Child India

January 2022

Monthly e-Newsletter of Indian Academy of Pediatrics

IAP EXECUTIVE COMMITTEE - 2022

PRESIDENT	DR REMESH KUMAR R
PRESIDENT-ELECT	DR UPENDRA S KINJAWADEKAR
IMM. PAST PRESIDENT	DR PIYUSH GUPTA
VICE-PRESIDENT (CENTRAL ZONE)	DR K RADHAKRISHNA
VICE-PRESIDENT (EAST ZONE)	DR SUDHIR MISHRA
VICE-PRESIDENT (NORTH ZONE)	DR HARINDER SINGH
VICE-PRESIDENT (SOUTH ZONE)	DR SUBRAMANYA NK
VICE-PRESIDENT (WEST ZONE)	DR CHETAN B SHAH
SECRETARY GENERAL	DR VINEET K SAXENA
TREASURER	DR SAMIR HASAN DALWAI
EDITOR-IN-CHIEF, IP	DR DEVENDRA MISHRA
EDITOR-IN-CHIEF, IJPP	DR S THANGAVELU
JT. SECRETARY - LIAISON	DR ALOK BHANDARI
JT. SECRETARY - ADMIN	DR PURNA A KURKURE

Executive Members

DR ANTHATI S KIREETI
DR P ANIL KUMAR
DR R RAMAKRISHNA PARAMAHAMSA
DR MRITUNJAY PAO
DR CHANDRA MOHAN KUMAR
DR SHEO BACHAN SINGH
DR ARUN PRASAD
DR SUSHIL KUMAR
DR AJAY KUMAR GUPTA
DR LALAN K BHARTI
DR MANISH GUPTA
DR VIRENDRA SADANAND GAOKAR
DR KANAKSINH U SURMA
DR RAMESH M BAJANIA
DR SAMIR R SHAH
DR AVS RAVI
DR MAHAVEER PRASAD JAIN
DR RAVINDER K GUPTA
DR JOY BHADURI
DR GEETA PATIL
DR MADHU S PUJAR
DR SN PRASHANTH
DR SHIVA PRAKASH SOSALE C
DR JOSE OUSEPH
DR M NARAYANAN
DR MK NANDAKUMAR
DR RIAZ I
DR KEWAL KISHORE ARORA
DR MAHESH MAHESHWARI
DR NARENDRA R NANIVADEKAR
DR PRAMOD M KULKARNI
DR RAMAKANT D PATIL
DR SANJAY B DESHMUKH
DR BELA VERMA
DR SHYAMKUMAR LAISHRAM
DR SANTANU DEB
DR PRASANT KUMAR SABOTH
DR SUSRUTA DAS
DR GURDEEP SINGH
DR HARPREET SINGH
DR ANURAG TOMAR
DR RAMESHWAR L SUMAN
DR A CHENTHIL
DR A SOMASUNDARAM
DR J BALAJI
DR R SOMASEKAR
DR G VIJAYA KUMAR
DR KOTTOORU SRISAILAM
DR S SRIKRISHNA
DR DHIRAJ M GUPTA
DR OM SHANKAR CHAURASIYA
DR RAJEEV KRASHAK
DR UTKARSH SHARMA
DR ASOK KUMAR DATTA
DR KAUSTAV NAYEK
DR SUTAPA GANGULY
DR SHEILA S MATHAI (SERVICES)

CONTENT

Editor's Note

Dear colleagues,

Greetings from Child India. On behalf of all of you we congratulate Dr Piyush Guta, Dr Basavaraj and Team IAP 2021 for all the good work done and wish Team 2022 lead by IAP President 2022 Dr Remesh Kumar and HSG Dr Vinet Saxena all the very best.

The 1st issue for this year is dedicated to Pediatric Genetics.

Medical genetics emerged from basic science only one half century ago. Scientists have accomplished many major advances in the study of genetic diseases in children starting in 1948 with the establishment of the American Society of Human Genetics. Even before the use of modern laboratory techniques, Pediatric departments spearheaded the clinical description of simple genetic disorders, syndromes, and major malformations. The burgeoning of medical genetics as a specialty and its tremendous growth in departments of pediatrics was stimulated by major technological advances, such as the ability to visualize human chromosomes, the development of methods to study biochemical variations in blood and urine, cell culture, somatic cell hybridization, and molecular technology, all of which allowed for the diagnosis, treatment, and prevention of genetic disorders in children. Many pediatricians sought training in genetics, and training programs in medical genetics flourished in departments of pediatrics. The explosion of knowledge concerning the metabolic and molecular causes of genetic disease and understanding of their pathogenesis has led to a variety of specific diagnostic, preventive, and therapeutic approaches for alleviating the symptoms or preventing the complications of many of these disorders. Medical genetics is now recognized as a distinct medical specialty.

We thank the contributors for sharing their expertise in the field of pediatric genetics to enlighten pediatricians and PGs of our country.

Happy reading,

Wishes for a Happy New Year to all,

Jai IAP!

Dr Jeeson C Unni
Editor-in-Chief

President's Address

Dear friends,

Greetings! The first issue of Child India this year starts from the genes!

Genetic defects can be causative for many diseases in childhood. These include abnormalities in a child's appearance (phenotypic anomalies), developmental delay or mental retardation, autism, speech delays, congenital hearing, and visual impairments, as well as congenital metabolic disorders or congenital neurological and muscular disorders.

Pediatricians had to browse through books like Smith's Recognizable Patterns of Human Malformation for information on malformation syndromes of environmental and genetic etiology, recognizable disorders of unknown cause and normal standards of measurement for an entire spectrum of disorders.

Present day pediatric practice is incomplete in almost all subspecialties without a pediatric geneticist consultation and many puzzling cases are confirmed by the appropriate genetic testing. Many genetic disorders are prevented by prenatal genetic testing. Pediatricians and our PGs need to understand the immense scope of this subspeciality and we hope this issue will be an eyeopener to this emerging field for all of us

Warm regards,

Dr Remesh Kumar
National President, IAP 2022

Secretary's Message

Dear Friends,

Greetings from your new HSG.

It's a great privilege to have the opportunity to serve this great Organization IAP, of 35,000 Members in this role.

If our organization is a force to reckon with and enjoys a great reputation across the world, it's because of the vision of great leaders of IAP and immense hard work of countless soldiers of IAP who always thought it worthwhile to put their innumerable man hours.

So many Guidelines, protocols, modules and teaching and resource materials have been the result of scholarly brilliance of our members. Sharing of knowledge multiplies it and that's how we have been able to prepare our members in a better way to deal with whatever problems come their way.

Friends, when everything seemed all set, we had to take a difficult decision of postponing the long awaited 59th National Conference, Pedicon 2022 at Noida. Health of our members and credibility of our Organization was far more important than any of the Financial losses or organizational difficulties faced in this process. If everything goes as per projected, we hope to meet each other physically after more than two-year gap in sometime late March or early April. The Organizing team of Noida is all geared up and making all necessary adjustments.

We will be making all efforts for not only Academically enriching but also socially relevant programs round the year. To reach out to the society is our one responsibility we shouldn't forget. We are all passing through the massive third wave of Covid 19. Though its much milder, we all must take adequate precautions as per protocols. I extend on behalf of IAP most sincere thanks to the Editorial team of Child India led by Dr Jesson Unni for putting so much efforts for bringing out this wonderful periodical show casing the activities of IAP.

Warm Regards

Dr Vineet Saxena
Hon. Secretary General 2022 & 23

Congratulations!!

IAP is proud of you

Lieutenant General Dr Madhuri Kanitkar was awarded the Param Vishisht Seva Medal on the 73rd Republic Day.

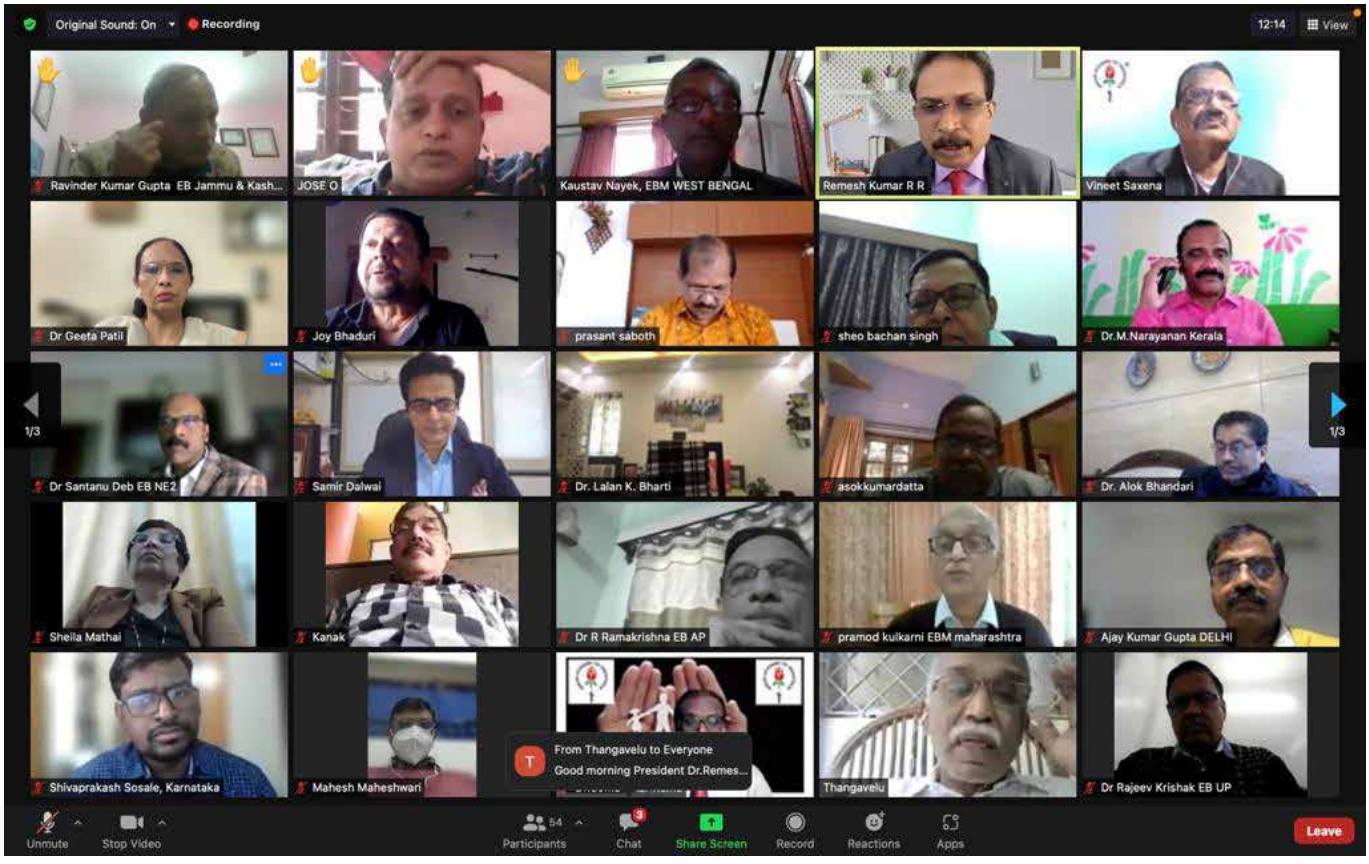
She is a retired General Officer in the Indian Army. She is the third woman in the Indian Armed Forces to be promoted to a Three-star rank, after Lieutenant General Punita Arora and Air Marshal Padma Bandopadhyay. She served as the Deputy Chief of Integrated Defence Staff (Medical) under the Chief of Defence Staff. Dr Kanitkar also serves on the Prime Minister's Science, Technology and Innovation Advisory Council (PM-STIAC)]

On 6 July 2021, she was appointed the Vice-Chancellor of the Maharashtra University of Health Sciences, Nashik by the Governor of Maharashtra. She took over this appointment after retiring from the Army in October 2021.

She has tenanted appointments of Associate Professor, Professor and Head of Department of Pediatrics at the Armed Forces Medical College, Pune. She has also served as a Professor at the Army College of Medical Sciences and at the Army Hospital (Research & Referral). Dr Kanitkar was instrumental in setting up the first Pediatric Nephrology service in the Army Medical Corps and has served as the President of The Indian Society of Pediatric Nephrology. She has served as the Deputy Director General Armed Forces Medical Services (Dy DGAFMS) in the office of the DGAFMS in New Delhi. She has served IAP as its Executive Board member (Defense Services) for 3 years, i.e., 2017, 2018, 2019.

Her husband, Lieutenant General Rajeev Kanitkar, a retired General Officer, last served as the Quartermaster General of the Indian Army. They are the first couple in the Indian Armed Forces to achieve the three-star rank.

First Executive Committee Meeting



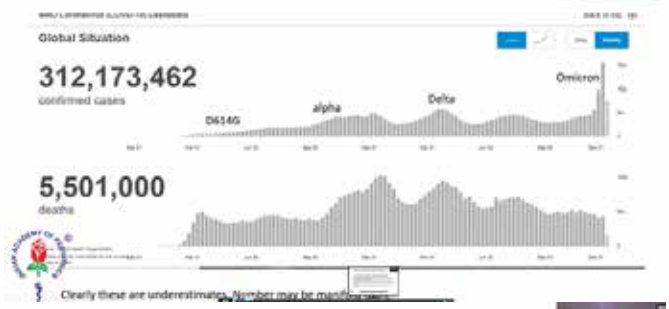
Team 2022 President Dr Remesh Kumar and HSG Dr.Vineet Saxena at IAP office

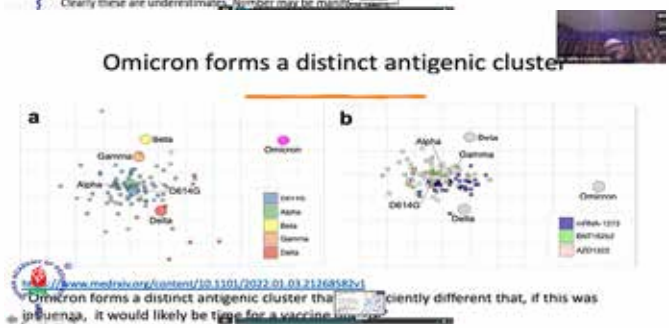
National Webinars by Indian Academy of Pediatrics

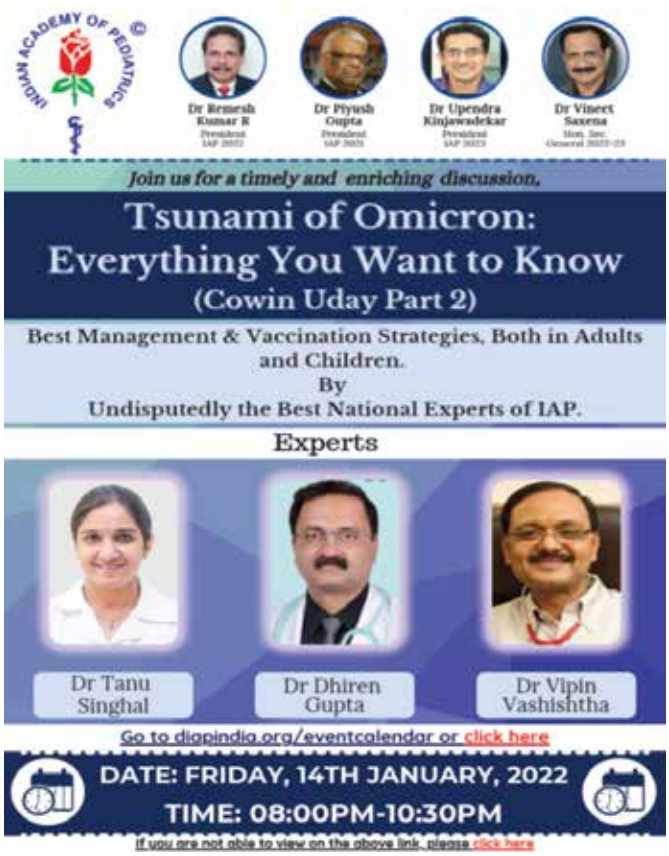
Tsunami Of Omicron: Everything You Want To Know

Dr. Tanu Singhal & Others

14th January 2022







Genetic Testing in Clinical Practice

Ambika Gupta, MD Pediatrics, DM Medical Genetics

Madhulika Kabra, MD Pediatrics
Department of Pediatrics
All India Institute of Medical Sciences
New Delhi

Introduction

Over the last two decades, the technological advancement in the field of molecular genetics and the availability of these have brought genetic testing from bench to bedside, thereby making it vital for the paediatrician to have a basic information on the rationale of ordering these tests, their utility as well as limitations. As these tests are expensive and interpretation of results may be challenging in many situations clinicians should understand the utility and pitfalls of these diagnostic modalities and use them judiciously.

Genetic testing was defined by, the Task Force on Genetic Testing as- the analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes. Such purposes include making a diagnosis, predicting risk of disease, identifying carriers, offering prenatal testing and for prognosis. Prenatal, new-born, and carrier screening, as well as testing in high-risk families, are included. In a pediatric clinic, the most common form of genetic testing is diagnostic testing and DNA based testing is the most common modality.

Importance of genetic testing

Even though the clinical diagnosis is certain in many disorders, molecular diagnosis is important because the type of variant may affect the disease severity and aids in appropriate disease prognostication. It also makes prenatal testing possible so that parents can decide whether or not they want to continue pregnancy with a similarly affected child . Knowing the variant may also direct therapeutic management. For example, in DMD certain types of out of frame deletions are amenable to exon skipping therapy. Another example is cystic fibrosis, in which, the common variant deltaF508, is amenable to Lumacaftor and those variants causing channel gating abnormalities, are amenable to Ivacaftor therapy.

The broad classification of genetic disorders includes 1. Chromosomal disorders 2. Single gene disorders 3. Multifactorial disorders. For multifactorial disorders (eg Neural tube defects

and cleft lip and palate) there is no specific diagnostic tests as the etiology includes multiple genes and environmental factors.

Chromosomal abnormalities

These include both numerical and structural aberrations (microscopic/ sub-microscopic). The traditional test to pick up chromosomal abnormalities is karyotype which picks up larger abnormalities. Structural aberrations which are sub-microscopic but larger then 1000 base pairs (1 kb) are referred to as "copy number variants" (CNV), these are picked up by other techniques described below.

