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Fetal Medicine and Genetics

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CHAPTER

Genetic Testing: Which and When

Ratna Dua Puri, Veronica Arora, Meena Lall, Sudha Kohli, Sudhisha Dubey

KEY POINTS

1. Genetic testing in obstetrics plays a pivotal role in prenatal care, offering insights into the genetic makeup of the fetus, thus aiding in the detection of chromosomal abnormalities and genetic disorders.
2. Fluorescence in situ hybridization (FISH) provides a quick turnaround but is limited by the need to know which specific abnormality to test for.
3. Karyotyping offers a broader view by examining the entire chromosome set for structural or numerical changes.
4. Chromosomal microarray analysis (CMA) has become a standard for identifying chromosomal imbalances at a higher resolution than karyotyping.
5. Indicated for individuals with developmental delays, congenital anomalies, or abnormal fetal ultrasound findings, CMA can detect copy number variations (CNVs) not visible on karyotype.
6. Multiplex ligation-dependent probe amplification (MLPA) is a technique used to detect copy number changes in specific genomic regions. It is particularly useful for conditions caused by deletions or duplications of small segments, such as certain hereditary cancer syndromes, Duchene muscular dystrophy, spinal muscular atrophy.
7. Sanger sequencing, the gold standard for precise genetic mutation identification, is utilized for the detection of single-gene disorders.
8. Exome sequencing (ES) examines the coding regions of genes (exome), where most known genetic variant mutations are found. Indicated for complex cases with suspected genetic disorders where other tests have failed to provide a diagnosis, it offers a comprehensive solution.
9. In prenatal ES a thorough knowledge is required for interpreting the variant of unknown significance.
10. The choice of test depends on the clinical context, with a thorough understanding of each method's capabilities and limitations being crucial for informed decision-making in prenatal care.

INTRODUCTION

The burden of genetic disorders in India is now being realized as the incidence of infectious diseases is decreasing with improved neonatal care facilities. In the absence of robust registry data, estimates of neonatal births with congenital anomalies and genetic disorder varies from 5.3 to 7.9% that translates to 15 lakhs per year.

Over the years, the scope of genetics has increased vastly with the availability of genetic and genomic tests along with

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enhanced imaging technology. Integration of baseline clinical information, imaging, genomic testing, and appropriate counseling now enables enhanced screening and diagnostic opportunities for prevention of birth defects and genetic disorders. This chapter focuses on the various genetic and genomic technologies and their application in the care of the unborn fetus. With the aid of examples, this primer for medical students and young doctors will focus on the requisite preliminary information that is imperative to decide the appropriate genetic test and the correct interpretation within the scope of fetal medicine practice.

In this chapter, we will address the basics of genetic testing, different types of genetic tests, indications of testing, and advantages and limitations of the tests with case examples.

BASICS OF GENETIC AND GENOMIC TESTING

Within the context of this chapter, this refers to the analysis of the human genome that comprises the nuclear content including chromosomes or the deoxyribonucleic acid (DNA). There are 23 pairs of chromosomes and each chromosome is composed of two chromatids made of condensed DNA strands attached at the centromere. Alternating combinations of the four nucleotides, ACGT (adenine, cytosine, guanine, and thymine), comprise the genes within the DNA strand. There are about 20,000 genes and these comprise only 1–2% of the genome.

TYPES OF GENETIC TESTS

Genetic tests in the practice of fetal genetics can be classified as screening tests and diagnostic tests.

- *Screening tests:* They include serum biomarkers, ultrasonographic soft markers associated with aneuploidy, fetal imaging for growth and malformations, and cell-free fetal DNA (cffDNA) analysis for rhesus (Rh) testing and aneuploidy.
- *Diagnostic tests:* These are confirmatory tests and include:
  - Tests for chromosomal disorders:
    - Fluorescence in situ hybridization
    - Karyotype
    - Chromosomal microarray analysis
  - Tests for monogenic disorders:
    - PCR-based tests
    - DNA sequencing
    - Next-generation sequencing

Screening Tests for Down Syndrome and Other Aneuploidies

The two main components of this screening are: Analysis of specific biomarkers in maternal serum and ultrasound markers to determine the likelihood for trisomy 21, 13, and 18 in the fetus. The risk for neural tube defect is also ascertained with maternal alpha-fetoprotein (AFP) levels.

The first-trimester combined screen is performed between 11 to 13 weeks and 6 days of gestation to assess the risk of trisomy 21, 18, and 13. Serum-free human chorionic gonadotropin [free beta human chorionic gonadotropin (fβ-hCG)] and pregnancy-associated plasma protein-A (PAPP-A) are tested. This test in conjunction with the nuchal translucency (NT) scan has a sensitivity of 85% for trisomy 21.

In the second trimester, screening is performed with the quadruple marker test, performed between 16 to 20 weeks of pregnancy and measures the levels AFP, hCG, estriol, and inhibin A. The test can detect 65–70% of the fetuses with trisomy 21. An added advantage is screening for open neural tube defects by the AFP component of the test. Triple test is not recommended these days for

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aneuploidy screening due to lower accuracy compared to other available screening tests.

It is important to note that these serum biomarkers are screening tests that can only identify pregnancies at higher risk for chromosomal abnormalities, but they cannot diagnose these conditions with certainty. If the results of these screening tests indicate an increased risk of chromosomal abnormalities, further testing such as chorionic villus sampling (CVS) or amniocentesis should be recommended to provide a definitive diagnosis.

Noninvasive Prenatal Screening

Noninvasive prenatal screening (NIPS) is based on the presence of cffDNA in the maternal blood. This DNA is released from the cytotrophoblasts into the circulation and sequenced to detect chromosome imbalances. The proportion of this DNA is adequate to be tested starting at 9 weeks' gestation and a maternal blood sample can undergo testing any time after this gestation.

