Volume 16 | Issue 4 | October - December 2023

Genetic Clinics

Official Publication of Society for Indian Academy of Medical Genetics
ISSN : 2454-8774

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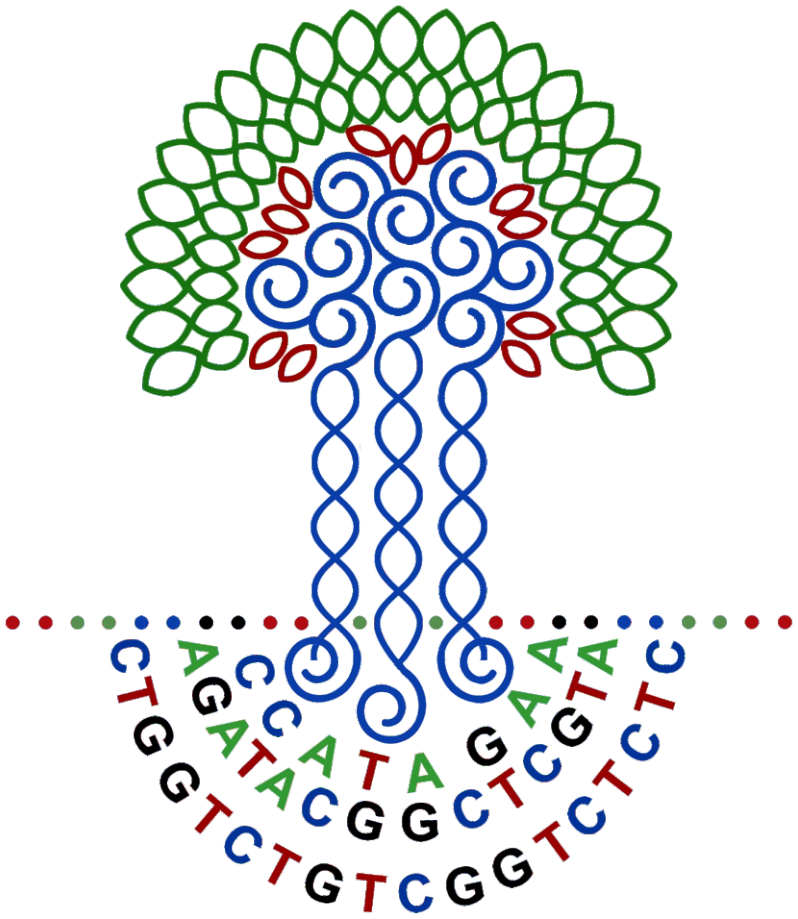

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PhotoQuiz - 62

Contributed by: Dr Haseena Sait

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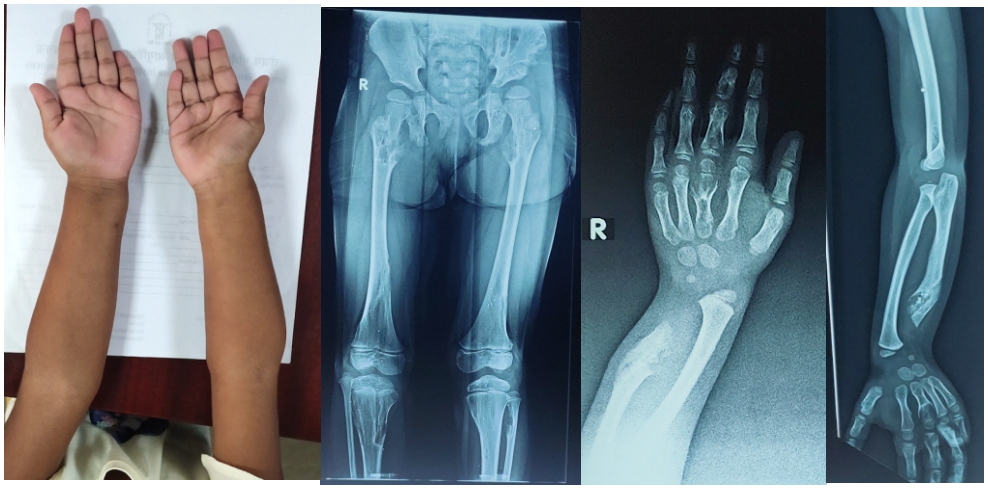
This 5-year-old female child born to non-consanguineous parents presented with complaints of bony swellings over the right forearm and right leg noted from 2 years of age. There is no history of pain. Examination revealed limb asymmetry. Skeletal radiographs are attached. Identify the condition.

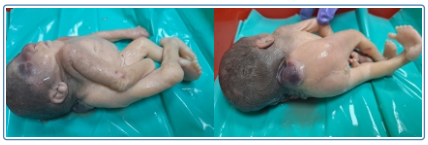
Please send your responses to editor@iamg.in
Or go to http://iamg.in/genetic_clinics/photoquiz_answers.php
to submit your answer.



Answer to PhotoQuiz 61

Iniencephaly

Iniencephaly is a rare type of neural tube defect (NTD) which involves a combination of occipital bone defect and partial or total absence (rachischisis) of the cervicothoracic vertebrae resulting in fixed and extreme retroflexion of the head. In most cases, it is perinatally lethal. The exact underlying etiology is not identified in majority of affected fetuses.



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2. Dr Vibha Jain, Anuvanshiki- The Genetic Clinic, Ghaziabad
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GeNeDit

Mirror, Mirror on the Wall – Show the Complete Picture to Us All!

Editorial

The Human Genome Project was an ambitious dream. Reading all our genomes became a reality in 2003! The gaps were filled and now we know the 'telomere to telomere' ATGC sequence of Homo sapiens. Now almost anyone and everyone can get their genome sequenced any time- before birth, at birth or after birth. The power of this technology is being harnessed by clinicians not only in diagnostics but also for developing treatments based on the understanding of genetic pathogenesis. Examples include personalized treatment for cystic fibrosis based on the disease-causing variants and antisense oligonucleotide therapies for various monogenic diseases as discussed in the review article in this issue. Curative treatment in the form of gene therapies for beta thalassemia, hemophilia, retinitis pigmentosa, and spinal muscular atrophy is a major milestone in the history of genetic disorders. At the same time the utilization of this technology has caused a paradigm change in the diagnosis of monogenic disorders. Diagnosis of rare disorders has become easier and more definitive than that of tuberculosis. Short-read/long-read whole genome sequencing and optical genome mapping have made the diagnosis of all types of genomic variations possible. At present these techniques are complementary to each other and can be enhanced by RNA-based studies to make the diagnosis of exonic and intronic variants, triplet repeat expansions, copy number variations, structural variants, and imprinting abnormalities in one go. We are close to a one-stop diagnostic solution for all genetic disorders. However, the development of disease-modifying treatments is still moving at a slow pace. Hence, prevention of disorders by prenatal diagnosis is an acceptable option and is being offered in a big way. Here, termination of the fetus affected with the disorder is the option if it is acceptable to the family. This puts a lot of responsibility on medical geneticists to give a definite answer as to whether the fetus carrying a variant will develop serious disease, and to predict the severity of the phenotype. This is very challenging as we continue to face the complexities of interpretation of genetic variants.

Technical marvels will keep on increasing the diagnostic yield of possible genetic disorders and may identify novel genes and maybe novel mechanisms for still-enigmatic genetic disorders. Though these technological advances provide accurate genetic diagnosis to many patients and shorten the diagnostic odyssey, many genetic mysteries continue to remain unsolved. Though the identification of a variant for a Mendelian disorder has become an easy job, the previously known issue about modifying factors, especially genetic modifiers, is confronting us in a big way. Why do some patients with the L444P (p.Leu483Pro) mutation in the *GBA* gene develop neurological manifestations of Gaucher disease and others do not? Among those with this *GBA* mutation who do not have oculomotor involvement during childhood, we cannot predict who will go on to develop neurological involvement later and when. The modifiers for thalassemia intermedia and spinal muscular atrophy are known but do not explain all the phenotypic variability. A large volume of data has confirmed that there is no genotype-phenotype correlation for most of the genetic disorders. There is great degree of intrafamilial phenotypic variability for autosomal dominant disorders. Exome sequencing has expanded the phenotypic spectrum of many monogenic disorders. Late-onset variants for many serious neurogenetic disorders like Krabbe disease, metachromatic leukodystrophy, and other inborn errors of metabolism have been observed. Many of these disorders, which we usually consider to be lethal, are being diagnosed in adults and some may present even after 50 years of age. Some of the individuals might have led fulfilling lives and contributed to society by then. Many of the genes like *LMNA* and collagen-encoding genes are associated with numerous phenotypes of varying severity. We can correlate the phenotype with the genotype and use it as supportive evidence for prediction of the pathogenicity of a genetic variant.

