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Chapter
1

Impact of Advances in Genetics on Prenatal Diagnosis

Priya Ranganath, Shagun Aggarwal

INTRODUCTION

Genetic disorders and birth defects are significant contributors to infant morbidity and mortality worldwide. Approximately 3% of newborns are affected by congenital or inherited disorders, equating to about 1 in every 33 births.[1] These conditions can lead to long-term disabilities, imposing substantial emotional and financial burdens on families and healthcare systems.[1]

In the Indian context, genetic disorders and birth defects represent a substantial public health concern due to the country's large population and high birth rate. Annually, over 1.7 million children in India are born with birth defects.[2] The prevalence of birth defects in India is estimated to range from 61 to 69.9 per 1,000 live births.[3]

Over the past decade, advances in genetic technologies have transformed prenatal screening and diagnosis, significantly improving early detection, risk assessment, and decision-making for expectant parents. Conventional prenatal screening methods, such as first-trimester combined screening include nuchal translucency measurement and maternal serum markers, which can detect approximately 85–90% of cases of Down syndrome and other aneuploidies, with a false-positive rate of about 5%.[4]

Noninvasive prenatal testing (NIPT), based on the analysis of cell-free fetal DNA (cffDNA) in maternal blood, has emerged as a highly accurate screening tool, detecting 99% of trisomy 21 cases with a false-positive rate of 0.1%. Next-generation sequencing (NGS) has further revolutionized carrier screening, enabling the detection of pathogenic variants associated with recessive and X-linked conditions, allowing for more informed reproductive decisions. Whole exome sequencing (WES) has enhanced the ability to diagnose rare monogenic disorders, particularly in cases with abnormal ultrasound findings.

Additionally, molecular cytogenetic techniques such as chromosomal microarray (CMA) provide higher resolution than conventional karyotyping, detecting submicroscopic deletions and duplications that may be missed by standard cytogenetic methods. These advances enable a more precise genetic diagnosis, guiding obstetricians in counseling parents about the prognosis and management options for affected pregnancies.

HISTORY AND PROGRESS

Amniocentesis was first performed for the diagnosis of genetic diseases, specifically for fetal sex determination, by Fuchs and Riis in 1956. However, its application dates back even earlier, particularly in the management of erythroblastosis fetalis, a condition caused by Rh incompatibility between Rh-negative mother and Rh-positive fetus.

A key discovery in 1949 by Canadian anatomist Murray Llewellyn Barr and his colleagues paved the way for broader applications of the procedure. They identified Barr bodies, small cellular structures that serve as markers of the inactive X chromosome, allowing for sex determination in addition to analyzing sex chromosomes.

The use of amniocentesis expanded in subsequent decades, particularly for the detection of chromosomal abnormalities such as Down syndrome. The introduction of karyotyping revolutionized prenatal cytogenetics, enabling direct visualization of fetal chromosomes. By the 1970s, the advent of chorionic villus sampling (CVS) alongside amniocentesis allowed for earlier and more comprehensive prenatal diagnosis, ushering in a new era of fetal genetic screening and diagnostics.[6]

In the 1980s and 1990s, fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR) technologies provided a leap forward by enabling more targeted detection of specific chromosomal and genetic conditions.[7] The early 2000s witnessed the emergence of chromosomal microarray (CMA), which could detect submicroscopic copy number variations (CNVs) with far greater resolution than karyotyping, uncovering conditions previously undetectable by conventional methods.

The past two decades have seen the introduction of noninvasive prenatal testing (NIPT), whole exome sequencing (WES), and other next-generation sequencing (NGS) technologies, which have dramatically reshaped prenatal diagnostic practices. The timeline illustrating key advancements in prenatal genetic testing technologies is illustrated in Fig. 1.1.

Noninvasive prenatal testing (NIPT) introduced in 2011, revolutionized prenatal screening by analyzing cell-free fetal DNA (cffDNA) in maternal plasma. It offers high sensitivity, specificity and extremely low false-positive rates for detecting common aneuploidies, such as trisomy 21.[8] This advancement has contributed to significantly reducing number of amniocentesis needed for confirmatory testing. Chromosomal microarray (CMA) enhanced the detection of clinically significant copy

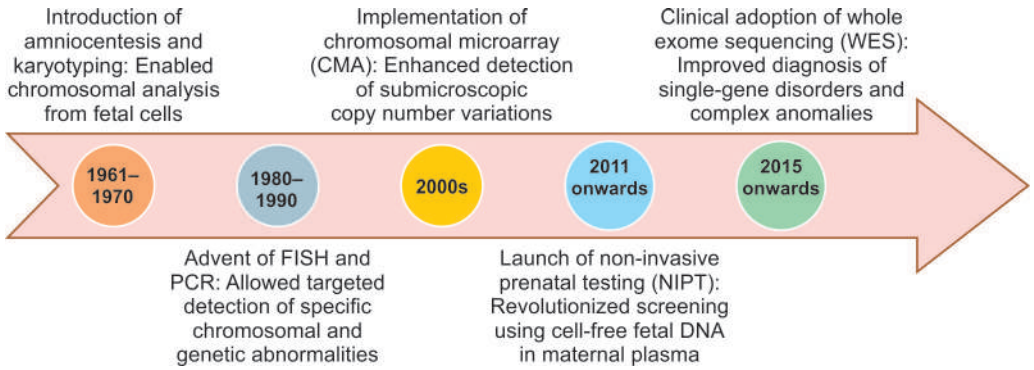


Fig. 1.1: Timeline illustrating key advancements in prenatal genetic testing technologies

number variations (CNVs), particularly when ultrasound detects fetal anomalies, but karyotyping results are normal. CMA is now a first-tier test in such cases. Whole exome sequencing (WES) adopted clinically from 2015, has expanded diagnostic capabilities by identifying single-gene disorders, especially in fetuses with unexplained structural anomalies. Additionally, preconception couple carrier screening is gaining importance, enabling the detection of carrier status for recessive genetic disorders, thereby facilitating informed reproductive decisions.[9]

This chapter aims to provide a concise overview of various genetic tests used in prenatal diagnosis including their principles, indications, strengths, and limitations. It will cover screening tests such as NIPT and biochemical screening, as well as diagnostic tests including karyotyping, fluorescence *in situ* hybridization (FISH), quantitative fluorescent PCR (QF-PCR), CMA, and next-generation sequencing-based approaches. By understanding the role of each test, clinicians can optimize risk assessment, diagnosis, and counseling for couples at risk of genetic disorders.

