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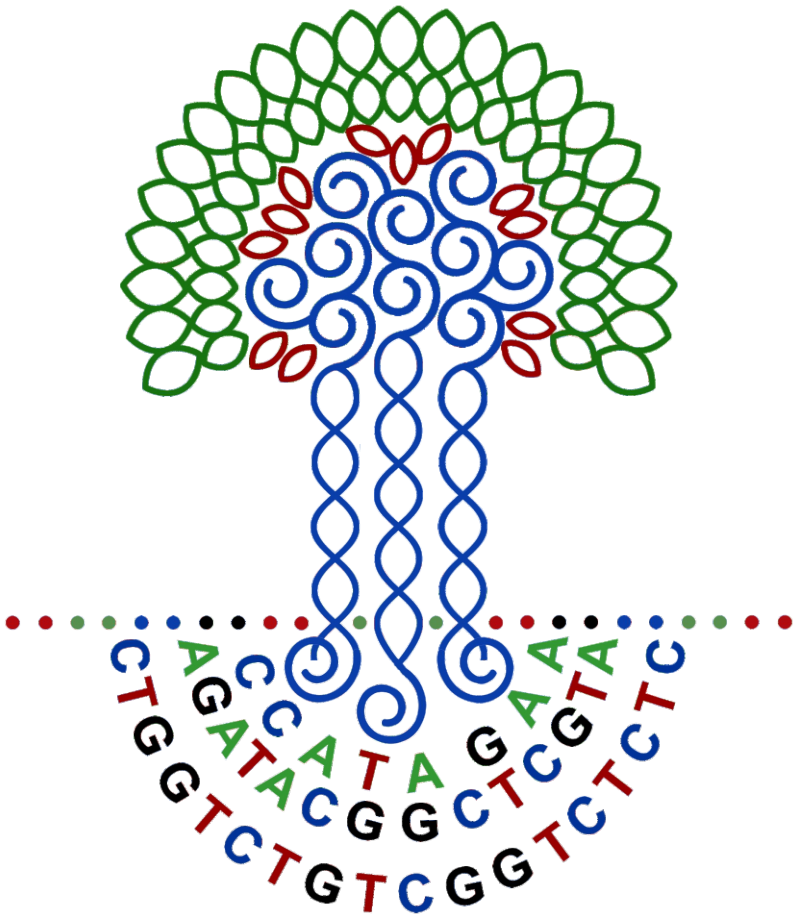


Address for correspondence
Dr Shubha R Phadke
Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow-226 014, EPABX: 0522-2668005-8 | Phone: 0522 249 4325, 4342 | E-mail: editor@iamg.in

PhotoQuiz - 65

Contributed by: Dr Shubha Phadke

Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, India

Correspondence to: Dr Shubha Phadke *Email: shubharaophadke@gmail.com*

This two-year-old boy was brought for evaluation of short lower limbs and cleft palate. Identify the condition.

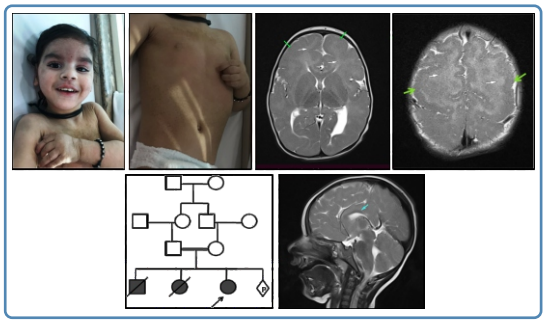
Answer to PhotoQuiz 64

Cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma syndrome (CEDNIK syndrome) (OMIM #609528)

CEDNIK syndrome is a rare autosomal recessive disorder caused by biallelic pathogenic variants in the *SNAP29* gene (OMIM * 604202). This disorder is associated with global developmental delay, intellectual disability, microcephaly, seizures, ocular anomalies, hearing loss, dysmorphic facial features, neuropathy, palmoplantar keratoderma and ichthyosis. Intracranial anomalies detected on neuroimaging include cerebral dysgenesis, absence of the corpus callosum, cortical dysplasia, and white matter abnormalities.

Correct responses to PhotoQuiz 64 were sent by:

1. Dr Ranjana Mishra. Yashoda Hospital and Research Centre, Ghaziabad, India.
2. Dr Surya G Krishnan. Centre for Human Genetics, Bangalore, India.



GeNeDit

Importance of Artificial Intelligence in Medical Genetics

Editorial

Artificial intelligence (AI) is making all areas of medicine swift, sharp, speedy and hence, effective. AI-based tools can do many things which human intelligence cannot. However, AI is not new for medical geneticists. AI-based next-generation sequencing (NGS) data analysis and variant interpretation has made life easy for medical and clinical geneticists. In this issue, the review article about the clinical implications of splice site variants has discussed the use of many AI-based tools such as SpliceAI. The functional effects of splice site variants can be tested in laboratories but may not be possible for every variant detected in clinical situations. Deep learning has been incorporated into bioinformatic algorithms for genome analysis, protein structure classification, prediction, regulatory genomics and cellular imaging. This is especially important in this era where sequencing three billion nucleotides is easy but the challenges of interpretation of variations of unknown significance are bigger than that of sequencing. Tools for changing uncertainties to certainties and help in decision making for patient care have been developed. The use of AI in various areas of genomics is facilitating wider applications of genomics in patient care and also in population screening.

With use of NGS-based testing for varied phenotypes presenting at all ages including antenatally detected anomalies to late-onset genetic disorders, for cancers and familial cancer predisposition syndromes, for population-based screening for monogenic disorders in neonates, for pharmacogenetics and for cancer screening, a large population is getting NGS-based testing for some or the other clinical reason. This not only needs high throughput and speedy results of NGS, but also needs clinicians empowered with clinical knowledge about when to order the test, deep and reverse phenotyping, and pretest and post-test counselling.

AI is going to help all clinicians in a great way as it is doing for NGS laboratories. The GenExpress in this issue highlights the various ways in which this can be done. Image analysis tools have greatly improved over the last decade and are being successfully used in getting diagnosis/ differential diagnosis for dysmorphology, diagnosis of retinal disorders, histopathological diagnosis etc. This helps 'non-experts' to get an expert opinion, spreading services to remote places. As medical genetics is encompassing all branches of medicine, the need for knowledge about this speciality in clinicians is increasing. There is not only the need of superspecialist medical geneticists but all clinicians to be empowered with the principles and tools of genetics in addition to the knowledge of genetic disorders.

Management of genetic disorders has genetic counselling as an important component. Genetic counselling is also essential before NGS-based testing and while communicating the positive, negative or uncertain reports. AI-based communication methods are being found to be suitable for these purposes. Studies have shown that the chatbot can be effectively used to provide pretest counselling. The GenExpress in this issue has covered two articles about successful and efficient use of chatbots for genetic counselling for breast cancer, etc. As the applications of genetic testing are increasing, the need for genetic counsellors is greatly increasing and even developed countries which have training programs in genetic counselling are not able to cope up with increasing demands. The use of AI-based counsellors appears to be a solution for providing personalized counselling. One article in GenExpress also talks about the efficient use of AI for selecting cases from the neonatology unit for whole genome sequencing.

On the one hand the application of genetics in medicine needs a high level of intelligence, while on the other hand interaction with patients needs a sensitive heart as well. AI cannot replace clinical geneticists when it comes to handling the emotional and ethical issues related to genetic diseases. The GeneFocus and HearToHearTalk articles in this issue talk about the emotional

strain clinicians carry, and the ethical dilemmas associated with prenatal diagnosis, which AI cannot address.

The human mind and intelligence and its power of imagination will find out many more applications of AI for genetics in medicine. We are passing through a revolution of molecular medicine and AI is becoming an important aid for clinicians and hospitals getting zapped with the data, novel diagnoses and new therapeutics. Hence the need for clinicians who are knowledgeable and comfortable with the new form of genetic medicine. The next generation of doctors in medical colleges need to get exposure and experience of this next-generation medicine. This will be possible if the teachers are trained in medical genetics as applied to their specialities. Medical colleges need departments of medical genetics and teachers should be empowered to provide genetics services to the patients. The whole world is thinking of ways to update genetic curricula and upscale the existing knowledge about genetics among medical and paramedical workers. In India, we have our training courses such as the GeneTOP (online) and ICMR Course in Medical Genetics & Genetic Counseling in addition to sessions on genetics in various conferences, and online teaching modules of the Indian Academy of Pediatrics and other societies. Many institutes like the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad are conducting courses on NGS analysis. The eyes of the medical fraternity in India have opened to the importance of genetics in medicine. We have to put our mind to this and see how to incorporate medical genetics in the medical care system of India and use our imagination to make the best use of AI.

Dr. Shubha Phadke
1st July, 2024

GeNeViSTA

Mosaicism in Clinical Genetics: Counselling Challenges and Diagnostic Dilemmas

Haseena Sait

Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

Correspondence to: Dr Haseena Sait *Email:* hasi.flower@gmail.com

Abstract

Mosaicism involves the presence of more than one genetically distinct cell line within a single organism, contributing to a range of pathologies from chromosomal disorders to various cancers. In clinical practice, mosaicism presents significant challenges, from suspicion to detection. Equally complex is the process of genetic counselling for mosaic disorders. This review focuses specifically on non-oncological conditions related to mosaicism. It outlines the clinical classification, mechanisms, and common presentations of mosaic conditions. Additionally, the article provides an overview of mosaicism in different clinical settings, including various diagnostic techniques and the uncertainties and challenges in genetic counselling related to mosaicism.

Keywords: Germline mosaicism, somatic mosaicism

Introduction

Mosaicism is defined as the occurrence of two or more cell lines with a different genetic composition derived from a single fertilised egg within a single individual. Mosaicism can have a wide range of effects, from early pregnancy loss to organ specific pathology, to modification of clinical syndromes.

Classification

The developmental timing and cell lineage affected, along with the phenotypic consequences of the mutation, ultimately determine the tissue and cell type distribution of mosaicism and also the patterns of disease recurrence within families. The broad clinical classification includes:

- ***Somatic***- occurring only in the cells of the body, but not including the germline.
- ***Germline***- occurring only in the germ cells or their precursors but not found elsewhere in the body.
- ***Mixed gonadal and somatic*** (Gonosomal)- occurring in both the cells of the body and the germline.

Mechanisms causing mosaicism (Wallace et al., 2022)

- ***De novo postzygotic genetic alteration*** (chromosomal or single nucleotide variation): This can occur in the embryo any time after the first cell division (**Figure 1a**) or in an actively dividing cell throughout the life of an individual resulting in somatic, germline, and/or placental mosaicism (**Figure 1b**).
- ***Epigenetic silencing***: This causes inactivation of select genes on the X chromosome in cells with more than one X chromosome. E.g., females are natural mosaics for X chromosome (**Figure 2**).
- ***Mosaic nullizygosity:*** A mosaic (or postzygotic) genetic alteration occurring in an actively dividing cell in trans with a germline pathogenic variant resulting in mosaic loss of the normal allele in a fraction of cells. This causes type 2 segmental mosaicism as seen in conditions like neurofibromatosis type 1, tuberous sclerosis (**Figure 3**).
- ***Rescue mechanisms*:**
  - Spontaneous ***postzygotic loss*** of a trisomic chromosome resulting in mosaic uniparental disomy (**Figure 4a**)

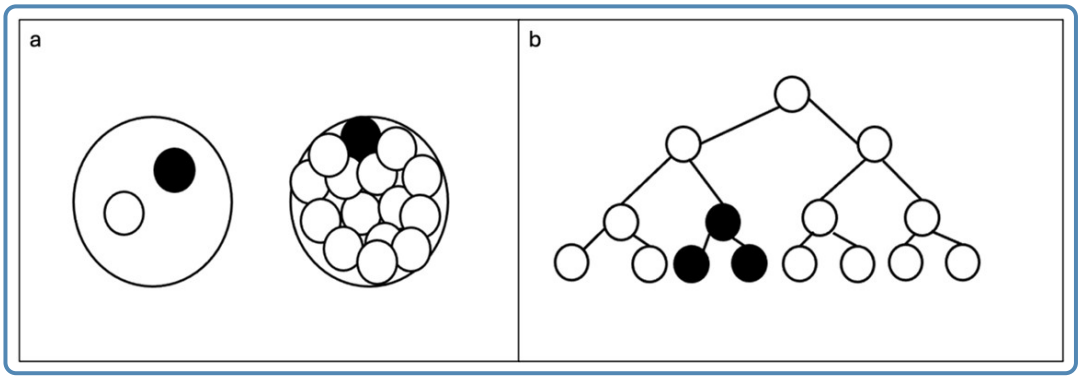


Figure 1 a) De novo postzygotic genetic alteration (dark circle) in two cell or multicellular stage of zygote b) De novo postzygotic genetic alteration (dark cell) in an actively dividing cell