But can we always accurately predict the phenotype of a known pathogenic variant if it is detected in an asymptomatic carrier parent, in the fetal DNA in maternal plasma, or in the amniotic

fluid sample? No, in many cases we cannot! If we identify a de novo pathogenic variant in the *FBN1* gene in the fetal DNA from maternal plasma, will we be able to explain the life of an individual with Marfan syndrome to the would-be parents from a non-medical background? Can they decide whether to give birth to a child with Marfan syndrome or not? Is termination justified for a disease where the life expectancy is likely to be normal/ near normal? If we feel the answers to all these questions are 'No', is screening of all couples for carrier status, of asymptomatic fetuses for pathogenic variants, of de novo mutations in fetal DNA from the mother's plasma justified at this point? For families with a child or an individual with a serious disorder with a poor outcome, prevention of recurrence appears to be justified at this time. The family is aware of the disorder, its clinical presentation and outcome. Prenatal diagnosis in this scenario will not have any significant error rate related to an uncertain outcome. But prenatal diagnosis for a likely pathogenic variant in a family without previous history of the disorder cannot give an idea about the outcome with certainty. We know this from our experience of counseling, for example in the case of a female fetus with a full mutation in the *FMR1* gene. How difficult is counseling and the decision! In fact, some pathogenic variants and homozygous loss-of-function variants are seen in databases of normal populations also.

The desire to have a normal, healthy, and beautiful child is normal. As clinicians we know that a 'normal child' free of disease cannot be guaranteed. Even when we do whole genome sequencing of the fetus, there is still a lot which cannot be seen and most importantly, much cannot be definitely interpreted. We, the clinicians and scientists, need to be aware of the limitations of interpretation of the currently available powerful sequencing technologies. Genome sequencing ability is a technological marvel, but we still do not know the interpretation of many variants and the role of modifying variants. These powerful technologies should be used for efficient postnatal diagnosis and research in understanding genotype-phenotype correlations, so that new therapeutic strategies can emerge. Along with these genome sequencing strategies we need to develop strong supporting knowledge of gene functions and a more in-depth understanding of protein-protein interactions, etc. Till we are able to definitely predict the phenotype based on the genotype, it may not be wise to take decisions about termination of pregnancies or even preimplantation diagnosis, based entirely on variants detected through carrier screening of couples or broad-spectrum testing of fetal DNA, without history of the disease in the family. Without foolproof evidence even a murderer cannot be hanged and here, a child who may not have a serious disease and who might have been able to live a long life might be terminated. Even preimplantation diagnosis increases the risk of birth defects and poor outcomes. Excessive parental control over the genome of the unborn baby, with blind faith in the power of genomic techniques and the ambitious confidence of scientists in prevention of genetic disorders by screening through next-generation sequencing (NGS) technology, is frightening. Though the intentions of geneticists are good, they may cause termination of normal fetuses. And what effect such population-based screening will have on the gene frequencies, if carried out on a large scale, cannot be foreseen. It is time to tell the parents that a disease-free 'normal baby' cannot be guaranteed and that though technology can read the genome completely, at present we are able to understand the meaning only partially. Half knowledge can be dangerous.

The mirror of genomic technology needs to be able to show multiple dimensions of interpretations of ATGC variants before it can be applied for prenatal or pre-conceptional screening of a normal population and of low-risk pregnancies.

Mirror-mirror on the wall
Please show the correct multi-dimensional picture to us all.
The ATGC story is ready to be read,
But a lot needs to be deciphered.
Show us not only the exterior image,
Not just the variants but also the mechanism causing the damage.
Based on which we can do correct interpretation,
And help families come to a meaningful conclusion.

(The views expressed here do not represent the views of the Indian Academy of Medical Genetics or of the Editorial Board of Genetic Clinics. These are the personal views of the author to open the minds of geneticists and clinicians to the complexities of the issue.)

Shubha Phadke
1st October, 2023

Clinical Vignette

A Novel Pathogenic Hemizygous Variant of *AP1S2* Gene in a Child with Dandy-Walker Malformation, Developmental Delay, and Autism

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Abstract

There is a high degree of genetic and phenotypic heterogeneity for X-linked intellectual disability. Pettigrew syndrome is a rare X-linked syndromic intellectual disorder that presents with hydrocephalus with or without Dandy-Walker malformation (DWM), basal ganglia calcification, developmental delay, autism, and seizures. This is a report of a male child who presented at the age of 7.5 years with global developmental delay, autism, and behavioral disturbances. Ultrasound during the antenatal period in the third trimester had revealed hydrocephalus. Magnetic resonance imaging (MRI) of the brain after birth showed features of hydrocephalus and DWM. The chromosomal microarray was normal. Trio whole-exome sequencing (WES) revealed a novel hemizygous pathogenic variant c.286dup (p.Ser96LysfsTer4) [NM_001369007.1] in exon 3 of the *AP1S2* gene related to Pettigrew syndrome and the mother was found to be heterozygous for the same variant. Though hydrocephalus and DWM are etiologically heterogeneous, *AP1S2* gene-related Pettigrew syndrome should be considered in individuals who are found to have these intracranial anomalies with autism and intellectual disability.

Keywords: Pettigrew syndrome, *AP1S2* gene, autism, Dandy-Walker malformation

Introduction

Pettigrew syndrome is a rare X-linked intellectual developmental disorder (Cacciagli et al., 2014) with variable phenotypic features. Global developmental delay, facial dysmorphism, and hydrocephalus are common findings of this disorder. Dysmorphic features include macrocephaly/microcephaly, high forehead, anteverted large ears, strabismus, long nose, and micrognathia. Behavioral abnormalities, autism, and abnormal gait have been reported in some patients. Cerebral calcification, iron deposition in basal ganglia, and choreoathetosis are uncommon findings. *AP1S2* gene contains five exons and codes for adaptor protein-1 (Baltes et al., 2014). *AP1S2* gene pathogenic variants and their association with Pettigrew syndrome were first identified in 2006. Till now only nine pathogenic variants have been identified in the *AP1S2* gene (Huo Let al., 2019). All these pathogenic variants are either nonsense variants or splice variants.

Clinical report

This male child, referred at the age of 7.5 years for evaluation of global developmental delay, autism, and behavioral disturbances, was born to a healthy non-consanguineous couple. The first and second pregnancies of the couple resulted in spontaneous abortions and one pregnancy was terminated due to unilateral hydrocephalus in the fetus. The boy was born by vaginal delivery with a birth weight of 3.5 kg. There was no history suggestive of teratogenic exposures or maternal comorbidities. The nuchal scan and detailed fetal anomaly scan were normal. A growth scan at 9 months of gestation revealed hydrocephalus (ventricular atrial diameter of 17 mm) in the fetus. Postnatally, ventriculoperitoneal (VP) shunt placement was done for hydrocephalus at the age of 3 months. There was no feeding difficulty or respiratory distress**.** The child had global developmental delay and autism and was not

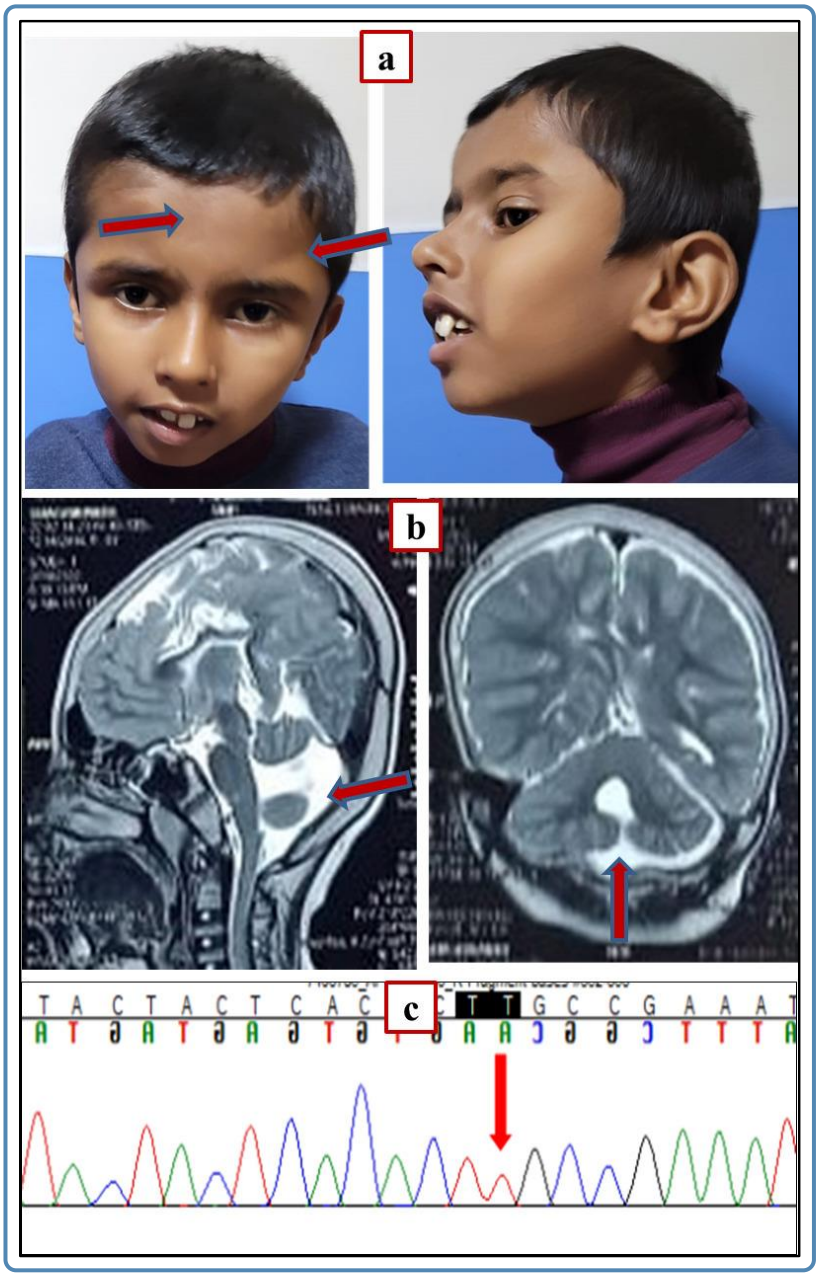

Figure 1 Clinical photographs depicting the facial, radiological, and genetic characteristics of the child