Genetic disorders can be broadly classified into chromosomal disorders and monogenic disorders, each requiring distinct diagnostic approaches. Chromosomal disorders arise from numerical or structural abnormalities in chromosomes, such as trisomies, monosomies, deletions, duplications, and translocations. These disorders can be detected using cytogenetic techniques like karyotyping and chromosomal microarray (CMA). In contrast, monogenic disorders result from mutations in a single gene and follow Mendelian inheritance patterns such as autosomal dominant, autosomal recessive, and X-linked inheritance. These disorders require molecular genetic techniques for diagnosis. An overview of these techniques has been depicted in Fig. 1.2. Sanger sequencing is commonly used for targeted analysis when a specific gene mutation is suspected, particularly in conditions with well-established genotype–phenotype correlations. However, for a broader evaluation, especially in cases with genetic heterogeneity, next-generation sequencing (NGS)-based approaches such as whole exome sequencing (WES) are preferred.

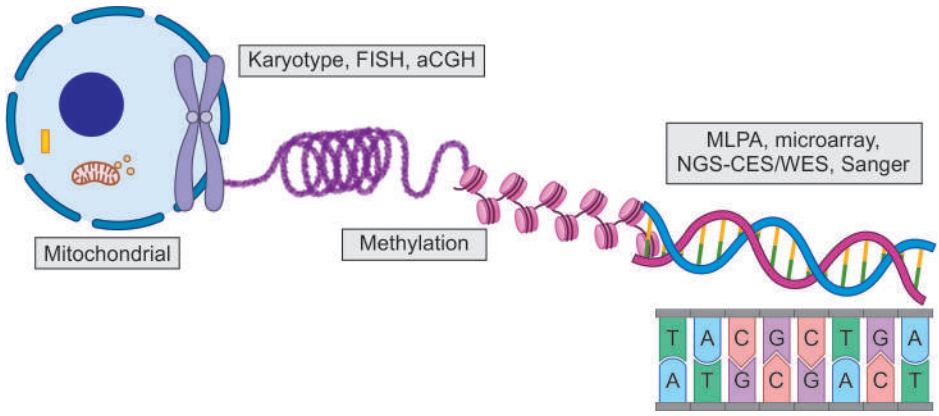


Fig. 1.2: Overview of genomic testing methodologies targeting various levels of the genome, from chromosomal and epigenetic changes to single-gene and nucleotide-level variations. (FISH: Fluorescence in situ hybridization; aCGH: Array comparative genomic hybridization; MLPA: Multiplex ligation-dependent probe amplification; NGS: Next-generation sequencing; CES: Clinical exome sequencing; WES: Whole exome sequencing)

TRADITIONAL CYTOGENETIC TESTING

Karyotyping

Karyotyping is a cytogenetic technique used to analyze the number and structure of chromosomes in fetal cells. It detects numerical and structural chromosomal abnormalities such as trisomies, monosomies, deletions, duplications, translocations, and inversions.[10]

Technique

The technique relies on staining metaphase chromosomes and arranging them in a standard format to identify abnormalities. The process begins with the collection of fetal cells, typically obtained through chorionic villus sampling (CVS) between 11 and 14 weeks of gestation or amniocentesis at 15 weeks or later. Once collected, the sample is cultured in a growth medium to encourage cell division. Cells are arrested in metaphase using colchicine, treated with a hypotonic solution to swell the nuclei, and then fixed. Staining techniques, such as Giemsa banding (G-banding), are used to visualize characteristic chromosomal patterns, allowing for the identification of chromosomal imbalances (Fig. 1.3). The chromosomes are then arranged in a karyogram and analyzed under a microscope to detect abnormalities.

Karyotyping remains the gold standard for detecting numerical chromosomal abnormalities including common aneuploidies such as trisomies 13, 18, and 21. It is also valuable in identifying structural chromosomal rearrangements such as translocations and inversions, which may impact reproductive outcomes. Another advantage of karyotyping is its ability to differentiate mosaicism, provided that at least 10–20% of cells

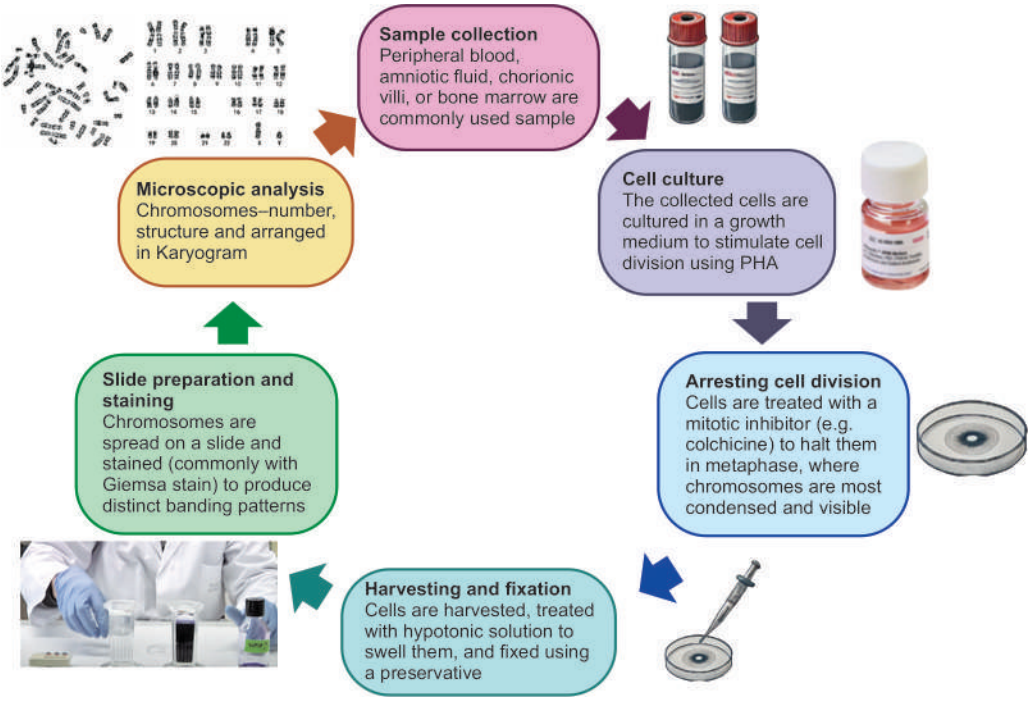


Fig. 1.3: Workflow of karyotyping—from sample collection to microscopic chromosomal analysis