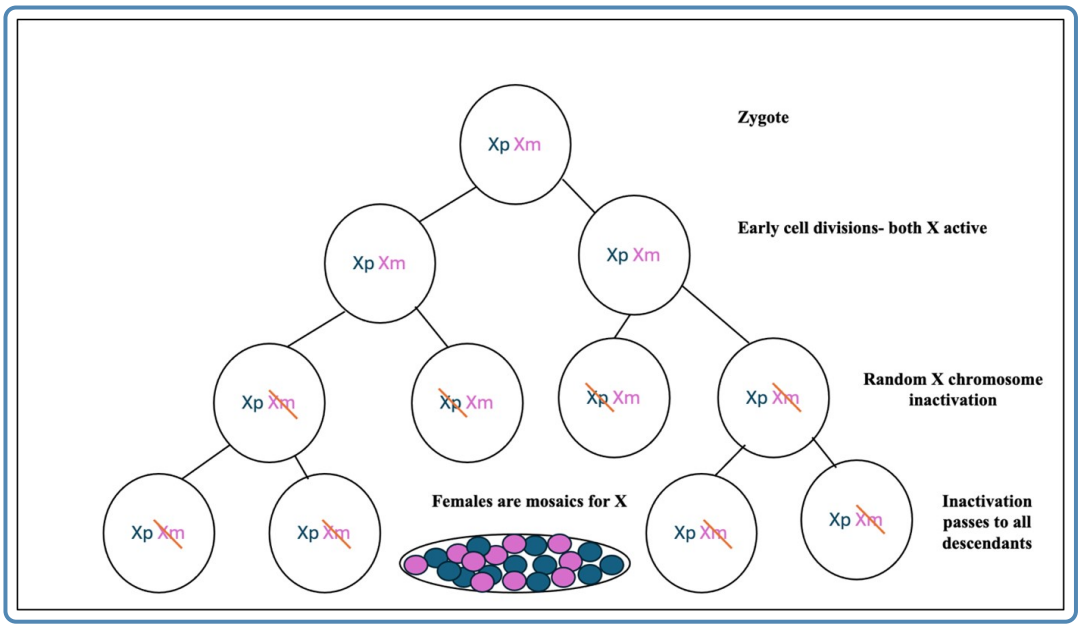


Figure 2 Random inactivation of one of the X chromosome in females through epigenetic silencing *Xp: paternally derived X chromosome, Xm: maternally derived X chromosome*

– Spontaneous ***reversion*** of a germline genetic alteration in a dividing cell that eliminates the germline variant (i.e., revertant mosaicism) (**Figure 4b**).

Common clinical presentations of mosaic disorders:

Mosaicism tends to present with the following clinical manifestations:

- Patchy distribution of pigmentary lesions (linear or whorled hyper- and/or hypopigmentation) is an important clinical clue. It may not be always obvious on external examination. For a few disorders like intellectual disability, the presentation can be mild and often overlooked.
- Growth abnormalities especially causing asymmetric, focal or segmental involvement.
- Asymmetric brain overgrowth and cortical malformations (e.g., focal cortical dysplasia, hemimegalencephaly)
- Vascular malformations (e.g., venous,

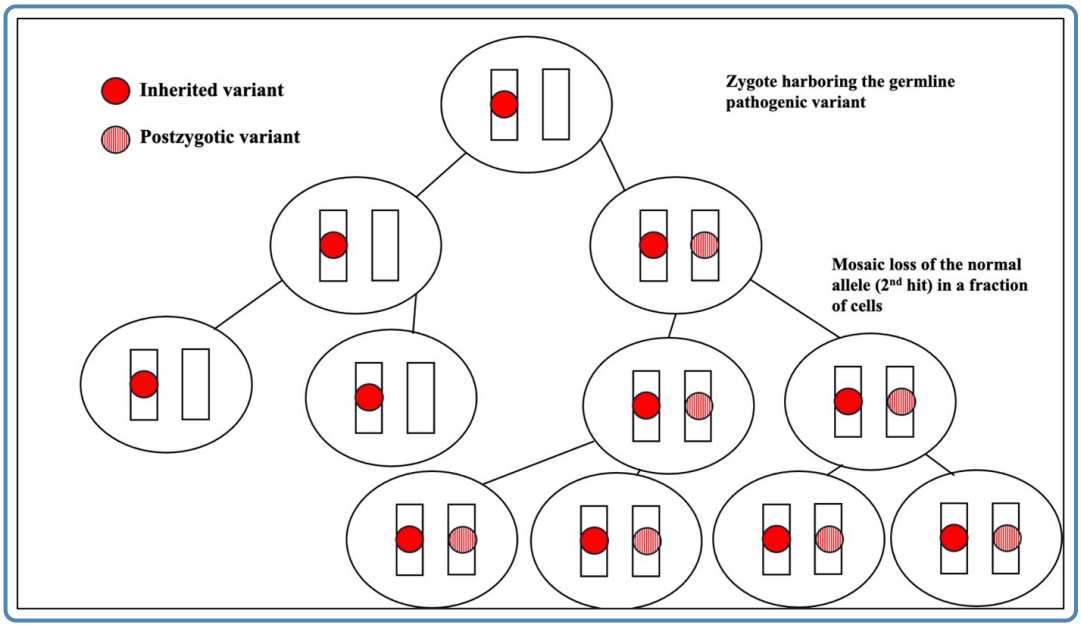


Figure 3 Mosaic nullizygosity- 2nd hit (genetic alteration) in a fraction of cells which already carry a germline variant

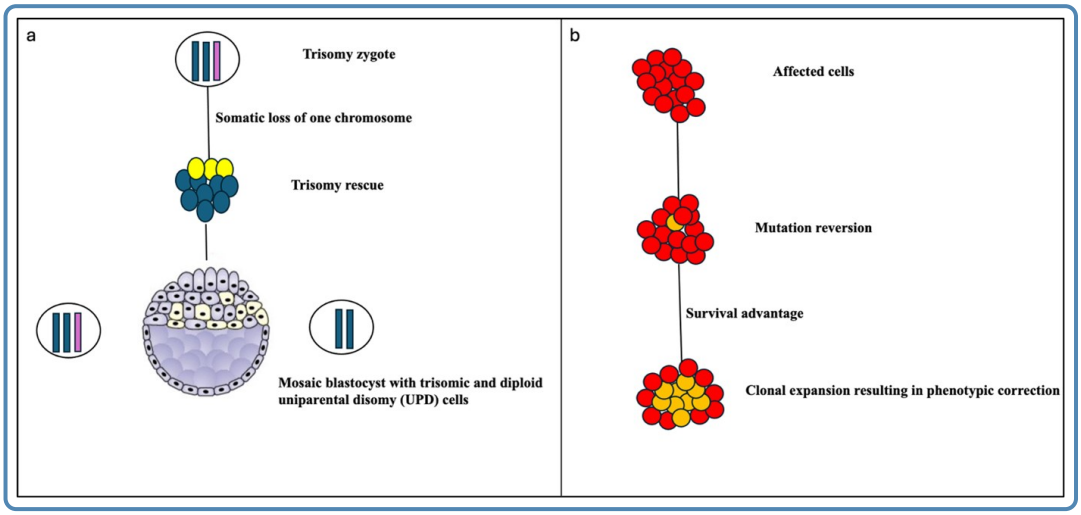


Figure 4 a) Mosaic blastocyst due to trisomy rescue, b) Revertant Mosaicism

capillary, mixed) and/or lymphatic malformations

- Benign and/or malignant tumours.

Mosaicism in different clinical contexts

Chromosomal mosaicism (Pre and postnatal scenarios)

Mosaicism for many types of chromosome abnormalities is well known in both abortuses and liveborn individuals. A constitutional gain or loss of only a few single chromosomes is compatible with viability: trisomy of chromosomes 13, 18, 21 and X. All of these trisomies or monosomies have also been detected as mosaic abnormalities, usually with a milder phenotype than the constitutional aneuploidy (**Figure 5**). Postnatally, the presence of patchy pigmentary abnormalities is a clue to possible chromosomal mosaicism. The presence

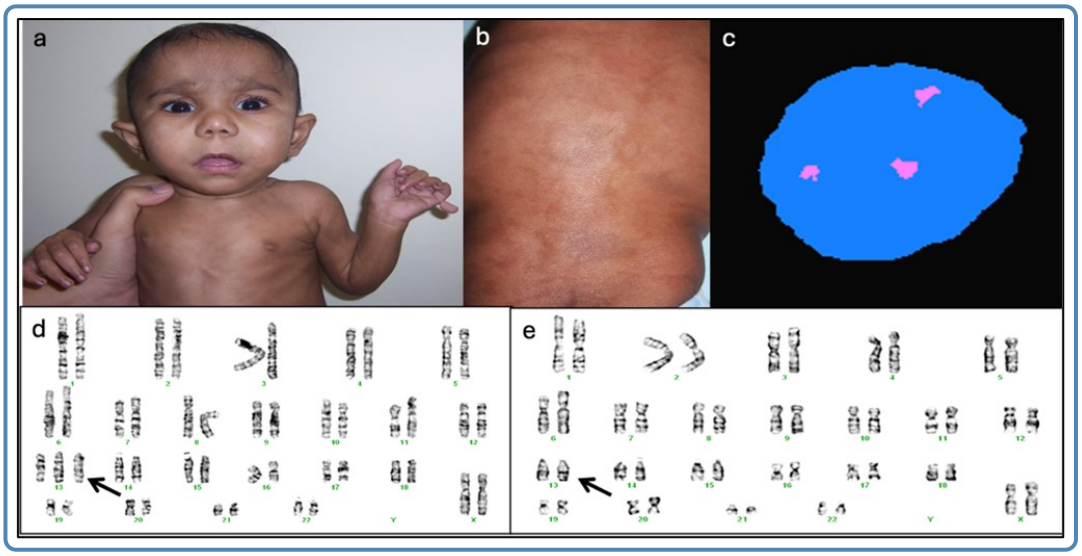


Figure 5 a) Child with global developmental delay and postaxial polydactyly, b) Patchy pigmentary abnormality of skin over back, c) Interphase FISH: 46,XX,+13 [80] / 46,XX [20], d and e: Karyotype: 47,XX,+13 [13] / 46,XX[7]. *Picture courtesy: Dr Shubha R Phadke*

of a normal cell line in patients with common trisomies, such as trisomy 21 or trisomy 18, may suggest better cognitive function, but there is no correlation between the level of mosaicism and the outcome.

An additional class of aneuploidies can be found only when they are mosaic, presumably due to selection against the aneuploid cells in a specific tissue or at a specific stage of development. These include trisomies of chromosomes 8, 9, 14, 17, and 22.

Mosaicism for various types of structural abnormalities has also been identified, including balanced and unbalanced translocations, deletions, duplications, inversions, ring chromosomes and isochromosomes (Biesecker et al., 2013). Isochromosomes are often supernumerary, presumably because the loss of even one copy of a chromosome arm can be lethal with a common exception of isochromosome Xq causing Turner syndrome which is well tolerated. Some of the commonly recognisable syndromes associated with isochromosomes include isochromosome 12p (Pallister– Killian syndrome), isochromosome 22q (cat eye syndrome), isochromosome 15q11 and isochromosome 18p.The isochromosome 12p seen in Pallister–Killian syndrome is always present in a mosaic form, as the abnormality would be lethal when constitutional. The mosaicism in these patients is almost always limited to cultured fibroblasts and is usually not seen in lymphocyte cultures. However, it can be detected sometimes with cytogenetic microarray techniques using DNA extracted from peripheral blood.

Prenatally, the effects of mosaic chromosomal aneuploidies on fetuses depend on the type of chromosome involved, mosaicism levels, and the tissues involved, the intrauterine phenotype and clinical outcomes. UPD and chromosomal aneuploidy often co-occur, so it is necessary to analyze them simultaneously when chromosomes with imprinted regions (chromosomes 6, 7, 11, 14, 15, 20) are involved or when there are known carriers of a recessive allele. The information provided during genetic counseling should include the affected chromosomes, mosaicism levels, mosaic tissues involved, methylation status, recessive gene variations on the UPD chromosome, intrauterine phenotypes, clinical manifestations of similar patients after birth, and current clinical treatments available.

Mosaic manifestations of Mendelian disorders

The mosaic manifestations of Mendelian disorders can be divided into three groups:

- Mosaicism for lethal mutations causing clinical pictures that exist only in mosaic form

- Mosaicism for mutations known in autosomal-dominant/X linked recessive disorders

- Rare mosaicism that causes aggravation of the phenotype in a segmental area due to a second mutation event on the other allele (usually loss of heterozygosity) in autosomal-dominant inherited disorders

Mosaicism for lethal mutations causes clinical pictures that exist only in mosaic form

Some mosaic disorders are caused by mutations that are seen only in mosaic form. These disorders are lethal in its constitutional state and hence cannot be passed on by the affected individuals to their offsprings. The classic example for this group of disorders is McCune–Albright syndrome (MAS) that occurs due to mosaic gain-of-function mutations in the *GNAS1* gene. Other classic examples include overgrowth disorders that are caused by activating mutations in *PIK3CA, AKT3* and *MTOR* pathways (Moog et al., 2020).