Noninvasive prenatal screening is highly accurate screening test with a sensitivity of 98.80% [95% confidence interval (CI) 97.81–99.34] and a specificity of 99.96% (95% CI 99.92–99.98) for trisomy 21 with a false-positive rate of 0.09%. It is an accurate screening test for the common aneuploidies of chromosome 21, 13, and 18. False-positive NIPS are reported and every screen-positive test must be followed by confirmatory diagnostic invasive testing. NIPS will not detect structural anomalies. There are various methods to implement NIPS—primary screen, though not preferred at a primary level in India due to cost, contingent to a prior positive aneuploidy screen. Academic societies confirm NIPS as an efficient screening modality for the above aneuploidies and allow individual decisions on the model to be implemented in countries. Rare autosomal trisomies and subchromosomal imbalances including microdeletions and microduplications testing by NIPS are not recommended.

Pre- and post-test genetic counseling is essential. This is not the test of choice in the presence of fetal structural malformations or increased nuchal translucency.

Rhesus genotyping of fetus by noninvasive testing (cell-free fetal DNA from maternal blood): Rhesus incompatibility occurs in RhD-negative mothers when they carry a RhD-positive fetus. If she is sensitized and produces anti-D antibodies (alloimmunization), these cross the placenta and can cause fetal anemia if the fetus is Rh (D) positive. This is the most common cause of fetal anemia. If the mother is RhD negative and father is RhD positive, there is a one-third chance that the fetus is RhD negative. The actual RhD status of the husband is ascertained by RhD genotyping. If the father is RhD±, then fetal RhD testing is performed in maternal plasma by cffDNA testing. In this situation, noninvasive fetal RhD testing in maternal blood is recommended. The sensitivity of this test is 97.2% and specificity is 96.8%.

If the fetus is RhD negative, no further monitoring is required for fetal anemia. If the fetus is RhD positive, then the pregnancy is followed up by antibody titers and middle cerebral artery (MCA) Doppler assessments as per guidelines. It is essential to do father's RhD genotype before fetal RhD testing.

TESTS FOR CHROMOSOMAL DISORDERS

Karyotype

A *karyotype* examines all 23 pairs of chromosomes. These are visualized under the microscope and arranged according

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to their G-bands, their descending order of size, and presence of the centromere position. Karyotype will examine the whole genome and identify abnormalities in chromosome number and large (>5–10 Mb) structural abnormalities. The latter include chromosomal deletions, duplications, isochromosomes, inversion, ring chromosomes, extra structural abnormal chromosome (ESACs or markers) and balanced and unbalanced translocations. Their diagnostic accuracy of karyotype in amniotic fluid (AF) and CVS being 99.4–99.8% and 97.5–99.6%, respectively (Shaffer et al., 2007).

Figure 1 depicts an abnormality of chromosomal number where the child has Down syndrome due to an extra chromosome 21.

Figure 2 depicts a structural chromosomal abnormality that is a translocation of genomic material between chromosomes 1 and 13.

Sample for Karyotype

- Peripheral blood in heparin anticoagulant vacutainer
- Chorionic villi or AF sample
- Skin fibroblasts

Indications of Karyotype in Fetal Medicine

- Screen-positive first- or second-trimester aneuploidy screen test
- A parent is a carrier of a balanced translocation.

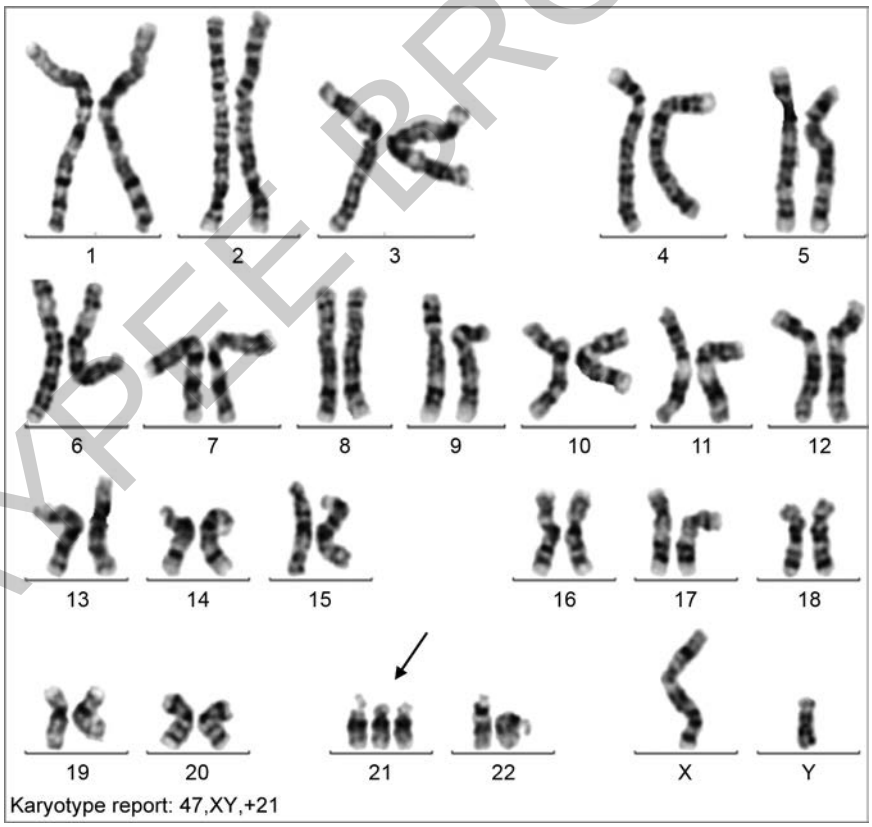


Fig. 1: Karyotype showing a chromosomal abnormality of number.