Table 1.1: Strengths and limitations of karyotyping

Strengths	*Limitations*
Gold standard for detecting numerical chromosomal abnormalities (trisomies, monosomies)	Requires longer turnaround time (10–14 days due to cell culture)
Can identify balanced and unbalanced structural rearrangements	Cannot detect submicroscopic deletions/duplications (<5 Mb)
Cost-effective compared to molecular techniques like chromosomal microarray (CMA)	Trained manpower is required
Can differentiate mosaicism (if present in ≥10–20% of cells)	Requires viable dividing cells, making sample quality crucial

in the sample exhibit an abnormal karyotype.[11] Furthermore, karyotyping is relatively cost-effective compared to molecular techniques such as chromosomal microarray (CMA) or next-generation sequencing.

Indications for Prenatal Karyotyping

- Advanced maternal age (≥35 years) due to increased risk of aneuploidies.
- Abnormal first/second-trimester screening (biochemical or NIPT).
- Ultrasound-detected fetal anomalies and/or high-risk/multiple soft markers (e.g. congenital heart defects, increased nuchal translucency).
  Parental chromosomal rearrangements (e.g. balanced translocations, inversions).

Strengths and limitations of karyotyping are described in Table 1.1.

Molecular Cytogenetic Testing

Rapid Aneuploidy Test

Various tests like FISH, MLPA or QF-PCR can be used to look for common aneuploidies, i.e. those of chromosome 13, 18, 21, X and Y in a rapid manner when time is a constraint, e.g. prenatal testing close to 20 weeks gestation or in couples very anxious to know results of prenatal tests. These tests may also be used in immediate neonatal period to guide treatment decisions.

1. **Quantitative fluorescent PCR (QF-PCR):** Amplifies specific short tandem repeats (STRs) to detect common aneuploidies (e.g. trisomies 13, 18, 21, and sex chromosome abnormalities). This method utilizes fluorescently labeled primers to

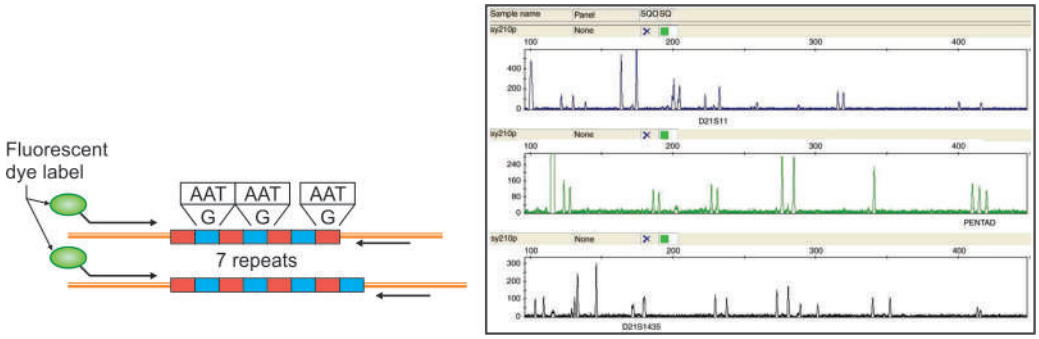


Fig. 1.4: Schematic representation of STR (short tandem repeat) analysis using fluorescently labeled primers and capillary electrophoresis for fragment size detection

Table 1.2: Advantages and drawbacks of QF-PCR	
Advantages	*Drawbacks*
• Small sample requirement • Rapid turnaround time and no culture requirement • Simultaneous assessment of maternal cell contamination • Automated workflow • Multiplexing of larger number of samples	• Commonly available panel detects aneuploidies associated only with chromosome 13, 18, 21, X and Y • Fails to detect other aneuploidies, structural abnormalities and mosaicism

amplify polymorphic STR markers specific to targeted chromosomes, allowing for quantification of chromosomal material based on the relative fluorescence intensity of amplified fragments (Fig. 1.4 and Table 1.2).

2. **Fluorescence *in situ* hybridization (FISH):** Fluorescence *in situ* hybridization (FISH) is a molecular cytogenetic technique that uses fluorescently labeled DNA probes to hybridize to specific chromosomal regions, allowing for the detection of numerical and structural chromosomal abnormalities. It is commonly used for rapid aneuploidy detection (trisomies 21, 18, 13, and sex chromosome aneuploidies) and for identifying microdeletion syndromes such as DiGeorge syndrome (22q11.2 deletion) and Prader-Willi/Angelman syndrome (15q11-q13 deletion/duplication). FISH can be performed on uncultured amniocytes or chorionic villi, providing results within 24–48 hours, making it valuable in time-sensitive prenatal cases. It is particularly useful to detect mosaicism (Fig. 1.5 and Table 1.3).