A chromosomal disorder should be suspected under following circumstances and appropriate genetic testing should be offered

1. Phenotype is suggestive of a known aneuploidy or a deletion/ duplication syndrome

   Example: Down's syndrome, Edward syndrome, Patau syndrome (Trisomy 21, 18 and 13 respectively) and Wolf Hirschhorn syndrome (4pdel), DiGeorge's syndrome (22q11.2del), 1p36 microdeletion and many more
2. Unexplained Global developmental delay or Intellectual disability with/ without dysmorphism and with/without malformations
3. Disorders of sex development (DSD)
4. A balanced chromosomal rearrangement may be identified in couples with recurrent pregnancy loss and/or birth of a child with one or more structural malformations
5. Unexplained infertility
6. Female with short stature and primary amenorrhoea where, Turner syndrome should be ruled out

Tests for chromosomal disorders

Karyotype - Numerical and larger structural (> 5MB) variations can be picked up by a karyotype, which is an ordered arrangement of homologous chromosome pairs starting from the largest to the smallest chromosome and the sex chromosome complement represented at the end.

Figure 1. shows translocation 14;21 in a child with Down syndrome . The extra chromosome 21 has translocated to chromosome 14

Fluorescence in situ hybridization (FISH)- This is a targeted test and uses fluorescent probes complimentary for a particular region of a chromosome , so the testing is based on suspicion of a particular deletion or duplication (eg Digeorge syndrome, Williams syndrome etc) Current utility of FISH for detection of known deletions and duplications has been largely superseded by MLPA and CMA but is still being used both for postnatal and rapid prenatal diagnosis ,identification of marker chromosome found on karyotype and also for balanced and unbalanced chromosomal re arrangements. The test is expensive and is labour intensive and low throughput.

Multiplex Ligation dependent probe amplification (MLPA) – This test is based on the principle of nucleic acid hybridization and absence of the amplified product correlates with the deletion of the exon/ region under consideration. It is a good test for detection of single/ multiple exon deletions as in some of the common disorders like Duchenne Muscular Dystrophy (DMD) and Spinal Muscle atrophy (SMA). The gene for DMD comprises of 79 exons and two MLPA probe sets are used to detect single/multiple exon deletions/duplications. For SMA, homozygous deletion of Exon7 of SMN1 is confirmatory. Additionally, MLPA also gives the number of SMN2 copies which helps in predicting the disease severity. Presently it is the recommended test for diagnosis of DMD (detects deletion/ duplications in about 70% of cases) and SMA.

This is a very reliable and a low cost test with a quick turnaround time. However, it is a targeted test and utility of the test is limited to a single gene or a group of genes with a known phenotype.. Variation at the probe binding site may cause false positive results. The technique requires a capillary electrophoresis sequencer.

Figure 2 shows MLPA results for a case of DMD

Chromosomal Microarray (CMA) - This technique is based on the principle of nucleic acid hybridization using fluorescently labelled probes to detect with a genome wide analysis of copy number variants and copy neutral changes like uniparental disomy which may be implicated in development of imprinting disorders.

As per the ACMG recommendations CMA is indicated in the following:

- Multiple anomalies not specific to a well-delineated genetic syndrome
- Apparently nonsyndromic developmental delay / Intellectual disability
- Autism spectrum disorders after first line evaluation by ruling out disorders like Fragile X syndrome , Rett syndrome etc

Figure 3 shows CMA results of a patient with Cri-du- Chat syndrome

Table 1 summarises techniques commonly used for detecting structural copy number variants

Testing for suspected monogenic disorder

In general, monogenic disorders are caused by sequence variants. In certain situations, however, they may be caused by single/ multiple exon deletions/duplications or whole gene deletions. Classical examples of these disorders are Thalassemia, DMD, SMA, Achondroplasia , Tuberous sclerosis, cystic fibrosis etc. Tests for monogenic disorders may be PCR based or DNA sequencing based. Molecular diagnosis of many common genetic disorders can be established by simple polymerase chain reaction (PCR) based tests.

PCR based tests

When the phenotype is highly suggestive of a particular aetiology with an established mode of inheritance, one can directly opt for targeted testing. For disorders in which a single common pathogenic variant (hotspot) in a given population is known, simple PCR based tests like allele specific oligonucleotide hybridization (dot blot), Amplification refractory mutation system (ARMS) PCR, Restriction fragment length polymorphism (RFLP) based testing may be done. These tests are relatively less costly and more readily available. Some common disorders for which these tests can be performed are Beta thalassemia, Sickle cell anemia, Achondroplasia, Gaucher disease, Cystic fibrosis etc

DNA sequencing-based tests

Sanger sequencing- This method is used when a patient is suspected of having a variant within a specific gene but that gene may have many different causative variants (allelic heterogeneity), as well as for the identification of hot spot variants. It is still considered as the "gold standard" for mutation screening. However, sequencing very large genes may be practically expensive and time consuming. It is also used to confirm the results of next generation sequencing. It is also prudent not to opt directly for Sanger Sequencing when the clinical suspicion is of a disorder which is caused by several genes (locus heterogeneity).

Next generation sequencing (NGS)- This primarily includes Whole Exome sequencing and Focused Exome or clinical exome sequencing. Next generation sequencing can also be used as targeted panels for a group of disorders with overlapping phenotypes (eg Inborn errors of metabolism, Rasopathies) or for a same disorder

caused by variations in multiple genes (eg nonsyndromic deafness). It has the advantage of a more specific gene coverage.

In focused exome sequencing,4500-6000 genes with known disease causation/ association are sequenced. This approach is more practical for disorders with a well- defined clinical phenotype with a clear monogenic aetiology. In Whole exome sequencing, on the other hand, all the known protein coding genes (~ 20,000) are sequenced and it is the test of choice for complex disease phenotype with a suspicion of monogenic aetiology. Whole genome sequencing, though available is not used routinely for clinical purposes.

Types of results from Next Generation Sequencing and Chromosomal Microarray analysis.

Variants reported in relation to the phenotypic description provided by the clinician to the laboratory are of five types:

1. Pathogenic	Most likely cause of the disease under consideration
2. Likely Pathogenic	
3. Variant of uncertain significance*	
4. Likely benign	Unlikely cause of the disease under consideration
5. Benign	

* A variant falling under this category, needs re-evaluation and referral to a geneticist should preferably be made for interpretation of these ambiguous results.

It is also important to consider that the variant/variants identified are commensurate with the mode of inheritance of the suspected disorder.

Figure 4 summarizes simplified approach to genetic testing in clinical practice.

Tests for special conditions

1. **Triplet primed PCR (TPPCR)**- This test is specifically used for the diagnosis of a special class of disorders called as triplet repeat disorders. These include Fragile X, Myotonic dystrophy, spinocerebellar ataxia, Friedrich's ataxia and Huntington's disease. Next generation sequencing techniques do not detect these.
2. **Methylation sensitive MLPA(MSMLPA)**- This test is an extension of MLPA technology in which comparison of the methylation ratios of specific probes between controls and patient sample is used to diagnose imprinting disorders especially Prader Willi Syndrome (PWS), Angelman syndrome (AS) involving imprinted regions on chromosome 15q11.2q13; Beckwith Weideman syndrome(BWS), Russell Silver syndrome(RSS) involving chromosomal region 11p15. MSMLPA can be offered as a first-tier investigation when suspecting one of these disorders.
3. **Inversion PCR**- This test is used for detection of intron 22 inversion in patients with severe Hemophilia A and if the test is negative, then sequencing of the entire Factor VIII gene is carried out.
4. **Chromosomal breakage studies**- Useful for some disorders associated with impaired DNA repair.

Sometimes, the phenotype is complex and it is difficult to delineate whether the cause is chromosomal or monogenic. In such cases, one may have to adopt a wider approach and go in a stepwise manner doing Chromosomal Microarray followed by Whole exome sequencing.

Samples used for genetic testing

1. Karyotype – Whole blood, at least 2 ml in a Heparinised vial (green cap), collected gently and free flowing maintaining strict asepsis (to prevent contamination) and used preferably within 4-6 hours.
2. DNA based testing- Unlike sensitive procedures like karyotyping, DNA is a robust molecule and can be obtained from blood preserved in

EDTA (preferably 5ml), tissues like muscle, skin, liver and even from buccal and uro-epithelial cells. Blood can easily be stored at 40 C for short term and at -200C for long term storage. Every effort should be made for storage of blood and/or tissue samples in critical undiagnosed cases with a suspected genetic aetiology.

Some important points to consider while offering a genetic test

1. Appropriate pre- test counseling – The first step is to offer an appropriate test, not to order one. While a detailed description of all the components of pre-test counseling are beyond the scope of this communication , one should always inform that the test may or may not be able to provide a definitive diagnosis, diagnosis may or may not affect the specific management, possibility of incidental findings, cost and turn around time which is usually 2-4 weeks for karyotype, 2-3 weeks for CMA, 2 weeks for MLPA and around 6 weeks for Exome sequencing. .
2. Analysis of a genetic test report in the light of patient's clinical presentation
3. Appropriate post- test counseling- If a diagnosis is made, the family should be informed about the course and prognosis of the disease. Specific treatments for the identified disorder should be discussed, their cost, availability, success rate should be explained. Risk of recurrence in subsequent pregnancies with option of prenatal testing and evaluation of at- risk family members should also be offered. If the cause is not identified, the family should be kept in follow up counseled empirically based on clinical diagnosis and likely inheritance .

The detection rate of the various test is not an absolute value and the diagnostic yield is always influenced by the phenotypic details including a detailed family history and ancillary investigations, provided by the clinician.

Conclusion

The progress in the field and the clinical utility of rational genetic testing makes it imperative for pediatricians to have a basic knowledge and understanding of these tests, so that timely diagnosis, appropriate management, counseling and referral can be offered to the patients and their families.

Suggested Reading

1. McPherson E. Genetic Diagnosis and Testing in Clinical Practice. Clin Med Res [Internet]. 2006 Jun [cited 2022 Jan 12];4(2):123. Available from: /pmc/articles/PMC1483893/
2. Elles, R., Wallace, A., 2010. Molecular diagnosis of genetic disease, third ed. Humana Press, Clifton, NJ.
3. Strachan, T., Read, A.P., 2011. Human molecular genetics, fourth ed. Garland Science, London.
4. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424. doi:10.1038/gim.2015.30
5. Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, Raca G, Ritter DI, South ST, Thorland EC, Pineda-Alvarez D, Aradhya S, Martin CL. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med. 2020 Feb;22(2):245-257. doi: 10.1038/s41436-019-0686-8. Epub 2019 Nov 6. Erratum in: Genet Med. 2021
6. Ranganath P., Lakshmi D., Aggarwal S,2017. Genetics Update for the Next Generation Clinician. The Society of Indian Academy of Medical Genetics.

Figure 1:Karyotye showing 14; 21 translocation :47,XX,+21,t(14;21)(q21;q21)

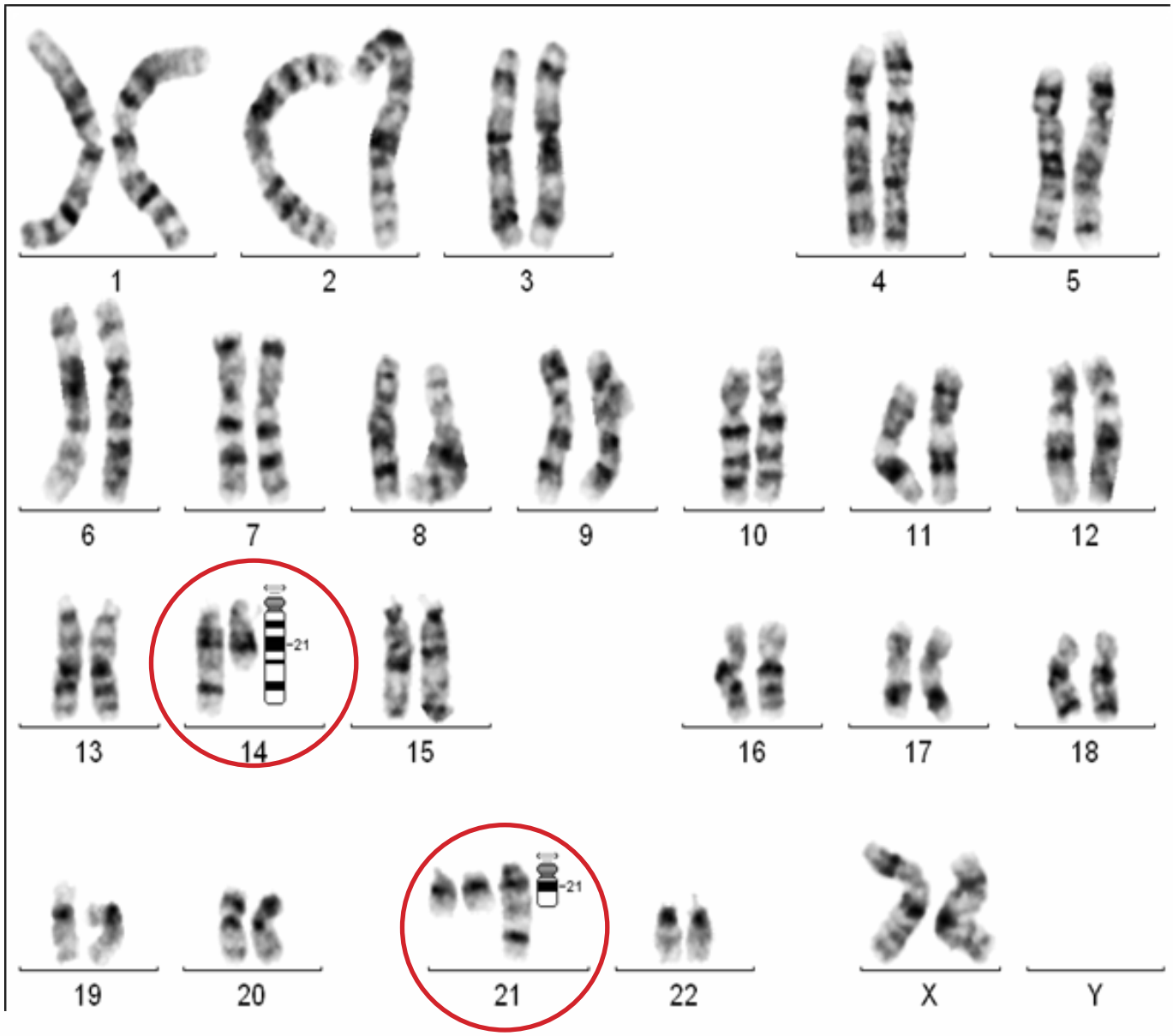


Figure 2 : Showing deletion of multiple exons in a case of DMD indicated by arrow

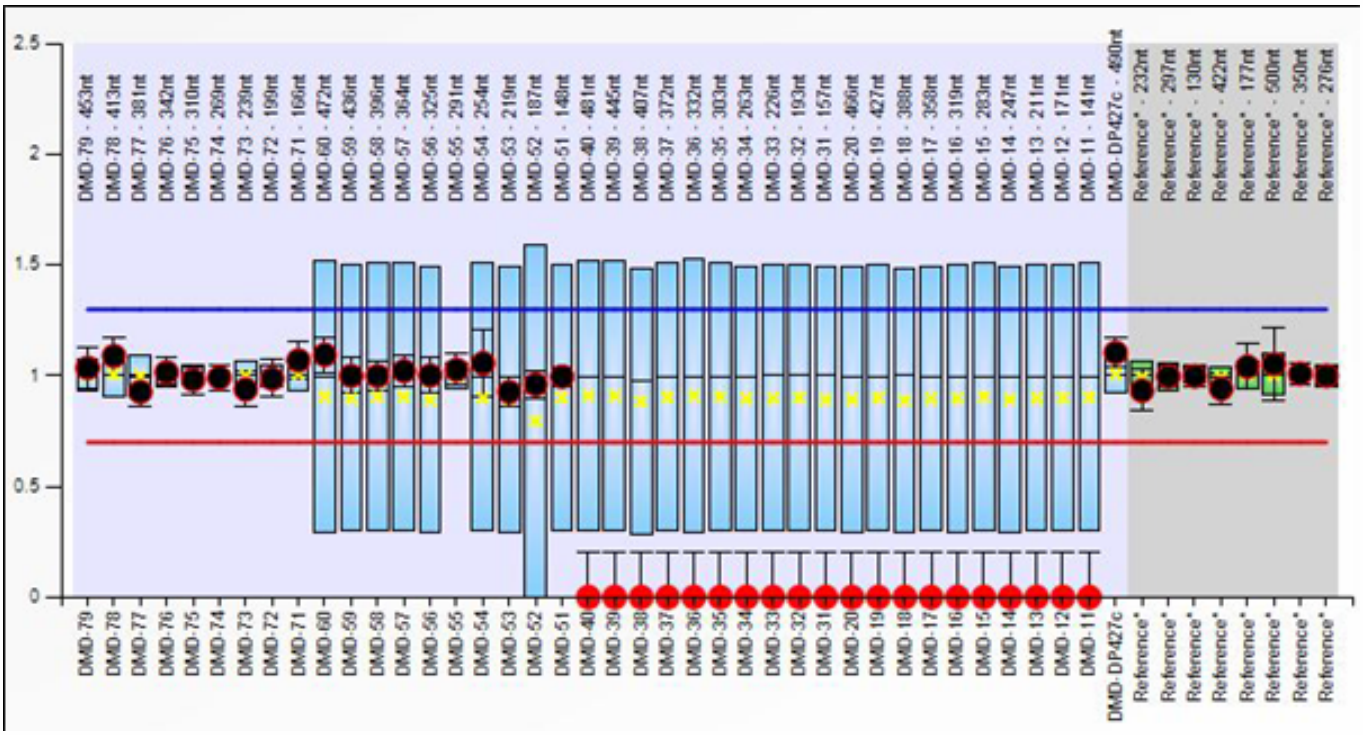


Figure 3: CMA showing deletion in the p arm of chromosome 5p15.33-p15.2 (Cri-du-Chat syndrome) . Picture of affected child is also shown

