal clinical care. For example, methods for the detection of paternally inherited fetal alleles in maternal plasma are so robust that they are now widely used for the prediction of fetal Rhesus D blood group status[32–34] and have also been used for the diagnosis of paternally inherited thalassemia and achondroplasia[35–37]. More recently, proof of concept manuscripts have appeared that describe the use of targeted genomic sequencing and maternal haplotype data[38].

Cell-Free Fetal DNA Analysis via Shotgun Next-Generation Sequencing

The most significant obstacle that prevented progress beyond the non-invasive detection of paternally-inherited alleles towards the goal of aneuploidy detection is the fact that maternally inherited fetal alleles are identical in primary sequence to their endogenous maternal counterparts. Therefore, it is extremely difficult to distinguish these two populations. Fan et al [39] first demonstrated proof of concept for the use of whole-genome sequencing of maternal plasma DNA for non-invasive aneuploidy detection in 2008. This was followed rapidly by reports from Chiu *et al*.[40] and Chu *et al*.[41] These methods utilized shotgun sequencing of plasma cell-free DNA. The basic principle of this is that DNA fragment libraries are generated from maternal plasma and then sequenced randomly (shotgun) to generate very large numbers of sequence tags that are then aligned computationally to the human genome. Perfectly aligned matching tags are then quantified in a chromosome-specific or region-specific fashion using sophisticated statistical methods. As a result one can generate accurate measurements of copy number for different chromosomes and it is therefore possible to identify tiny alterations in inter-chromosomal ratios that might be contributed by the presence of even a small number of aneuploid fetal genome equivalents present in maternal plasma. This is a powerful approach to the problem of analyzing fetal chromosome copy number that does not require fetal DNA fragments to be physically distinguished from their maternal counterparts (Fig. 5). For example, assuming 10% frequency of fetal genome equivalents in a plasma samples, and 90% contributed by the mother, one could expect to identify (0.9 x 2) + (0.1 x 3) = 1.1 x n copies of chromosome 21 for a trisomy 21 fetus versus (0.9 x 2) + (0.1 x 2) = 1.0 x n copies of chromosome 21 for a euploid fetus (where n = the total number of genomes present). This subtle change in chromosome 21 copy number of only 5% requires sensitive counting methods.

These initial proof of concept studies were followed by large scale demonstration that the whole genome maternal plasma DNA sequencing approach can be used routinely for the non-invasive detection of aneuploidies involving chromosomes 21, 18 and 13 and other disorders involving sex chromosome copy number anomalies. This method has now been widely adopted for clinical use, with both Sequenom and Verinata driving the commercialization and development of this approach, and a number of large case-control studies [42–46] have been carried out using their methods. These data have been comprehensively discussed in the excellent review by Benn *et al* [47]. Generally, they achieve close to 100% sensitivity and specificity for trisomy 21 and 18 and slightly less than this for trisomy 13. In 2011, Palomaki *et al*[43], carried out a blinded, nested case-control study using a cohort of 4664 pregnancies at high risk for Down syndrome in which they compared fetal karyotyping standard methods with whole-genome shotgun sequencing of maternal plasma DNA. The study, which included 212 trisomy 21 and 1484 matched euploid pregnancies was able to detect trisomy 21 at 98.6% (209/212) with a false-positive rate of 0.20% (3/1471), and the testing failed in 13 pregnancies (0.8%); all were euploid[43].

As the technology has matured, these promising results have been improved even further, largely because of developing sophistication at the level of the computational analysis of sequencing data. For example, Bianchi *et al*[44] analyzed 532 prenatal plasma samples by massively parallel DNA sequencing. This study correctly identified all trisomy 21 cases

(n=89, sensitivity 100%, 95% [confidence interval] CI 95.9–100), 35 out of 36 trisomy 18 cases (sensitivity 97.2%, 95% CI 85.5–99.9) and 11 out of 14 trisomy 13 cases (sensitivity 78.6%, 95% CI 49.2–99.9). No false-positive results were obtained for aneuploidies involving autosomes (100% specificity, 95% CI more than 98.5 to 100) and the authors were also able to correctly classify 15 out of 16 monosomy chromosome X cases (sensitivity 93.8%, 95% CI 69.8–99.8). Similarly, in a 2012 study, Palomaki *et al*[45] found that of 1,971 samples analyzed (out of a total of 1,988 for which results were obtained), observed trisomy 18 and 13 detection rates were 100% (59/59) and 91.7% (11/12) respectively. False-positive rates were 0.28% for trisomy 18 and 0.97% and for trisomy 13.