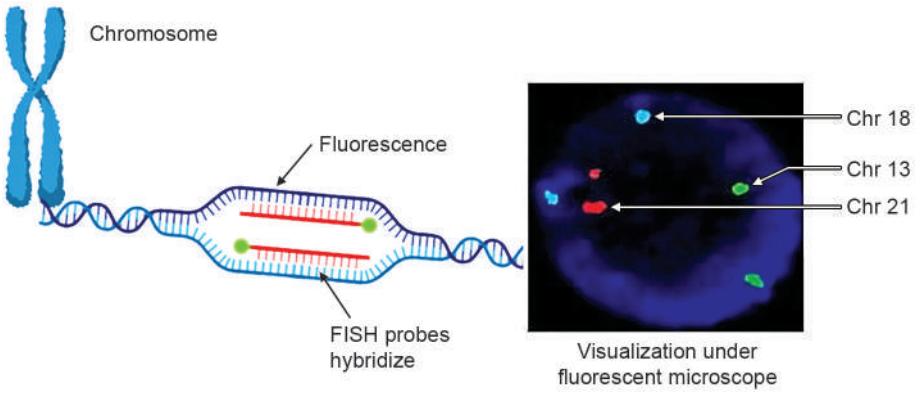


Fig. 1.5: Principle of fluorescence *in situ* hybridization (FISH)

Table 1.3: Advantages and drawbacks of FISH	
Advantages	*Drawbacks*
• Rapid detection of aneuploidies • Identification of specific known microdeletions (e.g. DiGeorge syndrome) • Useful to detect mosaicism	• Trained manpower • Can detect only common aneuploidies or specific microdeletions • Poor resolution for duplication • Susceptible for artifacts

Advanced Cytogenetic Testing

Chromosomal Microarray (CMA)

Chromosomal microarray (CMA) is a high-resolution molecular cytogenetic test that detects numerical chromosomal abnormalities and copy number variations (CNVs), including microdeletions and microduplications, which karyotyping may miss. Large studies and meta-analysis have shown 6–10% additional diagnostic yield of CMA for microdeletions/microduplications in case of structural fetal abnormalities positioning this as the first-tier genetic investigation of choice for this scenario.

CMA is also valuable in analyzing products of conception or fetuses/neonates undergoing post-mortem examination after unexplained pregnancy loss or stillbirth. It is also the first-line test for multiple malformation syndromes.[2] While primarily indicated for fetal anomalies, CMA may be offered as an alternative to karyotyping in cases of prenatal testing with normal ultrasound findings, providing a more comprehensive assessment of submicroscopic chromosomal imbalances and has been shown to have additional yield of 0.5–1% (Table 1.4).

Methodology

CMA uses either array comparative genomic hybridization (aCGH) or single nucleotide polymorphism (SNP) arrays, enabling precise detection of CNVs. SNP arrays additionally detect regions of homozygosity, uniparental disomy (UPD), and low-level mosaicism.[11]

Indications for prenatal CMA

- Structural anomalies detected on ultrasound
- Increased nuchal translucency or other soft markers
- Advanced maternal age or abnormal maternal serum screening
- Parents are balanced translocation carriers
- As an adjunct test when invasive prenatal testing is being offered for other indications
- Unexplained intrauterine fetal demise or stillbirth.

Table 1.4: Strengths and limitations of chromosomal microarray in prenatal diagnosis

Strengths	*Limitations*
• Higher resolution than karyotyping, capable of detecting submicroscopic deletions and duplications (>50–100 kb)	• Cannot detect balanced chromosomal rearrangements (e.g. translocations, inversions)
• Recommended as a first-line test for fetal anomalies on ultrasound, even when the karyotype is normal	• Cannot identify low-level mosaicism (<20%)
• Faster than karyotyping, as it does not require cell culture	• May detect variants of uncertain significance (VUS), which complicates counseling
• Detects uniparental disomy (UPD) and regions of absence of heterozygosity (AOH), which may be associated with recessive conditions or imprinting disorders	

- POC microarray—analysis after pregnancy loss to detect chromosomal abnormalities, including CNVs.

Traditional Molecular Genetic Testing

Sanger Sequencing

Sanger sequencing remains the gold standard for targeted genetic testing due to its high accuracy and reliability in detecting point mutations and small insertions or deletions (indels). In prenatal settings, it is primarily used to confirm specific genetic variations or diagnose suspected single-gene disorders, particularly when a known familial mutation has been identified.

Sanger sequencing is based on the dideoxy chain termination method, a widely used technique for precise DNA sequencing. The process begins with DNA extraction, where fetal DNA is isolated from amniotic fluid (amniocentesis) or chorionic villi (CVS). The target gene region is amplified using polymerase chain reaction (PCR) with specific primers designed to flank the region of interest. The amplified DNA is then subjected to sequencing reactions that include a mixture of deoxynucleotide triphosphates (dNTPs) and fluorescently labeled dideoxynucleotide triphosphates (ddNTPs). During DNA synthesis, the incorporation of ddNTPs leads to chain termination at specific nucleotides, producing a set of DNA fragments of varying lengths. These fragments are separated using capillary electrophoresis, where a laser detects the fluorescent labels, allowing for precise determination of the DNA sequence. The final sequence is analyzed and compared to reference sequences to identify mutations or variants of interest (Table 1.5).

Indications

- Known familial mutations established by proband testing or in couple carrier screening
- Confirmation of suspected point mutations or small indels in fetuses with specific ultrasound findings associated with hotspot mutation—achondroplasia, Apert syndrome.

Other Molecular Genetic Tests

Multiplex ligation-dependent probe amplification (MLPA): It is a molecular technique used in prenatal testing primarily for rapid aneuploidy detection and for assessing specific single-gene disorders like Duchenne muscular dystrophy (DMD) and spinal

Table 1.5: Advantages and drawbacks of Sanger sequencing

Advantages	*Drawbacks*
• Highly accurate for detecting point mutations and small indels	• Limited to single-gene or small targeted regions, not suitable for large-scale genetic screening
• Cost-effective and efficient for known mutations or hotspot regions	• Cannot detect large deletions, duplications, or structural rearrangements
• Low error rate, making it ideal for confirmatory testing	• Lower sensitivity for detecting low-level mosaicism
• Provides direct variant validation, unlike next-generation sequencing (NGS) which requires orthogonal confirmation	• Not suitable for high-throughput screening due to longer turnaround time

muscular atrophy (SMA). It is a versatile test and the probes can be also designed to be used for various known microdeletions and microduplications. Its advantages include high sensitivity, cost-effectiveness, and the ability to analyze multiple targets simultaneously. However, MLPA cannot detect balanced chromosomal rearrangements, single-nucleotide variants (SNVs), or low-level mosaicism.