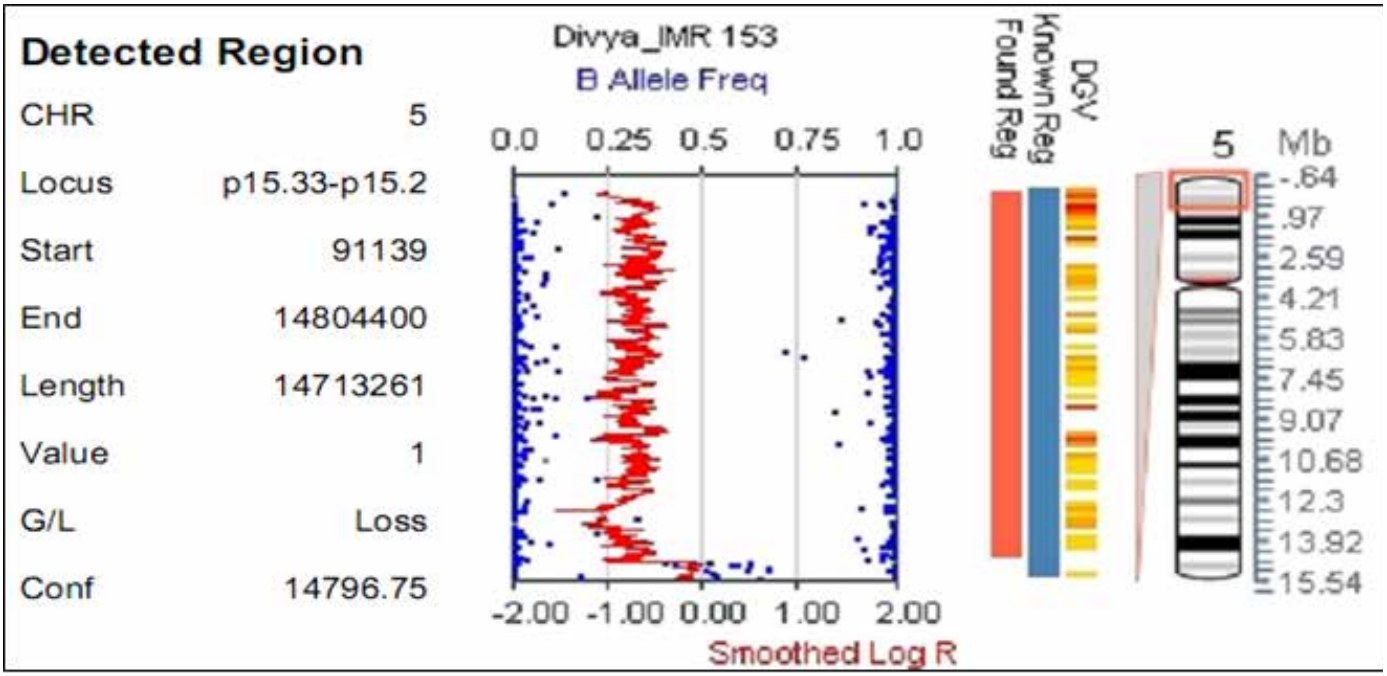


Figure 4. Approach to genetic testing in a clinical setting

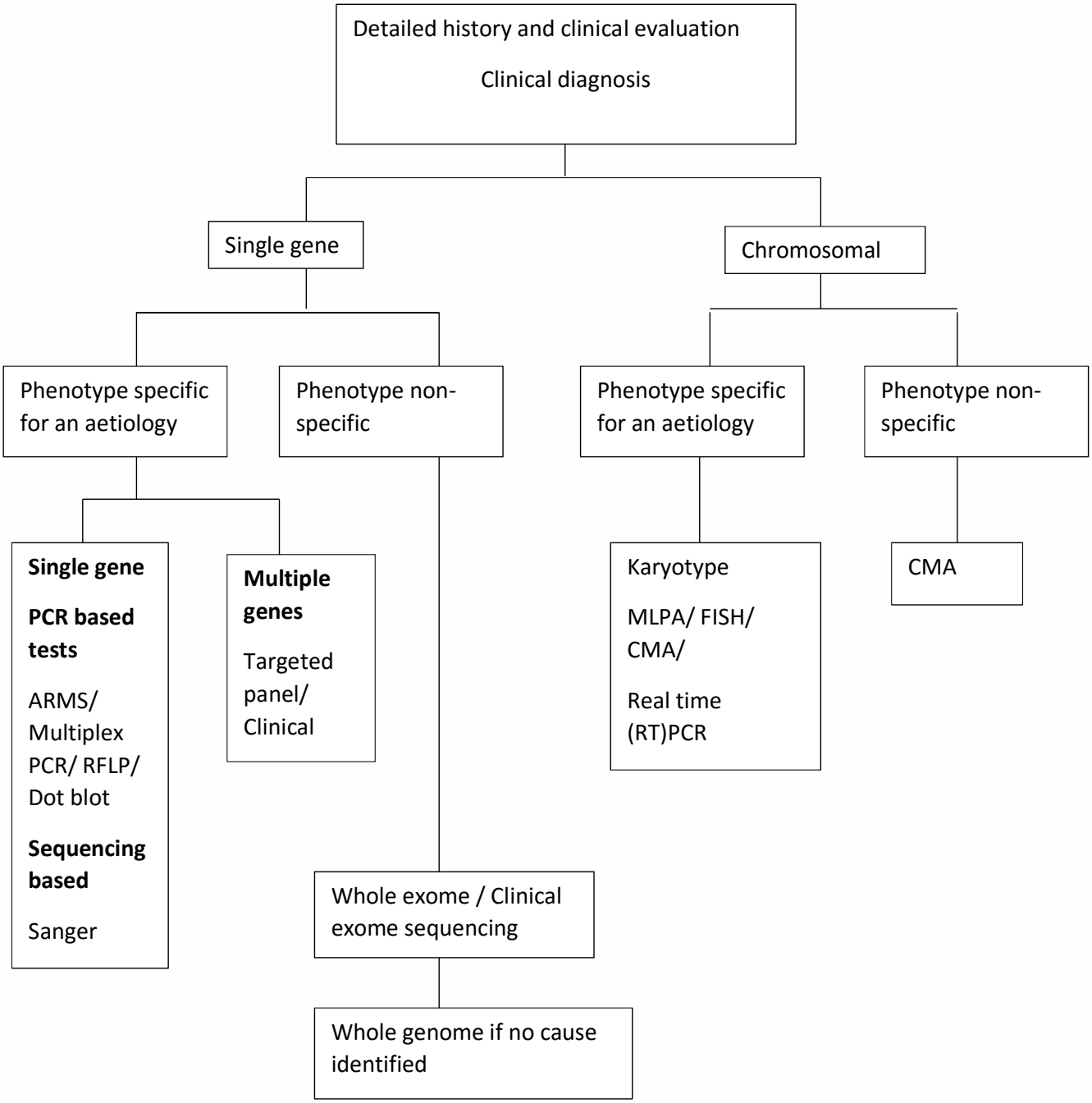


Table 1. Techniques commonly used for detection of copy number structural variants

Technology	Major utility	Strengths	Limitations
MLPA/ MSMLPA	DMD, SMA, PWS, Angelman, BWS, RSS, subtelomeric deletions in unexplained ID	40 different deletions can be studied using single kit, results within 48 hours	Does not indicate size of variant; only known variations detected
FISH	Microdeletions/ duplications/ aneuploidy (resolution 50-100kb)	Best for mosaicism, identification of marker chromosome, cancer cytogenetics	Does not indicate the exact size of deletion; only known variations detected
CMA	Unexplained ID/GDD + dysmorphism+ behaviour abnormality+structural malformations Resolution (resolution 200 kb or even more)	Both known and unknown variants detected	The results depend on probe density, result interpretation can be challenging
Multiplex PCR	DMD	Does not need any specific equipment other than PCR machine, up to 5 different deletions can be detected in a single tube	Does not indicate size of variant; only known variations detected
Gap PCR	Alpha thalassemia		Only known variations detected

Utility of Next Generation Sequencing in Clinical Practice

ANJANA KAR
ASHWIN DALAL
Diagnostics Division,
Centre for DNA Fingerprinting and Diagnostics,
Hyderabad

Genetic diseases are caused due to pathogenic variations in human DNA. These variations can be identified by elucidating the sequencing of A,T,G,C nucleotides in DNA using various sequencing strategies. Next Generation Sequencing (NGS) is a technique of massively parallel DNA sequencing, where millions of DNA fragments are sequenced in parallel. NGS aims for investigation of multiple genes or the whole exome/genome for finding genetic etiology explaining clinical features in the patient. NGS technologies are not only cost effective and rapid, but also highly accurate and reproducible. Let us understand more about utility of NGS in clinical practice:

Q1 : What are the various technologies used in sequencing of DNA?

The foundation of DNA sequencing was laid by Sanger Dideoxy Chain Termination Method (Sanger sequencing) and Maxam Gilbert Chemical Cleavage Method. Both these methods are useful to sequence short fragments of DNA of around 1000 bases. The second generation methods, termed as Next Generation Sequencing (NGS), is a process of massively parallel sequencing of millions to billions of short DNA fragments (35-500 bp). Determination of sequences is done either by taking high resolution images after incorporation of nucleotide e.g., Illumina sequencers or by determination of pH change on semiconductor chip e.g., Ion Torrent. The third-generation sequencing methods aim for longer sequencing reads (10-15kb) by using DNA fragment in a well which is bound to modified DNA Polymerase followed by detection of illumination during sequencing process e.g., PacBio. The fourth-generation techniques are based on passing of DNA fragments through a "pore" and measuring differing current with passage of each nucleotide e.g., Oxford Nanopore.

Q2 : What are the situations where Sanger sequencing alone can be used to achieve a diagnosis?

Sanger Sequencing is method of choice for DNA sequencing in the cases where there is clear diagnosis based on clinical features/examination with following scenarios (1) If the disease under investigation is known to be caused due to single or few mutations e.g., Achondroplasia, Sickle cell disease, Apert syndrome (2) Gene for the disease under question is small and can be sequenced completely with one or few Sanger sequencing processes e.g. Beta thalassemia is caused due t mutations in beta globin gene which has only 3 exons (3) Cases where genetic mutation for disease is available from previously affected child and prenatal testing is required in future pregnancies or for carrier analysis in family.

Q3 What are the situations where Sanger Sequencing is not suitable and Next Generation Sequencing is helpful?

Sanger sequencing is considered to be laborious, expensive and time consuming in certain situations and is not preferred. NGS is considered as a better choice in such situations like (1) Genetic diagnosis of diseases that are caused by pathogenic variants in more than one gene, e.g. Maple Syrup Urine disease (MSUD) can be caused due to mutations in 3 genes, Deafness/ Blindness can be caused due to mutations in more than 70 genes (2) Genetic diagnosis of diseases which are caused due to mutations in large genes, e.g. Osteogenesis imperfecta Type I can be caused due to mutations in COL1A1 and COL1A2 genes which consist of 101 exons. (3) Genetic diagnosis of patients where a definite clinical diagnosis based on biochemical/radiological investigations is not available, e.g. patient with intellectual disability and/or dysmorphic features (5) Carrier screening in couple with family history suggestive of genetic disorder but the exact disorder is not known

Q4 What are various ways in which NGS can be used in clinical practice?

NGS is majorly used in clinical practice for diagnostic evaluation to identify a genetic etiology of disease in patient which helps in early diagnosis and proper management of the disease as well as for better understanding of disease mechanisms. Currently different NGS approaches are in use i.e. Targeted Panels Sequencing (TPS), Whole Exome Sequencing (WES)/Clinical Exome Sequencing (CES) and Whole Genome Sequencing (WGS). TPS is used for diseases/phenotypes which are known to be caused by a group of genes wherein sequencing of a gene panel is done e.g. Genetic epilepsy panel, Muscular dystrophy panel. CES is a large panel of about 5500 genes wherein sequencing of genes known to cause single gene disorder in humans is done. WES is sequencing of all ~19500 genes in human genome. WGS refers to sequencing of entire genome including the coding and non-coding regions.

Q5 What is Targeted Panel Sequencing? What are advantages / disadvantages?

Targeted Panel Sequencing (TPS) is selective sequencing of few genes (20 -200) of clinical importance which are known to lead to a particular disease/phenotype. TPS is used for genetic diagnosis in group of diseases/phenotypes such as hearing loss, retinitis pigmentosa, inherited cancer etc. The advantages of TPS are that, it focuses on limited number of genes and sequencing is done with better coverage. This increases the analytical sensitivity and specificity of the test. TSP is cost effective compared to WES or WGS. It can also detect heterozygous variants with high confidence as well as mosaicism. Major disadvantages of TPS are that it cannot detect mutations in genes which are not included in the panel. Hence TPS should be mainly used in scenarios where a definitive diagnosis is available based on clinical/biochemical/radiological investigation and sequencing using sanger sequencing is going to be expensive. The large targeted panel commonly used in clinical practice consists of about 5500 genes which are known to cause human genetic disease. It is referred to as Clinical exome/Mendeliome/Focussed exome sequencing. The advantage of this panel is that it is cheaper than whole exome sequencing but the disadvantage is that it will not detect mutations in newly identified genes which are not included in the panel.

Q6 What is Whole Exome Sequencing? What are advantages / disadvantages?

Whole Exome Sequencing (WES) is sequencing of entire coding regions of genome (exons of ~20,000 genes), which is ~1% of the genome. It is known that 85% of Mendelian disorders are caused by pathogenic variants in protein coding regions. Hence WES is likely to give the genetic diagnosis in large proportion of patients. WES is widely used in patients with

suspected Mendelian disorder where a definitive diagnosis is not available e.g syndromic intellectual disability, multiple malformation syndrome etc. It is cost effective compared to WGS with diagnostic yield of 15–45%. The major disadvantages of WES include inability to identify variants in non-coding regions of genome (introns, intergenic regions) and to detect different genetic mechanisms like copy number variations, triplet repeat disorders, methylation disorders etc.

Q7 What is Whole Genome Sequencing? What are advantages / disadvantages?

Whole Genome Sequencing (WGS) is sequencing of entire human genome i.e., ~ 3 billion bases, including both coding and non-coding regions. WGS is mostly used as second line of investigation where WES/TPS are inconclusive. WGS is currently the most expensive method of sequencing for genetic diagnosis. WGS is advantageous over WES and TPS as it is single test for identification of single nucleotide coding variants, single nucleotide non-coding variants, small/ large insertions and deletions, copy number variations and structural variants. Further the sequencing coverage is uniform in WGS since not capture or amplification step is involved. However the disadvantages of WGS include generation of large amount of sequencing data, which requires extensive bioinformatics analysis for interpretation. Further, we still do not have enough knowledge for interpretation of variants in the non-coding regions.

Q8 How can I interpret a report of next generation sequencing?

It is very important that every pediatrician should be aware regarding interpretation of a NGS report since NGS based testing is being routinely used in clinical practice. It is easy to interpret the report if a systematic method is followed. NGS report mainly comprises of following segments:

Variant Information: Gene name, Transcript ID (most abundant mRNA of the gene), location of variant – chromosome number, nucleotide position on the chromosome, position on mRNA (c.) and position on protein (p.), Zygosity (presence in homozygous or heterozygous form in the patient).

Disease Information: Name of the disease, OMIM details, Segregation pattern of the disease

Variant frequency Information: indicates presence of variant in public databases like 1000 genomes, gnomAD etc with Minor allele frequency (MAF) or known mutation databases for known variants like Clinvar, OMIN etc.

Pathogenicity Information: indicates pathogenicity prediction by various tools for novel variants.

Based on the above information, the classification of variants is done using American College of Medical Genetics guidelines, that classify variants in 5 classes i.e. Pathogenic, Likely pathogenic, VOUS (Variant of Uncertain Significance), Likely benign and Benign.

A variant is classified as Pathogenic or Likely pathogenic variant, if the variant is found in a gene previously described with clinical features of the disease in the patient and is reported at very low frequency in normal population. The variant is classified as Benign/Likely Benign if the variant is present in normal population at high frequency and is not predicted to be damaging for the protein. However if we are not able to confidently classify a variant as Pathogenic/ Likely pathogenic or Benign/Likely Benign, then the variant is classified as a Variant of Uncertain Significance (VOUS).

Q9 What is a VOUS and what should I do next?

VOUS (Variant of Uncertain Significance) is a variant which cannot be confidently classified as Benign/Likely benign or Pathogenic/Likely pathogenic using the ACMG guidelines. These

mainly include novel variants in genes related to the phenotype in patient which are found at very low frequency in normal population. Reclassification of VOUS to Pathogenic or Benign can occur in future as more knowledge and information on genetics of the disease is obtained. Further study of such variants using mendelian segregation in family and functional analysis of effect on protein function using cell based or model organism based studies can help in reclassification of the variants. VOUS in NGS reports should be interpreted with caution by clinicians, and same should be explained to patients. VOUS should not be used for irreversible actions like genetic counseling, prenatal diagnosis or carrier screening. Once a VOUS is identified in the patient, the clinician can try to check the phenotype of patient in detail for genotype-phenotype correlation and keep the patient on follow up for any future reclassification of variant.

Q10 What is the role of NGS in prenatal diagnosis and carrier screening?

NGS technology has empowered the clinical implementation of non-invasive fetal aneuploidy screening for detection of trisomy 13,18 or 21 aneuploidies with higher sensitivity. However the use of NGS for prenatal diagnosis without a definite diagnosis in previous child or positive couple screening result, is controversial. If direct prenatal diagnosis is offered to a couple using NGS, then the couple needs to be counseled that only Pathogenic and Likely pathogenic variants will be identified and the test does not guarantee a healthy baby. VOUS should not be reported in prenatal diagnosis.

Primary prevention of genetic diseases can be done by carrier screening in couples planning for a pregnancy. WES/CES can be used for carrier screening with the basic objective to screen for presence of pathogenic variants in same gene in the couple, which can be transmitted to offspring. Prenatal diagnosis can be offered to the couple if a significant variant is identified. However it is important to note that only Pathogenic and Likely pathogenic variants should be reported in carrier screening and VOUS should not be reported.

Representative Case

A consanguineous family presented with two out of three children affected with intellectual disability and microcephaly (Fig 1 A,B). Since the phenotype in the patients can be caused due to large number of genes, we ordered for a whole exome sequencing. The exome sequencing resulted in identification of about 131974 variants in the patient. These variants were filtered based on population frequency and pathogenic potential, which ultimately led to identification of one disease causing variant (WDR62:NM_001083961.2:c.669delC:p.Phe223Glyfs*11) which was present in both the affected children (Fig 1C,D). The variant was a single base deletion of C which is likely to result in a frameshift and a premature termination codon after 11 amino acids. Mendelian segregation analysis using sanger sequencing in the family revealed that both the affected children are homozygous for the variant and both parents are carriers for the same variant which explains the autosomal recessive inheritance in the family (Fig 1E). The variant was classified a Pathogenic based on the ACMG criteria. The family was counseled regarding the cause of disease in the children and were informed about physiotherapy and special education. They were informed regarding the inheritance pattern of the disease and the possibility of prenatal diagnosis in next pregnancy to prevent occurrence of same disease in next child.