Recently, a large multi-centered trial compared the use of whole genome plasma DNA sequencing against the existing standard of prenatal screening in low risk patients[48]. Inclusion criteria for this study required that mothers be at least 18 years of age, have a singleton pregnancy, be at a minimum of 8 weeks gestation when enrolled (and blood drawn) and have undergone a standard prenatal screening involving serum analysis combined with ultrasound. Of 2042 patients recruited, 72 had no clinical outcome, 42 were lost to follow up, 24 did not result in a live birth which prevented the determination of fetal karyotype, 17 did not have a result after whole genome plasma sequencing and 39 did not have a result after the standard screening protocol. Of the remaining 1914 samples, sensitivity for the detection of trisomy 21 was found to be 100% (95% CI, 47.8–100) and specificity was 99.7% (95% CI, 93.3–99.9). Sensitivity for trisomy 18 was 100% (95% CI, 15.8–100) and specificity was 99.8 (95% CI, 99.6–100). Positive predictive value was found to be 45.5% (95% CI, 16.7–76.6) and 40.0% (95% CI, 5.3–85.3) for trisomy 21 and 18 respectively. Negative predictive value was 100% (95% CI, 99.8–100) in each case. When compared with the performance of the standard screening method, these results suggest that whole genome maternal plasma DNA sequencing out-performs the existing standard of care, although the study was limited by the low number of true trisomy 21 and 18 positives analyzed by the sequencing method (6 and 3 respectively) and the fact that approximately 1/3 of the plasma DNA samples sequenced were prepared from maternal blood drawn in the third trimester. This is significant because of the fact that the fetal DNA fraction increases throughout gestation, and the likelihood that the ease with which aneuploidy can be detected non-invasively increases in parallel with increasing fetal fraction.

Targeted Sequencing Methods for Aneuploidy Detection

In parallel to the clinical development and commercial success of whole genome plasma DNA sequencing, two targeted approaches have been commercialized. One of these involves a ligation mediated amplification method in which target chromosomes are sequenced to determine copy number in a fashion that is analogous to the whole genome approach except with reduced content, which potentially results in higher throughput and lower cost[49]. It has also been shown that a highly multiplex PCR approach in which the simultaneous amplification of >11,000 single nucleotide polymorphisms from maternal plasma DNA samples can be used to detect aneuploidy[50]. Sequence data is analyzed in a sophisticated manner such that it is hypothesized that the fetus is monosomic, disomic or trisomic at the target loci. After also evaluating the possibility that a recombination event has occurred, the method is able to identify the presence of fetal aneuploidy, triploidy or uniparental disomy.

This broad range of sensitivity to a variety of chromosomal abnormalities is a distinct advantage (the whole genome and non-SNP targeted methods cannot identify triploidy or uniparental disomy) and the method has been shown to be accurate and robust[51]. Of course, the SNP-based approach will detect non-paternity or consanguinity.

Beyond Aneuploidy: Microdeletions and Microduplications

There is considerable interest in extending the range of detection of NIPT methods to include other structural anomalies including microdeletions and microduplications. This is not surprising given that the incidence of aneuploidy in human pregnancy is between 1–2%, whereas the collective incidence of microdeletions and microduplications is 3.6%b [9, 11]. The majority of microdeletions and microduplications are <5Mb, which is below the resolution (5–10Mb) of traditional metaphase chromosome analysis. Because of the clinical significance of these disorders there has been considerable effort directed towards the development and validation of methods for their diagnosis. Array comparative genomic hybridization (aCGH) is a powerful tool for the high-resolution evaluation of many microdeletions/microduplications in parallel. aCGH is now considered to be the diagnostic standard of care for pregnancies with an abnormal ultrasound and will soon become the standard of care for all prenatal diagnostic testing. However, despite its clinical utility, aCGH has the major drawback that it must be performed utilizing AF or CVS. It is therefore essential that NIPT technologies be developed that enable the detection of a broad spectrum of microdeletions and microduplications. This will increase the likelihood that NIPT can be offered as a viable and risk-free alternative to invasive diagnostic procedures, which will ultimately allow the risk-free potential of NIPT to be realized by achieving a significant reduction in fetal mortality and morbidity.