Figure 1A: Frontal and lateral view of the face showing dysmorphism in the form of a long face, depressed metopic suture and supraorbital ridges, flat forehead, low-set left ear, long nose, and a short philtrum

Figure 1B: MRI brain showing Dandy-Walker malformation

Figure 1C: Targeted Sanger sequencing confirmed presence of the hemizygous variant *c.286dup* in exon 3 of the *AP1S2* gene in the child

able to speak. He had not attained bladder and bowel control. There were no seizures or visual abnormalities. Hearing was impaired as per the parents. The mother was pregnant again and had been referred at around 8 weeks of gestation for prenatal genetic counselling.

On examination of the child, the head circumference was 48.5 cm (5^{th}-10^{th} centile),

height was 122 cm (50th centile), and weight was 21 kg (25th-50th centile). There was craniofacial dysmorphism in the form of plagiocephaly, long face, depressed metopic suture and supraorbital ridges, flat forehead, low set left ear, long nose, and short philtrum (**Figure 1A**). The neck, chest, spine, and extremities were normal. The feet were flat. The child was not responding to sounds and there was no eye contact. There was hypotonia with normal deep tendon reflexes. Examination of other systems was normal. MRI brain was suggestive of communicating hydrocephalus with a Dandy-Walker malformation (**Figure 1B**). The electroencephalogram (EEG) was normal. The couple was counseled regarding the possibility of a genetic etiology. Differentials considered were either a copy number variation or a neurodevelopmental disorder with structural brain abnormalities.

Chromosomal microarray of the boy did not reveal any clinically significant copy number variants. Trio whole-exome sequencing (WES) revealed a novel hemizygous pathogenic variant (PM2, PVS1, PP3, PP4) c.286dup (p.Ser96LysfsTer4) [NM_001369007.1] in exon 3 of *AP1S2* gene related to Pettigrew syndrome. Targeted Sanger sequencing confirmed presence of the variant in hemizygous form in the child (**Figure 1C**) and revealed heterozygosity for the variant in the mother. The couple was counselled regarding the X-linked recessive inheritance of the disorder with a 50% risk of recurrence in each male offspring. They opted for prenatal genetic testing of the ongoing pregnancy and amniocentesis was done. Targeted testing of the *AP1S2* gene variant in the amniocyte DNA revealed absence of the variant in the fetus.

Discussion

In 1973, Fried and Sanger first reported a Scottish family with X-linked mental retardation with hydrocephalus (Fried & Sanger, 1973). Later, multiple individuals with intellectual disability and Dandy-Walker malformation (DWM) with or without hydrocephalus were categorized under X-linked intellectual disability disorder and the condition was named Pettigrew syndrome (PGS) (Pettigrew et al., 1991; Cowles et al., 1993; Carpenter et al., 1999; Turner et al., 2003; Wakeling et al., 2002). *AP1S2* gene pathogenic variants and their association with Pettigrew syndrome were first identified in 2006 in families with intellectual disability and abnormal behaviour (Tarpey et al., 2006). Basal ganglia calcification is also one of the requisite findings to recognize this syndrome (Saillour et al., 2007; Borck et al., 2008; Cacciagli et al., 2014). Till now, there are only 58 patients who have been diagnosed with PGS caused by *AP1S2* mutation. Intrafamilial and interfamilial variable expressivity has been observed. Though facial dysmorphism is seen in many cases with PGS there are no specific dysmorphic features. Macrocephaly, long face, high forehead, protruding ears, strabismus, long nose, and small pointed jaw are some of the common facial features reported with PGS (Huo et al., 2019).

Imaging features of the brain may be normal in early childhood or affected individuals may have hydrocephalus, cerebellar/ posterior fossa anomalies (Strain et al., 1997), and/or iron and calcium depositions in the basal ganglia. Periventricular nodular heterotopia has also been reported. Based on imaging and brain pathology in Pettigrew syndrome, neurodegeneration begins with iron deposition. Serial imaging of the brain would be required to identify the same. Table 1 compares the clinical features in previously reported cases and our case. Female carriers are usually asymptomatic. Mild intellectual disability and iron deposition with neuroaxonal dystrophy in the basal ganglia leading to presenile dementia have been reported in a few carrier females. This could be due to skewed X inactivation (Pettigrew et al., 1991).

AP1S2 gene has 5 exons and encodes the sigma-2 subunit of the heterotetrameric adaptor protein-1 (AP1) and plays a role in the assembly of endocytic vesicles and recognition of signals of transmembrane receptors (Baltes et al., 2014; Glyvuk et al., 2010). Till now 9 pathogenic loss-of-function variants (5 intronic variants and 4 exonic variants) have been reported (**Figure 2**). The variant identified in our patient is a novel variant. Intrafamilial and interfamilial variable expressivity are not explained by the type of pathogenic variants. Genotype-phenotype correlations are not well defined in this syndrome as there are only a few cases reported till now. Based on previous reports, those with nonsense mutations had a higher incidence of microcephaly; seizures were common in cases with splice site mutations. Our patient did not have microcephaly or seizures.

The recurrence risk estimation is by exact molecular diagnosis and carrier status of the mother. In our case as the mother was a carrier,

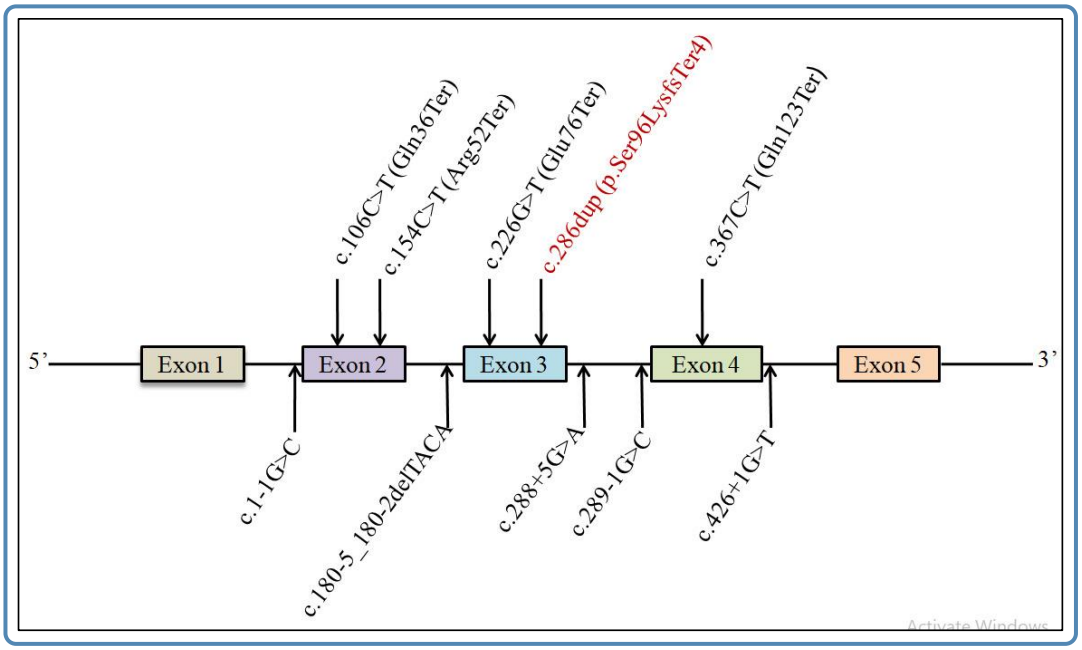

Figure 2 Pathogenic variants reported in the *AP1S2* gene; the variant identified in our patient is shown in red font

the recurrence risk was 50% for each male offspring. If the mother is not a carrier for the disorder, the empiric recurrence risk due to gonadal mosaicism is not more than 1%. Preimplantation genetic diagnosis and assisted reproduction with use of donor ovum (for female carriers) are other available reproductive options.