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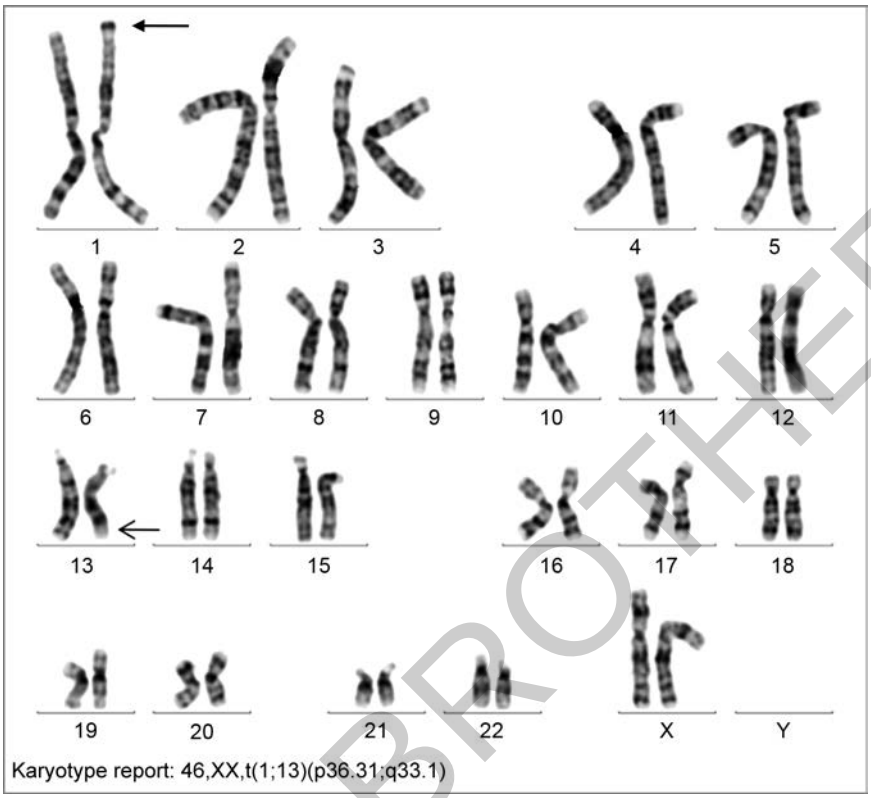


Fig. 2: Karyotype showing a balanced translocation, an abnormality of chromosome structure.

- Fetal malformations suggesting a specific disorder, e.g., duodenal atresia and risk for trisomy 21.
- Fetal malformations when CMA is not possible.
- Confirmation of fetal chromosomes after preimplantation genetic testing for structural rearrangement (PGT-SR).
- Other indications related to obstetric practice include primary amenorrhea, recurrent pregnancy loss, and infertility.

Limitations of Karyotype

Limitations include a resolution up to 5–10 million base pairs. Small deletions and duplications will not be identified by karyotype. Karyotyping requires cultured cells from blood, AF cell, or CVS to arrest dividing or mitotic cells at metaphase when the chromosomes are visible microscopically **(Fig. 3)**.

Fluorescent in situ Hybridization

Fluorescent in situ hybridization is a targeted approach when there is a specific suspicion of a particular chromosomal abnormality based on ultrasound findings or family history. The cells of prenatal samples (AF or CVS) or of the blood sample of the patient are immobilized on the glass slides and hybridized with the now commercially available DNA probes of the targeted genomic region to detect the particular disorder for which probes are used. This can provide rapid results compared to other techniques like karyotyping or CMA. Examples of common FISH tests with centromeric or locus-specific probes

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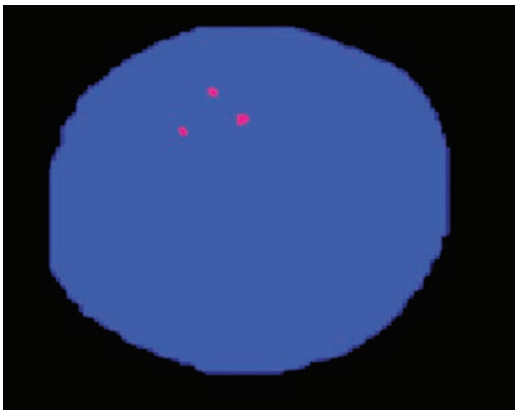


Fig. 3: FISH with Vysis DNA probe LSI 21/ spectrum orange showing three signals for trisomy 21 for chromosome 21 aneuploidy. (DNA: deoxyribonucleic acid; FISH: fluorescence in situ hybridization)

include five common aneuploidy detection for trisomies of 13, 18, and 21 and the sex chromosomes, targeted microdeletion syndromes like 22q11.2 deletion, Williams syndrome, and translocations involving various subtelomeres. Whole paint probes can help to delineate subtle suspected translocations on metaphase obtained from cultured cells. Cytogenetic evaluation of some structurally abnormal chromosomes, e.g., marker chromosomes is also possible in some cases. The sensitivity (83–100%) and specificity (99.8–100%) of interphase prenatal FISH tests has been summarized from multiple report (Shaffer et al., 2007). This should depend on the quality of the probes and preparation of samples for hybridization.

Sample

Same as for karyotype.

Indications of Fluorescent in situ Hybridization in Fetal Medicine

- As an adjunct to karyotype or microarray for a quick targeted result
- For a specific microdeletion syndrome.

Limitations

It requires prior knowledge of the specific chromosomal abnormality to be investigated. It is not a comprehensive test for all possible chromosomal abnormalities and it is usually used as an adjunct to a complete chromosomal analysis evaluation. These rapid aneuploidy detection (RAD) tests are adjunctive to the complete diagnostic tests of karyotyping or CMA.

Multiplex Ligation-dependent Probe Amplification

Multiplex ligation-dependent probe amplification is a DNA-based multiplex assay to detect deletions and duplications referred to as copy number variations (CNVs) in the chromosome or genes of the human genome.

Sample

Deoxyribonucleic acid-based test—blood in ethylenediaminetetraacetic acid (EDTA) coagulant vacutainer, fetal samples, and product of conception.

Indications

This method is used for identification of single or more than one exon deletions and duplications as in Duchenne muscular dystrophy **(Fig. 4)** and SMN1 and SMN2 copy numbers as in spinal muscular atrophy. Methylation-specific multiplex ligation-dependent probe amplification is used to determine methylation as well as copy number defects as for Angelman, Prader–Willi, Beckwith–Weidman, Silver–Russel syndromes as well as methylation of CpG islands in fragile X. A special type of MLPA called subtelomeric MLPA examines the common subtelomeric regions of all chromosomes which are known to cause recurrent deletions and duplications.