In 2012, Peters *et al* demonstrated for the first time a proof of concept for the detection of a fetal microdeletion syndrome in maternal plasma[52] and this has been followed by at least three other studies that support these initial observations[53–55]. Significant obstacles remain, however, and it is likely that such methods, in their current form, will suffer from high false positive rates that may hinder their translational potential. In light of these limitations, a significant improvement in sensitivity was recently demonstrated by using a region-targeting approach in which it was shown that a microdeletion of 100 kb can be readily detected with relatively low numbers (and therefore low cost) of single end sequencing reads[56].

Whole-Exome Sequencing (WES): the potentials in prenatal diagnosis and neonatal intensive care

Sanger sequencing is currently the gold standard for detection of point mutations and molecular diagnosis of well-known genetic conditions. However, when a patient presents with “atypical” manifestations or when there is locus heterogeneity and multiple genes are in question, Sanger sequencing may become unacceptably expensive. Next generation sequencing technologies (NGS) have revolutionized the current practice in genetic diagnosis[57]. The use of high-throughput platforms to perform the whole genome sequencing (WGS), whole exome sequencing (WES), or targeted exome capture (TEC) enable more accurate and cost-effective testing for multiple hereditary diseases in a single experiment

(Fig. 6). Sequencing of the whole exome, complete coding regions, is well justified as an efficient approach to reveal variants underlying rare monogenic (Mendelian) conditions. Although exonic regions encompass less than 2% of the genome, they contain almost 85% of disease-causing mutations. To date, nearly 6000 Mendelian disorders have been described, and genetic diagnosis of these conditions is of substantial interest to provide the best treatment, reproductive counseling and to reduce or prevent an associated risk of mortality and disability. Despite the rare nature of individual Mendelian disease, collectively they account for about 20% of infant mortality[58.59].

Presently, WES is mostly used in clinical diagnoses of individuals with undiagnosed diseases, children with multiple congenital anomalies, intellectual disabilities, seizures, metabolic disorders and mitochondrial diseases. To date, multiple reports of WES application in neonatal diagnosis and successful treatment of children with endocrine, immune, and neurodevelopmental disorders clearly demonstrate benefits of NGS technologies in clinical practice[60,61]. The genetic diagnosis of fetal structural abnormalities can be substantially improved through the application of NGS in prenatal care[62,63]. In a cohort of 30 fetuses and neonates with abnormal prenatal ultrasound and normal karyotype, WES has yielded pathogenic variants in 10% of the cases[63]. During pregnancy, non-invasive prenatal screening for most common aneuploidies is also achieved by NGS. Preconception carrier testing for severe recessive and X-linked disease mutations by NGS showed that the expense of screening analysis is much lower than the cost of management of the patients born with these disorders,[64,65]. In addition, comprehensive newborn screening for inborn errors of metabolism enables identification of asymptomatic neonates and immediate preventive intervention.

Targeted exome capture can provide an effective alternative to WES in detection of mitochondrial disorders and newborn screening for inborn errors of metabolism, replacing tandem mass spectroscopy for detection of genetic conditions. The potential advantages of WES in prenatal and neonatal populations include accurate genetic testing, enabling personalized diagnosis and therapy, risk assessment and preventive treatment, reproductive genetic counseling and family planning. The use of WES or TEC in routine neonatal practice is nascent, and NGS technology will become a standard of care for clinical diagnosis of newborns with congenital anomalies, neurodevelopmental or endocrine system dysfunction, and genetic screening.