Triplet repeat primed PCR (TP-PCR): It is a specialized molecular technique used to detect trinucleotide repeat expansions, which are the underlying cause of several repeat expansion disorders. TP-PCR enables rapid detection of expanded alleles, even in cases where conventional PCR fails due to excessively long repeat tracts.

Advanced Molecular Genetic Testing

Next-Generation Sequencing (NGS) in Prenatal Diagnosis

Next-generation sequencing (NGS) has revolutionized the field of prenatal genetics, allowing for high-throughput, high-resolution analysis of genetic variants. Unlike traditional cytogenetic methods such as karyotyping or chromosomal microarray (CMA), NGS enables the detection of single-nucleotide variants (SNVs), small insertions and deletions (indels), and copy number variations (CNVs) at an unprecedented scale. NGS-based technologies, including clinical exome sequencing (CES), whole exome sequencing (WES), and whole genome sequencing (WGS), offer different levels of genetic analysis, making them valuable tools for the prenatal diagnosis of monogenic disorders (Fig. 1.6).

The NGS process involves several key steps. First, fetal DNA is extracted from samples obtained through chorionic villus sampling (CVS) or amniocentesis. The extracted DNA is then fragmented into smaller pieces, and sequencing adapters are ligated to each fragment in preparation for sequencing. During sequencing, DNA fragments are amplified and sequenced using massive parallel sequencing platforms. The raw sequencing data undergo bioinformatics analysis, including quality control, alignment to a reference genome, variant calling, and annotation. Finally, identified variants are filtered and analyzed based on their population frequency, pathogenicity

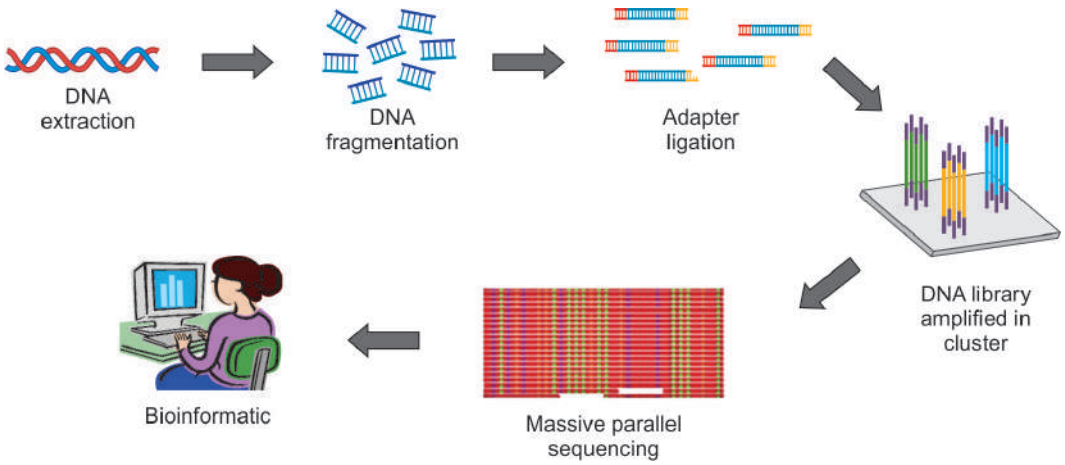


Fig. 1.6: Workflow of next-generation sequencing (NGS). (DNA: Deoxyribonucleic acid)

predictions, and clinical relevance, which help determine their potential disease association.

- **Clinical exome sequencing (CES):** It is a targeted NGS approach that sequences a curated panel of clinically relevant genes associated with genetic disorders. CES is useful in cases where there is a strong clinical suspicion of a genetic syndrome, allowing for a more focused and cost-effective approach compared to WES or WGS.
- **Whole exome sequencing (WES):** It involves sequencing all protein-coding regions (exons) of the genome, which comprise approximately 1–2% of the entire genome but harbor nearly 85% of disease-causing mutations. WES is particularly valuable in cases of ultrasound-detected anomalies where chromosomal microarray and karyotyping are normal, allowing for the identification of monogenic disorders that may explain the fetal phenotype.
- **Whole genome sequencing (WGS):** WGS sequences the entire genome, including coding and non-coding regions. This approach provides the most comprehensive genetic analysis and is useful in detecting complex genetic variations such as structural variants, repeat expansions, and deep intronic mutations. However, WGS remains costly and generates large amounts of data, making clinical interpretation challenging.

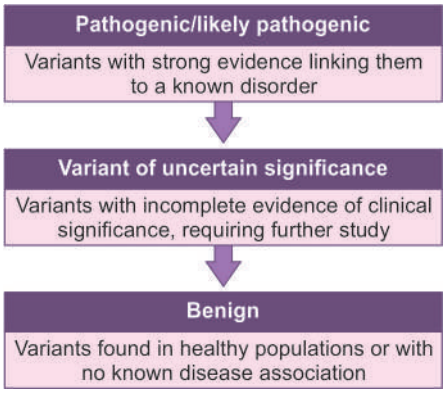
Common indications for WES in prenatal testing

- *CNS anomalies:* Ventriculomegaly, holoprosencephaly, agenesis of the corpus callosum.
- *Congenital heart defects:* Hypoplastic left heart syndrome, complex cardiac defects, situs abnormalities
- *Renal anomalies:* Cystic kidneys, renal agenesis, or dysplasia.
- *Non-immune fetal hydrops:* Unexplained fluid accumulation.
- *Skeletal dysplasia:* Short long bones, fractures, or bowing.
- *Multiple congenital anomalies:* Unexplained major structural anomalies affecting multiple systems.
- *Arthrogryposis multiplex:* Multiple joint contractures

Despite its advantages, NGS has several limitations. WES and WGS may miss structural rearrangements, repeat expansions, methylation abnormalities, and low-level mosaicism. Another significant challenge in prenatal genetic is the identification of variants of uncertain significance (VUS), which complicates interpretation and makes it difficult to provide definitive diagnoses. Additionally, incidental and secondary findings unrelated to the fetal phenotype may be identified, raising ethical and counseling challenges. Although costs are gradually decreasing, NGS remains expensive, and the analysis requires expertise, leading to delays in time-sensitive prenatal settings.[12]

Interpreting NGS results in a prenatal setting is complex due to the difficulty in correlating genetic variants with fetal phenotypes. Many genetic disorders have variable expressivity, making it hard to predict the severity of the disease in the fetus. Furthermore, prenatal genotype–phenotype correlations are still being developed, leading to gaps in knowledge when interpreting results. The presence of variants of uncertain significance (VUS) poses a dilemma in genetic counseling, as their impact on fetal development remains unclear.