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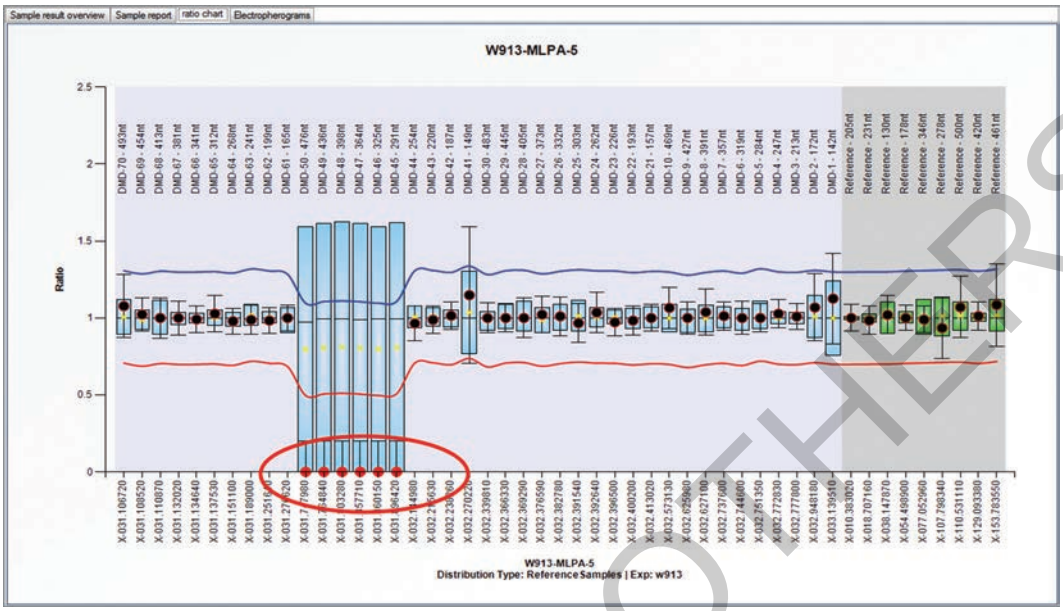


Fig. 4: Multiplex ligation-dependent probe amplification (MLPA) ratio plot showing hemizygous deletion of exon 45–50 in a male patient with Duchenne muscular dystrophy. Deletions are highlighted as red dots.

Limitations

This test will not identify balanced translocations, all unbalanced translocations, and single-variant nucleotide mutations in genes causative of disease.

Chromosomal Microarray Analysis

This technique enables the study of chromosomes at a higher resolution than gold standard karyotype. In simple terms, CMA is a multiplex FISH with hundreds and thousands of labeled oligonucleotides and SNPs from all over the genome. The number of probes used on the immobilized array slides on which the patient's DNA is hybridized with a differentially labeled dye and identifies chromosomal or genomic regions that are deleted or duplicated of at least 1,000 bp size, referred to as CNVs. This requires computerized software as the number of probes are many and cannot be assessed manually or microscopically. CMA has the ability to detect genomic deletions up to 20 kb and duplications up to 40 kb. The sensitivity and specificity of detection of the DNA dosage imbalances detected are dependent on the size of the CNV and the coverage and density of the clone of probes of the platform used for this test. Various databases (ClinVar, ClinGen, Decipher, UCSC, etc.) and published literature are used to interpret the CNVs as pathogenic, benign, and variations of uncertain significant (VUS). The benefit of CMA is that it interrogates various regions of the whole genome for pathogenic and benign CNVs at a much higher resolution than other cytogenetic and molecular cytogenetic tests.

Sample

DNA-based test—blood in EDTA coagulant vacutainer, fetal samples, and product of conception.

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Indications for Chromosomal Microarray Analysis

The indications of this test in fetal medicine include evaluation of a fetus with ultrasound abnormalities or multiple malformations where it has a 6–10% additional diagnostic yield compared to a karyotype analysis. CMA is also a very good technique for stillbirth and intrauterine death as the testing method does not require dividing cells. Even in the absence of a structural malformation in the fetus, it can provide additional information in 1–2% (16–18). Another indication for CMA in the fetal sample is when a parent is a heterozygous carrier of a balanced translocation.

Limitations

The limitation of a microarray includes the VUS for which literature evidence is not available. Therefore, expert genetic counseling is mandatory to help appropriate parental decisions for the pregnancy. A CMA is not the test of choice for monogenic disorders.

Case

The first pregnancy of a nonconsanguineously married couple—the anomaly scan identified fetal growth restriction, congenital cardiac malformation, and agenesis of corpus callosum. There was a family history on the paternal side of recurrent pregnancy losses. A fetal sample was tested for chromosomal CNVs by CMA. A 5.0 Mb deletion of chromosome 6p25.3p25.1 and a 23.8 Mb duplication of chromosome 4p16.3p15.2 was identified in the fetal sample **(Figs. 5A and B)**. In view of this report, a parental karyotype analysis was advised and performed and the father was identified to have a balanced chromosomal translocation between chromosome 4 and 6. The parents were rendered genetic counseling with details of about the options for future pregnancies.

BEYOND CHROMOSOMES, DISORDERS DUE TO GENE MUTATIONS

Case

A 27-year-old second gravida has one 4-year-old child with transfusion-dependent beta thalassemia major. She provides this history in her first antenatal history at 8 weeks gestation. What will be the genetic counseling provided and flow of events for this family?