Conclusion

This case highlights that *AP1S2*-related Pettigrew syndrome, though a rare cause of X-linked intellectual disability, should be considered in cases with prenatal presentation of hydrocephalus and Dandy-Walker malformation, and postnatal presentation with autism and intellectual disability.

Acknowledgments

The authors wish to thank the patient and family for their cooperation and for giving consent for photography.

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Table 1 Comparison of the clinical features of previously reported individuals with Pettigrew syndrome with those of our patient

Clinical feature/finding	n-58 (% of previously reported patients)	Our patient
Motor developmental delay	59%	+
Intellectual disability	100%	+
Autism	7%	+
Seizures	82%	-
Aggressive behaviour	52%	-
Self–abusive behavior	48%	-
Facial dysmorphism	52%	+
Microcephaly	72%	-
Hypotonia	53%	+
Abnormal gait	26%	-
Hydrocephalus	76%	+
Dandy-Walker malformation	17%	+
Cerebral calcification	12%	-
Iron deposition in basal ganglia	9%	-

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GeNeViSTA

RNA-based Therapies for Monogenic Disorders

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Abstract

Monogenic disorders are conditions caused by variants in single genes. The current treatment strategies for monogenic disorders include DNA, RNA, or protein-based therapies. RNA-based therapeutics can be divided into four main categories namely antisense oligonucleotides, RNA interference, aptamers, and messenger RNA (mRNA) based therapies. Here, we discuss the RNA therapies that have emerged as promising strategies for the treatment of several monogenic disorders in recent years.

Keywords: RNA-based therapies, antisense oligonucleotides, small interfering RNAs (siRNA), aptamers

Introduction

Monogenic disorders are hereditary disorders that are caused by variations in single genes. These disorders can follow autosomal dominant, autosomal recessive, or X-linked modes of inheritance. These conditions are rare, but cumulatively affect more than 6% of the world's population which accounts for hundreds of millions of people (Clarke et al., 2023). Thus, the management of these conditions presents a vast global challenge.

Currently, the key challenges in the treatment of monogenic disorders are the high cost of treatment. Moreover, these drugs are not targeted against all genotypes. Also, only 10% of the genes are estimated to produce druggable target proteins (Clarke et al., 2023).

Current therapeutic strategies for monogenic disorders

The treatment strategies, at present, are aimed to treat monogenic disorders by targeting the DNA (gene therapy, gene editing), RNA (antisense oligonucleotides, RNA interference, readthrough compounds), or the protein encoded by the mutated gene (Zhu et al., 2022). In this review, we will be focussing on RNA-based therapies for monogenic disorders.

An overview of the major developments in the RNA targeting field

RNA-based therapies have emerged as promising strategies for the treatment of a number of monogenic disorders in recent years. The vital role of RNA in the flow of genetic information was first described by Francis Crick in his study on the 'Central Dogma of Molecular Biology' and was later confirmed by the discovery of mRNA in 1961.

The first application of RNA base-pairing for therapeutic purposes was described by Stephenson and Zamecnik in 1978 when they designed an antisense oligonucleotide targeting the 35S RNA sequence of the Rous sarcoma virus (RSV) thereby inhibiting its replication. In the year 1998, the first antisense oligonucleotide-based drug (Fomivirsen) was approved by the United States Food and Drug Administration (US FDA) for the treatment of cytomegalovirus retinitis. The first aptamer drug (Pegaptanib) was approved by US FDA in 2004 for the treatment of neovascular age-related macular degeneration (AMD). Patisiran was the first siRNA-based drug that was approved by the US FDA (2018) for the treatment of hereditary transthyretin-related (hATTR) amyloidosis (Kim, 2022).

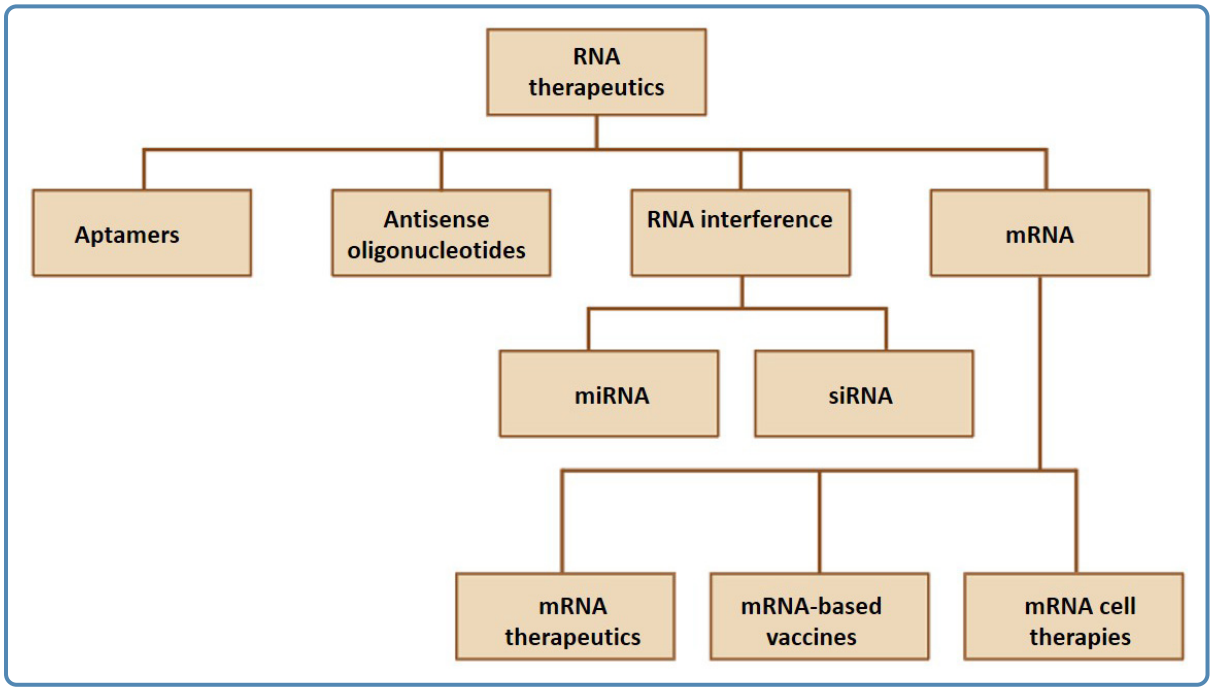

Figure 1 Classification of RNA-based therapies (Adapted from Zhou et al., 2019)

A few mRNA vaccines (Comirnaty by Pfizer, and BioNTech and Spikevax developed by Moderna) also got emergency approval in the year 2020 (final approval 2021) for use in the recent COVID-19 pandemic caused by SARS-CoV-2 (Kim, 2022).

Types of RNA therapies

Based on the structural characteristics and mode of action, RNA-based therapies can be broadly classified into four main categories namely, antisense oligonucleotides (ASO), RNA interference (siRNA, miRNA), aptamers, and messenger RNA-based therapeutics (**Figure 1**). The various FDA-approved RNA-based therapies for different genetic and non-genetic disorders are listed in **Table 1**.

Antisense oligonucleotides (ASO)

Antisense oligonucleotides (ASO) are single-stranded nucleotide sequences that modulate the expression of target RNAs via sequence-specific binding. Therapeutic ASOs range from 12 to 24 bp in length. Although the structure of these antisense oligonucleotides is determined primarily by their specific sequence, their chemistry can be modulated to produce novel effects with an increase in their specificity and stability (Zhu et al., 2023).

Antisense oligonucleotides that have been approved by the US FDA can be divided into two broad categories depending on their mechanism of action. The first group induces the cleavage of RNA by binding to the target sequence. Once these ASOs form a DNA-RNA duplex with their target RNA, it is recognized by the RNase H enzyme which leads to the degradation of the target RNA (**Figure 2**). Because these therapeutics utilize RNase H, which is active in both the nucleus and cytoplasm, these agents can be used to target noncoding elements as well (Dhuri et al.,2020). This provides an advantage over siRNA-based drugs which act primarily in the cytoplasm (Kim, 2022).