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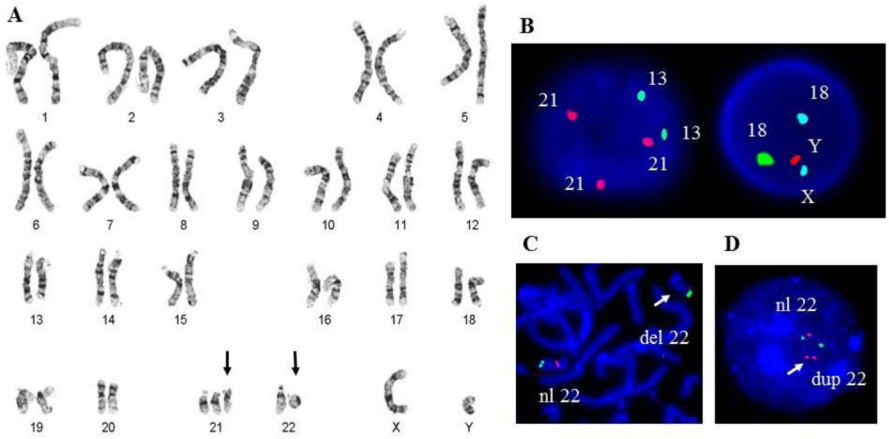

Fig 1. Prenatal and postnatal diagnosis of chromosome abnormalities

A. Classical chromosome analysis of product of conception. G-banded karyotype at the 500-band resolution showing a trisomy 21 (black arrow) and an abnormal chromosome 22 (black arrow) with a deletion in the long arm in a male fetus. **B**. Aneuvysion FISH analysis on the interphase cells showing two signals for chromosome 13 and 18, one signal for each chromosome X and Y, and three signals for chromosome 21- specific probe, indicating a trisomy 21 in a male fetus. **C**. FISH analysis on a metaphase spread showing a deletion (white arrow) in the DiGeorge/Velocardiofacial critical region on 22q11.2 in a newborn with congenital heart defect. **D**. Interphase FISH analysis demonstrated duplication (white arrow) in the 22q11.2 region in a stillborn.

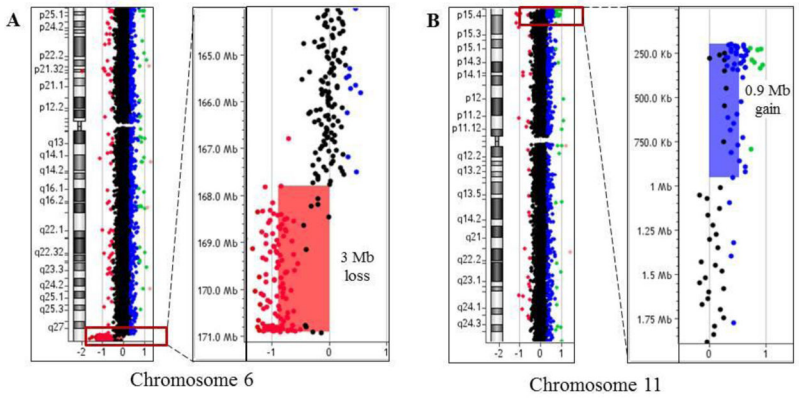

Fig 2. Detection of complex submicroscopic chromosome abnormalities using whole genome microarray in a neonate with multiple congenital anomalies and normal karyotype

A. On the left, idiogram and array-CGH plot of chromosome 6, showing a loss (red box) in the subtelomeric region of the long arm (6q27). On the right, a magnified view of chromosome 6 array-CGH plot demonstrates a loss (red shaded area) of ~3,000,000 base pairs (3 Mb). **B**. On the left, idiogram and array-CGH plot of chromosome 11, showing a gain (red box) in the terminal 11p region. On the right, a magnified view of chromosome 11p with a gain (blue shaded area) of ~900,000 base pairs (0.9 Mb). These results indicate the presence of a derivative chromosome 6 resulted from a translocation of 6q and 11p in the child.