Reproductive counselling

These disorders are not inherited and arise sporadically early in embryonic development. The risk for an affected sibling is expected to be the same as in the general population. There is no possibility of vertical transmission due to embryonic lethality.

Mosaicism for mutations known in autosomal-dominant/ X-linked recessive disorders

Some of the common monogenic disorders for which somatic/ gonadal mosaicism is well documented include Duchenne muscular dystrophy, osteogenesis imperfecta, neurofibromatosis, hemophilia, Marfan syndrome etc. Somatic mosaicism is reported in 25-33% and 6.5% of persons with neurofibromatosis type 2 and type 1 respectively (Ruggieri and Huson., 2001). Somatic/gonadal mosaicism is present in 16% of asymptomatic parents of autosomal dominant *COL1A1* and *COL1A2* osteogenesis imperfecta patients (Pyott et al., 2011). Somatic mosaicism is reported in as high as 43% of persons with *ADCY5* related dyskinesias (Raskind et al., 2017). Some diseases, such as Cornelia de Lange syndrome (CDLS), show markedly high rates of mosaicism, even across mutational mechanisms and genomic loci (somatic mosaicism is reported in 10-15% of persons with *NIPBL*-related CDLS; Huisman et al., 2013). Mosaicism for increased *APP* gene copy number has been identified in the brains of individuals with Alzheimer's dementia, suggesting that mosaicism for mutations that cause Mendelian disease when present constitutionally may also contribute to sporadic disease (Bushman et al., 2015).

The severity and clinical symptoms of postzygotic mosaicism depend on the timing of the mutation event, the type of cell in which the mutation occurs, the expansion of cells with mutations, the mutated gene, and the specific mutation. Depending on the timing of the mutation event, these mosaics can present in a disseminated manner, causing atypical or attenuated forms of a clinical picture, or localized as segmental mosaicism type I, which generally has milder effects, such as in segmental neurofibromatosis type 1 (NF1) or mosaic forms of tuberous sclerosis. Parents with segmental neurofibromatosis can produce offspring with a constitutional/generalized form of NF1 when gonosomal mosaicism underlies their disease (Consoli et al., 2005). Sometimes, mosaicism can result in phenotypes that differ significantly from the expected manifestation of the disorder, potentially presenting as a different disorder altogether. For example, mosaic RASopathies have a different phenotype compared to constitutional RASopathies, being based on postzygotic gain-of-function mutations in *RAS/RAF* genes, which would presumably be lethal if they were constitutional. (Hafner and Grosser., 2013)

Reproductive Counselling

Parents with somatic mosaicism can produce offspring with a constitutional form of the disorder if their gonadal tissue is also affected. On the other hand, pure germline mosaicism is typically identified when multiple siblings are born with a genetic condition, despite their parents being unaffected (**Figure 6**). This occurs because the mutation arises during the formation of the parent's germline cells, meaning it is confined to the germline and not present in the parent's somatic cells, leaving them asymptomatic. Since genetic testing in parents usually examines DNA from blood samples, it would generally reveal the wild-type variant in such cases. Although there is empirical data on recurrence risks for some sporadic forms of autosomal dominant or X-linked disorders, this information is lacking for many rare de novo conditions. Therefore, prenatal

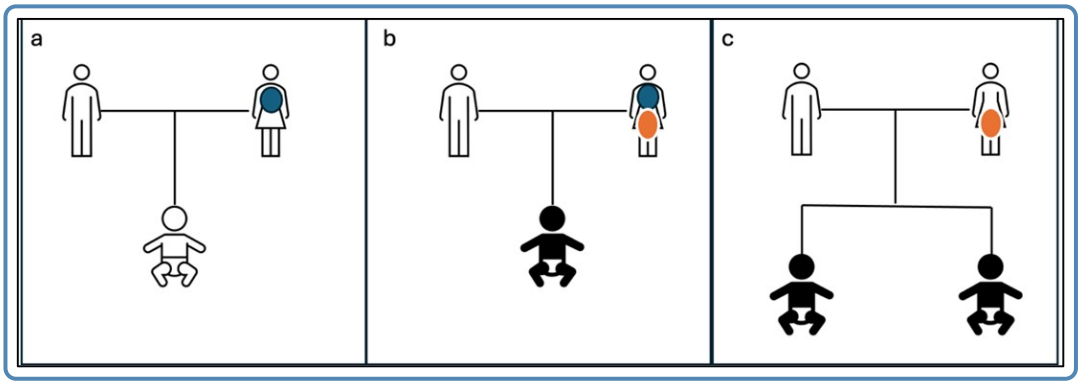


Figure 6 a: Mother carries mosaic variant in somatic cells and is mildly affected. Child is phenotypically normal. b: Mother carries mosaic mutation in both somatic and gonadal cells. Mother is mildly affected but child is affected with a generalized and severe form of the disease. c: Mosaic variant is restricted to gonadal cells in mother. Mother is phenotypically normal but multiple children are affected. *Blue circle: Somatic mosaicism, Orange circle: Gonadal mosaicism*

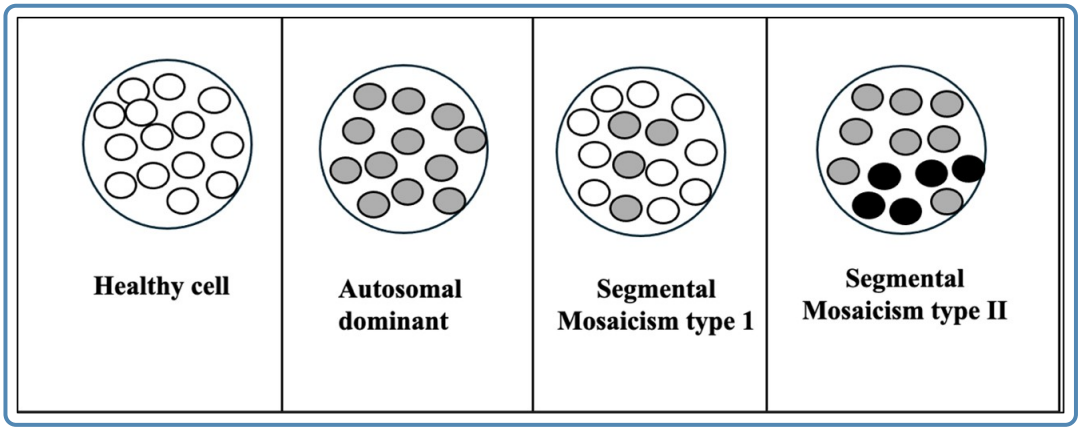


Figure 7 Segmental Mosaicism. Type 1: mosaic mutation in subset of cells, Type 2: second mutation in the subpopulation of precursor cells in an individual who already carries a germline mutation *White circle: Wild allele in homozygous state, Grey circle: Mutated allele in a heterozygous state, Dark circle: Two mutated alleles*

testing is currently justified for all families with sporadic cases of autosomal dominant or X-linked conditions.

Rare mosaicism that causes aggravation of the phenotype in a segmental area

Type II segmental mosaicism is a type of segmental mosaicism in which a second mutation occurs in the subpopulation of precursor cells in an individual who already carries a germline mutation. This leads to segments with more severe phenotypes. Examples of type II segmental mosaicism are manifestations of Hailey-Hailey and Dariers diseases with stripes of more severe cutaneous disease (**Figure 7**).

Epigenetic Mosaicism

Epigenetic mosaicism is defined as the presence of different epigenotypes in different cell populations within an organism developed from a single zygote. Epigenetic mosaicism is described in a number of genomic imprinting disorders, including hydatidiform mole, Angelman syndrome (AS), Prader–Willi syndrome (PWS), Silver–Russell syndrome (SRS), Beckwith–Wiedemann syndrome (BWS), Temple syndrome, pseudohypoparathyroidism 1B, and

transient neonatal diabetes mellitus (TNDM). Epigenetic mosaicism in genomic imprinting disorders seems to have different contributions to the formation of the clinical picture of the disorder. It was demonstrated that mosaicism in the case of BWS, PWS, and AS was associated with a milder phenotype and the appearance of clinical features were not characteristic of the given pathology (Sazhenova and Lebedev., 2019). The leading diagnostic features of AS include gait ataxia and absence of speech; whereas patients with SNURF-SNRPN imprinting centre mosaicism are able to speak single words, they have no stereotypic movements, and in some cases, there are clinical features overlapping with PWS (obesity and hyperphagia) (Le-Fevre et al., 2017). Mosaicism can therefore significantly obscure the typical clinical presentation of syndromes, making both clinical diagnosis and molecular genetic analysis challenging. For disorders like TNDM and SRS, the classical phenotype can result from even a small clone of epigenetically altered cells. In addition, epigenetic mosaicism was observed only in certain tissues in patients with TNDM (Sazhenova and Lebedev., 2019).

Fragile X syndrome is a triplet repeat disorder often characterized by the presence of mosaicism in *FMR1* variants. This mosaicism can manifest as either repeat size mosaicism or methylation mosaicism. Studies indicate that individuals with methylation mosaicism tend to perform at a higher intellectual level compared to those with fully methylated full mutations. This is because the production of FMR1 protein (FMRP) occurs from the transcription of the unmethylated alleles, which supports better cognitive functioning.

Revertant and Rescue Mosaicism

Revertant mosaicism is a phenomenon in which a mutation gets spontaneously corrected in a subset of cells in an affected organ perhaps driven by selective pressure. Revertant mosaicism can occur in the germline or in somatic cells. *In vivo* reversion of somatic cells tends to predominantly involve tissues with high cell proliferation rates including the skin, liver and the hematopoietic system (Lai-Cheong et al., 2011). The classic examples include epidermolysis bullosa, ichthyosis, adenosine deaminase deficiency, Wiskott-Aldrich syndrome, Bloom syndrome, Fanconi anemia, tyrosinemia I, etc. Germline revertant mosaicism has previously been described in myotonic dystrophy wherein the size of the CTG repeats in two unrelated healthy individuals, born to clinically affected parents, was normal despite having inherited the myotonic dystrophy DNA-marker haplotype (Brunner et al., 1993)

Revertant mosaicism often leads to phenotypic improvement and is therefore called “natural gene therapy”. It can also modify the phenotypic expression leading to atypical presentations of disease (Wada et al., 2008). The timing of reversion during development can also influence the extent of revertant mosaicism and the severity of the condition. Very rarely, unfavourable outcome due to overcorrection of mutants by somatic mosaicism has also been reported (Inaba and Nagamachi., 2021)

Confined placental mosaicism

Confined placental mosaicism (CPM) is defined as a chromosomally abnormal cell line restricted to the placenta, while the chromosomes of the fetus itself are normal. It arises either due to non-disjunction in a diploid conception as a post-zygotic error or by a trisomic rescue mechanism, wherein a viable trisomic conceptus loses one chromosome through anaphase lagging and produces a diploid cell line. CPM can be categorized into three subtypes (type 1, 2 and 3) depending on where the chromosomal abnormality is found in the placenta (Toutain et al., 2018).

Type 1: In type I CPM, the chromosomal abnormality is only found in the cytotrophoblast and can be found after examination of the short-term culture villi. Nowadays, this methodology is not in use as rapid results are available with other techniques like fluorescence in situ hybridization (FISH) and quantitative fluorescent polymerase chain reaction (QFPCR) for detection of aneuploidies.

Type 2: In type 2 CPM, the chromosomal abnormality is only found after long-term culture of villi and is restricted to the mesenchymal core of the chorionic villi.

Type 3: Type 3 CPM is characterized by the presence of the abnormality in both the mesenchymal core and cytotrophoblast and can be found after both long term and short-term cultures.

