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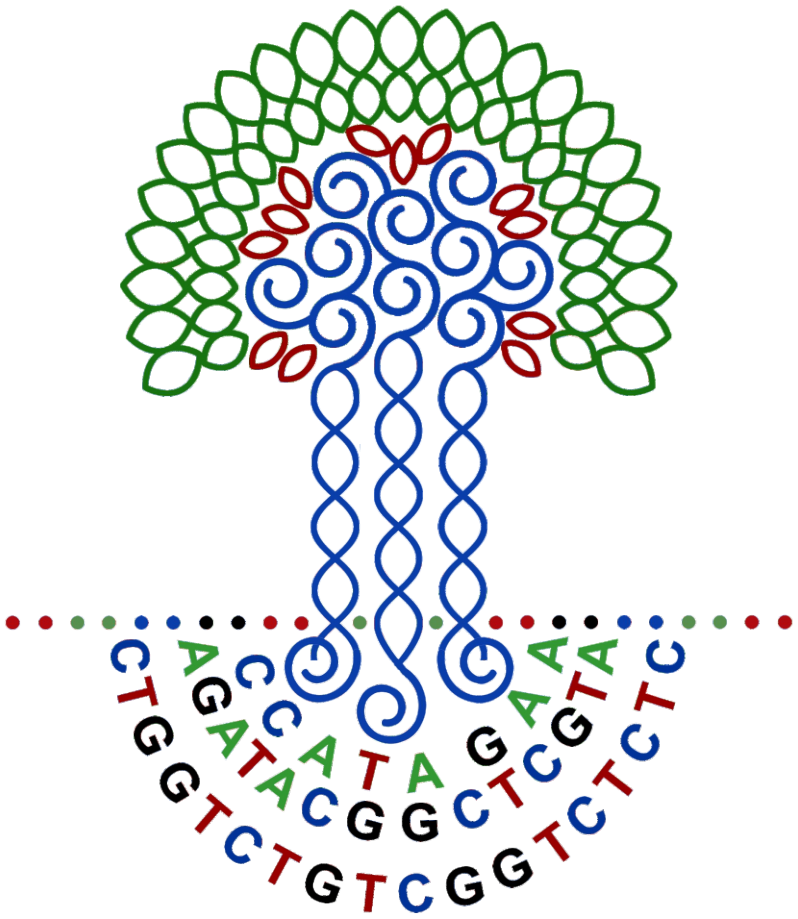


Address for correspondence
Dr Shubha R Phadke
Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow-226 014, EPABX: 0522-2668005-8 | Phone: 0522 249 4325, 4342 | E-mail: editor@iamg.in

PhotoQuiz - 64

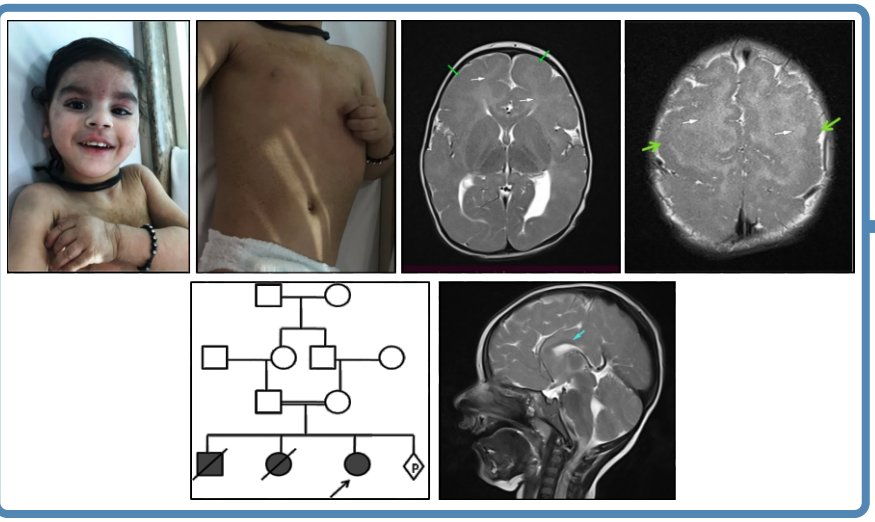
Contributed by: Dr Sunita Bijarnia–Mahay

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

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

This child was referred for evaluation of global developmental delay and a chronic skin disorder. Abnormalities noted in the MRI brain images (T2-weighted coronal and sagittal sections) are highlighted with arrows. 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.