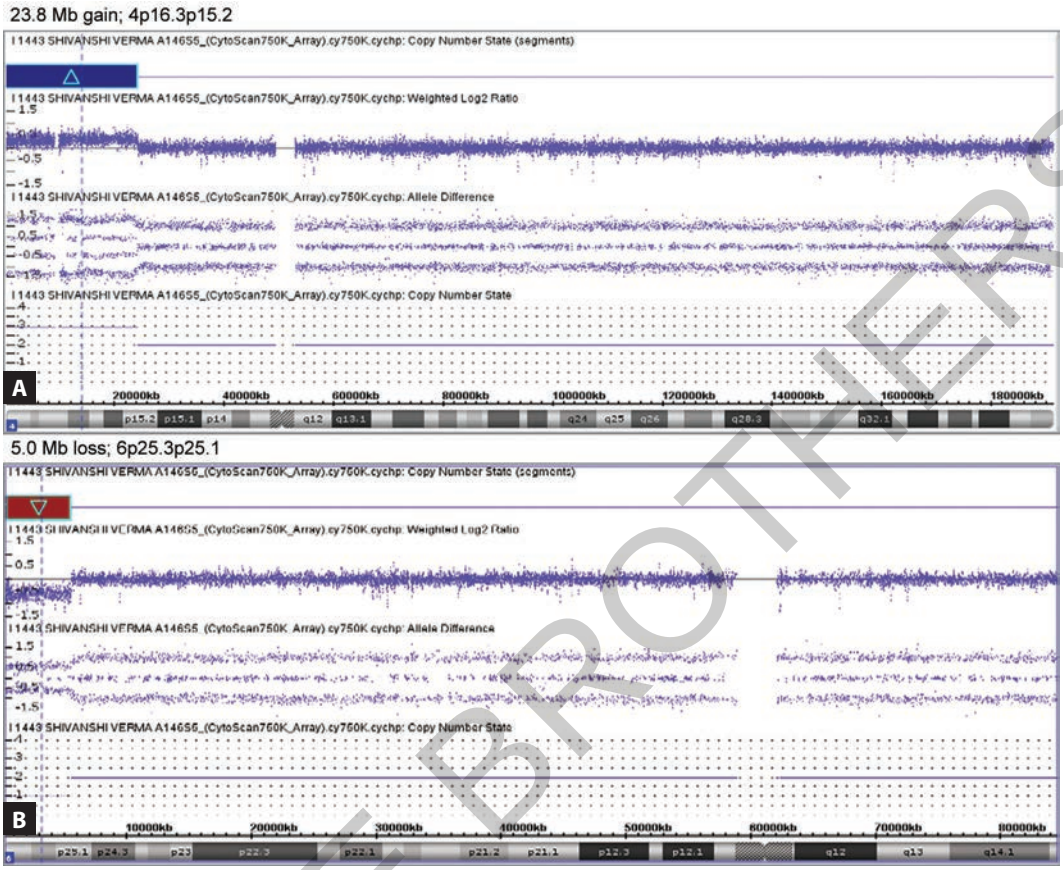
Beta thalassemia major is a disorder that occurs due to a mutation in both copies of the beta thalassemia gene, *HBB*. This is an autosomal recessive disorder that occurs due to mutations/variations in both copies of the *HBB* gene in the affected child. The parents are carriers in that one of their two copies have the mutation and thereby, they do not have any disease manifestations.

The steps for prenatal diagnosis for this family include:

- Confirming the clinical diagnosis in the proband and the carrier status in the parents
- Polymerase chain reaction-(PCR)-based testing or *HBB* gene sequencing in the proband and parents to identify the gene mutation causing the disorder in this family **(Figs. 6A to C)**.
- Genetic counseling to explain the about the disorder, recurrence risk, option of prenatal testing, procedure, risks, and limitations of the same.
- The specific mutation in the family must be identified prior to prenatal diagnostic procedure. In the fetal sample, the specific mutation relevant to the family is tested.

This *direct detection of the specific DNA variant* in the prenatal sample is the method

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Figs. 5A and B: arr[GRCh37] 4p16.3p15.2(68346_23955546)x3, 6p25.3p25.1(381118_5402772)x1.

of choice in all cases with a previously affected child or when a targeted disease-causing DNA variant is known in the couple. A DNA variant is defined as a nucleotide change from the reference sequence. These are simple, low throughput, most accurate diagnostic PCR-based tests.

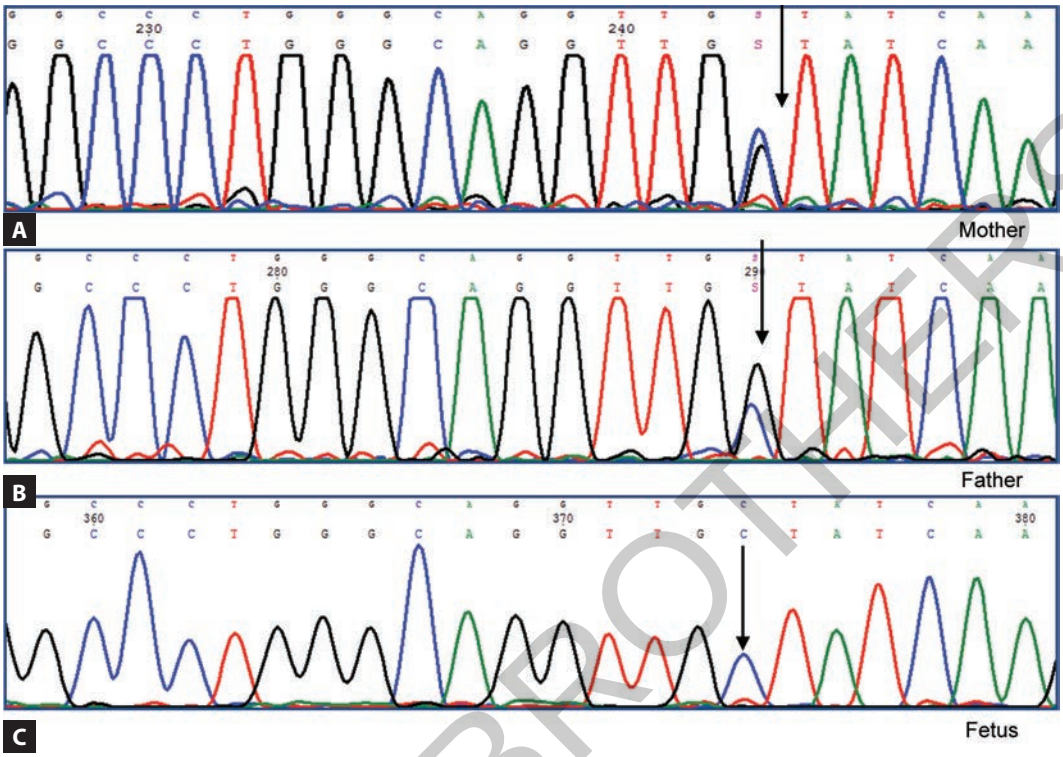
The *PCR-based methods* can be applied for many disorders including beta thalassemia, sickle cell anemia, achondroplasia, myotonic dystrophy, fragile X syndrome, Huntington disease, etc.