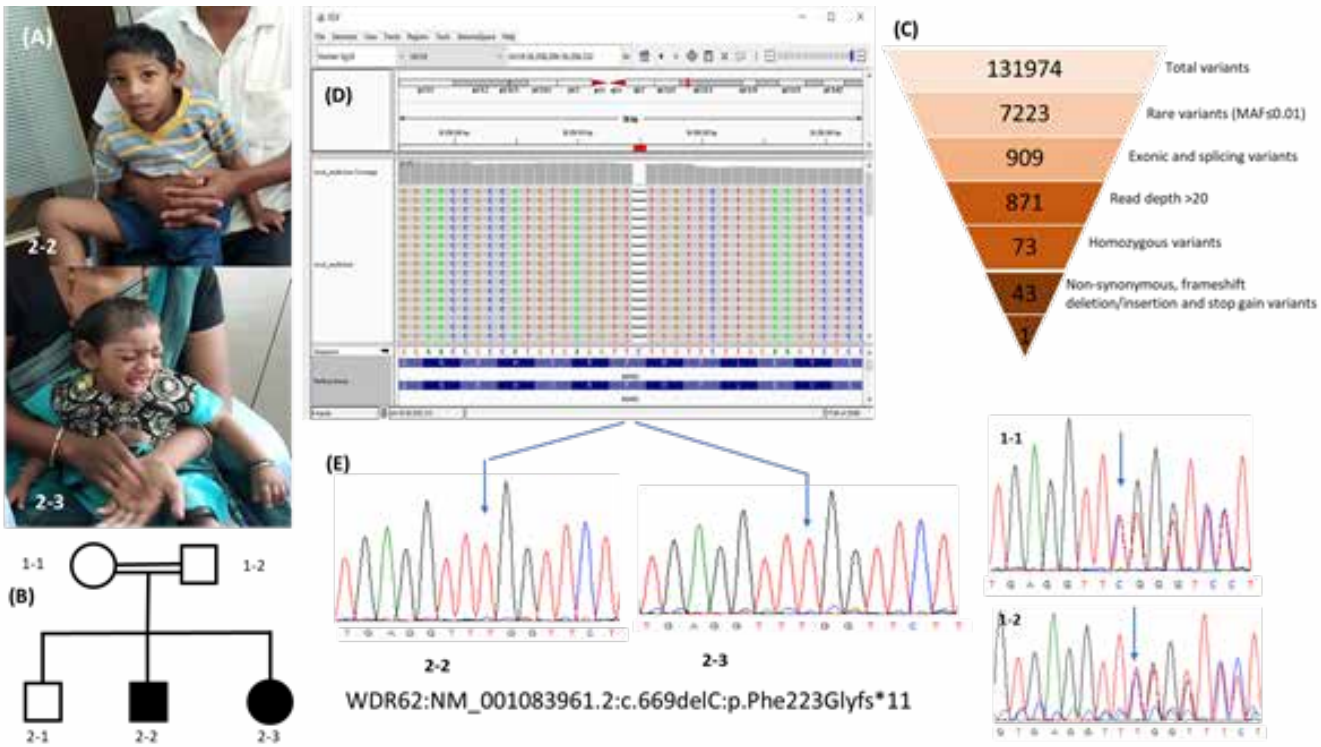


Figure1: (A) Photographs of proband and affected female sibling with microcephaly and intellectual disability (B) Pedigree of the family (C) Variant filtering strategy and corresponding variants in number identified through WES (Whole Exome Sequencing) (D) Schematic presentation of identified variant in WDR62 in proband via IGV (E) Schematic presentation of chromatogram (Sanger sequencing) in Proband 2-2, affected sibling 2-3, Mother 1-1 and Father 1-2.

NGS is not a magic bullet or "one tool for all diseases". But NGS has shown huge translational utility in diagnosis of genetic diseases and in finding genetic etiologies for unsolved cases. A judicious application of NGS based testing in clinical / diagnostic set up is likely to go a long way in helping the patients/families with genetic diseases. However it is very important for the pediatrician to understand the applications, advantages, disadvantages of each NGS based test as well as the principles of interpretation of the NGS result so that the patients and families are benefitted.

References:

Bamshad, M., Ng, S., Bidham, A. et al. Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet 12, 745 – 755 (2011).

Bhowmik, A. D. & Dalal, A. Exome Data Analysis for Clinicians: How & Why. Genetics Clinics 10, 5 – 10.

Chong, J. X., Bukingham, K. J., Jhangiani, S. N. et al. The Genetic Basis of Mendelian Phenotype: Discoveries, Challenges and Opportunities. Am J Hum Genet 97, 199 – 215 (2015).

Han, J. Y. & Lee, I. G. Genetic tests by next generation sequencing in children with developmental delay and/or intellectual disability. CEP 63(6), 195 – 202 (2020).

Posey, J. E., O'Donnell-Luria, A. H., Chong, J. X. et al. Insights into genetics, human biology, and disease gleaned from family based genomic studies. Genet in Medicine 21, 798 – 812 (2019).

Seaby, E. G., Pengelly, R. J. & Ennis, S. Exome sequencing explained: a practical guide to its clinical application. Brie in Func Genomics 15(5), 374 – 384 (2016).

Slatko, B. E., Gardner, A. F. & Anusubel, F. M. Overview of Next Generation Sequencing Technologies. Curr Protoc Mol Biol 122(1) (2018).

Down syndrome: One extra chromosome makes one extraordinary child

KSHITIJA PATIL
DR NEERJA GUPTA MD, DM
Additional Professor, Genetic Division,
Department of Paediatrics
AIIMS, New Delhi

Introduction:

Down syndrome was first described by an English physician John Langdon Down in 1866, but its association with chromosome 21 was established by Lejeune Ja French pediatric geneticist. The prevalence of Down syndrome in a recent study is reported to be approximately 1 in 792 live births1 in United states of America whereas in India it has been reported as 1 in 853 live births.[2]

Diagnostic criteria:

Down syndrome is diagnosed clinically based on the gestalt. Figure 1 shows facial features of Down syndrome children at various ages. The typical features include brachycephaly, flat face, epicanthal folds, upslanting palpebral fissures, small dysplastic ears, protruding tongue, open mouth, short neck, redundant skin, short 5th fingers and clinodactyly, single transverse palmar crease (simian crease), wide gap between 1st and 2nd toe(Figure 1). Brushfield spots of the iris are common. Chest examination may show signs of congenital heart disease. Dermatoglyphic analysis can demonstrate characteristic ulnar loops present on the index and middle fingers, radial loops on 4th and 5th fingers, and a distal palmar tri-radius. Radiograph of pelvis may demonstrate a hypoplastic and flared out iliac wings andflat acetabular angles. The gold standard for establishing diagnosis of Down syndrome isdemonstrating an extra copy ofchromosome 21 on karyotype analysis.

Fig 1 : Upper panel - Characteristic Down syndrome faciesat various ages - (A) 6 months (B) 1.5 years (C) 2 years (D) 3.5 years

Lower panel-(E)Single transverse palmar crease and fifth finger clinodactyly (F) Increased gap between first and second toe (G)Radiograph of pelvis showing hypoplastic and flared out iliac wings and flat acetabular angles

Etiology, Pathogenesis and Genetics:

Downsyndromeiscausedbychromosome21 trisomy.Mechanism of trisomy is nondisjunction in 95% cases. Robertsonian translocation and mosaicism account for remaining 5%, of which thetranslocation of 14 and 21 is most common with about half beingfamilial.

The characteristic features of Down syndrome may be relatednot only to dosage effects of genes onthe extra chromosome 21, but also epigenetic mechanisms.Multiple developmental pathwaysmay get disrupted by the extra genetic material. [3,4]

The most important determinant of nondisjunction trisomy 21 is advanced maternal age. Maternal meiotic errors account for 85–90% of cases whereas paternal errors are in 5–10%. Postzygotic mitotic nondisjunction is seen in5% of cases.[5]

For a mother of a child with trisomy 21, the risk of recurrence in a future pregnancy is estimatedat approximately 1%. The risk is higher in parents who are carriers of a translocation. Robertsonian translocation in the father has a 3–5% risk, and in mother has a 10–15% risk of recurrence of Down syndrome whereas a 21;21translocation in either, will have 100% risk of recurrence.6

Diagnostic tests:

Karyotype is the gold standard test for diagnosing Down syndrome. Other tests which may be used are fluorescence in situ hybridization(FISH) and quantitative fluorescent polymerasechain reaction (QF-PCR).For the early detection of Down syndrome,prenatal screening tests by ultrasound and biochemical tests in first and second trimester are available in majority of the cities in India. Non-invasive prenatal screening (NIPS) test using fetal cell free fetal DNA may be offered to the high risk groups. It has a high sensitivityas well as specificity of >99%.7 Alternatively, invasive test namely amniocentesis or chorionic villus sampling can be performed to obtain sample for karyotype.

Management:

Growth:

Down syndrome can lead to lower birth weight andlater growth rates may be slow. Hence, Down syndrome-specific growth charts developed in multiple countries should be used to record anthropometry.8-12Evaluation for growth failure should include tests for hypothyroidism, celiac disease and growth hormone deficiency. In case of feeding difficulty, vomiting or recurrent pneumonias, barium swallow should be planned to rule out gastroesophageal reflux or tracheoesophageal fistula.

Down syndrome is associated with increased risk for obesity in later age. A complete dietary history and work up for diabetes (serum insulin, HbA1c), hypothyroidism (free T4, TSH), and sleepdisordered breathing should be done.

Management:For feeding issues in neonates with Down syndrome, proper positioning and adequate burping should be explained. Appropriate referral to gastroenterologist for gastroesophageal reflux or celiac disease and anendocrinologist for growth failure related to growth hormone deficiency or hypothyroidism. There are no long-termstudies documenting benefit of growth hormone treatment in Down syndrome.13

Development:

Down syndrome is the commonest genetic cause of intellectualdisability.The degree of cognitive impairment can vary from mild (IQ of 50 –70), moderate (IQ of 35–50) tosevere (IQ of 20 –35).13Cognitivedevelopment is found to be influenced by maternal education, difference in temperament, school experiences andseverity of other associated medical conditions.14Besides, hypotonia can affect gross motor development. Fine motor skills are also delayed especially complex ones.15 Expressive languageability is generally more impaired than receptive language skills.Likewise, language usage in social context is better compared to use of syntax.16Attention deficit hyperactivity disorderis relatively common, seen in 34% cases.17 Almost 100% of Down syndrome develop Alzheimer disease by the age of 35–40 years.18

Management: Earlyintervention and physical therapy are beneficial.19 Over time, there have been claims of therapeutic value forsupplements, hormones, vitamins, glutamic acid, 5-OH-tryptophan,injectionof fetal cells etc. for improving cognitive abilities. However, most studies claiming benefit are anecdotal and properly controlled trials have not shown benefit. Screening test for autism and attention deficit hyperactivity disordershould be done before entering school. Psychotropic drugs should be used with caution.13

Cardiovascular:

Congenital heart disease is documented in approximately 50% of Down syndrome. The most commonheart disease in Down syndrome is endocardial cushion defect, followed by atrialseptal defect, ventricular septal defect, patent ductus arteriosus,coarctation of aorta, and tetralogy of Fallot.20

Management: Clinical examination, echocardiography and appropriate referral to cardiology for necessary medical and surgical intervention.13Down syndrome children with large ventricularseptal defects without pulmonaryblood flow obstructionshould be offered surgical repair before 4 months ofage to avoid complication of pulmonary artery hypertension.[13]

Endocrine:

Screening for neonatal hypothyroidism is important. A recent study demonstrated congenital hypothyroidism in 2% cases of Down syndrome whereas most hadsubclinical hypothyroidism (10%).[21] Borderline abnormality in thyroid function test on screening warrants repeating the tests in six weeksand referral to endocrinologist for starting L thyroxine if the values are abnormal.Another issue is related to sexual maturation, where it must be noted that any variation from normalis not expected and requires evaluationfor cause.

Management: Screening at birth and six monthly till one year and annually thereafter. Standard management of hypothyroidism under the guidance of an endocrinologist should be offered.13

Audiologic:

Sensorineural hearing loss due to inner ear abnormalities have been observed in manycasesof Down syndrome. Also, they are prone to conductive hearing loss due to recurrent otitis media which can be found in as many as 50-70% cases with Down syndrome.[22]

Management: Newborn screening for hearing by objective testing such as brainstem auditory evokedresponse (BAER). Initial 3 monthly follow up should be done and later 6 monthly or annually depending on risk of otitis media. Cases with hearing impairment should be referred to speech-language pathologist for early intervention.13

Ophthalmologic:

Cataracts occur in 15% and refractive error in 50%. Also, hyperopia, strabismus, astigmatism, and blepharitishave the high prevalence.Hypoplastic peripheral irides and Brushfield spots arecommon.[23]

Management: Initial evaluation by pediatric ophthalmologist should be within the first six months followed by annual or two-yearly eye examinations.13Correction of refractive error and cataract removal surgery can be planned as per requirement by ophthalmologist.

Musculoskeletal:

The incidence of occipito-atlantoaxial instability in Down syndrome is about 10–30%, and approximately 1% develop neurological symptoms. Other musculoskeletal issues are ligament laxity neck instabilities, hip subluxation or slipped capital femoralepiphysis, instability of patellofemoral joint, genu valgum, foot deformities like pes planus and spinal scoliosis.

Management: Children with signs and symptoms of myelopathy should be screened by radiograph of cervical spine and referred immediately to neurosurgeon24. Screening by radiograph of the cervical spine in asymptomatic children is not recommended. Parents shouldbe sensitized about the symptoms of myelopathy and about neck care to avoid excess flexion or extension. They should be explained the increased risk of cervical spinal cord injury due to contact sports such as football, diving from height, karate, boxing and gymnastics.Children should be encouraged for lower risk sports and neurologicalmonitoring must be done at every visit.[13]

Gastrointestinal:

There is increased prevalence of celiac disease in Down syndrome (up to 18.6%).25

Other known gastrointestinal disorders are tracheoesophageal fistula, duodenal atresia, Hirschsprung disease,annular pancreas,pyloric and anal stenosis.

Management: As discussed previously, barium swallow should be performed in cases with feeding difficulty and vomiting. Appropriate investigation for Hirschsprung disease like checking rectum for stool, abdominal x ray and if required, rectal biopsy should be done. Tissue transglutaminaseimmunoglobulin A (IgA) level and quantitative IgAmust be tested in symptomatic children to rule out celiac disease. If suggestive, plan to start on gluten free diet.[13]

Sleep disorder:

The presence of hypotonia and relative underdevelopment of midface causesan increased risk of obstructive sleep apnea in Down syndrome, even withnormal-sized adeno-tonsillar tissue. It can lead to depression, new onset mood disorder and decline in adaptive skills.[26]

Management: Parents should be counseled about symptoms like snoring, night awakening and subsequent daytime sleepiness, heavy breathing, and behavioral issues. All children with Down syndrome should have a sleepstudy by the age of 4 years old.13Some patients may require continuous positive airway pressure while others may require tonsillectomy or adenoidectomy.

Dental:

Common dental problems include delayed and asynchronous primary and secondary dentition, supernumerary teeth, partial anodontia, abnormal shaped crown and malocclusion.

Management: Parents should be instructed regarding dental hygiene and frequent visits to dentist. Those with congenital heart disease will require antibacterial prophylaxis against subacute bacterial endocarditis.

Dermatological:

Cutis marmorata occurs in about 8–13% of children whereas seborrheic dermatitis occurs in 30% cases with Down syndrome. Other dermatological disorders namely alopecia areata which is autoimmune has prevalence ranging from 1.3 to 11% in Down syndrome.27,28

Management: Thorough examination at every visit and referral to dermatologist, if required.

Immunologic:

There is a mild reductionin T-cell and B-cell counts in Down syndrome. Infants do not show normal lymphocyteexpansion and have variable size of thymus. Also, there is a reduction in naive T-cell percentages with suboptimalantibody responses to immunizations.The totaland specific immunoglobulin A are reduced and there is decreased chemotaxis of neutrophils.29It has been reportedthat Down syndrome cases have approximately 10hospital admissions in their lifetime,common reasons beingotitis media, upper or lower respiratory tract infection, cardiac disease with failure, and adenoid/tonsillitis.30

Management:Evaluate respiratory and heart disease during newborn examinationsand on follow up visits thereafter. Enable prompt treatment of any respiratory infections. Normal vaccination schedule should be followed. Special vaccines including influenza and pneumococcal vaccine can be advised.[13]

Neurologic:

Neuromuscularhypotonia, epilepsy, intellectual disability, neuropsychiatric problems, cervical cord compression, gait abnormalities, stereotypic movements and seizures are more common in Down syndrome compared to normal population.Five to 13% of casesof Down syndrome have seizures31, out of that, 40% will

have seizures (usually infantilespasms) before1 year.[32]

Management: Examination for focal deficits at every visit. Management of seizures as per protocol.

Urological:

Anomaliesreported arerenal anomalies or hypoplasia, hypospadias, posterior urethral-valves, micropenis, vesico-ureteric reflux, and chronic renal failure.

Management: Screening for renal anomalieswith ultrasound has been suggested.33Examination for cryptorchidism and periodic examination of testes.13

Neoplasia:

Individuals with Down syndrome are 20 times more prone for leukemia between 1–10 years of age. Acute lymphoblastic leukemia(DS-ALL) and acute megakaryocytic leukemia (AML-M7/AMKL) being the commonest leukemias noted. Transient myeloproliferative disorder is seen in 10% of neonates with Downsyndrome. Most cases regress spontaneously, however 20–30% go on to develop acutemegakaryocytic leukemia.34Solid tumors are infrequent, with testicular cancer being the only one with an increased standardized incidence compared to general population.

Management: Infants with transient myeloproliferative disorder or polycythemia should be managed by prompt referral to hematologist/oncologist. Parents must be counseled regarding the risk of leukemia. The testes should be examined periodically to rule out testicular germ cell tumors.Bone marrow transplant for acute myeloid leukemiais less effective compared to standard chemotherapy in these cases and hence avoided as thefirst line of management.34In July 2017, single-dose gene therapy called CAR-T cell therapy/Kymriah was approved by the FDAfor management of B cell-ALL in youngadults, where other treatment modalities havefailed.

Table 1 provides Down syndrome health supervision chart.

Conclusions

It is important for primary care pediatrician to recognize Down syndrome. A timely diagnosis is an essential step towards better outcome in these individuals. Monitoring for common childhood and adulthood issues and timely initiation of specific management is likely to improve the outcome. Early intervention results in better cognitive outcome. Guidance about occupational therapy provides opportunity for developing independent or semi independent skills. An understanding about the genetic mechanisms and availability of prenatal screening are helpful in appropriate genetic counseling and prenatal diagnosis.