CPM is believed to occur in roughly 1–2% of all placental tissue analysis. The presence

of chromosomally abnormal cells restricted to the placental areas that are not sampled by chorionic villus sampling (CVS) can go unnoticed. Non-invasive prenatal screening on the other hand appears to be more sensitive to detect CPM as compared to CVS, as the entire placental trophoblast sheds cfDNA into the maternal circulation (Brison et al., 2018; Van Opstal et al., 2018)

CPM significantly contributes to fetal growth restriction (FGR) (71.7%) and is associated with a high rate of premature births (31%). It is crucial to conduct thorough examinations of CPM pregnancies for structural fetal anomalies, given the notable 24.2% occurrence rate when analyzing placenta and fetal tissues. Investigations for uniparental disomy (UPD), notably involving chromosome 16, are advised due to their association with a higher frequency of FGR and premature delivery. High mosaicism levels in chorionic villus sampling (CVS) and UPD has been observed to result in adverse pregnancy outcomes (Eggenhuizen et al., 2021)

Overall, in cases of suspected CPM, pregnancy should be classified as high-risk, warranting intensive fetal growth monitoring from the first trimester, especially if trisomy involves chromosomes 2, 3, 7, 13, 15, 16, or 22. CPM especially involving chromosomes 21 and 8 poses a heightened risk for FGR. Counseling future parents should involve emphasis on the increased incidence of premature birth, structural fetal anomalies, FGR, and low birth weight. Conversely, CPM involving trisomies for chromosomes 9, 10, 12, 18, and 20 does not show indications of adverse pregnancy outcomes (Eggenhuizen et al., 2021)

Mosaicism in embryos detected by preimplantation genetic testing

With the advent of preimplantation genetic testing for aneuploidies (PGT-A), a new clinical scenario has emerged where mosaicism is detected in embryos. Embryos with an intermediate result after PGT-A between the range of euploidy and the range of aneuploidy, have been termed as 'mosaic embryos'. An embryo that is putatively mosaic is a frequent finding after PGT-A in recent times due to high sensitivity and resolution of NGS. A recent study showed a mosaic embryo rate of 2-13% with NGS analysis in trophectoderm cells and tends to decrease throughout pregnancy (Popovic et al., 2020)

Embryonic mosaicism can be classified using different parameters: grade of mosaicism (based on the percentage of aneuploidy), number of chromosomes involved (simple mosaic, complex mosaic or chaotic mosaic), cell lines affected [total, inner cell mass (ICM), trophoectoderm, ICM/trophoectoderm] or type of abnormality (whole-chromosome mosaic or segmental mosaic).The clinical decisions around transferring this type of embryo can be challenging, particularly when no chromosomally normal embryo is available.

Different international societies have published guidelines and position statements with their own set of recommendations regarding transfer priority in cases of embryo mosaicism detected through preimplantation genetic testing for aneuploidies (PGT-A). A summary of these recommendations is as follows:

a) Mosaic euploid/ monosomy mosaicism should be prioritized over euploid/ trisomy mosaicism [Preimplantation Genetic Diagnosis International Society (PGDIS) position statement 2016]

b) Low mosaicism level (20-40%) should be prioritized over high-level mosaicism (40-70%) [Controversies in Preconception, Preimplantation and Prenatal Genetic Diagnosis (CoGEN) 2017; PGDIS 2021 third statement]

c) Theoretical implications of the chromosome(s) involved should be considered (chromosomes not associated with a known chromosomal disorder should be favoured over chromosomes associated with uniparental disomy, intrauterine growth restriction or a viable chromosomal syndrome) (PGDIS 2016)

d) Avoid the transfer of mosaic embryos involving chromosomes 13, 14, 16, 18, 21 or 45,X as they carried the greatest risk of an affected viable pregnancy (PGDIS 2019 second statement, CoGEN 2017)

e) Segmental mosaicism prioritized over whole-chromosome mosaicism (PGDIS 2021 third statement)

f) When two embryos have identical qualities, a preference could be established based on their morphology (PGDIS 2021 third statement)

g) A new stimulation cycle is not advised when transferrable low-level mosaic embryos are available [European Society of Human Reproduction and Embryology (ESHRE) 2022]

There are conflicting reports regarding the clinical outcome of transferring mosaic embryos. While some have reported a reduced rate of implantation and an increased rate of miscarriage with mosaic embryo transfers, others have reported successful pregnancies following the transfer of embryos with mosaicism. However, data regarding the outcome for specific types of mosaicism are still limited and should be interpreted with caution. Each mosaic embryo should be evaluated individually, and in cases involving more than one mosaic embryo available for transfer, prioritization should be based on the most current evidence. According to current evidence, embryos with results within the mosaic range should not be discarded or disregarded for transfer, as this practice could negatively affect the cumulative live birth rate per cycle (Munoz et al., 2024).

Overall, preimplantation genetic testing for aneuploidies (PGT-A) is not without its limitations, including the potential for both false positive and false negative results. While some studies indicate a favorable impact of PGT-A on pregnancy outcomes, particularly in specific cases, the results are inconsistent, especially for women under 35 years old. Given these uncertainties, it is crucial to offer amniocentesis after the transfer of mosaic embryos to confirm the genetic status of the fetus. Pretest counseling is a critical component of incorporating PGT-A into assisted reproduction. During counseling, families should be thoroughly informed about the benefits, limitations, and potential risks associated with PGT-A. They should also be made aware of their right to refuse PGT-A, ensuring that their decision is well-informed and respects their autonomy.

Overview of challenges in genetic counselling in mosaicism

- Mosaic phenotypes may have incomplete syndromic features, which may stay unnoticed, especially in a low-grade mosaicism
- Mutation load in the material tested does not necessarily correlate with the severity of disease.
- Blood cells are an unstable source of genetic material given multiple rounds of self-renewal during haematopoiesis. Multiple sources of primarily obtained DNA needs to be examined. Ectodermal tissues can be sampled from buccal brushings or hair root bulbs, mesodermal tissues are available from blood or saliva, while endodermal origin DNA is available from urothelial cells collected in urine samples. Levels of mosaicism across tissues and body locations can show surprising variability, even within the same embryonic lineage. A common example includes the i(12p) (Pallister Killian syndrome) which is not usually observed in cultured peripheral blood Tcell lymphocytes analysed by Gbanding, but can generally be identified in cultured skin fibroblasts in a mosaic state.
- Low levels of somatic mosaicism may not be detectable by standard genetic sequencing and pure germline mosaicism will not be detectable by testing of specimen types routinely available to diagnostic laboratories.
- Usually more than one technique is needed to recognize mosaicism and an additional method, usually different than the first one used, is required to confirm the result (Table 1).
- Empiric recurrence risk estimates are only available for the most prevalent and well-studied diseases making counselling challenging for other rare genetic disorders (Table 2).

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Table 1 Genetic tests for detection of mosaicism (Campbell et al., 2015)

Technique	Clinical utility and Limitations
Karyotype	Cytogenetic analysis of 30 metaphase cells can identify 10% mosaicism with a confidence level of 95% in case of sex chromosomal mosaicism Poor resolution (5-10Mb), Cannot detect mosaicism < 10% .
FISH	Laborious; cannot detect mosaicism < 10%; can count hundreds of cells in interphase FISH with relative ease.
Array-based techniques	aCGH: mosaicism detection level as low as 10–20% SNP array: Mosaicism for CNVs of modest size can be detected as low as 5% by searching for slight deviation from expected allele frequencies, but resolution is limited by availability of known polymorphic SNPs
Sanger sequencing	Mosaicism for dominant alleles present in less than 25–35% or greater than 65–70% of cells can remain undetected
Pyro-sequencing	Offers better minimum detection limits (as low as 5%) with more quantitative results. Results can be impeded by sequence features of the target, specifically repetition of the same nucleotide.
Massively parallel sequencing	The ability to detect mosaicism is proportional to the read depth of coverage or number of reads that are available covering a given base position. Next-generation sequencing with deep sequence coverage enhances sensitivity and allows for accurate quantification of the level of mosaicism. Although NGS technologies allow for low-grade mosaicism detection, number of samples to be tested per one patient is limited because of the high cost.
Person-alised assays	For massively parallel sequencing, custom-capture reagents, which enrich the DNA library to be sequenced for template molecules of interest allow for greater sensitivity Digital PCR: Identify mosaic SNVs and indels at levels as low as 0.1% Molecular inversion probes: Identify mutant DNA at levels as low at 0.5%

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Table 2 *Overview of counselling regarding recurrence risk* * (Adopted from Wallace et al., 2022)

Genetic disorder	Counselling
Chromosome disorders that frequently occur in mosaic form	Recurrence risk in sibs is expected to be low in the absence of a genetic predisposing factor (such as biallelic *BUB1B* pathogenic variants) in parents.
Mosaic disorders due to postzygotic *de novo* heterozygous variants	The risk of recurrence in subsequent offspring of the parents of an affected child is not increased compared to the general population.
Monogenic disorders that frequently occur in mosaic form	The risk that a parent with germline mosaicism will transmit the pathogenic variant to offspring is <50%. When transmitted, the variant will be constitutional, and the child will be severely affected.
Disorders associated with epigenetic mosaicism	The recurrence risk for siblings would be expected to be as in general population.

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Spliceosome Variants: Insights into Disease Pathology, Current Detection Techniques, and Clinical Implications in Genetic Diseases

Prince Jacob[1#], Keerthana Kumari S[1], Rachanashree HN[1], Akila Prashant[1,2], Siddaramappa J Patil[3#]

[1]*Department of Medical Genetics, JSS Medical College, and JSS Hospital, JSS Academy of Higher Education and Research, Mysore, Karnataka, India*

[2]*Department of Biochemistry, JSS Medical College, and JSS Hospital, JSS Academy of Higher Education and Research, Mysore, Karnataka, India*

[3]*Division of Medical Genetics, Mazumdar Shaw Medical Center, Narayana Hrudayalaya Hospitals, Bangalore, India*

Correspondence to: Dr Siddaramappa J Patil *Email:* drsjpatil@gmail.com

& Dr Prince Jacob *Email:* princejacob@jssuni.edu.in

Abstract

The mechanism involved in pre-mRNA splicing represents a vital stage in eukaryotic gene expression, ensuring the precise excision of introns and the joining of exons to yield mature mRNA transcripts. Deviations in this process, often instigated by spliceosome variants, can result in irregular splicing patterns which impact gene function, and thus contribute to the development of various human diseases. This review examines the diverse forms of spliceosome variants and their implications in disease pathology, encompassing variants at donor and acceptor splice sites, deep intronic variants, exonic variants affecting splicing, and alterations in branch points. The array of methodologies, including bioinformatics tools, experimental procedures, and functional assays, utilized for the detection of spliceosome variants and the elucidation of their functional impacts, is discussed. A few variants identified at our centre in patients with different genetic disorders with a confirmed molecular diagnosis are enumerated in this study. Furthermore, the clinical significance of spliceosome variant detection in disease diagnosis, prognosis, and treatment is underscored in this study, emphasizing the potential of personalized medicine strategies. Finally, future avenues of research in spliceosome variant investigations are outlined, which underscores the necessity of interdisciplinary approaches and collaborative endeavors in advancing precision medicine and healthcare.

Keywords: Splice site variants, Spliceosome, pre-mRNA splicing, Disease pathology, Detection methodologies

Introduction

Pre-mRNA splicing, orchestrated by the spliceosome, is a fundamental process in eukaryotic gene expression. This highly regulated process involves the removal of intronic sequences and the ligation of exons to generate mature mRNA transcripts (Ares et al, 1999). The spliceosome, a dynamic macromolecular complex comprising both RNA and protein components, governs the fidelity and precision of splicing events. The splicing process, which occurs in the nucleus, relies on the interaction of cis and trans elements. Cis elements are DNA sequences crucial for splicing regulation, including donor and acceptor splice sites, branch points, polypyrimidine tracts, as well as splicing enhancers and silencers. These sequences collectively form consensus splice site sequences. The spliceosome, a complex composed of five small nuclear ribonucleoproteins (snRNPs) and

numerous proteins, catalyzes splicing. Through complementary RNA-RNA interactions facilitated by small nuclear RNAs (snRNAs) within snRNPs, the spliceosome accurately identifies specific splicing sites (Anna et al, 2018).

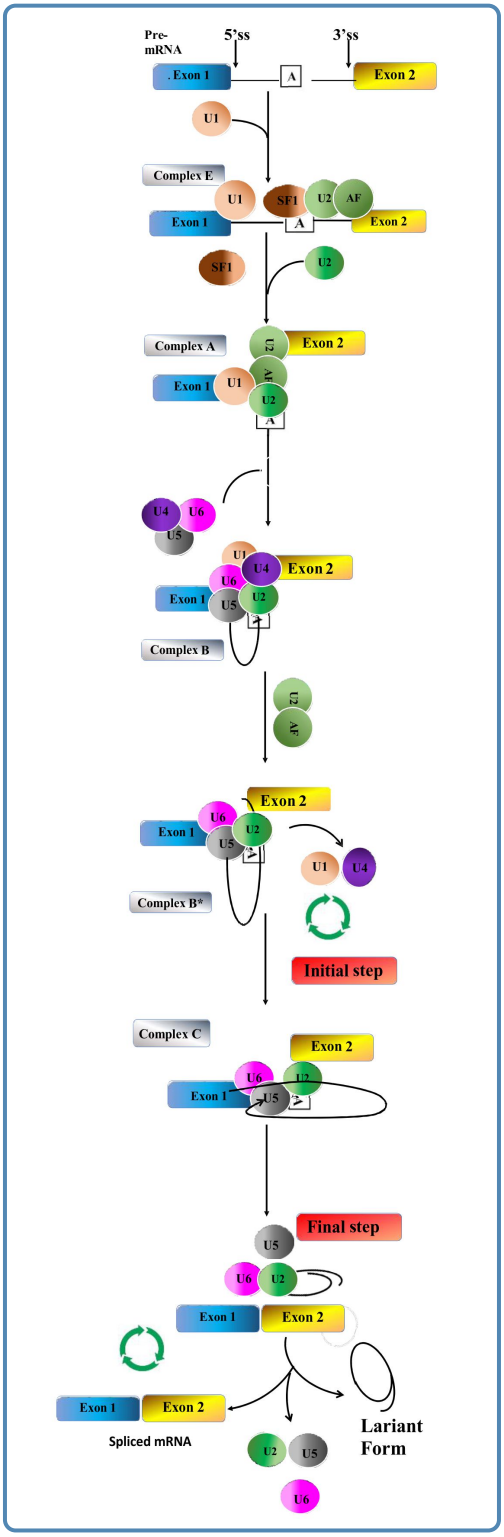

Figure 1 **Pictorial representation of the splicing process.** The process of splicing involves two main steps: recognizing the splicing sites at the intron/exon junctions and removing the introns while joining the exon ends. Initially, four complexes form between pre-mRNA and the spliceosome. The early (E) complex occurs when U1 snRNP binds to the AG-GU sequence at the 5' splice site, and SF1 binds to the branch point. This helps U2AF65 bind. U2 snRNP then displaces SF1 at the branch point, forming the ATP-dependent A complex. RNA helicases Prp5 and Sub2 stabilize these interactions, facilitating the recruitment of U4/5/6 tri-snRNP to form the B complex, or pre-catalytic spliceosome. Further RNA helicase activity rearranges the spliceosome, releasing U1 and U4 snRNPs and allowing U6 to interact with U2 snRNP, forming a pre-mRNA loop in the C complex. Within this complex, two transesterification reactions occur: the intron is removed, and the exon ends are joined.