Flowchart 1.1: Classification of a genetic variant as per ACMG criteria



To enhance accuracy in variant interpretation, standardized databases such as ClinVar, gnomAD, and HGMD should be utilized. In cases with limited data, referring to scientific literature and functional studies can provide insights into variant pathogenicity. Segregation analysis involving parental testing may help determine whether a variant is *de novo* or inherited, aiding in its classification. A multidisciplinary review, involving geneticists, fetal medicine specialists, and bioinformaticians, improves diagnostic precision and ensures well-informed decision-making.

A 2022 meta-analysis of 66 studies (4350 fetuses) found that the overall diagnostic yield of prenatal exome sequencing (ES) after a normal karyotype/CMA was about 31%, but this varied widely by the type of ultrasound-detected anomaly. Table 1.6 summarizes the pooled diagnostic yield (percentage of cases with a genetic diagnosis from ES) for different categories of fetal structural anomalies. The highest ES yields were observed in skeletal disorders, and the lowest in isolated nuchal translucency or

Table 1.6: Pooled diagnostic yield for different categories of fetal structural anomalies

Fetal structural anomaly	*Approximate diagnostic yield (%)*
Skeletal anomalies (e.g. limb shortening, skeletal dysplasias)	42–63%
Neuromuscular anomalies (e.g. fetal akinesia deformation sequence—FADS)	25–50%
Multisystem anomalies (multiple systems affected)	20–40%
Hydrops fetalis (non-immune)	10–35%
Central nervous system (CNS) anomalies	10–25%
Congenital heart defects (CHD)	5–20%
Craniofacial anomalies	5–15%
Renal/Urinary tract anomalies (CAKUT)	5–15%
Fetal growth restriction (FGR) (isolated cases)	1–8%
Increased nuchal translucency (NT) (isolated cases)	0–5%
Gastrointestinal anomalies	0–5%
Chest/Thoracic anomalies (lung/thorax)	0–2%
Abdominal wall defects	0–2%

isolated gastrointestinal anomalies, with no diagnoses found in small cohorts of chest or abdominal wall anomaly cases.[13]

The involvement of a clinical geneticist is critical in NGS-based prenatal testing. Their role includes determining the choice of right test and in genotype–phenotype correlation. Pre-test counseling is crucial to explaining test limitations, potential findings, and ethical considerations to parents. Geneticists play a key role in variant interpretation, assessing pathogenicity and providing clear diagnostic recommendations. After testing, post-test genetic counseling helps families understand results and navigate reproductive decision-making effectively.

Noninvasive prenatal screening (NIPS): Also known as noninvasive prenatal testing (NIPT), is a high performing screening test for detecting fetal aneuploidies, including trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome), and abnormalities of sex chromosomes (X and Y). The test can be performed from 10 weeks of gestation onwards and offers high sensitivity and specificity. Despite its accuracy, it is important to note that approximately 50% of conceptuses with aneuploidy spontaneously abort in the first trimester. While it is technically feasible to conduct screening earlier, the clinical utility of very early testing is limited due to this high rate of natural pregnancy loss. Hence, performing NIPT after first trimester NT scan is practically a more judicious use of this test.

Noninvasive prenatal testing (NIPT): Analyzes cell-free fetal DNA (cffDNA) in maternal blood from 10 weeks of gestation. The sample is processed to extract cffDNA, and sequencing techniques like massively parallel sequencing (MPS) or SNP-based analysis detect chromosomal abnormalities, including trisomies 21, 18, and 13. Results are classified as low-risk, high-risk (requiring confirmatory invasive testing) or no-call (requiring repeat testing) (Fig. 1.7).

A screen-negative result on NIPT significantly reduces the likelihood of aneuploidy for the screened chromosomes by more than 99%, offering considerable reassurance to expectant parents. However, a screen-positive result requires confirmation through invasive testing, such as chorionic villus sampling (CVS) or amniocentesis, before any clinical decisions can be made. Although NIPT outperforms conventional screening methods in detecting common aneuploidies, it has several limitations. The cost of the

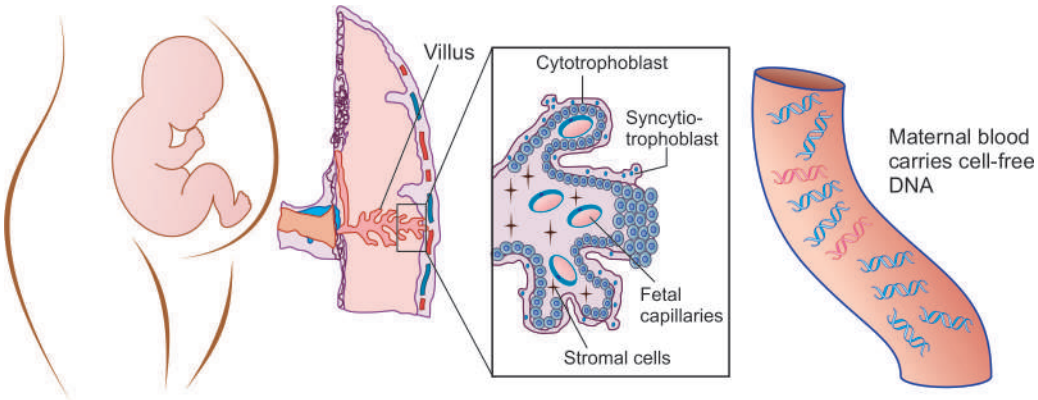


Fig. 1.7: Origin of cell-free fetal DNA (cffDNA) in maternal blood—released from the placental trophoblasts into maternal circulation, enabling non-invasive prenatal testing (NIPT)

test remains a significant barrier, preventing its widespread adoption as a universal screening tool. In addition, approximately 1% of cases result in test failures or uninformative results due to insufficient fetal DNA or technical limitations, necessitating repeat sampling.[14] Furthermore, NIPT does not screen for neural tube defects (NTDs) or pregnancy complications such as preeclampsia or fetal growth restriction. Its performance is also affected in specific scenarios, including pregnancies involving donor ovum, vanishing twin syndrome, maternal obesity, and multiple gestations, where the test may be less reliable.