References:

1. de Graaf G, Buckley F, Skotko BG (2015) Estimates of the live births,natural losses, and elective terminations with Down syndrome inthe United States. Am J Med Genet A 167A(4):756–767.
2. Kaur A, Singh J. Chromosomal abnormalities: Genetic disease burden in India. Int. J. Hum. Genet. 2010;10(1-3):1- 14.
3. Bartesaghi R, Haydar TF, Delabar JM, Dierssen M, Martinez-Cue C, Bianchi DW (2015) New Perspectives for the Rescue ofCognitive Disability in Down Syndrome. J Neurosci35(41):13843–13852.
4. Gardiner KJ (2015) Pharmacological approaches to improvingcognitive function in Down syndrome: current status and considerations.Drug Des Devel Ther 9:103–125.
5. Ghosh S, Feingold E, Dey SK (2009) Etiology of Down syndrome:Evidence for consistent association among altered meioticrecombination, nondisjunction, and maternal age across populations.Am J Med Genet A 149A(7):1415–1420.
6. Berr C, Borghi E, Rethore MO, Lejeune J, Alperovitch A (1990)Risk of Down syndrome in relatives of trisomy 21 children. Acase-control study. Annal Genet 33(3):137–

Table 1 Down syndrome health supervision chart[13].

Test	Birth-6mth	6mth-1 year	1 - 5 years	5 years- adolescence
Anthropometry*	✓	✓	Annually	
Karyotype*	✓			
Thyroid profile*	✓	✓	Annually	
Hearing screening (OAE)	✓			
Audiometry (BERA)*		✓	Annually	
Ear examination (for otitis media, upper airway obstruction, etc)*			Annually	
2D ECHO*	✓			
Ba swallow (In case of feeding difficulty, recurrent pneumonia, etc)	✓			
Eye exam for cataract, refractive error, strabismus,etc*	✓	✓	Annually	Every 2 years
Hemogram to r/o myeloproliferative disorder	✓			✓
Hemogram for Iron deficiency anaemia*		✓	Annually	
Neurological examination for myopathy*	✓	✓	Annually	
Formal Intelligence quotient testing and behaviour assessment			✓	
Sleep study			✓	
Celiac disease work up (if symptomatic)		✓		
Early stimulation, physiotherapy, Occupational therapy*	✓	✓	Every visit	

As all Down syndrome patients may not present at birth, tests with * should be advised at first visit.

140.

7. Norton ME, Wapner RJ (2015) Cell-free DNA Analysis for NoninvasiveExamination of Trisomy. N Engl J Med 373(26):2582.
8. Zemel BS, Pipan M, Stallings VA, Hall W, Schadt K, Freedman DS,Thorpe P (2015) Growth charts for children with Down syndromein the United States. Pediatrics 136:e1204–1211.
9. Afifi HH, Aglan MS, Zaki ME, Thomas MM, Tosson AM (2012)Growth charts of Down syndrome in Egypt: a study of 434 children0-36 months of age. Am J Med Genet A 158A(11):2647–2655.
10. Bertapelli F, Agiovlasitis S, Machado MR, do Val Roso R, Guerra-Junior G (2017) Growth charts for Brazilian children withDown syndrome: Birth to 20 years of age. J Epidemiol27(6):265–273.
11. Su X, Lau JT, Yu CM, Chow CB, Lee LP, But BW, Yam WKL, TSEPWT, Fung ELW, Choi KC (2014) Growth charts for ChineseDown syndrome children from birth to 14 years. Arch Dis Child 99(9):824–829.
12. Tuysuz B, Goknar NT, Ozturk B (2012) Growth charts of Turkishchildren with Down syndrome. Am J Med Genet A158A(11):2656–2664.
13. Bull MJ (2011) Health Supervision for Children With DownSyndrome. Pediatrics 128(2):393–406.
14. Couzens D, Haynes M, Cuskelly M (2012) Individual and environmentalcharacteristics associated with cognitive development inDown syndrome: a longitudinal study. J Appl Res IntellectDisabil 25(5):396–413.

15. Frank K, Esbensen AJ (2015) Fine motor and self-care milestonesfor individuals with Down syndrome using a RetrospectiveChart Review. J Intellect Disabil Res 59(8):719–729.
16. Abbeduto L, Warren SF, Conners FA (2007) Languagedevelopment in Down syndrome: from the prelinguistic periodto the acquisition of literacy. Mental Retard Dev Disabil ResRev 13(3):247–261.
17. Oxelgren UW, Myrelid Å, Annerén G, Ekstam B, Göransson C,Holmbom A, … Fernell E (2017) Prevalence of autism andattention-deficit–hyperactivity disorder in Down syndrome: apopulation-based study. Dev Med Child Neurol 59(3):276–283.
18. Head E, Powell D, Gold BT, Schmitt FA (2012) Alzheimer's diseasein Down syndrome. Eur J Neurodegener Dis 1(3):353–364.
19. Dreyer BP. Early Childhood Stimulation in the Developing andDeveloped World: If Not Now, When? 2011. Pediatrics127(5):975–977.
20. Stoll C, Dott B, Alembik Y, Roth MP (2015) Associated congenitalanomalies among cases with Down syndrome. Eur J Med Genet58(12):674–680.
21. Pierce MJ, LaFranchi SH, Pinter JD (2017) Characterization ofThyroid Abnormalities in a Large Cohort of Children withDown Syndrome. Horm Res Paediatr 87(3):170–178.
22. Maris M, Wojciechowski M, Van de Heyning P, Boudewyns A (2014)A cross-sectional analysis of otitis media with effusion in childrenwith Down syndrome. Eur J Pediatr 173(10):1319–1325.
23. Creavin AL, Brown RD (2009) Ophthalmic abnormalities in childrenwith Down syndrome. J PediatrOphthalmol Strabismus46(2):76–82.
24. Pizzutillo P, Herman M. Cervical spine issuesin Down syndrome. J PediatrOrthop. 2005;25(2):253–259.
25. Pavlovic M, Berenji K, Bukurov M (2017) Screening of celiac diseasein Down syndrome - Old and new dilemmas. World J ClinCases 5(7):264–269.
26. Capone GT, Aidikoff JM, Taylor K, Rykiel N (2013) Adolescentsand young adults with Down syndrome presenting to a medicalclinic with depression: co-morbid obstructive sleep apnea. Am JMed Genet A 161A(9):2188–2196.
27. Barankin B, Guenther L (2001) Dermatological manifestations ofDown's syndrome. J Cutaneous Med Surg 5(4):289–293.
28. Daneshpazhooh M, Nazemi TM, Bigdeloo L, Yoosefi M (2007)Mucocutaneous findings in 100 children with Down syndrome.Pediatr Dermatol 24(3):317–320.
29. Colvin KL, Yeager ME (2017) What people with Down Syndromecan teach us about cardiopulmonary disease. Eur Resp Rev26(143).
30. Fitzgerald P, Leonard H, Pikora TJ, Bourke J, Hammond G (2013)Hospital admissions in children with down syndrome: experienceof a population-based cohort followed from birth. PLoSOne 8(8):e70401.
31. Arya R, Kabra M, Gulati S. Epilepsy in children with Down syndrome. Epileptic Disord. 2011 Mar;13(1):1-7.
32. Pueschel SM, Louis S, McKnight P. Seizure disorders in Down syndrome. Arch Neurol. 1991 Mar;48(3):318-20.
33. Kupferman JC, Druschel CM, Kupchik GS (2009) Increased prevalenceof renal and urinary tract anomalies in children withDown syndrome. Pediatrics 124(4):e615–621.
34. Gamis AS, Smith FO (2012) Transient myeloproliferative disorderin children with Down syndrome: clarity to this enigmatic disorder.Br J Haematol 159(3):277–287.

A Clinical approach to skeletal dysplasia

DR SANKAR V H
MD,MNAMS (Paed) DM (Medical genetics)
Additional Professor & Geneticist
Department of paediatrics, SAT Hospital
In Charge, Genetic & Metabolic lab, CDC
Government Medical College, Thiruvananthapuram

Skeletal dysplasias, also known as osteochondrodysplasias, are a heterogeneous group of heritable disorders characterized by abnormalities of cartilage and bone growth, resulting in abnormal shape and size of the skeleton and disproportion of the long bones, spine, and head. Even though individually rare, they are common as a group (1 in 3000 to 1 in 5000 births) in paediatric practice and a major cause for chronic orthopaedic disability.

Early accurate diagnosis is important for the treatment of the affected patient and genetic counselling to the affected family. Many skeletal dysplasia can be detected during antenatal targeted ultrasonography. Apart from clinical history and thorough physical examination, skeletal radiography, biochemical tests and molecular tests will help in the definitive diagnosis of most of the skeletal dysplasia. The following article presents a simplified approach to skeletal dysplasia which help the clinician to categorise skeletal dysplasia clinically and radiologically.

Skeletal dysplasia can be inherited as autosomal dominant, autosomal recessive, or X-linked, Examples include the following:

- Autosomal dominant - Fibroblast growth factor 3 (FGFR3) disorders (achondroplasia, thanatophoric dysplasia, hypochondroplasia)
- Autosomal recessive - Cartilage-hair hypoplasia, Ellis-van Creveld syndrome, hypophosphatasia, osteopetrosis
- X-linked recessive - Chondrodysplasia punctata (CDP)

During evaluation of a suspected child with short stature for the possibility of skeletal dysplasia, ten step approach will help the clinician to arrive at a reasonable clinical diagnosis.

Diagnostic Step1: Presence of reduced height/length

First step in any child with short stature is plotting the height/length in a growth chart to confirm that it is less that 3rd centile for that age. Mid-parental height also should be plotted at 18 years to follow the height trajectory to differentiate pathological short stature from familial short stature.

Diagnostic step 2: Recognition of association with disproportion

The upper segment/lower segment ratio should be measured and compared with age specific values. The normal US/LS at birth is 1.7, at one year is 1.5 and gradually approaches to a value of 1 by 7 years. The disproportion can be due to short limbs as in achondroplasia where US/LS is more for the age or due to short trunk where US/LS is less than the age specific value.

Other clues to the presence of disproportion are a real or relative macrocephaly, bowing of the long bones, asymmetry and hypo plastic chest.

Diagnostic step 3: Determine body areas with greatest shortness

Once the disproportion has been recognised, it is important to establish which body areas are the shortest. Only the limb may be short , but more frequently the limbs and trunk have reduced length.

The short limbs can be Rhizomelic (short femur and humerus), mesomelic (short middle segment-tibia, fibula, radius, ulna) or acromelia (distal shortness). Rhizomelic shortening characteristically seen in achondroplasia where there will be increased skin folds in the arm/ thigh segment. Mesomelic shortening is seen in diaphyseal dysplasia and Langer mesomelic dysplasia. In acromelic shortening, there is some value in trying to clinically determine whether the carpel, metacarpal or phalangeal bones are equally or individually short. Generalised shortening of the fingers are called brachydactyly, whereas shortening of metacarpals is called brachymetacarpy.

When the trunk is short, it usually means some section of the spine is short. It can be due to flat vertebral bodies (platyspondyly), or have segmentation defects (hemivertebrae) or spine curvature like scoliosis or kyphosis. Skeletal dysplasia due to spine involvement is predominantly occurred in spondyloepi/ metaphyseal dysplasia.

Diagnostic step 4: Some important clues in the history

Some important clues in the antenatal history especially in lethal skeletal dysplasia are polyhydramnios, increased or decreased uterine size, decreased fetal movements, hydrops fetalis, joint contractures, dislocations and fractures. The mother's drug history of any teratogenic drugs like warfarin to be elicited which can cause phenotype similar to chondrodysplasia punctata. A maternal or family history of still births (malformed or otherwise) should be elicited. The details of the baby like photograph, radiograph, autopsy result or any genetic tests results should be reviewed. Family history of consanguinity is important since many skeletal dysplasia are autosomal recessive in nature. Family members should be scrutinised for any overtly features of skeletal dysplasia.

Diagnostic step 5: Identify the secondary defects

Identifying the secondary effects or deformations caused by the osseous abnormalities can simplify the diagnostic process and may aid in accurate categorization. Bowing of the long bone signifies a curvature that is due to shortened long bones or bones that lack adequate bone density. Skin dimples can develop over the area of sharpest angulation in conditions like campomelic dysplasia and diastrophic dysplasia. Joint contractures are seen in Diastrophic dysplasia whereas joint hypermobility in Morquio syndrome. Short ribs/hypoplastic thorax is seen in thoracic dystrophy and short rib polydactyly syndrome. Skull abnormalities like macrocephaly (achondroplasia), craniotabes (Osteogenesis imperfecta) and craniosynostosis (thantophoric dysplasia) also to be examined.

Diagnostic step 6: Associated malformations

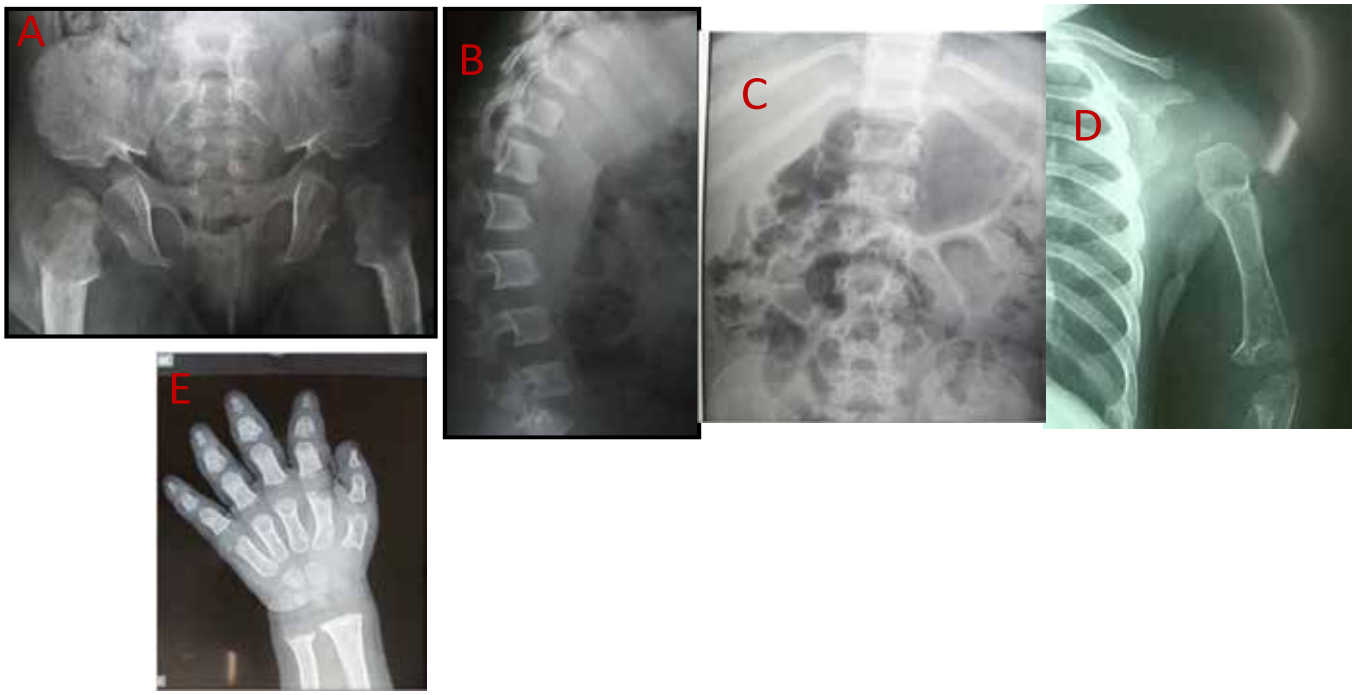
Significant proportion of skeletal dysplasia have associated malformations and will help the clinician to reach a practical differential diagnosis. Some malformation is so specific to reach a definitive diagnosis like cystic ears in diastrophic dysplasia and multiple gingival frenulae in Ellis-van Crevald syndrome. Table 1 gives examples of associated malformations in some skeletal dysplasia.

Table 1: Malformation that may be particularly useful in diagnosing specific skeletal dysplasia

Feature	Representative Example
Heart Defects (esp single atrium)	Ellis-van Crevald syndrome
Polydactyly , nail hypoplasia	Ellis-van Crevald syndrome
Cleft palate/lip	Diastrophic dysplasia
Hitchhikers thumb	Diastrophic dysplasia
CTEV	Diastrophic dysplasia
Blue sclera	Osteogenesis imperfecta
Sparse scalp hair	Cartilage hair hypoplasia
Cataracts	Chondrodysplasia punctate
Myopia/retinal detachment	Stickler syndrome
Severe nasal bridge hypoplasia	Acrodysostosis
Clavicular agenesis	Cleidocranial dysostosis
Camptodactyly	Campomelic dysplsia
Renal cyst	Majewaki , Saldino-Noonan syndrome,

Figure 1: Characteristic radiological features of Achondroplasia

(A) The iliac bones are short and round and horizontal flat acetabular roofs (squared pelvis); sharp spur at triradiate cartilage and narrow greater sciatic notch. Characteristic oval translucency in the upper part of femur. Short femoral neck. (B) lateral view of thoraco lumbar spine with short pedicles, scalloped posterior border and "bullet-shape" of vertebral bodies.(C)AP view showing interpeduncular distances narrow progressively from L1-L5 or are parallel.(D) wide metaphysis with normal epiphysis, short tubular bones (E) short metacarpals of equal length and short (bullet shaped) phalanges give a trident shape appearance.



Diagnostic step 7: Radiological evaluation

Radiological survey in a suspected skeletal dysplasia should include the following X-rays: Skull (AP& Lateral), Chest (PA), Thoracolumbar spine (AP & Lateral), Pelvis with Hip(AP), wrist with hand both sides (AP) and one upper limb and lower limb. Categorising radiological findings by areas of involvement will help in the pattern recognition. In radiological terms, physeal regions (epiphyseal, metaphyseal or diaphyseal), spine, hands, skull or any combination thereof. The epiphyseal involvement include delayed ossification, irregular ossification, flattened or small epiphysis or stippled epiphysis which is seen in multiple epiphyseal dysplasia. In metaphyseal dysplasia, metaphysis will be wide, cupped, irregular with spiculed lateral metaphyseal borders (similar to rickets). Sometimes you may identify some growths on or off the metaphyses, such as exostosis or enchondromas. Diaphyseal involvement as the only long bone finding is rare and seen in conditions with hyperostosis.

Spine involvement is examined by vertebral size, contour and basic anatomy. Most of the skeletal dysplasia involving spine will have reduced height or platyspondyly. The vertebral body architecture can be abnormal like anterior beaking in mucopolysaccharidosis, posterior concavity, coronal clefts, superior and inferior border irregularities. Anatomic defects like hemi vertebrae can be seen in some conditions like spondylo-thoracic dysplasia. In this condition ribs can be absent, bifid, hypoplastic or duplicated. Horizontally placed short ribs are seen in SRP syndrome. Multiple rib fractures and swellings in the costochondral junction is seen in osteogenesis imperfecta.