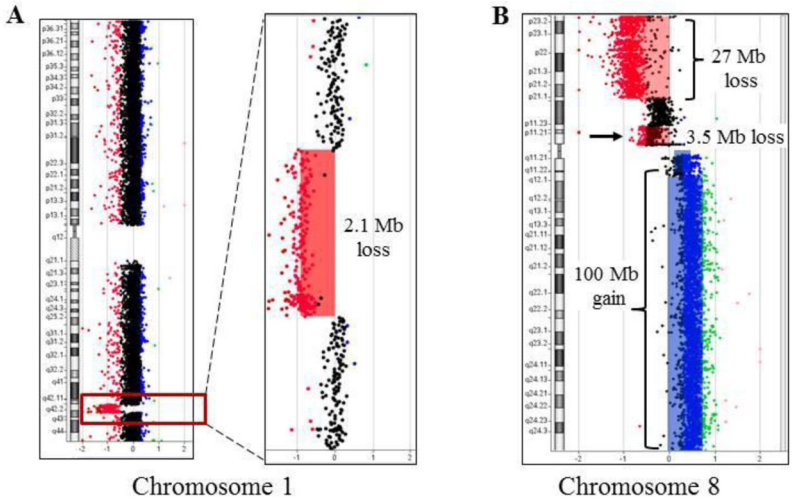

Fig 3.

A. Microarray analysis reveals submicroscopic chromosome alteration in a newborn with brain malformation. On the left, idiogram and array-CGH plot of chromosome 1, showing a loss (red box) in the 1q42-q43 chromosome region. On the right, a magnified view of a ~2.1 Mb deletion (red shaded area). **B.** Microarray analysis from a spontaneous abortion. Karyotype analysis was not available as the cells failed to grow in culture. Array CGH analysis showed complex alterations of chromosome 8, including a loss of ~27 Mb in short arm, a loss of ~3.5 Mb in the pericentromeric region of the 8p, and a gain of entire long arm of chromosome 8 (~100 Mb). These results are consistent with an isochromosome 8q in the fetus.

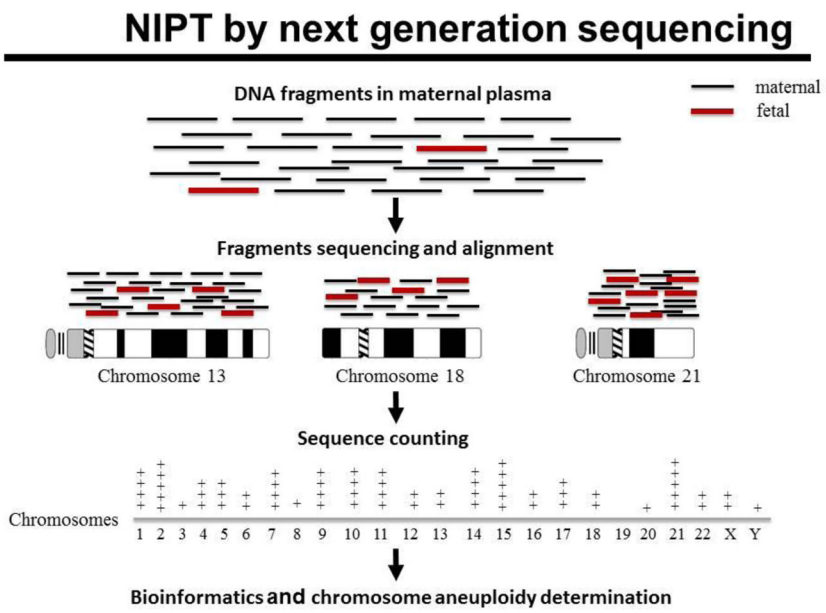

Fig 4. Regions of homozygosity detected by CGH+SNP combo array analysis can reveal copy number neutral chromosomal abnormalities

A. Contiguous regions of homozygosity (blue shaded areas) detected in a newborn with severe IUGR consistent with consanguineous parents and an increased risk for autosomal recessive disorder. **B**. CGH+SNP analysis in a newborn with hypotonia and undescended testes reveal absence of heterozygosity for the entire chromosome 15 (blue shaded area), consistent with uniparental disomy for chromosome 15 (UPD15).