Despite its high accuracy, NIPT is not the preferred test in certain high-risk situations where direct invasive testing is warranted. If ultrasound findings indicate fetal anomalies or if serum screening results suggest a highrisk of aneuploidy, invasive diagnostic testing should be considered as the primary approach. Similarly, in cases where one parent carries a known balanced chromosomal translocation, invasive testing is necessary, in view of the high possibility of unbalanced karyotype in fetus, which can be missed on NIPS. Certain high risk soft markers like nuchal fold thickness, ventriculomegaly where the likelihood of chromosomal abnormality is high, also warrant invasive diagnostic testing instead of NIPT, cost is another limiting factor; due to financial constraints, NIPT is not a viable option for all patients and is not yet recommended as a routine screening tool in many settings.[15]

Given the low false-positive rate of NIPT, it can be a valuable screening tool for families who wish to avoid invasive testing, particularly in cases where the pregnancy is highly valued, such as after a long period of infertility, recurrent miscarriages, or previous pregnancy loss. High-risk women who prefer to avoid invasive procedures may opt for NIPT despite their elevated risk. Additionally, couples with intermediate risk, such as those with advanced maternal age, a previous child with Down syndrome, or an isolated soft marker on ultrasound, may consider NIPT as an alternative to invasive testing. Even low-risk couples who do not have financial constraints or are excessively anxious about the risk of aneuploidy may choose NIPT for reassurance. However, pre-test and post-test counseling is essential to ensure that patients understand the limitations of the test, including the possibility of false-negative results and test failures requiring repeat sampling.

A woman who receives a positive screen result on NIPT should be promptly referred to a Clinical Geneticist or a Fetal Medicine Specialist for further evaluation and counseling. It is crucial to emphasize that no pregnancy should be terminated solely based on a high-risk NIPT result without confirmatory diagnostic testing through invasive methods. NIPT serves as a valuable screening tool, but its results must be interpreted cautiously, with appropriate follow-up testing when necessary.

ADVANCED GENETIC TESTING FOR CARRIER SCREENING

Carrier screening is a genetic test used to identify individuals who carry pathogenic variants in autosomal recessive or X-linked genes that could be passed on to offspring. The American College of Medical Genetics and Genomics (ACMG) recommends an expanded carrier screening (ECS) approach, which evaluates multiple genes regardless of ethnicity, replacing the traditional ethnicity-based model. Similarly, the Society of Indian Academy of Medical Genetics (SIAMG) has also released guidelines for carrier screening, emphasizing the need for universal carrier screening for β-thalassemia and

spinal muscular atrophy; and expanded carrier screening using NGS for couples with consanguinity, a family history of genetic disorders, or other high-risk setting.[16]

The core components of carrier screening include testing for thalassemia and spinal muscular atrophy (SMA), both of which are universally recommended. Additionally, screening encompasses other autosomal recessive and X-linked conditions associated with neonatal and childhood morbidity and mortality. Ideally, carrier screening should be based on the carrier frequency of a disorder within a given population. However, expanded carrier screening using next-generation sequencing can be adopted in high-risk scenarios like couple with previous child or close family member with confirmed/suspected genetic disease, bad obstetric history like late pregnancy losses, previous anomalous fetus, unexplained neonatal deaths, etc. Despite its advantages, carrier screening has limitations. Pathogenic variants in rare or technically challenging genes may not be detected, and variants of uncertain significance (VUS) can complicate interpretation. Furthermore, *de novo* mutations and polygenic conditions remain undetectable, leaving some genetic risks unaddressed. Also, carrier frequencies for autosomal recessive and X-linked recessive diseases for an ethnically diverse population like India are not available. Due to these limitations, at present NGS-based ECS is not recommended in low-risk couples.

APPROACH TO GENETIC TESTING IN PRENATAL MEDICINE

Pre-conceptional interventions play a crucial role in optimizing maternal and fetal outcomes. A thorough medical history should be taken to identify potential teratogenic exposures, including diabetes, prescription drugs (such as vitamin K antagonists, anti-epileptics, retinoids, and methotrexate), and substance use. If a teratogen is identified, safer alternatives should be prescribed, or doses minimized, while chronic conditions like diabetes must be well-controlled before conception. Additionally, a detailed three-generation family history (pedigree) should be obtained to assess for hereditary disorders such as hemolytic anemia, neuromuscular conditions, congenital malformations, and intellectual disabilities. If a genetic disorder is suspected, workup of index case is necessary for recurrence risk assessment and prenatal/pre-implantation diagnosis, and referral to a clinical geneticist is recommended.

Carrier screening should be offered to all couples, particularly for common genetic disorders like β-thalassemia (1 in 2 carriers) and spinal muscular atrophy (1 in 50 carriers). Thalassemia screening can be performed using HPLC and red cell indices, while SMA screening requires MLPA. Consanguineous couples, who have a higher risk of recessive disorders due to shared genetic background or positive family history, may be offered expanded carrier screening (ECS) via next-generation sequencing (NGS). Since ECS involves complex genetic analysis, it should be conducted under the guidance of a geneticist, with pre- and post-test counseling explaining its benefits and limitations. Additionally, rubella immunity should be confirmed using serum IgG levels, and non-immune women should receive rubella vaccination at least one month before conception. To prevent neural tube defects (NTDs), all women should take 400 µg of folic acid daily, ideally starting three months before conception and continuing until 12 weeks of pregnancy. Women with previous NTD-affected pregnancies, epilepsy, or diabetes require a higher dose (4–5 mg daily), which is safe for universal use.