The splicing process (**Figure 1**) involves two main steps: first, the recognition of splicing sites at intron/exon junctions, and second, the removal of introns and joining of exon ends. This process involves the formation of four complexes between the pre-mRNA and spliceosome. Initially, the early complex (E) forms are formed, where U1 snRNP binds to the donor splice site while SF1 and U2AF65 proteins bind to the branch point and polypyrimidine sequence, respectively. Subsequently, the ATP-dependent (A) complex is formed as SF1 is displaced by U2 snRNP. Stabilization of the branch point-U2snRNP interaction signals the recruitment of U4/5/6 tri-snRNP, leading to the formation of the B complex (pre-catalytic spliceosome). Further action by RNA helicases triggers spliceosome conformational changes, resulting in the release of U1 and U4 snRNPs and the formation of the C complex. Within this complex, two transesterification reactions occur, leading to intron removal and the joining of exon ends (Anna et al, 2018).

Disruption of spliceosome function, often

driven by variants, can lead to aberrant splicing patterns, resulting in the production of dysfunctional protein isoforms and contributing to the pathogenesis of various human diseases.

Canonical splice sites are characterized by the consensus sequences of GT (donor splice site) and AG (acceptor splice site) dinucleotide sequences (GT-AG) located at the 5' and 3' ends of introns. These sequences play a crucial role in accurately removing introns during mRNA maturation, with specific residues at positions +1 and +2 at the 5' donor splice site and positions -1 and -2 at the 3' acceptor splice site. Non-canonical splice site variants deviate from the usual GT-AG dinucleotide pairs found at the 5' and 3' ends of introns (donor and acceptor splice sites; GT-AG). These variants can disrupt normal splicing processes, affecting the maturation of mRNA. Research indicates that these non-canonical splice sites can produce abnormal transcripts, such as cryptic exons, with the extent of their occurrence influenced by the specific cellular environment. They are implicated in various genetic disorders such as congenital CD59 deficiency. Birt-Hogg-Dube syndrome, and ciliopathies. They are significant targets for identifying causative variants and enhancing understanding of disease mechanisms for better diagnosis and treatment (Anna et al, 2018; Chai et al., 2022). This article provides an overview of the current understanding regarding 'splice site variants', and techniques used for detecting these alterations in clinical diagnosis.

Different categories of splice site variants

Splice site variants can be categorized into various types depending on how they impact pre-mRNA splicing. These include:

Type 1: Variants at canonical splice sites and adjacent consensus sequences causing exon skipping

The first and most common category of splicing variants occur at the canonical splice sites, and lead to complete or partial exon skipping (**Figure 2A**). The most common variants typically impact the residues positioned one or two bases ahead of the 5' donor splice site and one or two bases before the 3' acceptor splice site. An analysis of splicing variants revealed a higher occurrence of variants at the donor splice site compared to those at the acceptor splice site when considering individual genes. Specifically, within the *NF1* gene, it was observed that variants affecting the 5' splice site were more frequent, accounting for 65% of cases, while variants affecting the 3' splice site occurred in 35% of cases (Anna et al., 2018). The impact of the variant at the canonical splice site may vary based on factors such as the strength of the splicing site, the presence of cryptic splice sites, the density of exonic splicing enhancers (ESE) and exonic splicing silencers (ESS), and the secondary structures formed by the pre-mRNA. The splicing complex primarily recognizes robust splice sites, and if the canonical splice site undergoes mutation, there is a higher likelihood of activating cryptic splice sites. In instances of weak splice sites, the likelihood of complete exon skipping is higher than the utilization of alternative splicing motifs (Anna et al., 2018).

Type 2: Intronic variants deep within the gene leading to the inclusion of a pseudo exon

The second category includes deep intronic variations that result in the inclusion of an intron fragment, the so-called cryptic exon or pseudo exon, into the mature transcript (**Figure 2B**). Functionally, such variants create novel acceptor /donor sites that are identified by the splicing and are used in combination with the existing intronic cryptic splice sites. One of the most common and well-known deep intronic change is a c.3718-2477C>T variant being one of the most frequent variants in *CFTR* gene responsible for cystic fibrosis (CF) (Anna et al., 2018).

Individuals with the c.3718-2477C>T variant in CF patients frequently exhibit a relatively mild phenotype, demonstrating variable disease expression. It has been observed that for the patients with CF the disease severity shows an inverse correlation with the abundance of accurately spliced transcripts, indicating that splicing regulation could serve as a significant modifier of the clinical course of cystic fibrosis in the presence of intronic variants (Anna et al., 2018).

Type 3: Variants in coding regions resulting in the loss of an exonic segment

Single nucleotide variants within exons can create new splice sites and can lead to the exclusion of an exon fragment (**Figure 2C**). These variants can establish a novel 5' or 3' splice site or activate a cryptic one that proves more robust than the original, thereby altering pre-mRNA processing and the loss of an exon fragment, referred to as type III splicing variant (Anna et al., 2018). It is important to note that variants in exons that lead to splicing changes are prone to

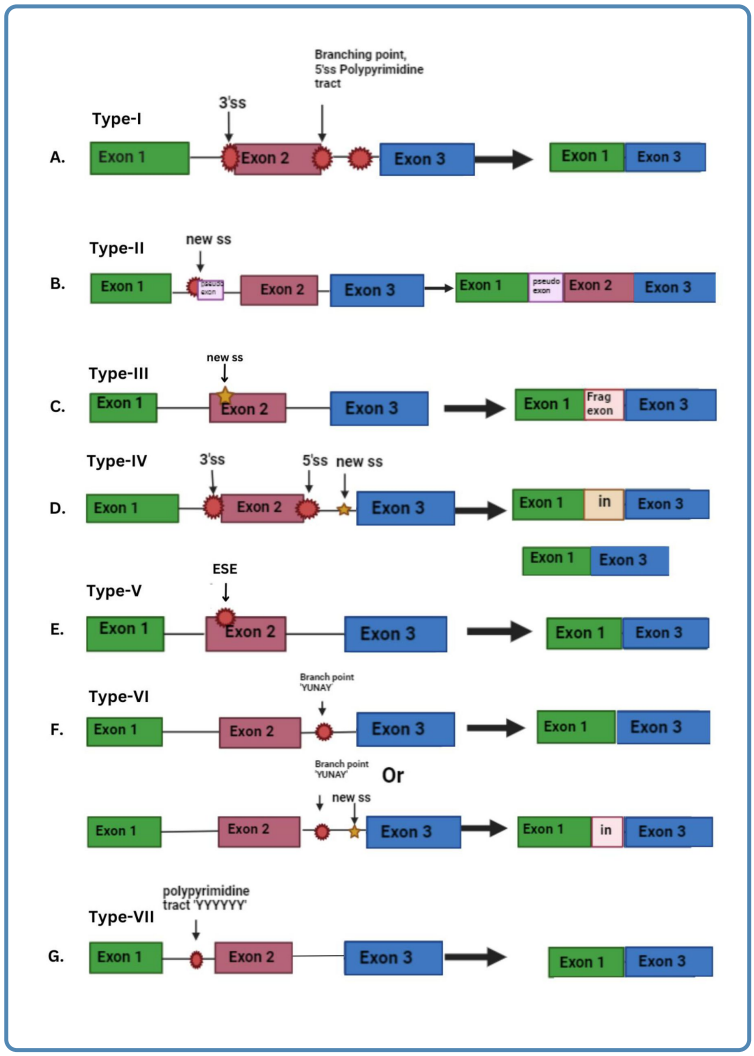

Figure 2 **Seven different types of splice site variants.** (A) Variants at canonical splice sites causing exon skipping. (B) Intronic variants deep within the gene leading to the inclusion of a pseudo exon. (C) Variants in coding regions results in the loss of an exonic segment. (D) Variants at canonical splice sites cause intron exclusion and skipping of exons. (E) Variant that disrupts ESE causes exon skipping or intron retention. (F) Variant at the branch point (YUNAY sequence) results in exon skipping. (G) Variant in Polypyrimidine Tract sequence [(Y)12–17] may lead to splicing alterations.

misclassification as synonymous, missense, or nonsense variants. Typically, the existence of these variants gives rise to two distinct transcripts from a mutated allele: one maintains the correct length but possesses a modified nucleotide, while the other is shorter and lacks an entire exon or a portion of it due to the nonspecific activity of the splicing complex (Ares et al.,1999). A good example of this is an exonic missense variant, c.887G>A in *MED12* resulting simultaneously in substitution of Arginine to Glutamine at codon position 296 and an aberrant splicing process leading to in-frame deletion of 42 bps in exon 7, r.847_888del (**Table 1**). Thus, through loss of the exonic segment and substitution, this missense variant leads to X-linked Ohdo syndrome (Togi et al., 2024).

Type 4: Variants at canonical splice sites cause intron exclusion and skipping of exons

Variants occurring in the canonical splice sites can alternatively (in contrast to type 1) lead to the activation of cryptic exonic or intronic splice sites. This activation results in the inclusion of an intron

fragment or the skipping of an exon fragment (**Figure 2D**). On analysis of the c.1525-1G>A variant in intron 9 of the *CFTR* gene, three distinct mRNA isoforms employing alternative splice sites within intron 10 and exon 10, positions c.1610–1611 and c.1678–1679 were identified. These isoforms were also found to lack the entire exon 10 or part of the fragment (Anna et al., 2018).

Type 5: Variants that disrupt ESE and lead to skipping of exon

Variants occurring within the exon can also (in contrast to type 3) cause the disruption of exonic splicing enhancers (ESE), which in turn leads to complete exon skipping (**Figure 2E**). Hence, the presence of exonic changes that result in the interference with exonic splicing enhancers is known as a type V splicing mutation. The utilization of RNA/cDNA sequencing in the diagnosis of genetic diseases is instrumental in identifying type V splicing variants (Ares et al., 1999; Anna et al., 2018). On analysis of *NF1* variants in the Leiden Open Variation Database (LOVD), it was found that 69% of the exonic variants were predicted to disrupt ESEs (Anna et al., 2018).

Type 6: Variants at the branch point (YUNAY sequence) results in exon skipping or intron retention

The branch point motif plays a crucial role in the early formation of the spliceosome complex. Changes in the branch point sequence can impact splicing accuracy. The branch point motif, positioned between −9 and − 400 base pairs downstream from the acceptor site, holds significance in humans for early spliceosome complex formation. It carries the consensus sequence YUNAY. Because branch point sequences are inherently variable, variants in this region may result in exon skipping. This occurs due to the improper binding of snRNP splicing proteins, leading to the disruption of the natural acceptor splicing site. Additionally, variants in the branch point sequence can induce intron retention if they generate a new 3' splice site (**Figure 2F**). Variants affecting the branch point sequence have been identified in the neurofibromatosis type 1 (*NF1*) gene. For instance, the variant 2410-18C>G in *NF1* leads to the partial retention (17 base pairs) of intron 15. This variant disrupts the original branch point sequence while creating a potential exonic splicing enhancer (ESE). Other splicing variants near this position include 2410-16A>G, 2410-15A>G, and 2410-12T>G. These findings underscore the critical role of this intronic fragment in facilitating the proper splicing of exons 15 and 16 (Anna et al., 2018).