It is important to check for *maternal cell contamination* in the fetal sample as it can result in erroneous reporting of the fetus. Maternal cell contamination is ruled out by quantitative fluorescent PCR (QF-PCR) of various microsatellite markers present on the various chromosomes **(Figs. 7A and B)**.

TRIPLET-REPEAT DISORDERS

The expansion of a tandemly repeated trinucleotide sequence, cytosine, adenine, and guanine (CAG), is the mutational mechanism for several human genetic diseases such as myotonic dystrophy, Huntington's disease, fragile X, Friedreich ataxia, and spinocerebellar ataxias. Detection of expansion employs PCR amplification method using a fluorescently labeled locus-specific primer, flanking the CAG/CGG/GAA, etc. repeats together with paired primers

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Figs. 6A to C: Partial chromatogram of *HBB* gene. Both mother and father are heterozygous for IVS 1–5 G > C (c.92 + 5 G > C) and fetus is homozygous (both alleles with the same DNA change) for IVS 1–5 G > C (c.92 + 5 G > C). Mutation is marked by arrows.

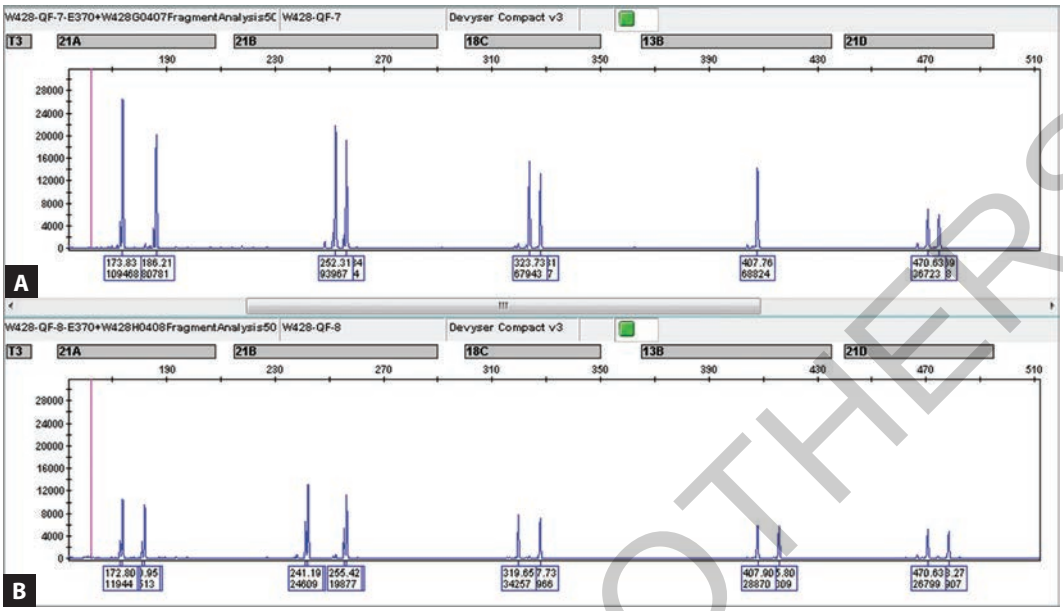
amplifying from multiple priming sites within the repeats. Triplet-repeat primed PCR (TP PCR) gives a characteristic ladder on the fluorescence trace, enabling the rapid identification of large pathogenetic repeats that cannot be amplified using flanking primers **(Fig. 8)**.

NEXT-GENERATION SEQUENCING

Next-generation sequencing is a high throughput method of analysis of a large number of genes together on one platform and at one time. This technique is very good for disorders or phenotypes that occur due to mutations in any one of many genes, e.g., Noonan disorder and RASopathies, hypertrophic cardiomyopathy, and ciliopathy group of disorders. The options available when using this technology include testing all 20,00 genes (ES), testing only those genes that are relevant to the phenotype (panel testing), or testing all genes for which there is disease phenotype correlation, about 5,000 genes of the Online Mendelian Inheritance in Man (OMIM morbid genes) as a clinical exome. The variant identified by this test is assigned a pathogenic, likely pathogenic, benign, likely benign, and variant of unknown significance category based on standard criteria for variant interpretation and classification.

Exome sequencing interrogates about 1.5–2% of the genome and includes testing for mutations in only the exons. This test

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Figs. 7A and B: Microsatellites marker analysis of mother (A) and fetus (B). The presence of only one maternal allele in the fetal sample indicates the absence of MCC and confirms that the sample is a pure fetal DNA sample and the accuracy of PND is reliable. (DNA: deoxyribonucleic acid; MCC: maternal cell contamination; PND: prenatal diagnosis)

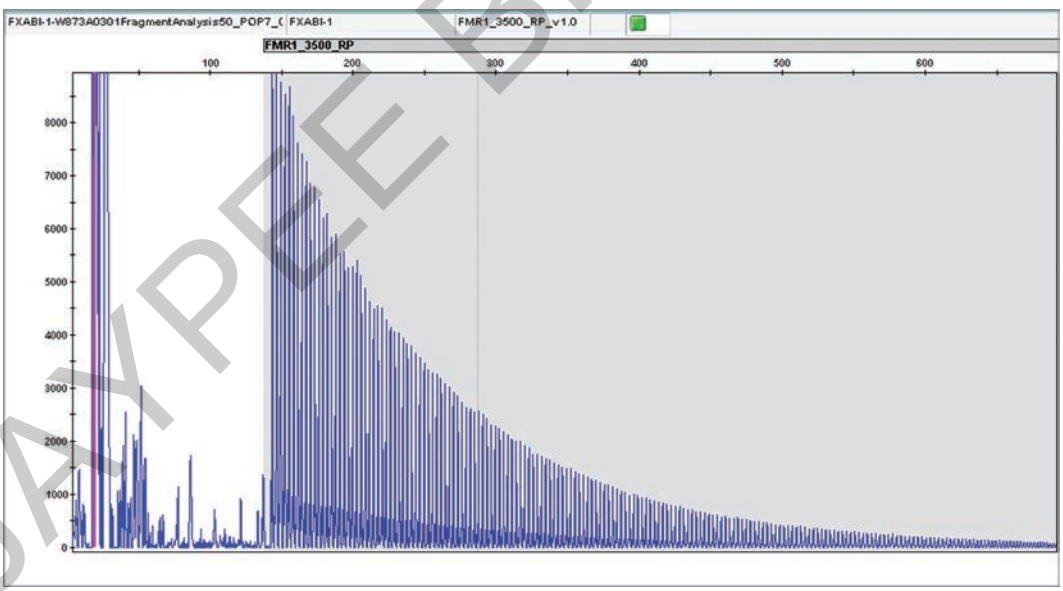


Fig. 8: CGG repeat expansion detected by triplet-primed polymerase chain reaction (PCR) in a male sample.

is indicated for use in the presence of fetal anomalies with normal CMA. Some salient features of this are highlighted further.