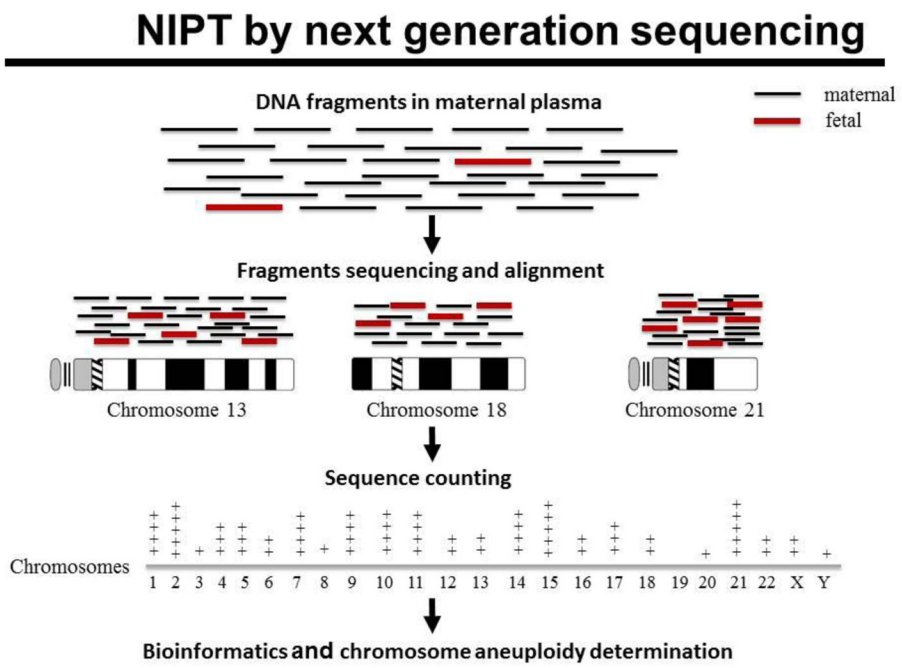

Fig 5. Overview of noninvasive aneuploidy detection using next-generation sequencing of maternal plasma

Maternal plasma contains small fraction of fetal DNA (red fragments), while maternal DNA (black fragments) represent 80–95% of circulating DNA. Both maternal and fetal fragments, obtained from maternal plasma sample, are sequenced and aligned to a specific chromosome positions according to the human reference genome. Using bioinformatics analysis, the total number of sequences mapped to each chromosome is counted and quantified to assess copy number for each chromosome. The number of DNA fragments from an aneuploid chromosome is expected to be higher (in cases with trisomy) and lower (in pregnancies with monosomy) in comparison to a normal diploid fetus.