Type 7: Variants in the polypyrimidine tract sequence [(Y)12–17] may lead to splicing alterations

Variants occurring in the polypyrimidine tract (situated upstream of the 3' splice site) or the pyrimidine-rich region (situated downstream of the 5' splice site) can lead to splicing alterations. These types of variants are rare. This sequence is crucial for binding the U2AF65 spliceosome subunit and the polypyrimidine tract-binding protein, both of which play a role in the regulation of alternative splicing (**Figure 2G**). Variants within the polypyrimidine tract have been observed in Hemophilia B, such as the variant c.253-19_253-16del in *F9*. This variant leads to the reduction of the polypyrimidine tract length from 24 nucleotides to 20. Consequently, this alteration causes inefficient splicing, resulting in the skipping of exon 3 (Anna et al., 2018).

Point variants at the branch point and polypyrimidine tract are infrequent and challenging to detect when analyzing genomic DNA, especially in coding sequences. Identifying their precise location is difficult, making it challenging to draw conclusions about the potential impact of a specific variant in these regions solely through genomic DNA analysis. To address this, RNA/cDNA sequencing is often employed, or their effects are evaluated through functional studies, such as minigene assays (Anna et al., 2018).

Synonymous variations in splice sites can cause diseases

Synonymous variants change the DNA sequence of a gene without affecting the amino acid sequence of the encoded protein. Though these types of variants are considered non-pathogenic, some synonymous variants can affect RNA splicing, translational efficiency, and mRNA stability. Synonymous variants can occur in a gene that has been directly associated with disease pathogenesis, as has been shown in the case of Treacher–Collins syndrome 17, Xlinked infantile spinal muscular atrophy 19, Seckel syndrome 20 and cystic fibrosis (Sauna et al., 2011). One investigation revealed that a synonymous variant within the *IL2RG* gene resulted in an abnormal splice pattern, leading to decreased expression of the common gamma chain (γc) and the onset of late onset combined

Table 1 Analysis of different splice site variants from our centre using *in-silico* prediction tools.

Gene	Variant details	Zygosity	Splice site tool predictions		OMIM Disease [MIM#]
			Splice AI (Δ score)	Human splice finder	
BTD	NM_001370658.1: c.400-3T>G	Homozygous	Moderate (0.2)	Alteration of the WT acceptor site, most probably affecting splicing	Biotinidase deficiency [253260]
CREEBP	NM_004380.3: c.3779+5G>C	Heterozygous	Strong (0.53)	Alteration of the WT donor site, most probably affecting splicing	Rubinstein-Taybi syndrome 1 [180849]
SMPD1	NM_000543.5: c.1341-10_1363dup	Compound heterozygous with another variant in *cis*	Strong acceptor gain (0.84)	No significant impact on splicing signals	Niemann-Pick disease, type A [257200]
VPS33B	NM_018668.5: c.96G>A p.Gln32=	Homozygous	Donor loss (0.37)	Alteration of the WT donor site, most probably affecting splicing	Keratoderma-ichthyosis-deafness syndrome, autosomal recessive [620009]
MED12	NM_005120.3:c.887G>A p.Arg296Gln; r.847_888del	Hemizygous	Acceptor gain (0.38)	Alteration of auxiliary sequences: Significant alteration of ESE / ESS motifs ratio (-9)	Ohdo syndrome, X-linked [300895]

immunodeficiency. Another study examined both disease-causing and neutral exonic point variants, concluding that synonymous variants primarily induce disease phenotypes by disrupting splicing. Furthermore, computational predictors were utilized to pinpoint splice-disruptive variants, encompassing missense or synonymous variants. Notably, deep learning-based predictors trained on gene model annotations exhibited the most effective performance in distinguishing disruptive from neutral variants. These findings underscore the significance of considering synonymous variations at splice sites in the investigation of disease genetics (Ares et al.,1999). We identified a synonymous variant, c.96G>A (p. Gln32=) in *VPS33B* leading to autosomal recessive keratoderma-ichthyosis-deafness (KID) syndrome. The prediction tools were consistent in predicting that this variant could potentially disrupt the WT donor site, and thus be causative (**Table 1**). It is worth noting here that according to the revised American College of Genetics and Genomics/ Association for Molecular Pathology (ACMG- AMP) recommendations (Walker et al., 2023), synonymous variants which are present in the first or last three bases of the exon and are predicted to impact splicing can be considered as disease-causing, and BP7 criteria should not be applied for the same. This variant in *VPS33B* mentioned in Table 1 is present in the last base of exon 1, and was thus considered for diagnosis, as the patient had a concordant phenotype.

Techniques/ Technologies for Detecting Spliceosome Variants

Bioinformatic approaches

Current methods for detecting and interpreting splice site variants include *in-silico* tools utilizing machine learning algorithms. Bioinformatics tools such as SpliceFinder integrate functional annotation tools and splice site prediction programs to analyze next-generation sequencing (NGS) data. Position Weight Matrix-based tools are effective in predicting the consequences of variants on mRNA splicing. Recent studies have shown that machine learning classifiers, particularly Random Forest (RF), outperform Support Vector Machine (SVM) in splice site prediction. Additionally, Convolutional Neural Network (CNN) architectures have been developed to predict splice sites and evaluate the impact of genomic variants on splicing. These technologies offer precise and reliable approaches for identifying splice site variants, aiding in the recognition of disease-causing variants and their influence on mRNA splicing (Anna et al., 2018)

These tools were initially created for research purposes but can potentially be integrated into routine diagnostics. They vary in their algorithms, focusing on consensus splicing sites and requiring sequence input within specific positions. Additionally, there are tools designed to assess the impact of distant variants on splicing, predict exon skipping, cryptic site activation, or the generation of aberrant transcripts, as well as algorithms specifically tailored to predict the influence of single nucleotide variants on branch site sequences or polypyrimidine tracts, such as the Branch Site Analyzer and SVM-BP finder (Anna et al., 2018)

When dealing with exonic variants, it is crucial to evaluate their potential effects on exon splicing enhancers (ESEs) or silencers (ESSs). Various algorithms are available for this assessment, such as ESE Finder, and ESRsearch employing a unanimous enrichment approach with hexameric sequence frequencies. Some models like FAS-ESS are based on functional analyses of random sequences through minigene assays, while others like SpliceAid2 rely on the direct interaction between splicing factors and RNA target motifs. Additionally, bioinformatic programs such as mFold or pFold can be employed to predict whether a variant might impact mRNA secondary structure (Chai et al., 2022)

To enhance user convenience, various programs employing different algorithms have been developed and are accessible via websites. Prominent examples include Human Splicing Finder (HSF), Splice AI and SROOGLE, which predict the presence of *cis*-splicing elements in provided sequences or offer predictions for specific variants in particular genes. Additionally, MutPredSplice is an online tool capable of analyzing individual variants or sets of variants uploaded in a VCF file format. Advanced tools used for annotating variants, particularly those derived from next-generation sequencing data, often integrate splicing prediction algorithms. For instance, the Variant Effect Predictor tool, accessible online, incorporates specialized plugins for splicing analysis utilizing the MaxEntScan model and the dbscSNV matrix from the dbNSFP database (Chai et al., 2022). We have enlisted few examples of splice site variants (**Table 1**) identified in patients from our centre with the scores of analysis from two commonly used and efficient *in-silico* prediction tools.

Experimental Techniques

Experimental approaches, including polymerase chain reaction (PCR)-based methods, RNA sequencing (RNA-seq), and mass spectrometry, provide complementary strategies for detecting spliceosome variants at the transcriptomic and proteomic levels. PCR-based assays, such as allele-specific PCR, enable the targeted amplification and quantification of splicing isoforms harboring specific variants. These assays offer high sensitivity and specificity for detecting spliceosome variants in patient samples, facilitating the identification of disease-associated variants and their correlation with clinical phenotypes (Togi et al., 2024)

RNA-seq, on the other hand, offers a genome-wide perspective on alternative splicing events and allows for the identification of novel spliceosome variants in disease-relevant tissues. By profiling the transcriptome of patient samples, researchers can identify dysregulated splicing events and prioritize candidate genes for further functional characterization. Moreover, RNA-seq enables the detection of fusion transcripts resulting from gene fusions and alternative splicing events, providing insights into the molecular mechanisms driving disease pathogenesis (Togi et al., 2024)

Mass spectrometry-based proteomics facilitates the characterization of spliceosome protein complexes and enables the detection

of post-translational modifications associated with spliceosome dysfunction. By profiling the proteome of spliceosome complexes, researchers can identify disease-associated variants and elucidate their functional consequences on spliceosome assembly and activity. Furthermore, mass spectrometry enables the quantification of protein expression levels and the identification of dysregulated splicing factors in disease states, providing insights into the molecular mechanisms underlying spliceosome-mediated splicing regulation (Sauna et al., 2011)

Functional assays

Bioinformatic algorithms serve as valuable tools for evaluating potential effects of identified changes. However, it is important to emphasize that these tests provide predictive outcomes, and the precise impact of the variant must be confirmed through functional studies. Another approach to validate the pathogenic effect of a specific splicing variant is to analyze its segregation with the disease in affected and unaffected family members at the DNA level. Nonetheless, laboratory testing is still necessary to ascertain the exact splicing effect (Walker et al., 2023)

The most straightforward and efficient functional assay to ascertain if the chosen variant impacts splicing involves analyzing RNA extracted from pertinent patient tissue or cell lines derived from patient cells. Sequencing RNA/cDNA following reverse transcription PCR (RT-PCR) enables confirmation of whether the identified variant affects the mRNA sequence. However, a significant challenge with this method is the potential occurrence of nonsense-mediated decay (NMD), which could obscure the effect of the presumed splicing mutation. To mitigate this limitation, patient cells can be treated with NMD inhibitors like puromycin, which halt RNA degradation (Walker et al., 2023)

If suitable material for functional RNA sequencing is not accessible, an alternative option is a minigene assay, a laboratory technique that acts as an in vitro hybrid system enabling ”exon trapping.” This method proves particularly beneficial for analyzing genes with low expression levels in leukocytes or fibroblasts. In the minigene assay, a fragment of the gene under scrutiny, such as a specific exon along with adjacent intronic sequences with and without variants, is amplified and then inserted into a specialized expression plasmid, facilitating the examination of pre-mRNA splicing. This approach serves to validate whether the potential splicing variant impacts splicing efficiency or triggers the activation of alternative cryptic splicing sites. Additionally, it allows for the investigation of the role of cis-acting elements in splicing regulation (Thanapattheerakul et al., 2020)

Lastly, CRISPR-based genome editing technologies enable the generation of isogenic cell lines carrying precise spliceosome variants, allowing for the elucidation of genotype-phenotype correlations and the identification of therapeutic targets. By introducing specific variants into the endogenous genome, researchers can assess the functional consequences of spliceosome variants on pre-mRNA splicing and gene expression regulation. Moreover, CRISPR-based genome editing enables the development of cellular models for studying disease pathogenesis and evaluating therapeutic interventions, paving the way for personalized medicine approaches (Jian et al., 2014) A comprehensive list of all the approaches is enlisted in **Table 2**.

Clinical Implications and Future Directions

The detection of spliceosome variants holds significant clinical implications for disease diagnosis, prognosis and therapeutic intervention. Characterization of spliceosome variant profiles in patient populations can inform personalized treatment strategies and guide the selection of targeted therapies tailored to individual molecular profiles. Furthermore, the integration of spliceosome variant detection into routine clinical practice has the potential to revolutionize precision medicine approaches and improve patient outcomes (Hu et al., 2013)

For example, small molecule modulators of spliceosome function, such as splice-switching oligonucleotides and small molecule splicing modulators, hold promise as therapeutic interventions for diseases characterized by aberrant splicing patterns. By targeting specific spliceosome components or splicing regulatory elements, these compounds can modulate splicing efficiency and restore normal gene expression patterns. Clinical trials evaluating the efficacy of spliceosome modulators in various disease settings are currently underway, with promising results reported in preclinical studies (Hu et al., 2013).

Moreover, the identification of spliceosome variants as prognostic biomarkers in cancer and other diseases has important implications for

Table 2 Different approaches for analysis of splice site mutations

Approaches	Tools/ techniques	Website links
1.Bioinformatic approaches (In-silico prediction tools)	Human Splice Finder	www.umd.be/HSF/
	Splice AI	https://spliceailookup.broadinstitute.org/
	Splice site prediction program	www.fruitfly.org/seq_tools/splice.html
	SPANER	http://tools.genes.toronto.edu/
	SpliceAid2	http://193.206.120.249/splicing_tissue.html
	NetGene2	http://www.cbs.dtu.dk/services/NetGene2/
	MutPredSplice	http://www.mutdb.org/mutpredsplice/submit.htm
	ESE finder	http://exon.cshl.org/ESE
2.Experimental Approaches	Polymerase chain reaction-based method	https://doi/10.1002/wrna.1364
	RNA sequencing (RNA-seq)	https://doi.org/10.1038/s41598-021-89938-2
	Mass spectrometry	https://doi.org/10.1371/journal.pone.0265766
3.Functional assays	Minigene splicing assay	https://doi.org/10.1002/humu.22624
	Reverse transcription PCR analysis	https://doi.org/10.1186/s12867-016-0060-1
	Protein truncation test (PTT)	https://10.1007/978-1-59745-388-2_8
	CRISPR-based genome editing technologies	https://doi.org/10.1002/gcc.22784

disease management and treatment planning. Patients harboring specific spliceosome variants may benefit from targeted therapies aimed at correcting splicing defects and restoring normal gene expression patterns. Furthermore, the development of companion diagnostic tests for detecting spliceosome variants could enable the stratification of patient populations and facilitate the selection of appropriate therapeutic interventions (Zhai et al., 2013).