- Trio prenatal ES (pES) of fetus and parents is preferred to allow for timely pathogenic variant evaluation. If solo pES

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is performed, segregation of the identified pathogenic/likely pathogenic variants is important before assigning them as causative of fetal phenotype.

- Limited information of fetal genotype–phenotype correlation
- Adequate and detailed clinical information is to be provided to the laboratory while ordering the test. This includes detailed fetal imaging reports such as sonograms, magnetic resonance imaging (MRI), and/or fetal cardiac ultrasound, prior fetal prenatal test results, and/or clinical laboratory report, parental past medical history, ethnicity, reproductive history, and family history, including parental consanguinity.
- Pretest and post-test genetic counseling is best provided by trained personnel or geneticists who understand the phenotype and can apply the appropriate test for the clinical situation, interpret the test results and explain to the family as per guidelines. Informed consent is to be undertaken before genetic testing for any fetal indication.
- Turnaround time—needs to be rapid during pregnancy and is best.
- The greatest challenge of pES is the variants of unknown significance identified on sequencing. Great experience and expertise is required to analyze these variants.
- The laboratory performing the genomic tests must be accredited and have experience in variant interpretation and fetal phenotype correlation.

At the present time, there are no data supporting the clinical use for ES for other reproductive indications, such as the identification of sonographic markers suggestive of aneuploidy or a history of recurrent unexplained pregnancy loss.

Indications of Prenatal Exome Sequencing in Fetal Medicine

- Multiple fetal malformations with normal CMA or single major malformation suggestive of a genetic disorder
- Fetal malformations highly specific of a single gene disorder, e.g., rhabdomyoma
- Family history of a monogenic disorder
- Severe fetal growth restriction with normal CMA
- Increased nuchal translucency with normal CMA.

Limitations of Prenatal Exome Sequencing

- Variants of uncertain significance (VOUS) are difficult to interpret due to evolving fetal phenotype.
- There is always a race against time due to turnaround time being more than 3 weeks.
- There is possibility of a blending of two or more phenotypes, wherein pES evaluation is very challenging.

As these genomic technologies are rapidly developing, these statements need to be reviewed frequently.

Case

Consanguineous family with second-affected child with short long bones and decreased bone density suggestive of osteogenesis imperfecta (OI) on the level II scan. OI is a skeletal dysplasia caused by mutations in more than one gene. This is referred to as genetic heterogeneity. Recurrence in a consanguineous family is most likely due to an autosomal recessive inheritance. A close differential diagnosis for OI is hypophosphatasia, a similar autosomal recessive disorder. Postnatal treatment is available for both disorders with variable outcomes. The couples are counseled and the above details discussed with them. In this clinical scenario, the option of pES can be offered to confirm the diagnosis and

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genotype–phenotype correlation of informed counseling **(Figs. 9 and 10)**.

A pictorial representation of the confirmatory prenatal tests with indications of each is summarized in **Figure 10**. A summary of the prenatal tests with advantages and disadvantages of each is summarized in **Table 1**.

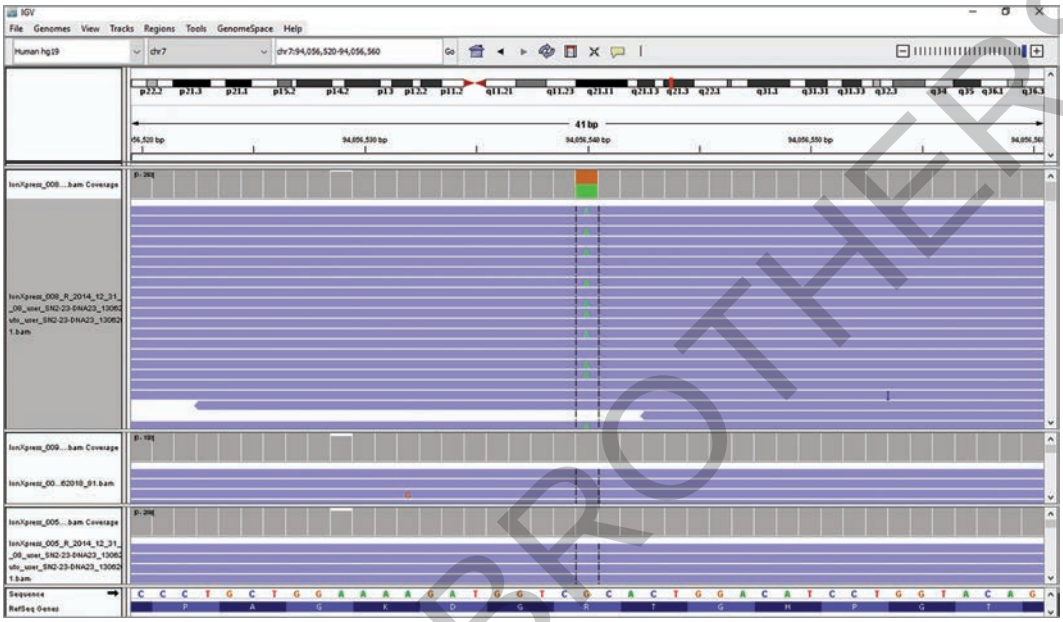


Fig. 9: Integrative Genomics Viewer (IGV) snapshot showing heterozygous variant: c.3200G > A; p.(Arg1067His) in *COL1A2* gene.