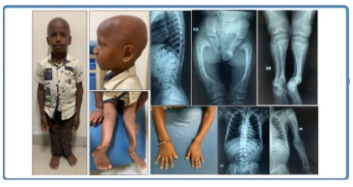


Answer to PhotoQuiz 63

Vitamin D Dependent Rickets Type 2A (VDDR2A) (OMIM #277440)

Vitamin D dependent rickets type 2A (VDDR2A) is an autosomal recessive disorder caused by biallelic pathogenic variants in the *VDR* gene (OMIM * 601769). The disorder is characterized by progressive rickets which usually manifests in early childhood with poor growth and skeletal deformities. Alopecia is present in almost two-thirds of affected individuals and is an important diagnostic clue. Biallelic mutations in the *VDR* (vitamin D receptor) gene reduce the action of the receptor which leads to an impaired intestinal absorption of calcium and phosphate. Biochemical findings include severe hypocalcemia, hypophosphatemia, secondary hyperparathyroidism with high levels of serum alkaline phosphatase, and elevated serum levels of calcitriol (1,25-dihydroxyvitamin D3). Radiological features include severe rachitic changes, osteomalacia, and decreased bone mineralization. Treatment includes daily administration of high doses of oral calcitriol and oral calcium, with intravenous calcium infusions to manage severe hypocalcemia.

Correct responses to PhotoQuiz 63 were sent by:



1. Dr Sunita Bijarnia-Mahay, Sir Ganga Ram Hospital, New Delhi
2. Dr Suvarna Ghansham Magar, MGM Medical College, Aurangabad
3. Dr Sheeba Farooqui, Kasturba Medical College, Manipal, Karnataka
4. Dr Surya G Krishnan. Centre for Human Genetics, Bangalore.

GeNeDit

Advanced Approaches for Rare Diseases

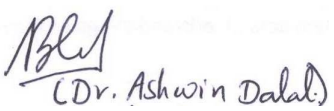
Editorial

Throughout history, humanity has grappled with various diseases, each presenting unique challenges. As medical advancements continue to evolve, our focus shifts accordingly. Thanks to breakthroughs in vaccines and antibiotics, we have triumphed over several infectious diseases and continue to combat others. Consequently, attention is increasingly directed towards non-communicable diseases (NCDs). India, too, is experiencing this transition, with a notable rise in NCDs. Among these, rare genetic disorders emerge as a significant concern. Rare diseases often pose significant challenges due to limited understanding, diagnosis, and treatment options. Patients and their families frequently endure long journeys marked by misdiagnoses, lack of effective therapies, and social isolation.

Rare Disease Day, observed annually on the last day of February, is a global initiative aimed at raising awareness about rare diseases and the challenges faced by those affected by them. This day serves as a platform to amplify the voices of individuals living with rare diseases, as well as their families and caregivers, highlighting the need for greater research, access to treatment, and support networks. This year, the Rare Disease Day falls on 29th February, which is itself a rare occurrence, occurring once in 4 years. Rare diseases often present unique and complex medical, social, and financial burdens, making it crucial to foster understanding and solidarity within communities worldwide. Through education, advocacy, and research, we all need to collectively strive to promote inclusivity, empowerment, and progress towards improved diagnosis, treatment, and ultimately, a better quality of life for patients impacted by rare diseases. A number of such events were conducted in association with SIAMG recently and few glimpses are present in the GenEvent section.

Technological advances in DNA sequencing have revolutionized the field of genetic diagnostics. It is very easy to obtain an exome or genome sequence of an individual in order to detect disease-causing variants even in individuals with atypical phenotypes. This is exemplified by the case reports in this issue on detection of a homozygous *TH* variant in a child presenting with hypotonia, and a homozygous *SOD1* variant in a child with infantile-onset motor regression. However, these high throughput technologies have led to new challenges with respect to interpretation of the pathogenic potential of the variants identified. There is a need for high throughput approaches to functionally characterize the variants in disease-causing genes so that the results can be interpreted with confidence and use for genetic counselling, prenatal diagnosis, and therapy. The GenExpress in this issue deals with few of such high throughput platforms for functional validation of genetic variants.