Future research directions in spliceosome variant studies encompass a broad spectrum of interdisciplinary approaches, including the development of novel detection

methods, elucidation of disease-specific splicing signatures, and exploration of therapeutic modalities targeting spliceosome dysfunction. Integration of multi-omics data, including genomics, transcriptomics, and proteomics, will facilitate a comprehensive understanding of spliceosome-mediated splicing regulation and its implications for human health and disease. Moreover, collaborative efforts between academia, industry, and regulatory agencies are essential for translating basic research findings into clinical applications and improving patient outcomes (Zhai et al., 2013)

Conclusion

Splice site variants can cause diseases by disrupting the proper recognition of exons and altering mRNA splicing. These variants can result in exon skipping, the formation of new exon/intron boundaries, or the activation of cryptic exons. Synonymous variants can also affect splicing by disrupting consensus sequences. To detect splice site variants, various technologies can be used. Bioinformatic algorithms can be applied to predict the effect of identified changes, but functional studies are necessary to confirm the exact impact of specific variants. In vitro transcription and variant analysis via a hybrid minigene system are commonly used methods for functional studies. These approaches can help in the diagnosis of splice site variants and provide insights into the mechanisms underlying splicing-related diseases. By leveraging cutting-edge technologies and interdisciplinary approaches, researchers can elucidate the molecular mechanisms underlying spliceosome-associated diseases and pave the way for innovative diagnostic and therapeutic strategies. Continued investment in spliceosome variant research holds the promise of transformative advances in precision medicine and personalized healthcare.

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A Day in the Life of a Geneticist

Sunita Bijarnia-Mahay

Institute of Medical Genetics & Genomics, Sir Ganga Ram Hospital, New Delhi

Correspondence to: Dr Sunita Bijarnia-Mahay *Email:* sunitabijarnia@sgrh.com

As I board my metro train to go home after a seemingly heavy day, I start reflecting on the day's proceedings. It started with a message on WhatsApp- with news of the death of a baby whom I had seen a few weeks ago. The baby had come from another country, with a hope for treatment. He was brought to our Institute for a specific reason. The child had maple syrup urine disease (MSUD) and had been on dietary management. The family was looking for a one-time solution. I had guided them regarding the liver transplant, which was not an easy option, with all its difficulties and risks. Perhaps the family accepted this option as a way out and therefore went for it. Little did they know of its outcome! The baby suffered portal vein thrombosis, a known complication and could not survive (Zanetto et al., 2018).

As I opened my email, I was in for another setback. A family from Jammu had been waiting anxiously. The 12-year-old boy was diagnosed with X-linked adrenoleukodystrophy (XLALD), having presented with a history of gradual loss of vision for a few months. A typical pattern of magnetic resonance imaging (MRI) of the brain involving the posterior white matter with gadolinium enhancement had provided the diagnosis, confirmed by a subsequent abnormal very long chain fatty acid (VLCFA) analysis and genetic testing, revealing a pathogenic variant in the *ABCD1* gene. In light of recent knowledge of a 'new drug' for XLALD, Leriglitazone (https://www.minoryx.com/), we (i.e., the family and I) were very optimistic. We reached out to experts abroad and looking at the well-preserved clinical status of the child, with a calculated Loes score of <15, there was hope for approval of the drug 'on compassionate grounds'. The email, however, said otherwise - the MRI reviewed by the experts abroad revealed a Loes score well above 15, thus disallowing him for the drug. Another hope dashed to the ground.

In the course of the day, we learnt about another patient, who had received Zolgensma therapy for spinal muscular atrophy about six weeks back, struggling with severe liver dysfunction and failure. This was the sad reality after almost six months of struggle getting him to receive the magic 'gene therapy'.

As the day rolled on, another patient's consultation came up – an afternoon video consultation for a 20- month-old boy with inherited L-asparagine synthetase (ASNS) deficiency. The little boy had been diagnosed with ASNS deficiency at another hospital in South India and had reached out to us in June last year. He was optimistically started on L- asparagine therapy, after consultation with experts abroad, even though the literature evidence for its utility was scant (Sprute et al., 2019). The baby had shown improvement in the first 4 months with cessation of seizures and gain of few milestones (neck control). However, I received another blow when the parents reported a resurgence of seizures, albeit brief ones, and no further gain in milestones or weight.

Another 5-month-old patient admitted in the ward was under investigation for progressive spleno-hepatomegaly and a squint. The elevated biomarker chitotriosidase had provided a clue leading to detection of deficient beta-glucosidase activity, and a diagnosis of neuronopathic Gaucher disease. Treatment for Gaucher disease is available, however the practicality of it makes it difficult - so near yet so far. The struggle to provide enzyme replacement therapy would start now.

To end my story, another delightfully cute baby came in for a follow up. Resident of Madhya Pradesh, the now 8-month-old boy had presented at 4 months of age with a cholestatic liver disease. Upon extensive work up, including an elevated serum chitotriosidase as an extremely useful clue, the baby was finally diagnosed, via whole exome sequencing to have Niemann-Pick type C. This little baby is doing well. Though the organomegaly is

Table 1 Brief description of the genetic disorders mentioned in the article

S. No.	Name of the disorder	Etiology	Salient clinical features	Treatment options
1.	Maple syrup urine disease (MSUD)	Inborn error of branched chain amino acid catabolism, due to deficiency of the branched chain keto acid dehydrogenase (BCKDH) enzyme.	Classical presentation is with episodic encephalopathy, seizures, life threatening.	Special diet highly restricted in branched chain amino acids; management of acute crisis; liver transplantation
2.	X-linked Adrenoleukodys-trophy (ALD)	Deficiency of ABCD1 transporter in peroxisomes	Cerebral ALD - Progressive neurodegeneration; adrenal insufficiency	Supportive therapy; hematopoietic stem cell transplantation; hormonal replacement therapy; gene therapy or oral Leriglitazone (clinical trials underway)
3.	Gaucher disease	Deficiency of lysosomal beta-glucosidase enzyme	Type 1 visceral type – progressive splenohepatomegaly, hematological and bone manifestations. Type 2 and 3 – acute and chronic neuronopathic forms along with manifestations as in type 1.	Enzyme replacement therapy for type 1 and 3; substrate reduction therapy
4.	Niemann-Pick disease type C	Defect in lysosomal lipid trafficking	Progressive storage of lipids (sphingosine) with splenohepatomegaly, and progressive neurological features such as cognitive, ataxia, gaze palsy etc.	Substrate reduction therapy with Miglustat, an oral reversible inhibitor of glucosylceramide synthase
5.	Spinal muscular atrophy	Absence or reduced functioning of survivor motor neuron (SMN) protein	Primary involvement of the motor neuron, with hypotonia, involving the axial/appendicular skeleton and respiratory system. Four types, with type 1 being most severe with onset in early infancy and death by 2 years of age.	Intravenous gene therapy with onasemnogene abeparvovec (Zolgensma); intrathecal antisense oligonucleotide (ASO) therapy Nusinersen; oral exon skipping therapy Risdiplam
6.	Asparagine synthase deficiency	Deficiency of the enzyme asparagine (non-essential amino acid) synthase	Prenatal / early onset microcephaly, seizures, delayed developmental milestones	Supplementation with oral synthetic L-asparagine (limited evidence)

persistent, he is gaining milestones and feeding well, the cholestasis having subsided. This leads us to wonder about his future. Treatment with miglustat may be applicable to him in the near

future (Pineda et al., 2018), and may be available too after some efforts and with funding support through the National Policy for Rare Diseases. But, at the moment, the future remains uncertain.....

Details of the disorders and the related therapies mentioned in this article are provided in **Table 1**.

We have witnessed the changing eras – from 'only a clinical diagnosis', to the ecstasy of cytogenomic or molecular confirmation, and now, the era heralding therapies at a fast pace. Like the above cases, all families and patients come to us with hope, but are we able to do justice? The 21st century ushered promises with newer advanced therapies becoming a reality, but the struggle is still there. The struggle of availability, affordability, procurement, procedural expertise and uncertainty of long-term outcomes! Cure versus stabilization and the challenge of evolving phenotypes as patients on definitive therapy survive beyond the understood natural history of the disorder. It is the era of hope for patients and an exciting challenge for geneticists to think 'out of the box', but with a word of caution to "look before you leap" and to remember the Hippocratic oath "First do no harm".

We march on, guided by our mentors, for the sake of our patients and for the future generations of physicians, who will have learnt from our struggles and mistakes, to give their patients a bright and healthy life.

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GeNeXpreSS

Applications of Artificial Intelligence (AI) in Medical Genetics: From Patient Counseling to Training of Students.

Pooja Motwani

Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, India

Correspondence to: Dr Pooja Motwani *Email:* pjmotwani1@gmail.com

Automated prioritization of sick newborns for whole genome sequencing using clinical natural language processing and machine learning

(Peterson et al., 2023)

Human Phenotype Ontology (HPO)-based phenotypic descriptions for whole genome sequencing (WGS) in the neonatal intensive care unit (NICU) is an established practice. It is done manually and at one or two isolated points of time. Constant vigilance for changes in laboratory results, diagnostic imaging, and clinical trajectories is crucial to identify infants most likely to benefit from WGS. It is labour-intensive and requires experts, making it a bottleneck. Automated clinical natural language processing (CNLP) technologies can help generate structured descriptions from unstructured clinical notes, aiding in triaging neonates for WGS.

The Mendelian Phenotype Search Engine (MPSE) combines the CNLP workflow with a machine learning-based prioritization tool, facilitating patient review. Diagnostic rates for CNLP datasets were consistently at or above the cohort diagnostic fraction of 43% at every MPSE score percentile. In contrast, MPSE scores from manually curated phenotypes showed weaker diagnostic performance. This difference can be from the average number of phenotype terms derived from each method. The CNLP method produced an average of 114.8 and 64.5 phenotype terms, compared to 4.1 and 9.5 from manual curation in two independent cohorts.

The CNLP/MPSE workflow prioritized patients for rapid WGS (rWGS) with high accuracy [area under the curve (AUC)=0.86], enriching diagnostic yields in the top-scoring quartile. MPSE automatically surveys all NICU admissions and updates their scores daily based on health record content. These MPSE score cutoffs can be used to prioritize patients for further physician review.

A randomized trial comparing the effectiveness of pretest genetic counseling using an artificial intelligence automated chatbot and traditional in-person genetic counseling in women newly diagnosed with breast cancer

(Al-Hilli et al, 2023)

Alternative service delivery models are critically needed to address the increasing demand for genetics services and the limited supply of genetics experts available to provide pre-test counseling. Al-Hilli et al. conducted a prospective randomized controlled trial of women with stage 0 to III breast cancer not meeting the National Comprehensive Cancer Network (NCCN) criteria for genetic testing. Patients were randomized to pretest counseling with a chatbot or a certified genetic counselor (GC). Nineteen were randomized to the chatbot and 18 to traditional genetic counseling. Out of the total number of participants, 38.2% had a family member with breast cancer but did not meet the NCCN criteria. Participants completed a questionnaire assessing their knowledge of breast cancer genetics and a survey assessing satisfaction with their decision regarding pretest counseling. After the pretest counseling, all patients opted to undergo genetic testing. There were no significant differences in the median knowledge score between the chatbot and traditional counseling (11 vs. 12, $p = 0.09$) or median patient satisfaction score (30 vs. 30, $p = 0.19$). No patients had a delay in time-to-treatment due to genetic testing turnaround time, nor did any patients undergo

additional risk-reducing surgery. The scores of satisfaction and comprehension in these patients using either an automated hatbot or an in-person Genetic counselor did not significantly differ. The study suggests that utilizing a chatbot for pretest counseling is as effective as traditional counseling by a certified genetic counselor regarding patient knowledge, satisfaction, and comprehension. This alternative approach can alleviate the strain on genetic counseling services by providing a viable, efficient option for pretest counseling, especially when genetic experts are in limited supply.