Many couples in India seek medical care only after conception, making early prenatal interventions essential. Such couples should undergo a genetic risk assessment similar to those in the pre-conceptional period. However, as genetic tests take 1–3 months, timely results may not always be available for the ongoing pregnancy. All women should be offered aneuploidy screening for conditions like Down syndrome (trisomy 21), trisomies 18 and 13. First-trimester screening (11–14 weeks) includes a combination of nuchal translucency (NT) ultrasound and biochemical markers (PAPP-A, free β-hCG), while second-trimester screening (15–20 weeks) includes the quadruple test (αFP, Inhibin-A, uE3, free β-hCG). Since these screenings have limited predictive value, any positive result should be confirmed via invasive testing before any pregnancy termination decision.

Noninvasive prenatal testing (NIPT) is a highly sensitive method for detecting trisomies 21, 18, and 13, as well as sex chromosome aneuploidies, from 10 weeks of pregnancy. However, NIPT has limitations, including high costs, potential test failures (1%), and reduced accuracy in cases such as donor egg pregnancies, multiple gestations, and maternal obesity. NIPT should not replace diagnostic testing in high-risk pregnancies. A screen-positive NIPT result must be confirmed by invasive testing, and termination should never be based on NIPT alone.

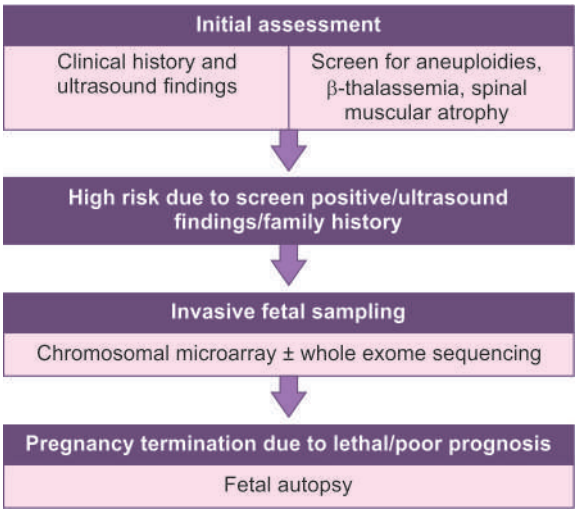
Ultrasound evaluations are critical in prenatal screening. The first-trimester ultrasound (11–14 weeks) assesses structural abnormalities, NT thickness, and multiple pregnancies. The targeted anomaly scan at 18–20 weeks helps identify structural defects and soft markers of genetic syndromes. If abnormalities are detected, referral to a geneticist or fetal medicine specialist is required. Additionally, maternal serum α-fetoprotein (MSAFP) can be measured at 15–20 weeks for NTD risk assessment, particularly in cases where first-trimester screening or NIPT has been performed, especially if access to good quality ultrasound is limited.[14]

Invasive genetic testing, such as chorionic villus sampling or amniocentesis, should be offered for confirmatory diagnosis when screening results indicate increased risk. Rapid aneuploidy tests (QF-PCR/FISH/MLPA) should be accompanied by karyotyping and/or chromosomal microarray (CMA). In case of ultrasound abnormalities, a combination of CMA and WES is advised to look for chromosomal as well as single gene disorders.

Ultrasound abnormalities and pregnancy losses may indicate an underlying genetic disorder. Any pregnancy with unexplained growth abnormalities, soft markers, or congenital malformations should be referred for genetic evaluation, as these conditions often require advanced genetic testing. Pregnancy losses, including stillbirths and terminations due to fetal anomalies, necessitate genetic investigations. Post-mortem examination and collection of fetal samples (cord blood, skin biopsy, umbilical cord) should be performed to aid in recurrence risk prediction and early prenatal diagnosis in future pregnancies. Recurrent early miscarriages (before 12 weeks) warrant parental karyotyping, while testing the product of conception is usually reserved for cases where parental chromosomal abnormalities have been ruled out.

If fetal autopsy is not feasible due to religious or personal reasons, alternative documentation such as photographs, radiographs, and external examination notes should be maintained. Emerging technologies like fetal MRI and minimally invasive autopsy may also be considered in such cases. These interventions ensure comprehensive genetic risk assessment, enabling informed reproductive decisions and improved pregnancy outcomes.

Flowchart 1.2: Diagnostic algorithm of genetic testing in prenatal cases



CONCLUSION

Advances in genomic technologies have revolutionized prenatal diagnostics, offering unparalleled insights into the genetic underpinnings of fetal anomalies. From targeted methods like Sanger sequencing to comprehensive approaches such as whole exome sequencing (WES), these tools enable early and accurate identification of genetic disorders. Techniques like chromosomal microarray (CMA) and SNP arrays have improved the resolution of chromosomal analysis, while WES has expanded the diagnostic yield for monogenic conditions underlying structural anomalies detected by prenatal ultrasound. Despite their transformative potential, limitations such as the inability to detect balanced rearrangements, low-level mosaicism, or non-coding variants, highlight the continued need for careful test selection, validation, and interpretation.

Comprehensive prenatal testing, complemented by robust genetic counseling, enables personalized care and informed decision-making for families. The integration of these technologies into clinical practice underscores the importance of a multidisciplinary approach, including obstetricians, geneticists, and fetal medicine specialists, to optimize outcomes. Ongoing advancements in sequencing methodologies and bioinformatics are poised to bridge current gaps, further enhancing diagnostic precision and clinical utility. As we navigate this genomic era, maintaining ethical standards and clear communication with patients will remain central to the responsible application of these powerful tools.

KEY POINTS

- **Early referral:** Refer cases with anomalies or family history to a geneticist promptly for evaluation.
- **Tailored resting:** Use tests wisely on a case to case basis, including CMA, WES, NIPT as indicated.
- **Expert interpretation:** Collaborate with geneticists to understand results and handle variants like VUS accurately.

- **Counseling is critical:** Provide genetic counseling to ensure families are informed and supported.
- **Teamwork matters:** Work closely with geneticists and counselors for comprehensive, patient-centered care.

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