The skull radiology shows reduced calvarial bone density, seen in osteogenesis imperfecta . Wormian bones are irregular, linear areas of translucency found primarily in occipital and parietal regions in conditions with reduced bone density. The most common iliac abnormalities include hypoplastic iliac bones, excessive iliac wing flare, squared ilia (achondroplasia), and short/narrow ilial base.

Other bony abnormalities not fitting into the scheme like short femoral neck, long distal fibula, bifid distal humerus and medial slant and constriction of the distal radial epiphyses (Madelung deformity) are few examples that can be found in certain skeletal dysplasia. Repeated radiological evaluation over time in instances of questionable diagnosis is important to identify the changes contributing to final diagnosis.

Diagnostic step 8: Categorization of the skeletal dysplasia

Considering the clinical and radiological features, the clinical decision can be made. The phenotype can be classified into pure skeletal dysplasia, Multiple congenital anomaly-skeletal dysplasia or multiple congenital anomaly/MR/ Skeletal dysplasia.The examples of three types are given in the Table 2.

Table 2: Categorization of Skeletal dysplasia with examples

Pure Skeletal Dysplasia	Malformation with Skeletal dysplasia	Malformation/MR and Skeletal dysplasia
Achondroplasia	Stickler syndrome	Campomelic dysplasia
Spondyloepiphyseal dysplasia	Ellis-van Crevald syndrome	Chondrodysplasia punctata
Osteogenesis imperfecta	Diastrophic dysplasia	Acrodysostosis

Diagnostic Step 9: Other laboratory test

Some skeletal disorders show abnormalities of calcium, phosphorous and alkaline phosphatase. In suspected lysosomal storage disorders like mucopolysccharidosis, enzyme analysis should be performed. Chromosomal study is usually normal in skeletal dysplasia except in campomelic dysplasia, in which sex reversal is common (XY with phenotypic female). Immunological evaluation is indicated when associated with immunodeficiency like cartilage hair hypoplasia (metaphyseal dysplasia). Autopsy studies in cases of lethal skeletal dysplasia is important which will identify other internal malformation to establish a definitive diagnosis. Histopathology and electron microscopy of bone tissue can identify the abnormality, however not very useful in the routine clinical practice.

Diagnostic step 10: Molecular Diagnosis

Molecular diagnostic techniques have led to the identification of the underlying gene disorders in most of the common skeletal dysplasia. Some examples are Achondroplasia (FGFR3), Osteogenesis imperfecta (COL1A1 &COL1A2) and Ellis-van Crevald syndrome (EVC1&EVC2). If the diagnosis is confirmed clinically/radiologically, sequencing of the gene involved can be done (in achondroplasia targeted mutation testing or sequencing of FGFR3). If multiple genes are involved for a phenotype like osteogenesis imperfecta (more than 15 genes), panel testing or clinical exom is recommended. Genetic confirmation will help the geneticist for genetic counselling including prenatal diagnosis in next pregnancy.

This clinical approach and classification system is more practical and useful for the paediatrician for arriving at a reasonable diagnosis. The genetics of these individually rare disorders can be established by genetic testing. Genetic counselling should be offered in this patients and their families.

Further Reading

1. Tournis S, Dede AD. Osteogenesis imperfecta - A clinical update. Metabolism. 2018 Mar;80:27-37.
2. Geister KA, Camper SA. Advances in Skeletal Dysplasia Genetics. Annu Rev Genomics Hum Genet. 2015;16:199-227..
3. Offiah AC. Skeletal Dysplasias: An Overview. Endocr Dev. 2015;28:259-276.
4. Alanay Y, Lachman RS. A review of the principles of radiological assessment of skeletal dysplasias. J Clin Res Pediatr Endocrinol. 2011;3(4):163-78.
5. Dighe M, Fligner C, Cheng E, Warren B, Dubinsky T. Fetal skeletal dysplasia: an approach to diagnosis with illustrative cases. Radiographics. 2008 Jul-Aug;28(4):1061-77. doi: 10.1148/rg.284075122. Erratum in: Radiographics. 2009 Mar-Apr;29(2):638. PMID: 18635629.
6. Hall BD. Approach to skeletal dysplasia. Pediatr Clin North Am. 1992 Apr;39(2):279-305.

Spinal Muscular Atrophy- An Update

DR GAYATRI N
(DGO, DNB , DNB Medical Genetics)
Consultant Medical Geneticist

DR PRAJNYA RANGANATH MD DM
Additional Professor and Head

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disorder characterized by progressive symmetrical proximal muscle atrophy and weakness. It is one of the most common genetic neuromuscular disorders, with the worldwide incidence ranging from 1 in 6000 to 10,000 live births [1]. The onset of manifestations can vary from the prenatal period to adulthood depending on the type of SMA. The phenotype is caused by biallelic loss of function variants of the SMN1 (survival of motor neuron 1) gene (OMIM * 600354), which lead to irreversible atrophy and degeneration of the anterior horn cells in the spinal cord and brain stem.

Molecular Mechanism and Pathophysiology

The SMN1 gene, which is present on the chromosome 5q13 locus, has nine exons and codes for the SMN protein. This 294-amino acid containing SMN protein is a ribonucleic acid (RNA)-binding protein, which plays an important role in the efficient assembly of small nuclear ribonucleoprotein (snRNP) complexes and in messenger RNA (mRNA) splicing. Pathogenic variants in the SMN1 gene lead to absence/reduction of the SMN protein, which in turn causes disruption in the formation of snRNPs and affects the genes involved in motor neuron circuits [2]. SMN protein is ubiquitously present in all cells including neurons; therefore, the reasons underlying selective restriction of the SMA phenotype to lower motor neuron dysfunction is unclear. It is hypothesized that the function of the SMN protein is either specific to lower motor neurons or lower motor neurons are more sensitive to reduced levels of SMN protein resulting in degeneration of the neurons [3].

Ninety-five to ninety-eight percent of patients with SMA have homozygous deletion of exon 7 ± exon 8, while two to five percent patients are compound heterozygous for one exon 7/8 deletion mutation and one pathogenic sequence variant in the SMN1 gene.

Another gene called SMN2 which is also present on the chromosome 5q13 locus, has a

1 Fernandez Hospital, Hyderabad, Telangana, India

2 Department of Medical Genetics, Nizam's Institute of Medical Sciences, Hyderabad 500082, Telangana, India.

* Corresponding author

nucleotide sequence very similar to SMN1, and differs from SMN1 in only eight nucleotides (5 intronic and 3 exonic- 1 each within exons 6, 7, and 8). The variation in position 6 of exon 7 [cytosine (C) in SMN1 versus thymine (T) in SMN2] results in altered splicing of SMN2. Most of the mRNA transcripts from the SMN2 gene are unstable and produce a less stable/ truncated non-functional form of the SMN protein which lacks exon 7; only a small amount of the SMN2 transcripts (around 10%) are full-length transcripts which include exon 7 and translate into normal SMN protein (Figure 1). The number of SMN2 copies in an individual can range from zero to five [2].

When both copies of the SMN1 gene are mutated (i.e., there are biallelic mutations in SMN1), very less functional SMN protein is produced and SMN2 cannot fully compensate for loss of SMN1-produced protein, which leads to the disease phenotype of SMA. However, when the number of copies of SMN2 is increased, the amount of functional protein generated by SMN2 is able to partially compensate for lack of SMN1-produced protein, leading to a milder (type II/ III/ IV) phenotype of SMA. Thus, the number of copies of SMN2 acts as a modifier of severity of the disease phenotype; the more the number of copies of SMN2, the milder will be the phenotype. Variants in the PLS3 gene which increase the expression of plastin 3 have also been found to be associated with a reduction in SMA disease severity [2].

Clinical Presentation

The clinical diagnosis of SMA is suspected in an infant with floppiness, paucity of limb movements, motor developmental delay, and areflexia with/ without tongue fasciculations, and in an older child or adult with proximal muscle weakness, hypotonia, and reduced/ absent deep tendon reflexes. Depending on the age of onset, clinical presentation, progression of symptoms and severity, SMA is classified into types 0, I, II, III and IV, as mentioned in Table 1.

Table 1. Clinical classification of spinal muscular atrophy

Type of SMA	Age of onset	Motor milestones attained	Clinical features	Survival
SMA 0	Prenatal	Nil	• Severe hypotonia and muscle weakness from birth • Facial weakness • Respiratory failure at birth • Antenatal period – reduced fetal movements and polyhydramnios • Arthrogryposis	Less than 6 months
SMA I	< 6 months	Partial head control; do not attain ability to sit without support	• Floppy infant • Hypotonia and areflexia • Paucity of limb movements • Difficulty in sucking and swallowing • Tongue fasciculations • Facial weakness may or may not be present	8 to 18 mths
SMA II	6 – 18 months	Can sit independently; may walk with support	• Motor developmental delay • Hypotonia and areflexia/ hyporeflexia • Tongue fasciculations • Proximal muscle weakness • Hand tremors	Up to around 25 years
SMA III	> 18 months	Attain independent ambulation, but have waddling gait; difficulty in climbing stairs	• Proximal muscle weakness • Hyporeflexia • Hand tremors • Easy fatiguability	Normal
SMA IV	Adulthood	Normal milestones	• Proximal muscle weakness • Easy fatiguability	Normal

Other neuromuscular disorders which must be considered in the differential diagnoses for a floppy infant include the congenital myopathies, congenital muscular dystrophies, congenital myotonic dystrophy, Prader-Willi syndrome, congenital myasthenic syndromes, and metabolic myopathies including Pompe disease and mitochondrial myopathies. Conditions which can have clinical presentations similar to SMA type III include Duchenne muscular dystrophy and limb girdle muscular dystrophies (LGMDs). Amyotrophic lateral sclerosis and Kennedy disease (spinal and bulbar muscular atrophy) can have features overlapping with SMA type IV.

Laboratory Diagnosis

Serum creatine phosphokinase (CPK) may be normal or mildly elevated (within two to three-fold) in SMA. Electromyography (EMG) is difficult to perform in a young infant, but in an older child or adult, features suggestive of motor neuron or motor axonal involvement are seen in the form of active denervation and compensatory changes of reinnervation and motor unit action potential enlargement. Abnormal spontaneous activity in the form of fibrillation potentials is also usually seen. Confirmation of the diagnosis is by molecular genetic testing of the SMN1 gene. As 95 to 98% of patients with SMA have homozygous deletion of exon 7 ± exon 8, the multiplex ligation-dependent probe amplification (MLPA) technique is used as the first line genetic test (Figure 2). In patients with a strong suspicion of SMA, where MLPA shows only heterozygous deletion of exon 7/8, SMN1 gene sequencing has to be done to look for a pathogenic sequence variant in the second copy of the SMN1 gene. Prior to SMN1 gene sequencing, selective amplification of the gene has to be done to ensure that the nucleotide sequence corresponding to the very similar SMN2 gene is not included. If MLPA of the SMN1 gene does not show even one (heterozygous) deletion of exon 7/8, the diagnosis of SMA is unlikely, and an alternative diagnosis with a similar phenotype as discussed above, must be considered. The stepwise approach to the laboratory diagnosis of SMA is given in Figure 3.

If the previous child of a couple with an SMA-like phenotype has succumbed to the illness and if the DNA sample of the child is not preserved, the diagnosis can be established by carrier testing through MLPA of the SMN1 gene in the couple. Most carriers have a heterozygous (one copy) deletion of exon 7/8 of SMN1, i.e., they have one normal copy of SMN1 on one chromosome 5 and one copy with exon 7/8 deletion on the other chromosome 5. This is referred to as the 1/0 genotype. However, in about 5%-8% of carriers with a heterozygous deletion, two copies of SMN1 can be present on the other chromosome; this is referred to as the 2/0 genotype. Carriers with the 2/0 genotype, will show false negative results on carrier screening, as MLPA will detect two non-deleted copies of SMN1, similar to that found in non-carriers. In such cases, testing of the parents of the carrier with the suspected 2/0 SMN1 genotype will give additional information; one of the parents will reveal a 1/0 SMN1 genotype (one normal copy) and the other parent will have a 2/1 SMN1 genotype (three normal copies). Carriers for non-deletional sequence variants in SMN1 will also test negative through MLPA and would require SMN1 gene sequencing to identify the pathogenic variant.

Management

Until recently, only supportive and symptomatic management was possible for patients with SMA in the form of regular physiotherapy and multidisciplinary care for respiratory failure, nutritional compromise, scoliosis and joint contractures. Over the past few years, some targeted therapies have become available for SMA, and several newer therapeutic approaches are being investigated. These treatment strategies can be classified as SMN-dependent approaches and SMN-independent approaches.

SMN-independent approaches do not

involve the SMN protein and are directed towards improving muscle strength through a different pathway. Phase 2 studies for two such drugs i.e., reldesemtiv, a tropinin complex activator which slows the rate of calcium release from the regulatory troponin complex of fast skeletal muscle fibres, and SRK-015, a myostatin inhibitor, have suggested some potential clinical benefit, but further studies to establish their efficacy are required. Other modalities such as pyridostigmine (neuromuscular junction/ synaptic function enhancer), gabapentin (neuroprotector), and stem cell therapy (with induced pluripotent stem cells, iPSC), have been tried, but have shown very limited benefits.

Most of the recently approved therapies and ongoing clinical trials for SMA are based on SMN-dependent/ SMN2-targeted approaches [4]. Initially, aminoglycosides (SMN protein stabilizer) and histone deacetylase inhibitors–HDACIs (SMN2 transcript upregulators) were tried, but no significant improvement in the phenotype was noted with these drugs. Later developments such as nusinersen and risdiplam which cause altered splicing of SMN2, and SMN1 gene replacement therapy, have shown significant clinical benefit and have now been approved for treatment of SMA patients [4]. The concept of maximum benefit from a combination of different available therapeutic strategies is also being explored.

Nusinersen (marketed as Spinraza® by Biogen, Massachusetts, United States), was approved for treatment of all types of SMA, by the United States Food and Drug Administration (US FDA) in 2016 and by the European Medicines Agency (EMA) in 2017. It is an antisense oligonucleotide i.e., a short single-stranded RNA molecule, which binds to complementary sequences in the SMN2 transcript and alters its splicing, which results in generation of more full-length transcripts that include exon 7. Phase II and III clinical trials with nusinersen have proved its safety and shown that it leads to significant motor function improvement in all types of SMA and improves survival of patients with infantile and late-onset SMA [5]. It is administered intrathecally, with the recommended dosage being 12 mg per administration. A total of four loading doses are administered with the first three loading doses at 14 day-intervals and the fourth loading dose 1 month after the third dose. Thereafter, the maintenance dose is administered once every 4 months. The use of Ommaya reservoirs has been reported to be safe for repeat infusions in selected cases, specifically those with spinal deformities or scoliosis to avoid repeated difficult lumbar punctures [6].

Onasemnogene abeparvovec-xioi (marketed as Zolgensma® by Novartis, Chicago, United States) is an adeno-associated virus vector-based gene therapy which has been approved for use in SMA patients less than 2 years of age, by the US FDA in 2019. At present it is recommended to be given as a one-time slow intravenous infusion; trials using single-dose intrathecal administration are ongoing [7]. The recommended dose is 1.1 × 1014 vector genomes (vg) per kg of body weight. It is reported to result in significant improvement in motor milestones and to prolong survival without ventilator-dependence. However, it is very expensive at present, and is one of the most expensive therapies to be approved by the US FDA, till date. The adverse effects reported with Zolgensma include thrombocytopenia, elevated troponin-I, acute serious liver injury, elevated liver enzymes and vomiting.

Risdiplam (marketed as Evrysdi® by Genentech, Roche, California, United States) and Branaplam (an experimental drug being developed by Novartis, Chicago, United States), are small molecule drugs that act as SMN2 splicing modifiers. Risdiplam was approved by the US FDA in 2020 for use in patients older than 2 months, with types I, II and III SMA. It is given orally, once a day. The recommended dosage is 0.2 mg/kg/day for patients aged 2 months to 2 years, 0.25 mg/kg/day for patients older than 2 years but weighing less than 20 kgs, and 5 mg/day for patients weighing more than 20 kgs. The

long-term safety and efficacy of Risdiplam have not been established at present [8],[9].

Beyond SMN replacement, preclinical research is targeting dysregulated pathways which are independent of SMN deficiency, as future therapeutic targets for personalized medicine in patients with SMA [7].

With the advent of newer therapies for SMA, efforts are being made to implement protocols to screen for SMA in neonates through newborn screening programs, to initiate treatment as early as possible. From 2018, newborn screening (NBS) for SMA has been added to the 'Recommended Uniform Screening Panel' (RUSP) in the US and is available in a few US states at present. Newborn screening for SMA has also been introduced in several other countries.

In addition, efforts are ongoing to identify specific biomarkers that would help in assessment of disease progression and response to treatment. Several biomarkers like SMN2 copy number, SMN protein level, levels of neurofilament proteins, creatine kinase (CPK) and creatinine levels, electrophysiological studies, and imaging measures have been found to provide insights into disease severity, progression, and treatment efficacy. Combinations of these biomarkers rather than single markers are likely to prove to be helpful aids in the assessment of treatment efficacy [10].

Genetic counselling and prenatal genetic testing

Spinal muscular atrophy is inherited in an autosomal recessive manner. In almost 98% of couples with an affected child, both partners are heterozygous carriers for one SMN1 gene mutation each. Only in around 2% of cases, only one partner may be a carrier and the other mutation may have arisen de novo in the child. When both partners are carriers, there is a 25% risk of recurrence of the disorder in each offspring. Recurrence risk is presumed to be low if the proband has one de novo pathogenic variant and one heterozygous pathogenic variant inherited from a carrier parent.

Accurate genetic counseling and definitive prenatal genetic testing can be done based on the exact molecular diagnosis identified in the proband and/or the carrier couple. Prenatal sampling is done through chorionic villus sampling at 11-13 weeks gestation or through amniocentesis at 16-18 weeks gestation, and targeted mutation analysis is done in the fetal DNA through SMN1 gene MLPA (for the exon 7/8 deletion mutation) and/or targeted Sanger sequencing (for sequence variants in the SMN1 gene).

Carrier screening of extended family members is also suggested when an individual is diagnosed to have SMA in the family. In fact, as the carrier frequency of SMA in the general population is quite high worldwide (around 1 in 50), the American College of Medical Genetics and Genomics (ACMG) and the American College of Obstetrics and Gynecology (ACOG) have recommended routine preconception carrier screening for SMA for all couples, irrespective of family history of SMA.