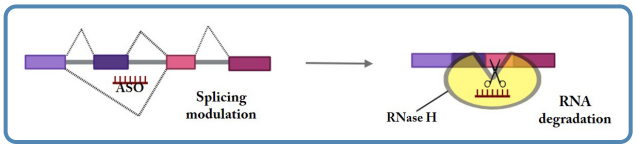

Figure 2 Antisense oligonucleotide-based RNA drugs that induce RNase H-mediated degradation of target mRNA (Adapted from Dhuri et al., 2020)

Several antisense oligonucleotide drugs using this type of cleavage have been approved

Table 1 FDA-approved RNA-based therapies (Adapted from Zhang et al., 2023; Kim, 2022)

RNA-based drug	Brand name	Year of FDA approval	Mechanism of action	Target disease
Antisense oligonucleotides				
Fomivirsen	Vitravene	1998	Inhibition of translation of viral mRNA encoding IE2 protein	CMV retinitis
Mipomersen	Kynamro	2013	Induction of the degradation of ApoB-100 mRNA	Homozygous familial hypercholesterolemia (HoFH)
Nusinersen	Spinraza	2016	Induction of exon 7 inclusion in SMN2 mRNA	Spinal muscular atrophy
Eteplirsen	Exondys 51	2016	Induction of exon 51 skipping in DMD mRNA	Duchenne muscular dystrophy
Inotersen	Tegsedi	2018	Induction of the degradation of TTR mRNA	Hereditary transthyretin-mediated (hATTR) amyloidosis
Golodirsen	Vyondys 53	2019	Induction of exon 53 skipping in DMD mRNA	Duchenne muscular dystrophy
Small interfering RNAs				
Patisiran	Onpattro	2018	RNA interference-mediated cleavage of TTR mRNA	Hereditary transthyretin-mediated (hATTR) amyloidosis
Givosiran	Givlaari	2019	RNA interference-mediated cleavage of ALAS1 mRNA	Acute hepatic porphyria
Lumasiran	Oxlumo	2020	RNA interference-mediated cleavage of HAO1 mRNA	Primary hyperoxaluria type 1
Inclisiran	Leqvio	2021	RNA interference-mediated cleavage of PCSK9 mRNA	Heterozygous familial hypercholesterolemia (HeFH)
RNA aptamers				
Pegaptanib	Macugen	2004	Antagonistic binding to VEGF protein	Neovascular age-related macular degeneration

by the US FDA including mipomersen and inotersen. Mipomersen is the second antisense oligonucleotide drug approved by the FDA (2013) as an adjunct to lipid-lowering therapy for the treatment of homozygous familial hypercholesterolemia (HoFH) (**Figure 3**). Apolipoprotein B-100 (ApoB-100) is the main component of low-density lipoprotein and its precursor very low-density lipoprotein (VLDL). Mipomersen binds to ApoB-100 mRNA and cleaves

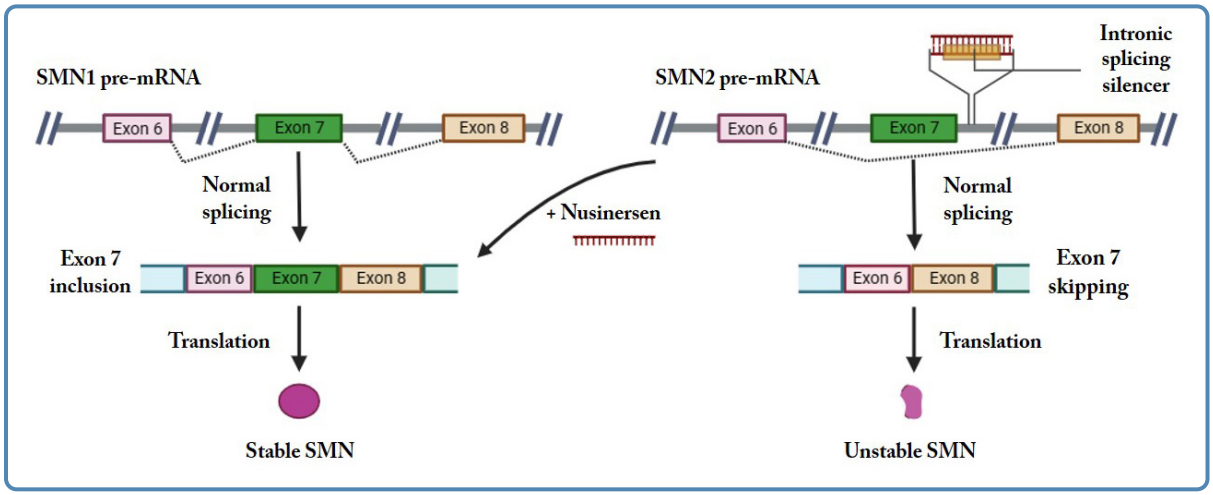

Figure 3 Antisense oligonucleotide-based RNA drug (Nusinersen) that modulates pre-mRNA splicing (Adapted from Kim, 2022)

its sequence, thereby reducing lipid levels in these individuals (Clarke et al.,2023).

The second group of antisense oligonucleotide drugs primarily regulates the splicing of pre-mRNAs by utilizing a steric hindrance-based mechanism. These ASOs bind to specific sequences within the pre-mRNA transcripts, and subsequently, modulate the other splicing factors to produce alternative splicing (**Figure 3**). A few FDA-approved ASOs which work on this mechanism include nusinersen, eteplirsen, golodirsen, and casimersen (Kim, 2022).

Individuals with spinal muscular atrophy have variations in *SMN1,* which encodes the survival motor neuron (SMN) protein. These variations prevent the expression of a functional SMN protein from the SMN1 gene locus but some amount of SMN protein is still produced from the *SMN2* locus. However, this protein product is smaller and less stable because of exon 7 skipping in the SMN2 pre-mRNA. As a result, there is loss of motor neurons eventually leading to muscle wasting. The intron splicing enhancer located between exons 7 and 8 in SMN2 pre-mRNA leads to this exon 7 skipping during the splicing of SMN2 pre-mRNA. Nusinersen is an antisense oligonucleotide that binds to this element and blocks its recognition by the splicing factors. As a result, the SMN2 pre-mRNA is spliced like SMN1 pre-mRNA leading to the production of a more stable SMN protein. This drug has shown improved motor function in infants with spinal muscular atrophy in a phase III clinical trial (Clarke et al.,2023) and was approved by the US FDA for clinical use in 2016.

Exon skipping approach has been utilized by a series of antisense oligonucleotide-based drugs such as eteplirsen, golodirsen, and casimersen that have been developed for the treatment of Duchenne muscular dystrophy (DMD) (Zhang et al.,2023). In individuals with DMD, the mRNA coding for dystrophin protein usually harbours a variation that can alter the reading frame, thereby producing a truncated (non-functional) protein in these individuals. Eteplirsen binds to the exonic splicing enhancer present in exon 51 of DMD pre-mRNA. Exon splicing enhancer element is required for the inclusion of this exon in the mature mRNA. Thus, binding of eteplirsen to this element leads to exon 51 skipping and correcting the reading frame in the mature mRNA. As a result, there is production of a short but functional dystrophin protein in these individuals. Exon 51 skipping may be beneficial for individuals with DMD with exon deletions ending at exon 50 or starting at exon 52 (Łoboda et al.,2020). Golodirsen and casimersen also utilize a similar mechanism in a different subset of individuals. Golodirsen induces exon 53 skipping while casimersen leads to exon 45 skipping to produce a functional version of the dystrophin protein (Kim, 2022).

Several clinical trials using antisense oligonucleotides such as IONIS-HTTRx for Hungtington disease and Tofersen, which targets the SOD1 protein frequently implicated in familial amyotrophic lateral sclerosis are underway (Clarke

et al.,2023).

Small interfering RNAs (siRNA) Small interfering RNAs (siRNAs) and micro RNAs (miRNAs) are small duplex RNA molecules that target messenger RNA (mRNA) leading to post-transcriptional gene silencing. siRNAs and miRNAs utilize the RNA interference (RNAi) pathway to modulate the expression of their target mRNA. RNAi is the endogenous and intrinsic defence mechanism of the body against invading viruses and transposable elements (Chen et al.,2018). In the RNAi pathway, endogenous small RNAs (siRNA or miRNA) form a complex with Argonaute 2 protein (Ago2) to produce an RNA-induced silencing complex (RISC) (**Figure 4**). This complex binds to the target mRNA via sequence-specific binding leading to mRNA cleavage (Zhang et al.,2023).

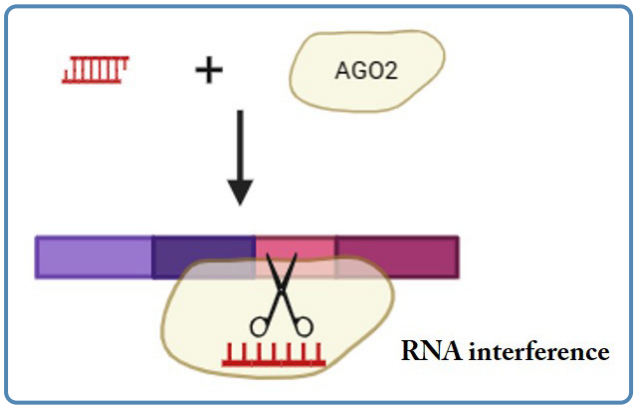

Figure 4 Mechanism of action of small interfering RNA-based drugs (Adapted from Kim, 2022)

siRNAs are double-stranded RNA molecules about 18 to 25 nucleotides in length. Four siRNA-based drugs have been approved by the US FDA namely patisiran, givosiran, inclisiran, and lumasiran for the treatment of hereditary transthyretin-mediated (hATTR) amyloidosis, acute hepatic porphyria, heterozygous familial hypercholesterolemia (HeFH) and primary hyperoxaluria type 1, respectively (Kim, 2022). However, no miRNA-based drug has been by approved so far by the US FDA for the treatment of monogenic disorders.