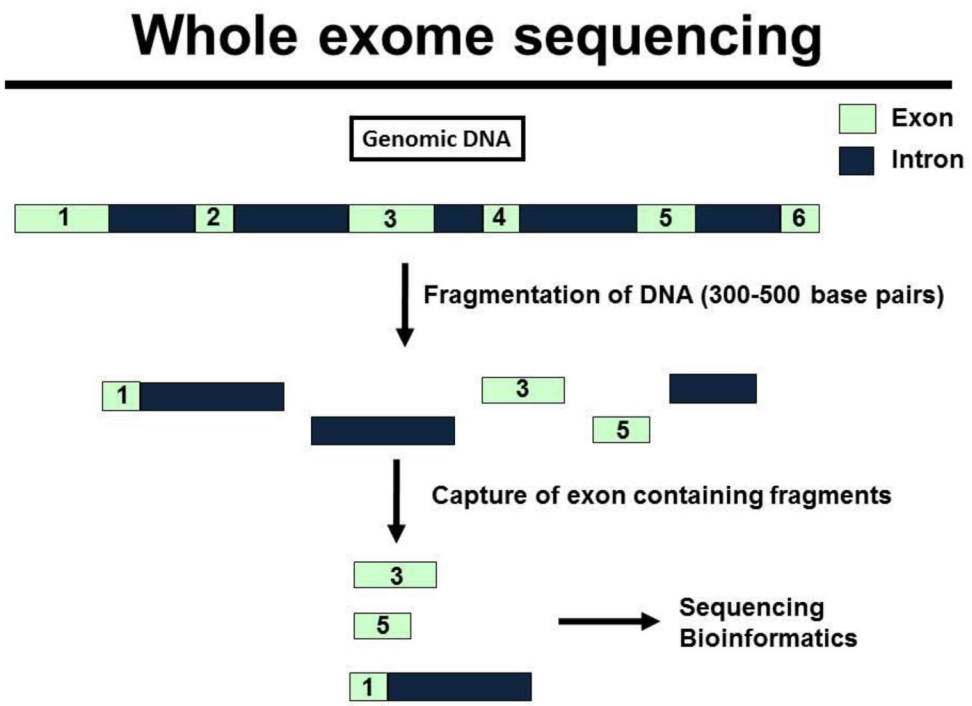

Fig 6. Schematic illustration of the whole exome sequencing

In whole exome sequencing, genomic DNA is fragmented, and specially designed baits are used to capture fragments of DNA that contain exons. Exons constitute only 1% of genomic DNA. The exon containing fragments are eluted, amplified by PCR and sequenced. Large amounts of data are generated and require specialized programs to align the sequences against the reference genome, to determine nucleotide variants that differ from the reference, and to identify potentially pathogenic mutations.

Table 1

Testing methodologies in perinatal diagnosis.

Method	Ploidy	Aneusomy	Balanced chromosome aberrations	Unbalanced chromosome rearrangements	Copy number alterations (micro-deletion,/duplication)	Point mutations	Resolution	Molar pregnancy	Uniparental disomy, homozygosity detection	Sample for testing	Viable cells for culturing	Examples
Karyotype	+	+	+	+	−	−	>5–10 Mb	−	−	AF, CVS, FB, CB, PB	+	Fig 1A
Aneuvysion FISH	+	+*	−	−	−	−	Whole chromosome*	−	−	AF, CVS, FB, CB, PB FFPE	−	Fig 1B (chromosomes 13, 18, 21, X, Y)
Locus-specific FISH	−	−	+	+^	+	−	150–200 kb	−	−	AF, CVS, FB, CB, PB	−/+	Fig 1C, D (DiGeorge syndrome)
Array CGH	−	+	−	+	+	−	10–100 kb	−	−	AF, CVS,FB, CB, PB FFPE	−/+	Fig 2
CGH+SNP, SNP arrays	+	+	−	+	+	−	CNV 10–100kb, ROH 5–10 Mb	+	+	AF, CVS, FB, CB, PB	−/+	Fig 2 Fig 3 Fig 4
NIPT	−	+*	−	−*&	−*&	−	Whole chromosome*, ~3–5 Mb*	−	−	MPS	−	(chromosomes 13, 18, 21, X, Y), deletion 5p, 22q11.2
Sanger sequencing	−	−	−	+	+	+	1 bp, single gene	−	−	Fetal DNA, CB, PB	−/+	Mendelian disease (cystic fibrosis)
WES, WGS	−	−	−	+	+	+	1 bp,multiple genes	−	−	Fetal DNA, CB, PB	−/+	Mendelian diseases

AF - amniotic fluid; bp - base pair; CB – cord blood; CGH - comparative genomic hybridization; CVS - chorionic villi sample; FB - fetal blood; FFPE - formalin fixed paraffin embedded tissue; FISH - fluorescence *in situ* hybridization; kb – kilobases (thousand base pairs); Mb – megabases (million base pairs); MPS – maternal plasma serum; NIPT – non- invasive prenatal testing; PB – peripheral blood; SNP arrays – array containing single nucleotide polymorphism probes; WES - whole exome sequencing, WGS – whole genome sequencing;

*limited to the selected chromosomes (13, 18, 21, X and Y) or selected regions;

&large rearrangements of chromosomes 13, 18, 21, or X might be revealed accidentally

^balanced rearrangements can be detected by use of locus-specific probe on cultured metaphase cells;

−/+might be necessary to obtain sufficient amount of fetal cells or DNA.