Key messages

- SMA is one of the most common genetic neuromuscular disorders.
- Onset of disease manifestations can vary from the prenatal period to adulthood, based on which SMA is classified into types 0, I, II, III, or IV.
- Majority of SMA cases (95%-98%) are due to homozygous deletion of exon 7 ± exon 8 in the SMN1 gene. Therefore, MLPA of the SMN1 gene is the recommended first line test for diagnosis and carrier testing of SMA.
- The carrier frequency of SMA is relatively high in the general population (~1 in 50); therefore, carrier screening for SMA should be done on a routine basis preconceptionally,

especially if the couple is consanguineous.

- Disease-specific therapies which have been approved for SMA include nusinersen, risdiplam and gene therapy, but they are all prohibitively expensive at present.
- There is an urgent need to further understand the pathophysiology of SMA and develop more cost effective and more efficacious therapies for SMA as well as to identify biomarkers that can be used for more effective monitoring of disease course and response to therapy.

References

1. Verhaart IEC, Robertson A, Wilson IJ, Aartsma-Rus A, Cameron S, Jones CC, et al. Prevalence, incidence and carrier frequency of 5q-linked spinal muscular atrophy - a literature review. Orphanet J Rare Dis. 2017; 12: 124.
2. Kolb SJ, Kissel JT. Spinal muscular atrophy: A timely review. Arch Neurol. 2011; 68: 979-983.
3. Arnold WD, Kassar D, Kissel JT. Spinal muscular atrophy: diagnosis and management in a new therapeutic era. Muscle Nerve. 2015; 51:157-167.
4. Chen TH. New and Developing Therapies in Spinal Muscular Atrophy: From Genotype to Phenotype to Treatment and Where Do We Stand? Int J Mol Sci. 2020; 21: 3297.
5. Hoy SM. Nusinersen: A Review in 5q Spinal Muscular Atrophy. CNS Drugs. 2021; 35: 1317-1328.
6. Papaliagkas V, Foroglou N, Toulios P, Moschou M, Gavriilaki M, Notas K, et al. Intrathecal Administration of Nusinersen Using the Ommaya Reservoir in an Adult with 5q-Related Spinal Muscular Atrophy Type 1 and Severe Spinal Deformity. Case Rep Neurol. 2021; 13: 710–715.
7. Chaytow H, Faller KME, Huang YT, Gillingwater TH. Spinal muscular atrophy: From approved therapies to future therapeutic targets for personalized medicine. Cell Rep Med. 2021; 2(7): 100346.
8. Mercuri E, Deconinck N, Mazzone ES, Nascimento A, Oskoui M, Saito K, et al. Safety and efficacy of once-daily Risdiplam in type 2 and non-ambulant type 3 spinal muscular atrophy (SUNFISH part 2): a phase 3, double-blind, randomized, placebo-controlled trial. Lancet Neurol. 2022; 21: 42-52.
9. Fowler S, Brink A, Cleary Y, Günther A, Heinig K, Husser C. Addressing Today's Absorption, Distribution, Metabolism, and Excretion (ADME) Challenges in the Translation of In Vitro ADME Characteristics to Humans: A Case Study of the SMN2 mRNA Splicing Modifier Risdiplam. Drug Metab Dispos. 2022; 50: 65-75.
10. Pino MG, Rich KA, Kolb SJ. Update on Biomarkers in Spinal Muscular Atrophy. Biomarker Insights 2021; 16: 11772719211035643.

Figure 1:
Diagrammatic representation of mRNA splicing of SMN1 and SMN2 genes

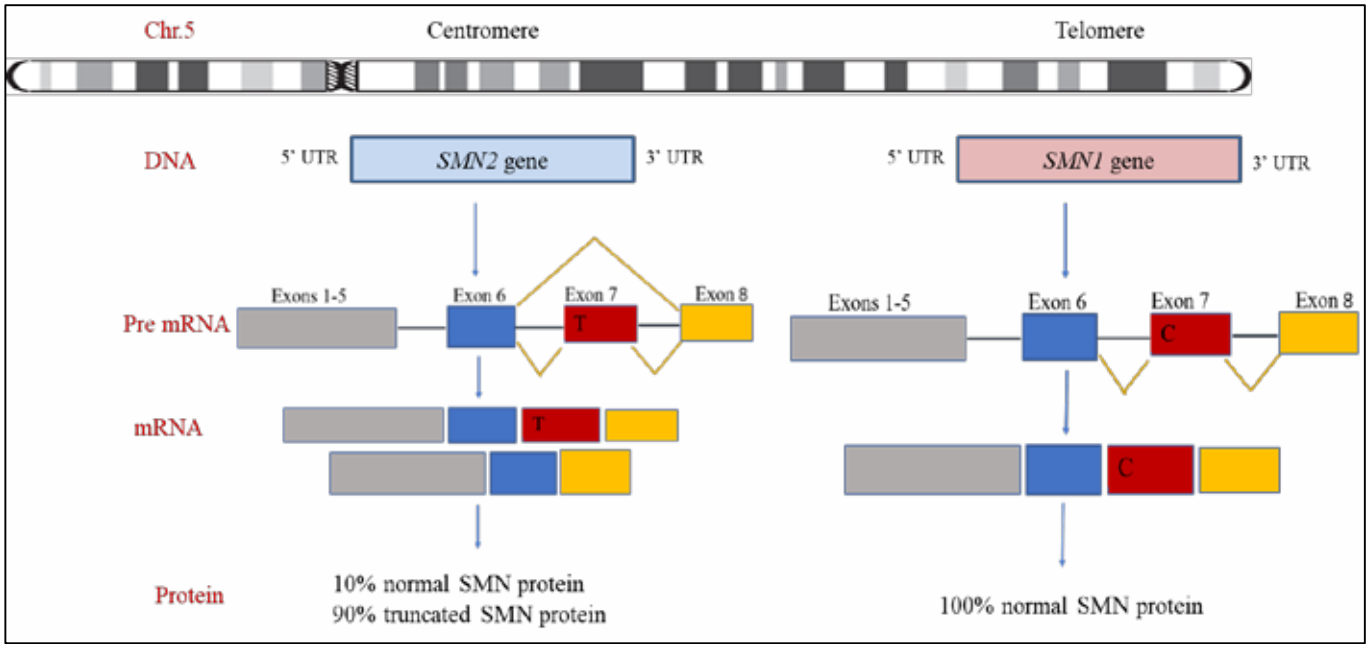


Figure 2:
Multiplex ligation-dependent probe amplification (MLPA) of the SMN1 gene showing homozygous deletion of exon 7 and exon 8 in a child affected with spinal muscular atrophy

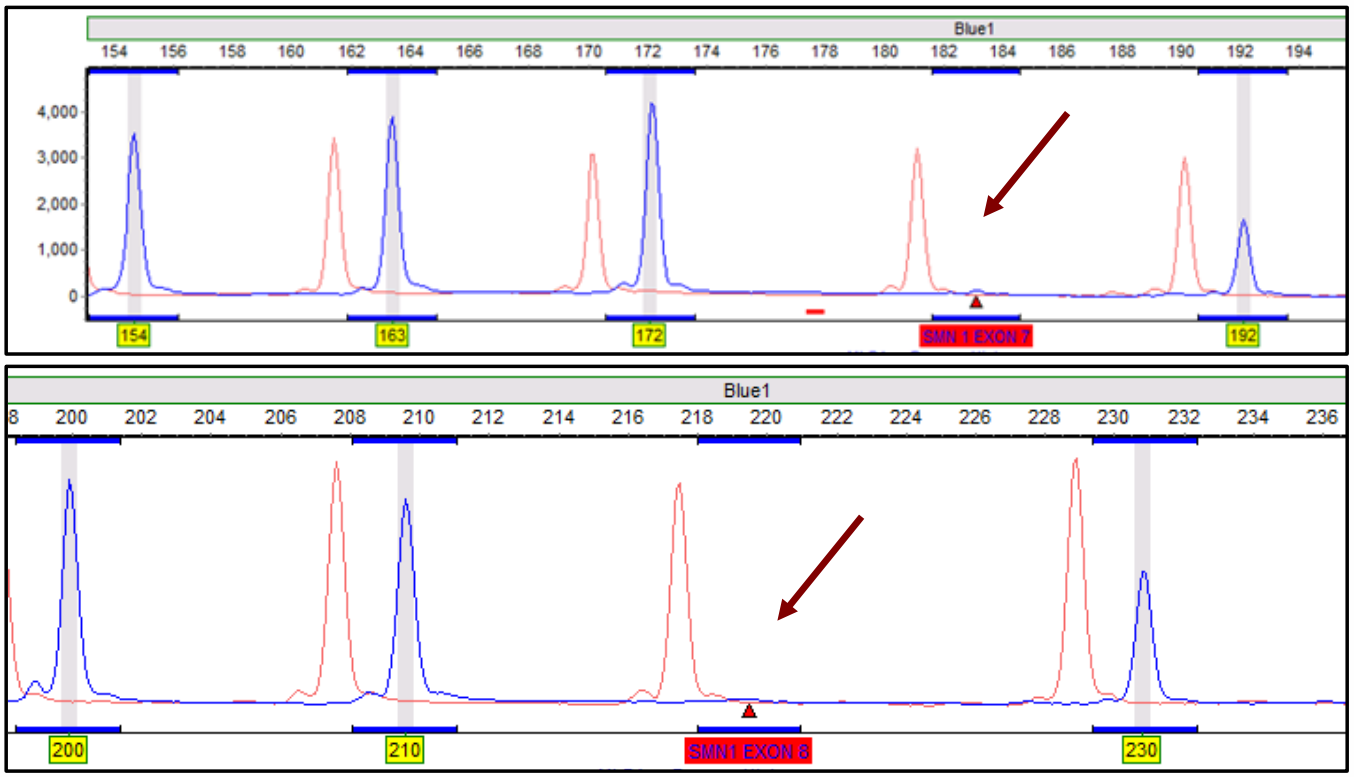
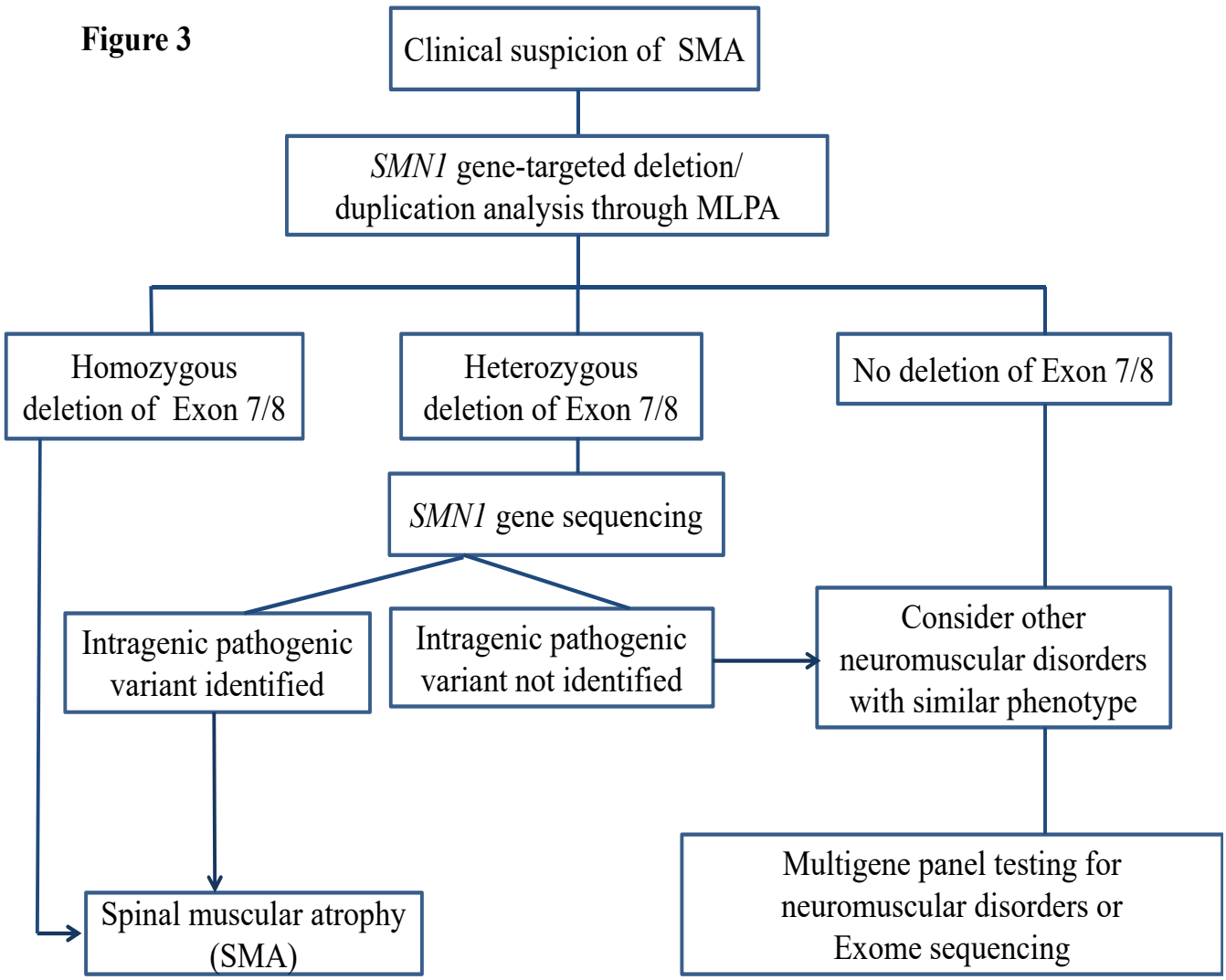


Figure 3:
Stepwise evaluation of a patient clinically suspected to have spinal muscular atrophy



Genetic Counseling: A must 'Tool - Skill' for every Paediatrician

DR SHUBHA PHADKE
MD [Pediatrics], D M [Medical Genetics]
Professor & Head, Department of Medical Genetics
Sanjay Gandhi Postgraduate Institute of Medical Sciences
Lucknow

Many genetic disorders present to paediatricians and are diagnosed and managed by paediatricians. In addition to the curative or supportive treatment of the patient genetic counselling of the family is an important component of management of genetic disorders. Genetic counselling is very important as many genetic disorders are still untreatable or the treatments are cumbersome or too costly.

Need of Genetic Counselling

Many genetic disorders are handicapping and associated with high mortality or morbidity. Offering genetic counselling or referring the family to medical genetics centre for diagnosis and counselling is the responsibility of every paediatrician. The objective of genetic counselling is to provide to help the patient and family to adjust with the disorder in concern by knowing information about the medical and genetic aspects of the disorder in concern, to know about the possibility of occurrence or recurrence of the disease and to take appropriate reproductive decisions, carrier testing, pre-symptomatic diagnosis, etc. It means educating the patient and the family about the disease and help them to cope up with the problem of occurrence or recurrence of the disease. Though information about recurrence risk and prevention of recurrence, if possible are important components of genetic counselling; it is not only limited to that. Many families who are not planning any pregnancy in future or have completed their family also wish to know about the cause of the genetic disease in concern and understand about the nature of the disease, if it could have been prevented, etc. Getting all the information about the disease helps the family by way of getting answers to the questions in their mind so that they can concentrate of the available treatment or supportive care of the child.

The common genetic disorders and common clinical presentations requiring genetic counseling are given in tables I and II. Diagnosis of genetic disorders does not always need DNA based testing. For example, diagnosis of beta thalassemia and hemophilia A are done based on quantification of fetal haemoglobin and factor VIII respectively. Radiological features of spine, pelvis and long bones in case of achondroplasia are diagnostic. However, mutation detection should be done in each case of monogenic disorder as it is essential for carrier screening and prenatal diagnosis. Similar is the case of Down syndrome where clinical diagnosis is possible. But the chromosomal anomalies in different cas-

Table I : Common genetic disorders presenting in paediatric practice

Genetic disorders	Comment
Chromosomal Disorders	
Down syndrome (Trisomy 21)	Breaking the news of Down syndrome in a neonate is a challenge and very important
Turner syndrome (45,X)	Need to investigate all girls with oedema of hands and feet during neonatal period and isolated short stature in pre-pubertal girl
22q microdeletion	Need to evaluate all children with congenital heart defect
Monogenic disorders	
Thalassemia major	Mutation detection by DNA studies of each patient with thalassemia major or thalassemia intermedia is necessary so that the family can be provided prenatal diagnosis during her next pregnancy
Hemophilia A	Mutation detection of one patient from each family is important for providing carrier detection and prenatal diagnosis to mother, sisters and other female relatives on maternal side. Inversion mutation accounts for about half of the cases of hemophilia A and hence, genetic testing is easy.
Wilson disease	Molecular diagnosis is very important for providing prenatal and pre-symptomatic diagnosis to the siblings. Each family has a different mutation and testing based on Next Generation Sequencing (NGS) is useful.
Metachromatic leukodystrophy	Many of these children are labled as cerebral palsy and not investigated. Presence of normal development during first year, presence of consanguinity or similarly affected child may be the clue to the possibility of neurodegenerative disorder. Mutation detection is necessary but confirmation by enzyme assay is essential.
Achondroplasia	Diagnosis is based on clinical and radiological findings. One common mutation accounts for 98% cases and hence, the genetic testing does not require costly NGS. In sporadic case born to normal parents, empiric risk of recurrence in siblings is 1 in 400 and hence, DNA based prenatal diagnosis should be provided. Ultrasonographic based diagnosis by measuring long bones is not possible before third trimester.
Epidermolysis bullosa (EB)	Even if the baby is sick and unlikely to survive DNA based diagnosis is essential as severe types of EB are autosomal recessive and prenatal diagnosis is indicated. Storage of 1 ml of blood in EDTA or a piece of umbilical cord can be used for DNA diagnosis at later date.
Retinoblastoma	Retinoblastoma is a prototype of familial cancers and needs genetic evaluation and genetic counseling
Mucopolysaccharidois I (MPS I)	Coarse facial features, joint contractures, corneal clouding and hepatosplenomegaly are characteristic features of MPS I. However, similar overlapping features are seen in other types of MPS and lysosomal storage disorders (LSD). Diagnosis needs to be confirmed by enzyme assay and also, by mutation testing. Accurate diagnosis of each type of LSD is essential not only for genetic counseling but some LSDs can be treated with enzyme replacement therapy and outcomes are good.

es of Down syndrome can be different. The cases with trisomy 21 due to translocation need karyotyping of parents and the risk of recurrence may vary from 5% to 100% depending on the type of translocation.