Patisiran is the first FDA-approved siRNA-based drug (2018) for treating hereditary transthyretin-mediated (hATTR) amyloidosis. This condition is caused by variation in the transthyretin (TTR) gene leading to the production of a misfolded transthyretin protein and it eventually leads to amyloid deposition in various tissues of the body. Patisiran utilizes a lipid nanoparticle-based delivery system and is injected into the body via intravenous infusion. These particles enter the hepatocytes via ApoE (apolipoprotein E) receptors. In the hepatocytes, patisiran combines with RISC and this complex binds to the 3' untranslated regions (UTR) of both the wild-type as well as mutant TTR mRNA leading to the suppression of TTR protein translation and an overall reduction in the amyloid deposition in the tissues (Zhang et al.,2023).

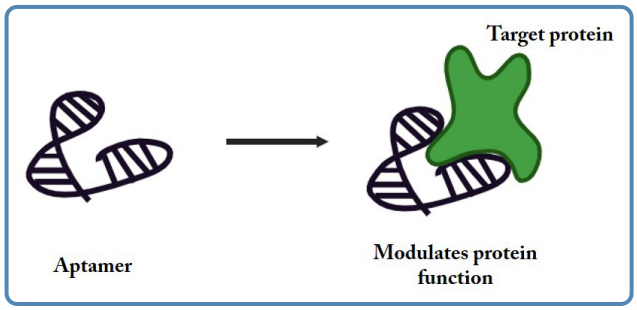

Figure 5 Mechanism of action of aptamer-based drugs (Adapted from Kim, 2022)

Aptamers Aptamers are small, single-stranded oligonucleotides (DNA or RNA) that bind to their targets (proteins, peptides, or nucleic acids) with high specificity and affinity and modulate their functions (**Figure 5**). Pegaptanib is the only aptamer drug that has been approved by the US FDA. It is a 28-nucleotide construct with two polyethylene glycol moieties (PEG) attached at its end. It binds to the vascular endothelial growth factor (VEGF) thereby inhibiting the interaction of VEGF with its receptor leading to the suppression of downstream VEGF signalling and cell proliferation. Pegaptanib was developed for the treatment of neovascular age-related macular degeneration, but it is rarely used nowadays because of the availability of several antibody-based drugs with similar efficacy. Nevertheless, it is a promising therapeutic strategy, and several RNA-based aptamers are under development for the treatment of various monogenic disorders (Zhu et al.,2022; Kim, 2022).

Conclusion

After several decades of development, RNA-based therapeutics are now becoming a clinical reality.

The field of RNA-based therapy is undergoing a major expansion and the underlying potential of these therapies for personalized medicine will certainly ensure the continued development of RNA-based therapeutics for years to come.

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GeNeViSTA

Validation of MLPA-detected Single Exon Deletion of the *DMD* Gene by Multiplex PCR

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Abstract

Multiplex ligation-dependent probe amplification (MLPA) is the most widely used technique to detect deletions and duplications of the *DMD* (dystrophin) gene that constitute two third of the cases of Duchenne (DMD)/ Becker muscular dystrophy (BMD). However, MLPA can yield false positive results for single exon deletions owing to the presence of single nucleotide polymorphisms (SNPs) at the ligation site. This study aimed to validate single exon deletions detected by MLPA in DMD/BMD individuals by multiplex polymerase chain reaction (PCR). The data of patients with DMD/BMD diagnosed by MLPA between January 2016 to February 2021 was collated and those with apparent single exon deletions were recruited. MLPA was performed using the P034/P035 DMD (MRC Holland) kit. Multiplex PCR was performed using Chamberlain and Beggs primer sets with appropriate controls. Sequencing of the relevant exon of the *DMD* gene was planned for discordant results between MLPA and multiplex PCR. SNPs within 8bp from ligation sites on MLPA probes and its frequency in the South Asian population was ascertained from appropriate databases. Single exon deletion was present in 166 (20.4%) of the affected individuals. Validation of MLPA results by multiplex PCR was performed in 135 affected individuals for the following exons- 4, 8, 43, 44, 45, 46, 48, 50, 51, 52 and 53. We obtained 100% concordant results for single exon deletions by MLPA and multiplex PCR probably due to zero frequency of alternate alleles for SNPs in the South Asian population. However, confirmation of MLPA-detected single exon deletions by an alternate technique is still essential due to emerging novel single nucleotide polymorphisms.

Keywords: Duchenne muscular dystrophy, Becker muscular dystrophy, Multiplex ligation-dependent probe amplification, multiplex polymerase chain reaction

Introduction

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked neuromuscular disorders caused by mutations in the dystrophin gene. The *DMD* gene is the largest known human gene containing 79 exons and spans about 2.5 Mbp of genomic sequence at the Xp21 locus (Barzegar et al., 2015). Approximately, two thirds of variants are large gene deletions or duplications, and the remainder are sequence variations (Flanigan et al., 2009). Previous techniques used for analysis of copy number changes like Southern blotting and quantitative polymerase chain reaction (qPCR) can detect fewer regions in specific genomic areas. At present, Multiplex Ligation dependent Probe Amplification (MLPA) is the most widely used technique to detect deletions and duplications in disorders like DMD due to its high degree of multiplexing. However, the ability of MLPA to detect single exon deletions can be varyingly affected based on population-specific single nucleotide polymorphisms (SNP) especially the CA mismatch between the 3' end of the left probe oligonucleotide and the target and this in turn can result in false-positive exon deletion. This is considered as one of the major pitfalls of MLPA as highlighted by various studies (Kim et al., 2016; Wang et al., 2008). It is therefore suggested that employment of a different technique like multiplex PCR, array comparative genomic hybridisation (aCGH) or Southern blotting is required to confirm single exon deletions detected by MLPA.

In this study, we tried to validate the results of

single exon deletions in the *DMD* (dystrophin) gene detected by MLPA in Indian patients with DMD by utilising an alternative multiplex PCR technique.

Methodology

This is a retrospective analysis conducted in our institute. The Institutional Ethics Committee approval was obtained. The data of patients with DMD/BMD diagnosed by the MLPA technique between the period January 2016 to February 2021 was collated. A subset of patients with apparent single exon deletions were recruited for further evaluation. Female carriers with deletion or duplication of dystrophin gene were excluded from the study.

DNA extraction and MLPA analysis

Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The DNA was quantified using a QIAxpert (Qiagen, Hilden, Germany) and stored at 4ºC. MLPA assay was performed using the P034/P035 DMD Kit with version B1 for P035 and B2 for P036 kit (MRC Holland, Amsterdam, Netherlands). Amplified products were analysed using an ABI 3100 analyser (Thermo Fisher Scientific, Massachusetts, USA). Peak heights were normalized, and a hemizygous deletion was confirmed when the normalized peak ratio value was 0 for male individuals.

Multiplex PCR analysis: DNA quality of all the stored samples was assessed by measuring its concentration in UV-Vis Spectrophotometer Q5000 (Quawell, USA). The exons tested by multiplex PCR included 4, 8, 43, 44, 45, 46, 48, 50, 51, 52 and 53 of the *DMD* gene. Chamberlain and Beggs primer sets were used. An external control and a single exon with different base pair size that was not deleted in the patient was used as an internal control in the patient's sample. The reaction mix contained 200 ng genomic DNA, 1µL of each primers, 10µL master mix and nuclease free water to make a final concentration of 20µL. Cycling conditions were 35 cycles of denaturation at 96°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 5 min. The PCR product (20µL) was electrophoresed in 2% agarose gel, stained with 2µL of ethidium bromide and run for 20-30 mins at 120V. The bands were visualized under an ultraviolet transilluminator.

Sequencing of the gene was planned if results were discordant between MLPA and multiplex PCR. National Center for Biotechnology Information dbSNP (https://www.ncbi.nlm.nih.gov/snp/) database was used to search for the single-nucleotide polymorphisms (SNP) within 8bp from the ligation sites on probes included in the P034/P035 DMD Kit. Allele frequency of these SNPs in the South Asian population was also ascertained from gnomAD (https://gnomad.broadinstitute.org), ALFA project (https://thealfaproject.com) and the recently published Indian database of 1455 individuals (Kausthubham et al., 2021)

Results

A total of 1051 patients were clinically diagnosed with DMD/BMD during the study period. The MLPA technique detected deletions and duplications in 813 (77%) individuals during the study period. Amongst them, single exon deletions were identified in 166 (20.4%) individuals. While single exon deletions identified in our cohort involved the following exons: 2, 4, 8, 9, 11, 13, 17, 18, 20, 43, 44, 45, 46, 48, 50, 51, 52, 53, 54, 55 and 57, we analysed only those individuals with 4, 8, 43, 44, 45, 46, 48, 50, 51, 52 and 53 exon deletion of the *DMD* gene. This was due to non-availability of few primers, insufficient number of patient samples in a single exon group and insufficient quantity and concentration of DNA in a few individuals. The distribution of single exon deletions of our cohort and those exon deletions analysed by multiplex PCR are depicted in **Figure 1**. Finally, DNA samples from a total of 135 patients with various single exon deletions were evaluated by the multiplex PCR technique. Amongst these, exon 45 followed by exon 51 were the commonest single exons found to be deleted in DMD/BMD patients.