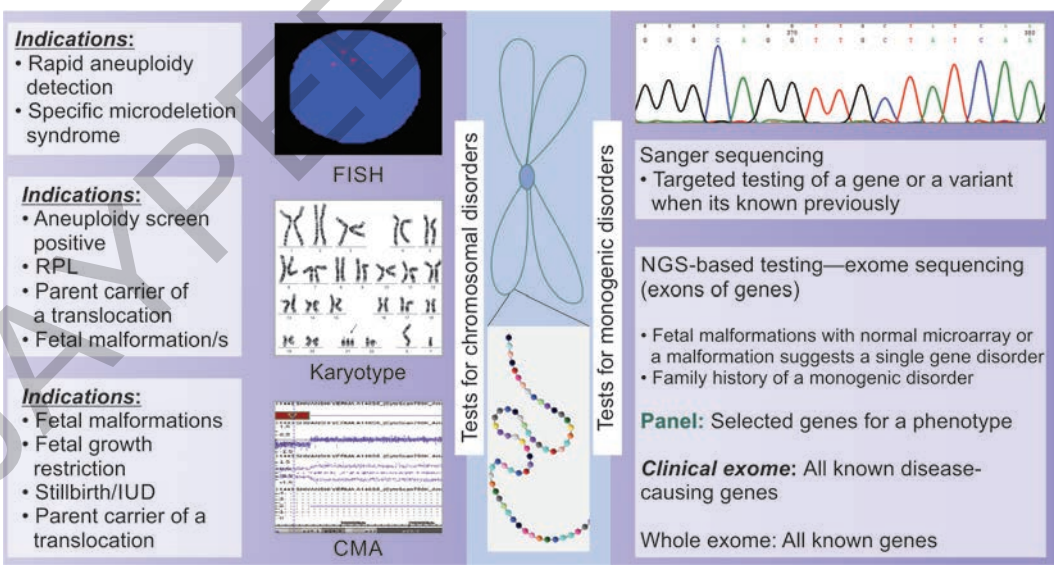


Fig. 10: Genetic tests in prental diagnosis at glance. (CMA: chromosomal microarray analysis; FISH: fluorescence in situ hybridization; IUD: intrauterine death; NGS: next-generation sequencing; RPL: recurrent pregnancy loss)

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TABLE 1: Different testing methodologies, indications, advantages, and limitations.

Test name	*Detection spectrum*	*Reporting time*	*Resolution*	*Advantages*	*Limitations*	*Indications in prenatal testing*
Karyotype	*Chromosomal abnormalities*: Loss/gain of chromosomal material, inversions, translocations, ring chromosomes, isochromosome, marker, mosaicism	2–3 weeks	>5–10 Mb	• Gold standard test for aneuploidies, polyploidies, and other structural aberrations • Affordable	Cannot detect microdeletions, microduplications, and very low-level mosaicism, or maternal cell contamination	• Risk for trisomy 21, 13, and 18 on antenatal screening tests • Advanced maternal age • Fetal malformations consistent with a specific chromosomal disorder • Family history of a chromosomal disorder • Positive cffDNA screen
FISH	Targeted areas of the genome	2–3 days	0.5 Mb	Quick results	Expensive, needs targeted probes, cannot screen all genomic regions together, does not detect maternal cell contamination	• Rapid aneuploidy testing • Specific microdeletion disorder, e.g., 22q11.2 microdeletion syndrome, can be used for validation of CNVs found in CMA
CMA	Chromosomal abnormalities	7–10 days	20 kb losses 40 kb gains	Detects small deletions or duplications, characterizes abnormalities such as markers and ring	Cannot detect balanced translocation or cannot differentiate between free trisomy 21 or translocation Down syndrome. Some platforms miss triploidy	• Fetal malformations • Multiple soft markers • Family history of chromosomal disorders • As an adjuvant to FISH or QF-PCR, it gives an additional 2% of diagnostic yield when performed for patients with normal USG

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Test name	*Detection spectrum*	*Reporting time*	*Resolution*	*Advantages*	*Limitations*	*Indications in prenatal testing*
MLPA	Exon/gene deletions and duplications	5 days	Exon level	Gold standard for exon level CNVs. Can detect methylation	• Commercial kits not available for all genes • Not detect balanced rearrangements or SNVs	• Family history of DMD, SMA, Alpha thalassemia • Subtelomeric MLPA for RPL
Direct DNA variant detection	SNVs, triplet-repeat disorders	1–2 weeks	Base pair level	Cheap, gold standard for the specific pathogenic variant	Requires a prior pathogenic variant identified in the family	Family history of a genetic disorder or one/both partners are carriers of a pathogenic genetic variant
Sanger sequencing	SNVs	1–2 weeks	Base pair level	Gold standard for SNV detection	Suitable for small genes. Does not identify deletions/ duplications	Beta thalassemia, connexin 26 related deafness, achondroplasia, hypochondroplasia
Exome	Monogenic disorders	3–6 weeks	Base pair level	Picks up SNVs, small deletions and duplications in coding region	Does not identify large CNVs, triplet-repeat disorders, methylation disorders, balanced rearrangements	• Multiple fetal malformations with normal CMA • Fetal malformations highly specific of a single-gene disorder • Severe FGR with normal CMA

(cffDNA: cell-free fetal deoxyribonucleic acid; CMA: chromosomal microarray analysis; CNVs: copy number variations; DMD: Duchenne muscular dystrophy; FGR: fetal growth restriction; FISH: fluorescent in situ hybridization; MLPA: multiplex ligation-dependent probe amplification; QF-PCR: quantitative fluorescent polymerase chain reaction; RPL: recurrent pregnancy loss; SMA: spinal muscular atrophy; SNVs: single nucleotide variants; USG: ultrasonogram)

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SUGGESTED READING

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Fetal Medicine and Genetics

As fetal medicine involves various disciplines, we as fetal medicine experts have struggled to gain knowledge regarding managing fetus as patient from different books, articles, and lectures. Hence, we felt an urgent need for a comprehensive book on relevant topics of fetal medicine together in the form of one book. All authors of the book are experts and pioneers of fetal medicine in India. They have given their precious time and have put forth their experiences regarding the must-know of every topic in lucid language and compact form. We hope you can find it a handy reference and will rouse your interest in fetal medicine and genetics as a professional career!



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