Table II lists some common presentations which can be due to genetic disorders. These presentations may be due to non-genetic aetiologies as well and high level of suspicion is necessary for evaluation the child with possibility of genetic disorder. Presence of consanguinity, similarly affected sibling, and unusual features (Fig 1) should suggest the possibility of genetic disorder. Even if the genetic testing is not possible immediately, sample should be stored for DNA analysis which can be done as per convenience. In this era of molecular medicine, recurrence of genetic disorder in a family for lack of genetic counseling may be considered a matter of medical negligence. Consultation of medical geneticist may be sought in appropriate clinical situations. The scenarios mentioned in table II are very common situations in day to day practice and stress the need to look at each case with genetics perspective. Stillbirths and intrauterine deaths are ignored by paediatrician and obstetricians. Clinical evaluation of such babies and if possible, autopsy of all is very essential as the cause can be identified. Identification of cause of stillbirth is very important for obstetrician and the family and can provide information about the risk of recurrence. Photograph and radiograph can correctly diagnose lethal skeletal dysplasia which is not uncommon cause of stillbirths (Fig 2).

Other common presentations of genetic disorders are jaundice during infancy (Gilbert syndrome, cystic fibrosis, Niemann Pick disease, Hereditary spherocytosis), short stature (Turner syndrome, Russel Silver syndrome, Hypochondroplasia, osteogenenesis imperfecta), seizures (Tuberous sclerosis, epileptiform encephalopathy, neuronal migration disorders, neuronal ceroid lipofuscinosis). Intellectual disability, autism and other neurodevelopmental disorders need etiological diagnosis and genetic counseling for preventing recurrence due to handicapping nature of these disorders.

Table II: Common Clinical Presentations which can be due to Genetic Disorders (Representative Disorders)

Acute sick neonate or infant	Neonate with hypotonia or respiratory failure	Stillbirth with or without malformations / Intrauterine death
1. Maple syrup urine disease	1. Congenital myopathy	1. Lethal skeletal dysplasia*
1. Urea cycle disorders	2. Prader Willi syndrome	2. Chromosomal disorder
2. Fatty acid oxidation defects	3. Spinal muscular atrophy	3. Long QT syndrome
3. Immunodeficiency disorder	4. Myotonic dystrophy in mother	4. Non-immune hydrop
4. Mitochondrial disorders	5. Myesthenia gravis in mother	5. Arthrygryposis syndrome

Fig 1: A Child with Multicentric Osteolysis, Nodulosis and Arthropathy: Note the Oseolysis of carpal bones, nodules on the sole of the feet and coarse facial features. The child was being treated as rheumatoid arthritis.

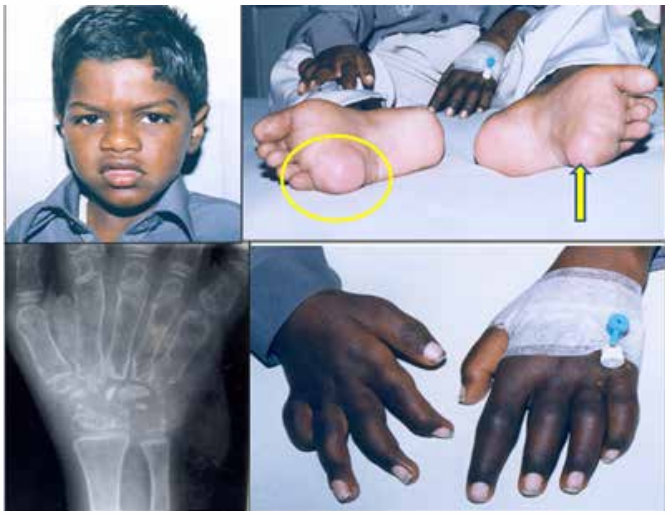
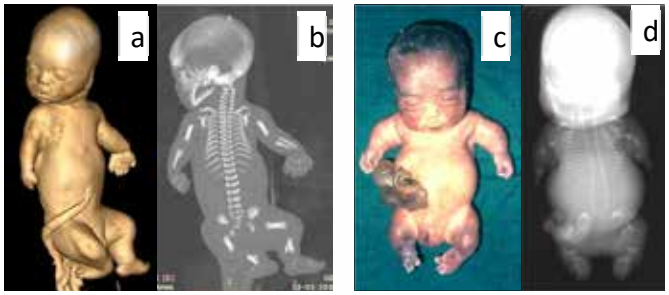


Fig 2: Lethal skeletal dysplasia [a] Short rib polydactyly syndrome – Note short horizontal ribs [b] Achondrogenesis – Note the absence of ossification of vertebral bodies



Illustrative Case Scenario of Genetic Counseling – Prelingual deafness

A three year old boy (proband) born to a consanguineous couple was deaf. His parents (consutands) came for genetic counseling as they were planning to the next child. According to the family history their first daughter was 5 year old and was also deaf and dumb. According to them, she was having some visual problems as well. The first step of genetic counseling is accurate and etiological diagnosis of the proband. Prelingual deafness is extremely heterogeneous condition (Table III and Fig 3). In this family the proband and his sister were evaluated by a paediatrician, audiometrist and an ophthalmologist.

Table III: Aetiologies of deafness

1. Non genetic: Congenital Rubella, neonatal meningitis
2. Isolated monogenic deafness:
   - Autosomal recessive (103 known genes)
   - X- linked – 7 genes
   - Autosomal dominant – 66 genes (may not express in some mutation carrier parents due to incomplete penetrance)
3. Syndromic deafness with malformations: Microtia, Treacher Collins syndrome, Waardenburg syndrome (Fig 3)
4. Syndromic deafness with abnormalities of other systems: Retinitis pigmentosa in Usher syndrome, Goitre in Pendred syndrome, Long QT in Jervell and Lange-Nielsen syndrome, B1 responsive megaloblastic anaemia, renal involvement in Alport syndrome, distal renal tubular acidosis in ATP6V1B1 related deafness syndrome

Fig 3: [a] Microtia [b] Waardenburg syndrome, type II showing light coloured iris and hypo pigmented patch of hair [coloured red artificially]

Diagnosis of the Proband

The evaluation of proband and his sister confirmed sensorineural deafness and identified pigmentary changes in retina. Electroretinogram confirmed retinitis pigmentosa. This confirms that the deafness in this family is not isolated but a part of Usher syndrome. Isolated deafness is treatable with cochlear implantation if detected on new-born screening. And prenatal diagnosis for isolated deafness may be debatable. However, blindness associated with Usher syndrome is not treatable and hence, prenatal diagnosis for Usher syndrome is important. For the information about genetic of Usher syndrome, visit Online Mendelian Inheritance in Man (OMIM) which is a catalogue of genes and genetic disorders and provides an up-to-date information < https://www.omim.org/ >. Usher syndromes are of two types, with and without vestibular involvement.

Genetic Counseling for the Family

Deafness in type I Usher syndrome is profound and they have defective vestibular function. Both types of Usher syndrome are genetically heterogeneous and recessively inherited hence the risk of recurrence in the next pregnancy is 25% or 1 in 4. It means that the possibility that the next child being normal is 75% or 3 in 4. However, it should be made clear to the family that the chance does not have memory and in some families consecutive 2, 3 (¼X ¼ X ¼= 1/64) or more children may be affected with autosomal recessive disorder. Because of heterogeneous nature of this condition, mutation (known as causative pathogenic variation) detection in the proband can easily be done by exome sequencing using next generation sequencing technique. If there are no cost constraints then, both the affected children with both the parents (who are obligate carriers) should be subjected to exome sequencing. This increases the diagnostic yield and also increases reliability of the results. If the causative pathogenic or likely pathogenic variation is detected in some gene for Usher syndrome, the family can be informed that the prenatal diagnosis during the next pregnancy of the mother will be possible by doing chorionic villus sampling at 12 weeks of gestation.

If the causative mutation is not identified in the family by exome sequencing, the prenatal diagnosis can not be provided. The information about risk of recurrence remains the same. Further testing by whole genome sequencing may provide mutation in some other gene or non-coding parts of the known genes for Usher syndrome as they were not evaluated by exome sequencing. This being costly the family may not be able to afford.

What next? In case the mutation is not identified, the family may decide to go ahead with pregnancy without prenatal diagnosis and see what happens. The other options like gamete donation (artificial insemination with sperm from unrelated donor in this case) may be discussed with sensitivity.

Pre-pregnancy Counseling

Evaluation of proband and genetic counseling should be before planning next pregnancy preferably as it allows time for investigations, discussion and decision making. For every such family who has come for pre-pregnancy counseling or have come during first trimester; screening for common genetic disorders, beta thalassemia & hemoglobinopathies, spinal muscular atrophy and fragile X syndrome should be offered as is done for every family planning pregnancy. In such families with consanguinity and a serious autosomal recessive disorder, it may be useful to educate them about the role of consanguinity. Most of the consanguineous families have normal offspring. But if there is history of autosomal recessive disorder, the family may educate family members and consider an option of avoiding further consanguineous marriages. Or offer carrier screening based on the

information of mutation in the proband. Write up of the information provided should be given for their records and to give the information to the referring physician who can follow up the case and provide necessary care. It is important to organize available management, supportive care and put the family in touch with appropriate patient support group.

Requisites of Genetic Counseling

The case discussed above highlights principles and steps of genetic counseling (Table IV).

Table IV: Steps of Genetic Counseling

Pedigree drawing and collecting family history
Evaluation and investigations of the proband
Genetic testing for detection of causative genetic abnormality
Collection of up-to-date information
Communication of information about the disease, natural course, treatment available and outcome
Discussion about reproductive options
Write up of the information discussed
Follow up visits to catch up with developments, answering new queries and issues

The importance of accurate diagnosis of the proband need to be stressed and is the prerequisite for genetic counseling. Hence, all genetic counseling should be done in supervision of a medical geneticist or a specialist clinician. The information which needs to be communicated to the family is complex, scientific and very important. Hence, genetic counseling session needs adequate time, quiet place and advance preparation on the part of genetic counsellor. The concerned family members should be present and preferable both of the couple should be present. Good communication skills and command over local language is essential. Also the psycho-social background, religious beliefs and expectations of the family should be understood for successful genetic counseling. Patient privacy should be taken care of and sometimes intra-familial privacy also needs to be respected. The scientific and medical information can be simplified without distorting it and use of diagrams may be used. The concepts of probability are difficult to understand and the counsellor should try to see if the concepts are clearly understood. Some family members may be in stage of denial or emotionally upset and the counsellor needs to be patient and able to deal with emotional complexities of human mind. Good listening skills, empathy, good eye contact, etc. are the qualities which a genetic counsellor need to develop.

Truthfulness and non-directiveness are the pillars of genetic counseling and needs to be strictly adhered to. The decisions about planning pregnancy, prenatal diagnosis and termination of pregnancy are personal decisions based on medical information. Hence, the decisions should be left to the family and genetic counsellor should provide complete and correct information to help the family take reproductive decisions which are appropriate for them. Patronizing attitude and misleading information should be avoided.

Outcome of Genetic Counseling

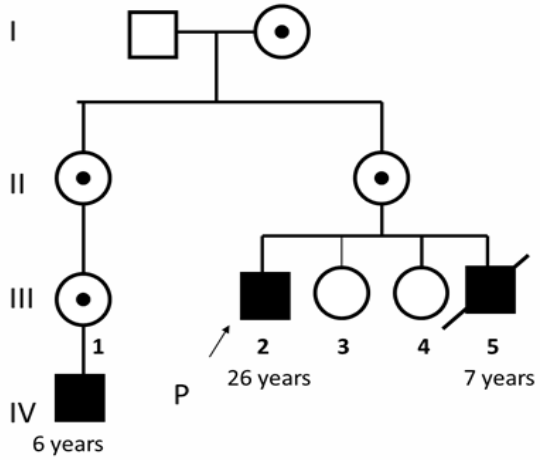
The goal of genetic counseling is to help the family to cope up with the possibility of occurrence or recurrence of a genetic disease in the family. The definition of genetic counseling as per the website of National Human Genome Research Institute, NIH, USA is as follows:

- Genetic counseling is the professional interaction between a healthcare provider with specialized knowledge of genetics and an individual or family.

- The genetic counsellor determines whether a condition in the family may be genetic and estimates the chances that another relative may be affected.
- Genetic counsellors also offer and interpret genetic tests that may help to estimate risk of disease.
- The genetic counsellor conveys information in an effort to address concerns of the client and provides psychological counseling to help families adapt to their condition or risk.

Based on the definition it is obvious that there are no simple measures to evaluate the outcome of genetic counseling. What can be assessed post genetic counseling is that if the family has correctly understood and can remember the facts provided and whether they feel satisfied with the information and interaction with genetic counseling. The decision of have children depends on many other issues than only the risk of recurrence or availability of prenatal diagnosis. Most of the families with no normal child tend to consider an option of next pregnancy even if risk of recurrence may appear high and prenatal diagnosis is not available. The reproductive options also depend on the perceived burden of the disorder. In fig 4, there are multiple male members with haemophilia A which is an X-linked disorder. The son of mother III-1 is well managed and is on factor replacement therapy with the funding of government haemophilia program and is doing very well. While the cousins of III-1, III-3 & III-4 was to undergo carrier testing and have decided to plan pregnancy only when definite prenatal diagnosis can be provided or only if they are not found to be haemophilia carriers. This is because their experience with haemophilia is not good. They have lost a brother due to intracranial bleeding and another brother has physical handicap due to recurrent joint bleeding episodes and chronic arthropathy. This highlights that there may be different decisions of different family members or different families depending on their perception of the disease and its burden. Availability of new treatments also changes the need of prenatal diagnosis.

Fig 4: A Pedigree of a Family with Hemophilia A



Genetic Counseling in Next Generation Sequencing (NGS) Era

High-throughput genetic technique like NGS and cytogenetic microarray has revolutionized genetic diagnosis. These powerful techniques have many far-reaching consequences due to their ability to look at all genes in one go. Important issues are uncertainties about causative nature of some variants (variations of uncertain significance) and detection of some pathogenic variations in genes for diseases which may manifest later and are unrelated to the current phenotype in concern. These issues need good communication and make pre-test and post-test counseling very important. Interpretation of reports of NGS based exome testing also needs knowledge of genetics and genetic counsellors can play an important role with clinical specialists like cardiologists, neurologists, etc. in communicating results to the patients and clinicians. The time is not far when the first tests which a neonate undergoes is sequencing of all genes and all clinicians needs

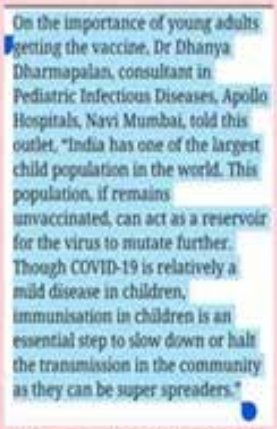
to be empowered with the knowledge of basic genetics and molecular vocabulary.

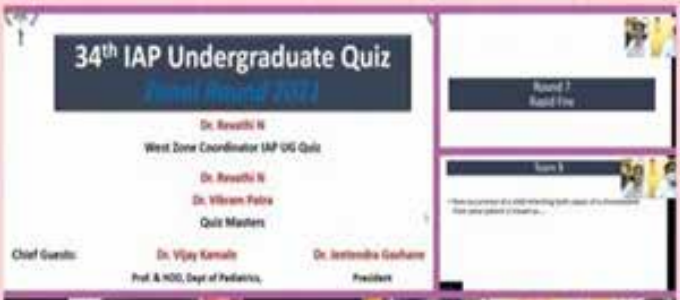
Suggested reading

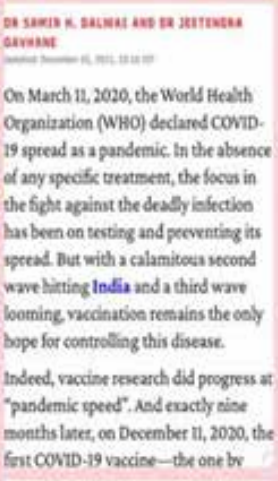
- Phadke SR. Genetic counseling. Indian J Pediatr. 2004 Feb;71(2):151-6
- Phadke SR, Pandey A, Puri RD, Patil SJ. Genetic counseling: the impact in Indian milieu. Indian J Pediatr. 2004 Dec;71(12):1079-82
- Phadke S, Gowda M. Genetic testing in children. Indian Pediatr. 2013 Sep;50(9):823-7.

IAP Navi Mumbai

IAP Navi Mumbai







IAP Navi Mumbai

IAP Navi Mumbai

IAP Kerala

IAP Kerala


NEWS 18 കേരളം
ശിശുരോഗ വിദഗ്ധൻ
ആരോഗ്യവിദഗ്ധൻ
ആരോഗ്യവിദഗ്ധൻ
പ്രിൻസിപ്പൽ
BRUMEX
സ്യൂസ് സെന്റർ
കുട്ടികളിലേക്ക് വാക്സിൻ എത്തുമ്പോൾ
വാക്സിനേഷൻ എന്തൊക്കെ തയ്യാറെടുപ്പുകൾ വേണം?


ARK@
my
ഡൽഹിയിൽ യെല്ലോ അലർട്ട് പ്രഖ്യാപിച്ച് മുഖ്യമന്ത്രി അരവിന്ദ് കെജ്രിവാൾ

IAP Kerala


IAP MALANAD


CALICUT MEDICAL COLLEGE -
GOVT. MEDICAL COLLEGE


PEDICON


IAP MALANAD

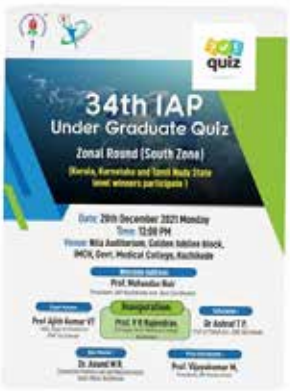
IAP Kerala

IAP Kerala



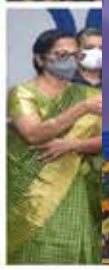



IAP THIRUVANANTHAPURAM
NRP THIRUVANANTHAPURAM
13 DEC
NRP FGM
PROVIDER COURSE
BNRP
FIRST


quiz
34th IAP
Under Graduate Quiz
Zonal Round (South Zone)







PEDICON KERALA 2021
02 33


IAP Jalandhar

Jalandhar Academy of Paediatrics along with IMA Jalandhar celebrated Republic day in full zeal.

Civil surgeon unfurled the National Flag. JAP Patrons Dr. S L Chawla, Dr. Anil Sud, Dr. Kunj Lalwani, Dr. Lalit Tandon, Dr. Pankaj Paul, Dr. Munish Singal, Dr.Jatinder, President Dr Gautam Chawla , Secretary Dr Saloni Bansal, Treasurer Dr Gaganpreet Singh joined the celebrations.

A message for our upcoming generations "Hum laaye hai toofan se kishti nikal kar, is desh to rakna mere bacho sambhal kar "