Amongst the evaluated samples, we did not find any discrepancy between the results of MLPA and multiplex PCR for all the evaluated samples confirming the true positivity of MLPA results in our cohort (**Figure 2a & 2b**). The SNPs within 8bp from the ligation sites on probes of the exons and the frequency of alternate alleles in South Asian population were obtained from gnomAD, ALFA project and the recently published Indian database of 1455 individuals. These details are represented in **Table 1** for all the exons where single exon deletion was identified by MLPA. The alternate allele frequency of all these SNPs was zero in the South Asian population including the

Indian database.

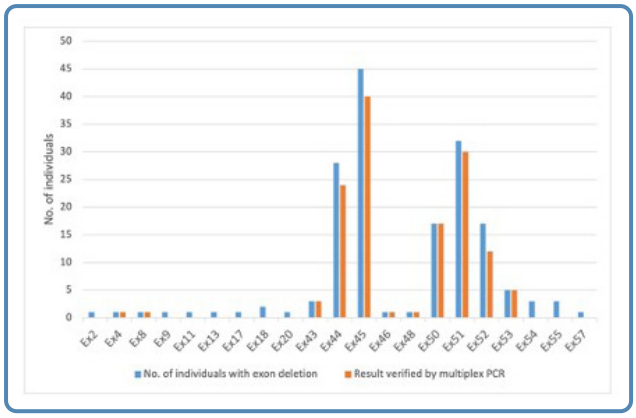

Figure 1 Total number of individuals with various single exon deletions identified by MLPA in our cohort versus those which were verified by multiplex PCR.

Discussion

Deletions and duplications of the dystrophin gene are the most frequently observed molecular events in DMD/BMD. In our study, single exon deletions constituted 20.4% of overall deletions/duplication events. Exon 45 followed by 51 was the frequent single exon deletion observed. These findings were similar to previous studies from India (Basumatary et al., 2013; Deepha et al., 2017) . In this study, we obtained 100% concordant results between MLPA and multiplex PCR technique for single exon deletion. Analysis of the alternate allele frequency for SNPs within 8bp of binding site on probes suggested absence of these alternate alleles in South Asian population.

Amongst various molecular techniques available to detect copy number variations (CNVs), MLPA offers important advantages as it can be used to identify female carriers, detect duplications and to map deletion and duplication borders which has important implications for prognosis and management. (Janssen et al., 2005). However, one of the frequent pitfalls of MLPA reported in literature (Kim et al., 2016) is false deletion results by sequence variations on probe-binding sites especially for single exon deletions. According to the MLPA design protocol, sequence variations within 8bp from ligation sites can affect the hybridization or ligation of the MLPA probe (MRC Holland, Amsterdam, Netherlands).

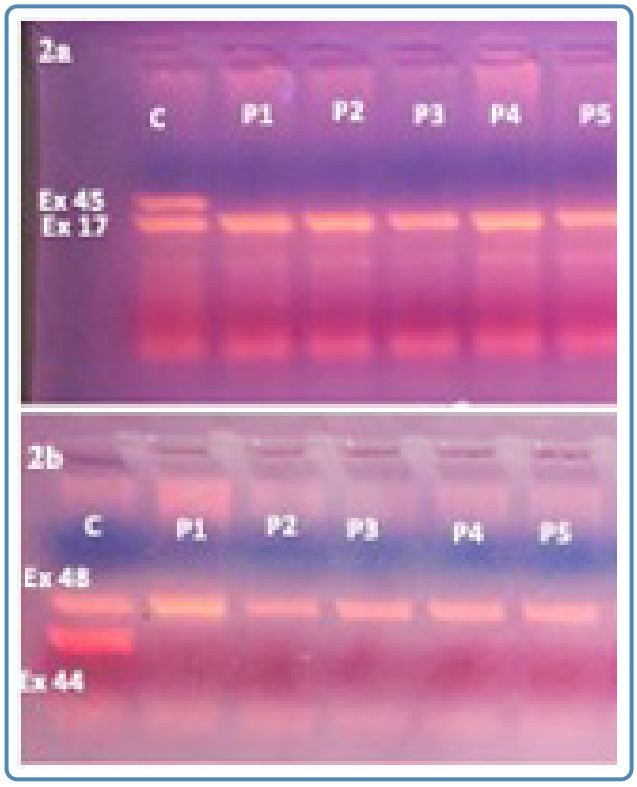

Figure 2 Multiplex PCR products run on gel electrophoresis.
2a: Top row showing band of exon 45 product and bottom row showing band of exon 17 product (internal control band).1st lane showing presence of both bands exon 45 and 17 in a control individual and rest of the lanes shows absence of band for exon 45 only, suggestive of exon 45 deletion in DMD patients.
2b: Top row showing band of exon 48 product (internal control band) and bottom row showing band of exon 44 product.1st lane showing presence of both bands exon 44 and 48 in a control individual and rest of the lanes shows absence of band for exon 44 only, suggestive of exon 44 deletion in DMD patients.

Also, CA mismatch between the 3' end of left probe oligonucleotide and the target is found to be associated with the lowest probe signal among single-nucleotide mismatches and studies have postulated the reason to presence of a single hydrogen bond for C and A nucleotide (Janssen et al., 2005; Aboul-ela et al., 1985). While efforts are

continuously being taken to prevent designing of probes in those regions, CA mismatch due to new mutagenesis cannot be prevented and hence interpretation of single exon deletions by MLPA have to be considered always with caution.

In our study, we did not observe any false positive results for single exon deletions detected by MLPA and multiplex PCR confirmed the presence of these single exon deletions. Next, we enlisted the SNPs especially those causing CA mismatches on binding regions of probes within 8bp from ligation sites by obtaining data from dbSNP. The databases like ALFA project, gnomAD and the recently published Indian database of 1455 samples were analysed for determining the frequency of alternate alleles. While considerable number of SNPs including those causing CA mismatches especially between the 3' end of left probe oligonucleotide and the target were identified, their allele frequency was zero in South Asian population. This probably explains the concordance of single exon deletion results by MLPA and multiplex PCR. A previous study in Korean patients has detected a false positive rate of 14.7% (11/75) for single exon deletions identified by MLPA and factors like CA mismatches in the binding region of the probe and probe melting temperature (Tm) ≤75°C were found to be causative for false positive MLPA results (Kim et al., 2016).

Various studies have proven that novel sequence variants (especially C to T transition) account for a considerable portion of point mutations for DMD gene and hence the probability of new CA mismatches can increase (Buzin et al., 2005). One solution to partially solve this issue is to upgrade the versions of MLPA regularly by designing new probes based on the occurrence of new SNPs. Another easier option is to cross check the results of the single exon deletion detected by MLPA with a simple alternate technique like multiplex PCR.

Conclusion

To conclude, this is the first study in literature that exclusively validated the results of MLPA-detected single exon deletions of the dystrophin gene by the multiplex PCR technique. Absence of false-positive results for single exon deletion by MLPA could probably be due to zero frequency of alternate alleles for SNPs. Confirmation of the results of single exon deletions obtained from MLPA by an alternate technique is however still warranted due to emerging novel single nucleotide polymorphisms.

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Table 1 SNPs within 8 bp from ligation sites on probes and their allele frequency in the South Asian population for the detected exons from gnomAD, ALFA project and in-house exome data of 727 samples.