Evaluation of the Rosa Chatbot providing genetic information to patients at risk of hereditary breast and ovarian cancer: Qualitative interview study

(Siglen et al, 2023)

Genetic testing has become integral for patients with breast or ovarian cancer, necessitating reliable access to genetic information. To meet this demand, a chatbot named Rosa was developed for human-like conversations about testing of the BRCA genes. This study aimed to evaluate the perceived utility and trust in Rosa among healthy individuals at risk of hereditary cancer and its influence on their handling of sensitive information. A total of 175 at-risk individuals were invited to test Rosa before and after genetic counseling, recruited from all cancer genetic clinics in Norway to ensure diversity. Among them, 61 (34.9%) consented to individual interviews, with a selected subgroup of 16 (26%) participating in in-depth video interviews. These semi-structured interviews explored usability, perceived usefulness, trust in the information, the chatbot's influence, and future digital tool use in healthcare. The findings indicated that participants welcomed Rosa, valuing its 24/7 availability and role in preparing for and reviewing genetic counseling sessions. The information provided by Rosa, created by healthcare professionals, was considered medically accurate, making it more reliable than general internet searches. Key themes emerged: “Anytime, anywhere”; “In addition, not instead”; and “Trustworthy and true.” Notably, none of the participants reported increased worry after using Rosa.

In conclusion, Rosa offers easy access to consistent, quality-assured genetic information, reassuring patients at risk of hereditary breast and ovarian cancer. The participants did not support its use as a replacement for genetic counseling when hereditary cancer is confirmed. Thus, Rosa serves as a complementary tool, enhancing but not replacing traditional genetic counselling.

Recognition of genetic conditions after learning with images created using generative artificial intelligence

(Waikel et al., 2024)

The study aimed to compare the ability of pediatric residents to recognize Kabuki syndrome (KS) and Noonan syndrome (NS) after exposure to one of four educational interventions, including generative artificial intelligence (AI) methods. Participants categorized 20 images following exposure to one of four educational interventions: text-only descriptions, authentic images, and two types of AI-generated images. For KS, sensitivity with text descriptions was 48.5%, not significantly different from random guessing. Sensitivity improved with natural images (60.3%) and AI-generated images (57.0% and 59.6%). For NS, text descriptions had a sensitivity of 65.3%, compared to 74.3% with authentic images and 68.0% and 71.0% with AI-generated images. In terms of specificity, none of the interventions showed a significant difference from the text-only approach. For KS, the number of participants unsure about diagnostic features decreased from 52.8% to 7.6%, and for NS, it decreased from 24.5% to 4.7%. There was a significant correlation between confidence levels and sensitivity for real and AI-generated images.

In conclusion, the study found that real and AI-generated images enhanced the recognition of KS and NS among pediatric residents, with real photos proving most effective. While slightly less effective than real images, AI-generated images were not inferior and could serve as a valuable adjunctive tool, particularly for educating about rare conditions. It highlights the potential role of AI-generated images in medical education to improve the recognition of genetic syndromes.

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Prenatal Diagnosis after Twenty-Four Weeks of Gestation: The Question is What Next?

Shubha Phadke

Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India.

Correspondence to: Dr Shubha Phadke *Email:* shubharaophadke@gmail.com

Prenatal diagnosis is not only a technically advanced process but needs equally competent counselling expertise. At present very few prenatally diagnosed disorders can be treated with a complete normal outcome. Hence, termination is the only option to prevent the financial and emotional burden of serious disorders on the family. But decision of termination is also not easy, especially at later gestation and for the disorders with mild disability or variable outcomes. Here, two real, contrasting scenarios are presented and the various issues originating are presented for introspection individually and further discussions amongst stakeholders at various forums.

Family 1

I was travelling to the United Kingdom (UK) and could not participate in a meeting of the committee to decide upon an application for termination of pregnancy after 24 weeks. The family asked for my time so that they could talk to me over phone when I reach UK. I got a call as soon as I reached the hotel room. The couple were well educated and working in good positions and were very disturbed as the fetus in the ongoing pregnancy at around 28 weeks gestation was detected to have aplasia of corpus callosum (ACC). Counseling for prenatally detected aplasia of corpus callosum is a very challenging situation. Detection usually around 24 weeks of gestation compounds the great variability of outcomes from normal to severe neurodevelopmental disability, and the inability to predict based on prenatal findings alone. Presence of other malformations and/ or chromosomal abnormalities almost confirms the possibility of neurodevelopmental disability. Available publications over the years have provided outcomes of prenatally detected isolated ACC. Though the various studies cannot be combined because of variables like age at evaluation, etc. the available follow ups up to 10 years show that the cognitive function is normal in 65 to 80% children. Ten to fifteen percent have moderate to severe neurodevelopmental deficits while similar numbers may have borderline intelligence quotient (IQ) or mild learning disability. Some so called 'isolated' ACC cases detected prenatally may have other anomalies detected by postnatal magnetic resonance imaging (MRI) of the brain or monogenic disorders detected by exome sequencing. Prenatal sampling and microarray can look for chromosomal imbalances which may be present in 5 to 7%. Evaluation for monogenic causes by prenatal sampling is necessary but it may be difficult to get the results in the required time frame.

Leaving aside the figures, it is obvious that the outcome of more than two-thirds or more of the children with prenatally detected isolated ACC is normal or satisfactory. The couple said they cannot take 'any risk' of a child with intellectual disability to be born. Being 'very intelligent', 'with high academic achievements' and 'busy with successful careers', they said they could not take such risks and wanted termination of pregnancy. On reminding them that the possibility that the child will have normal intelligence is 60 to 80%, they again asked, "Can you guarantee this?".

May be being achievers, they had never faced failures and did not know or could not consider that nothing in life is 'guaranteed'. We had some more discussions. I also presented the uncertainties of outcomes with normal ultrasound and many diseases which may come up after birth, background risks, etc. Many times, especially when in person, such discussions continue for a long time and one has to make attempts to wind up after the final word about 'uncertainty of outcome'.

Family 2

This case was easy as compared to the previous one. A less educated woman from a lower

socio-economic strata, a mother of 2 children, was evaluated for oligohydramnios. She was 23 weeks pregnant with ultrasonographically detected anhydramnios, enlarged bilateral multicystic kidneys and non-visualization of urinary bladder in the fetus. I told the outcome is definitely poor and survival after birth is unlikely. I discussed the option of terminating the pregnancy. She told she was aware of these facts, but she wanted to continue and not terminate the pregnancy as it was quite advanced according to her. She had come from another district and she said that the doctor there was insisting, and sort of forcing her to terminate. She did not want to do so and hence she had come to us for a second opinion. She was happy with my non-directive counselling and knowing that nobody can compel her to discontinue the pregnancy.

Analysis

As mentioned in the two cases above, there are different people with different perspectives, sensitivities and priorities. Prenatal diagnostics is improving and becoming widely available, but expertise in diagnostic technology and counselling is variable. Trained genetic counsellors and medical doctors with training in genetic counselling are very few. In general, many Indians have a low threshold for termination; there is no data regarding this, but this is a subjective impression based on personal experience. The pace of development of therapies is slow and parents and families are feeling strong control on the baby in the womb and are feeling confident about technology to assess the fetus. Conferences need not only include technology-based talks about the field of prenatal diagnosis but panel discussions on the issues of ethics surrounding prenatal diagnosis.

Situations, outcomes, and gestational age vary and are beyond our control; so are the views, perspectives and goals of life on which the decisions depend. Prenatal evaluation opens a Pandora's box leading sometimes to more dilemmas than solutions. Even after extending the legal limit for termination of pregnancy from 20 weeks to 24 weeks, the issues related to late terminations are not getting solved. Some anomalies get detected during the third trimester for various reasons. Microcephaly, some cases of ventriculomegaly, non-lethal skeletal dysplasias, hydrops, heart block, etc. may manifest during the third trimester and may not be picked up in a malformation scan at around 20 weeks gestation. Some lethal anomalies like anencephaly, iniencephaly and lethal skeletal dysplasias may come to notice during the third trimester as ultrasonography was not done in earlier gestation or not done by an expert.

As per the recently modified law, a specially formulated medical board can give decisions about termination after 24 weeks of gestation for such lethal disorders. However, for non-lethal disorders the decisions are difficult as the child born after induction may survive and may have added complications of prematurity. Some surgically treatable disorders like esophageal atresia, diaphragmatic hernia, and cardiac anomalies have variable outcomes of surgery and also a variable prognosis based on the underlying etiology. Families asking for termination of pregnancy for disorders such as Noonan syndrome or non-lethal skeletal dysplasias have been seen and these situations will become more frequent as imaging and sequencing gets better and easier. Many of the prenatally detected disorders may not have treatment but the outcome may be normal to near normal in some. The major issues are uncertainty about the outcome and the understanding of the family about the disorder and its effect on the quality of life. This makes genetic counseling difficult. Non-directiveness is the pillar of genetic counselling. But the decision of termination of the pregnancy has to be within the legal framework. Hence, the options for the family get restricted after 24 weeks of gestation. Like in the case of Family 2, the option of continuation even if the fetal disorder is not compatible with postnatal survival is totally under the control of the family. But sometimes it is otherwise and the disorder in concern is compatible with survival postnatally and the family wishes to terminate the pregnancy. Whether the mother's right to decide the fate of pregnancy because of the possibility of effect on her mental health can overrule the right of the fetus to be born is the debate. Someone has to plead for the fetus. The amendment in the medical termination of pregnancy (MTP) laws made in 2021 allows termination after 24 weeks gestation for substantial fetal anomalies after approval by the medical board. What is a substantial anomaly and what will happen if the fetus survives after early delivery after 24 weeks has not been described in the law. For example, can a fetus with trisomy 21 detected at 28 weeks because of ventricular septal

defect be delivered prematurely and left to die?

Synthesis

Prenatal diagnosis of disorders with uncertain outcomes varying from normal outcome with or without treatment to handicap or lethality is not uncommon during earlier part of the pregnancy. Likelihood of handicap like intellectual disability, short stature, physical handicap and magnitude of the severity can not be exactly predicted by prenatal testing in most of the situations. But before 24 weeks the fetus has less identity in the minds of many of us and termination is legally possible. Decision of termination in such situations, especially if there is uncertainty about the outcome is difficult and painful for the family. Though there is no specific point of gestation which gives the fetus a separate identity, advancing gestation does increase the fetal identity as an independent individual. In India, for those who are ready to accept the option of termination, the law has given the limit of 24 weeks.

Because of this legally approved option of termination and increasing availability of prenatal diagnosis the families feel empowered to have control over the child they want and more about what type of child they do not want. Everyone wants a normal child, and this desire is acceptable. But here prevention of disability or birth defect involves termination of pregnancy and hence, it has to be taken with great sensitivity by the families in concern and the team of doctors including obstetricians, medical geneticists and fetal medicine specialists. Many a times, other organ-based specialists and pediatric surgeons are involved. The family facing such a situation of prenatally diagnosed disorder is usually facing the problem for the first time and for them who were expecting a normal child, it is a blow from nowhere. At that time, they may be exposed to the option of termination and as we saw in the two cases above, the reactions may vary greatly. The medical doctors including medical geneticists, fetal medicine specialists and others need to provide detailed information about possible outcomes with and without treatment, their likelihoods, and available treatments including the cost and availability. Giving a real picture of long-term outcomes and the magnitude of the burden is practically impossible and involves a lot of uncertainty.

Conclusion

After 24 weeks what should be the approach of clinicians for non-lethal disorders needs to be discussed by the clinicians not only as medical practitioners but also as responsible and learned citizens. The society's ethical framework is built by its members and is very delicate. Its strength is the responsible and conscientious members. The inputs of leaders and representatives from various backgrounds like teachers, religious gurus, social scientists, etc. need to build the ethical guidelines which sometimes get misdirected by other forces like modernization, concepts of individuality, industry and money. A clinician's responsibility is at two levels; the first is when the disorder is diagnosed, and the issues are presented to the family. Whether the option of termination has to be discussed should be clear to the obstetricians, fetal medicine specialists and medical geneticists. Secondly, the medical board members who are from various other medical specialities also should understand the issues to be considered while giving case-based decisions about termination after 24 weeks of gestation.

To me, this is a very important issue and clinicians involved in the counselling for prenatal diagnosis and in the medical board need to be aware and discuss and debate the issue. The decision needs not only information about the disorder and its prognosis but ethical principles guiding the decisions. As said above, the identity of fetus as an independent individual increases with age and the law has identified 24 weeks of gestation as the cut-off. After that gestation, the decisions about pregnancy should be similar to what one would take for a liveborn neonate. Though the law gives a woman the reproductive rights after 24 weeks, the law also has to protect the fetus as well. The mother's desire to have a healthy child is acceptable but the right to avoid the birth of a child with a birth defect by discontinuing pregnancy cannot be only her decision after 24 weeks.

It is necessary to awaken the society to the fact that for every disorder detected prenatally, termination may not be the option. Our society needs to guide people to help them take right decisions. Fetuses with non-lethal disorders diagnosed after 24 weeks of gestation have right for postnatal treatment and a mother's tender loving care!

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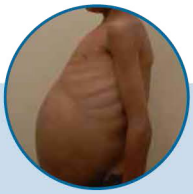

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