S no.	Exon number	Nucleotide change (NM_004006.3)	SNPs	Partial probe sequence adjacent to ligation site	Alternate Allele Frequency in South Asia (gnomAD and ALFA project)	Alternate Allele Frequency in South Asia (Indian database [5])
1	Exon 2	c.62T>C	rs398124010	TTCAAAAGAAAA-CAT***T***CACAAAAT	NA	0.000
2	Exon 4[#]	c.230T>A	rs886042956	TGCCCTGAACAA-TG***T***CAACAAGGC	NA	0.000
3	Exon 8[#]	-	-	AGGTAAAGTGTG-TAAAGGACAGCT	-	-
4	Exon 9	c. 861G>A	rs1284806719	GCACAGGGATAT-GA***G***A***G***AACTTCT	0.00[a]	0.000
		c.863G>T/C	rs767304770		0.00[a] 0.000[b]	0.000
5	Exon 11	c.1173C>T/G	rs1057522737	ATTTGACAGC***C***C-A***T***CAGGGCCGGG	0.00[a] 0.000[b]	0.000
		c.1176T>C	rs1057521572		0.00[a]	0.000
6	Exon 13	c.1594C>T/G	rs763936813	AATAATCTG***A***C***C***-TTAA***G***TT***G***TTCT	NA	0.000
		c.1597C>T	rs1556834538		NA	0.000
		c.1604T>C	rs770149373		NA	0.000
		c.1602G>A	rs1556834513		NA	0.000
7	Exon 17	c.2084T>G	rs1556780761	GGAA***C***AG***A***TCC***T***-GGTAAAGCATGC	NA	0.000
		c.2080A>T	rs777042810		0.00[a]	0.000
		c.2077C>T	rs1060502661		NA	0.000
8	Exon 18	c.2226G>A	rs775246170	AGAAGCTG***T***GTT-***G***CAGAGTCCTGA	0.00[a] 0.000[b]	0.000
		c.2222T>C	rs886039135		0.00[a]	0.000
9	Exon 20	c.2436G>A	rs398123889	CGGTG***G***ATCGAA-TTCTG***C***CAGTTG	NA	0.000
		c.2448C>A	rs1603634746		NA	0.000
10	Exon 43[#]	c.6195T>C	rs398124006	GCATTGCAAA***GT***-***G***CAA***CG***CCTGTG	NA	0.000
		c.6194G>T	rs767872954		NA	0.000

		c.6196G>T	rs1468473458		0.00[a] 0.000[b]	0.000
		c.6200C>T	rs1405599353		0.00[a]	0.000
		c.6201G>A	rs969925680		0.00[a] 0.000[b]	0.000
11	Exon 44[#]	c.6373C>T	rs128626251	GAA***C***AGTTTCTC-AGAAAGACACAA	NA	0.000
12	Exon 45[#]	c.6535A>T/G/C	rs1361261157	ACAGATGCC***A***GT-ATTCTA***CA***GGAA	NA	0.000
		c.6545A>G	rs749804804		0.00[a] 0.000[b]	0.000
		c.6544C>T	rs1557038061		NA	0.000
13	Exon 46[#]	c.6704C>G/T	rs1316893006	AACATTGCTA***G***T-ATCCCACTTGAA	0.00[a] 0.000[b]	0.000
14	Exon 48[#]	c.7024C>T	rs749996618	CAGTTAAATC***A***T-C***T***GCT***GC***TGTGG	0.00[a] 0.000[b]	0.000
		c.7016A>G	rs398124041		0.00[a] 0.000[b]	0.000
		c.7019T>C	rs1569492076		NA	0.000
		c.7023G>T	rs886042910		NA	0.000
15	Exon 50[#]	-	-	AGAGAATGGGAT-CCAGTATACTTA	-	-
16	Exon 51[#]	c.7427A>G	rs757394130	GCTCTGGCAGAT-TTCA***A***C***C***GGGCT	0.00[a] 0.000[b]	0.000
		c.7429C>T	rs759274835		0.00[a] 0.000[b]	0.000
17	Exon 52[#]	-	-	CTAGCCTCTTGA-TTGCTGGTCTTG	-	-
18	Exon 53[#]	c.7781A>G	rs777178221	CTGAGC***A***G***G***TCT-TAGGACAGGCCA	0.00[a] 0.000[b]	0.000
		c.7783G>T	rs1254164861		NA	0.000
19	Exon 54	c.7914T>A	rs754997935	TGGCAGACAAAT-GTAGA***T***GTGGCA	0.00[a] 0.000[b]	0.000
20	Exon 55	c.8069T>G	rs398124061	AAACTCATAGAT-***T***ACTGCA***A***CAGT	NA	0.000
		c.8076A>G	rs144518527		0.00[a] 0.000[b]	0.000
21	Exon 57	-	-	CTGAAAGATGAT-GAATTAAGCCGG	-	-

a: ALFA project, b: gnomAD, NA: frequency data not available, - indicates absence of SNPs within 8bp from ligation sites on MLPA probes, # indicates those MLPA-detected exon deletions that have been confirmed by multiplex PCR.

GeNeXpreSS

Optical Genome Mapping and Long Read Sequencing: Mirror of the Genome

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Assessment of Optical Genome Mapping for Analysis of Structural Variants in Constitutional Postnatal Cases (Iqbal et al., 2023)

This study compared current standard of care (SOC) methods used in clinical cytogenetics (chromosomal microarray, karyotyping, fluorescent in situ hybridization, Southern blot analysis, and PCR) with optical genome mapping (OGM). The authors evaluated a total of 409 samples (50 negative controls and 359 individuals with suspected genetic disorders who were referred for cytogenetic testing. Structural variants including copy number variants (CNVs), aneuploidies, regions of homozygosity, contractions in facioscapulohumeral dystrophy 1, and repeat expansion in *FMR1* were analyzed. The American College of Medical Genetics and Genomics (ACMG) guidelines were used for the classification of variants. Intrasite and intersite reproducibility, concordance of technical and clinical classification, and ability to provide additional clinically relevant information were used as measures for comparing OGM with SOC. Majority of the samples (98%) yielded successful data for interpretation and analysis. Technical concordance with OGM was 99.5% and replicative analysis showed 100% concordance. Blinded analysis and variant classification agreement was 97.6% between SOC and OGM. Thus, the authors concluded that OGM is an alternative to SOC for rapid diagnosis of postnatal constitutional disorders due to its analytic validity and clinical utility.

16p13.11p11.2 triplication syndrome: a new recognizable genomic disorder characterized by optical genome mapping and whole genome sequencing (Nicolle et al., 2022)

The short arm of chromosome 16 contains several highly identical segmental duplications (SDs). These account for over 5% of the human genome. Non-allelic homologous recombination (NAHR) leads to recurrent chromosomal rearrangements. SDS are susceptibility factors for these rearrangements. Several genomic disorders involving 16p have been identified by chromosomal microarray (CMA). However, there are limitations to the resolution of CMA and short reads of whole genome sequencing (WGS). These are major hurdles in the characterization of more complex chromosomal rearrangements. In this study, the authors have reported two unrelated patients with de novo 16p13.11p11.2 triplication with a 16p11.2 duplication detected by CMA. The two patients had similar clinical features (hypotonia, severe developmental delay, hyperactive behaviour, facial dysmorphism, and conductive hearing loss). The rearrangement breakpoints could not be mapped precisely with short-read WGS. Thus, optical genome mapping (OGM) was used to determine the genomic positions of breakpoints and relative orientation of triplicated and duplicated segments. Thus, the authors identified a mechanism involving recombination between allelic SDs and an NAHR event and reported a new genomic disorder. They concluded that OGM can be used to detect mechanisms of complex chromosomal rearrangements involving SDs.

High diagnostic potential of short and long read genome sequencing with transcriptome analysis in exome–negative developmental disorders

(Lecoquierre et al., 2023)

Nowadays, exome sequencing (ES) is the method of choice for the diagnosis of rare diseases. The authors conducted a pilot study of five individuals with neurodevelopmental disorders (NDD). They performed trio-based short-read genome sequencing (srGS), long-read genome sequencing (lrGS), and case only transcriptome sequencing (TS) and identified three new genetic variants in three individuals. A case of Perching syndrome caused by a homozygous deep intronic variant in the *KLHL7* gene resulting in a neo-exon inclusion was identified by srGS. A case of Sotos syndrome which had a balanced inversion in *NSD1* was identified by lrGS. A de novo mosaic intronic 22-bp deletion in *KMT2D* causing Kabuki syndrome was also identified by srGS. TS of these 3 cases showed monoallelic expression, decreased gene expression, and splicing defects respectively, thus validating the effect of these variants. The study highlights the utility and complexities of these technologies.

Diagnosis of Prader–Willi syndrome and Angelman syndrome by targeted nanopore long–read sequencing

(Yamada et al., 2022)

Detection of abnormal methylation in the promoter of *SNRPN* is the basis of molecular diagnosis of PWS and AS. Nanopore sequencing is a unique, scalable technology that enables direct, real-time analysis of long DNA or RNA fragments. It works by monitoring changes to an electrical current as nucleic acids are passed through a protein nanopore. CpG methylation is detected through differences in electrical current intensities produced from nanopore reads of unmethylated and methylated bases. The authors successfully diagnosed four Prader-Willi syndrome patients and three Angelman syndrome patients by targeting differentially methylated regions. Concurrent copy number analysis, homozygosity analysis, and structural variant analysis enabled precise delineation of the underlying pathogenic mechanisms, including gross deletion, uniparental heterodisomy, uniparental isodisomy, or imprinting defect.

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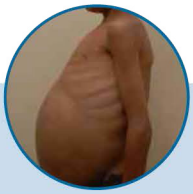

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