Recently, we heard the sad news of the passing of Dr I C Verma, a true stalwart in the field of rare diseases. Dr Verma dedicated his life to advancing our understanding and knowledge of rare diseases, leaving an indelible mark on the medical community and countless lives. His unwavering commitment, pioneering research, and tireless advocacy have inspired generations of clinicians, researchers, and patients alike. He leaves behind a legacy of compassion, resilience, and unwavering dedication that will continue to guide and inspire us all. We would like to dedicate this issue to his memory that we will cherish for a very long time.



Dr Ashwin Dalal
1st April, 2024

Clinical Vignette

Superoxide Dismutase Deficiency due to Biallelic *SOD1* Variants

Pallavi Sinha[1], Sunita Bijarnia–Mahay[1],
Hemlata Wadhwani Bhatia[2], Saurabh Chopra[3]

[1] *Institute of Medical Genetics and Genomics, Sir Ganga Ram Hospital, New Delhi, India*

[2] *Department of Medical Genomics, Max Superspeciality Hospital, Shalimar Bagh, New Delhi, India*

[3] *Department of Pediatric Neurology, Apollo Hospital, Sarita Vihar, New Delhi, India*

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

Abstract

Gain-of-function mutations in superoxide dismutase 1 (*SOD1*) are typically associated with familial Amyotrophic lateral sclerosis (ALS). Recently a distinct neurodegenerative disorder has been described, occurring due to biallelic loss of function in *SOD1*, manifesting as spastic tetraplegia with axial hypotonia in childhood. Debate exists regarding its classification, as to whether it is a distinct disorder or a part of the ALS spectrum.

Keywords: *SOD1*, Amyotrophic lateral sclerosis, biallelic *SOD1* variant, childhood-onset neurodegenerative disorder

Introduction

Superoxide dismutase (SOD) facilitates the transformation of the superoxide anion into hydrogen peroxide and oxygen and plays an important role in the cellular antioxidant defense. In humans there are three different isoforms of SODs: human Cu–Zn SOD (SOD1), the mitochondrial MnSOD (SOD2) and the extracellular Cu–Zn SOD (SOD3). Impairment of their antioxidant function or overactivity due to gain-of-function molecular mechanisms, represents a major pathophysiological role in the development of human neurodegenerative disorders (primarily, Amyotrophic Lateral Sclerosis) and cancer linked to SOD1 abnormalities (Eleutherio et al., 2021).

Mutations in *SOD1* are known to be associated with familial autosomal dominant Amyotrophic Lateral Sclerosis (ALS) mainly due to gain-of-function mechanism. Recently, Andersen et al. (2019), Park et al. (2019) and de Souza et al. (2021) reported paediatric patients with a severe neuromuscular disorder characterized by progressive motor neuron disease with hypotonia, spastic tetraplegia and loss of motor function due to homozygous biallelic pathogenic variants in *SOD1*. With no family history of ALS conforming to autosomal dominant inheritance, it was concluded that this disorder is distinct from *SOD1*- related ALS and constitutes a new clinical entity.

We present the clinical and genetic findings of a patient with biallelic pathogenic variant in *SOD1* associate with an early neurodegenerative phenotype.

Patient Details

A 1-year-old male child, third born to consanguineous parents from Uzbekistan, was referred for genetic evaluation. He was born at term gestation, by lower segment Cesarean section (LSCS), with a birth weight of 3.7 kg. The neonatal period was uneventful. He achieved all developmental milestones at an appropriate age until the age of 7 months after which he was noted to develop a gradual motor decline, with tightness of lower limbs being noted at around nine months of age. At 12 months of age, there was further motor regression with loss of ability to crawl or sit with support. There were no concerns regarding social, communication and emotional responses. There was no history of seizures, and visual or hearing impairment.

The proband's elder sister, who is 10 years old, was reported to be similarly affected (**Figure 1**). Parents noted a gradual motor decline for her, after 5-6 months of age. Currently, she was

bedridden and was not brought for evaluation. There was absence of speech in her. Social and communication domains were seemingly preserved. Magnetic resonance imaging (MRI) of her brain showed evidence of cerebellar atrophy.

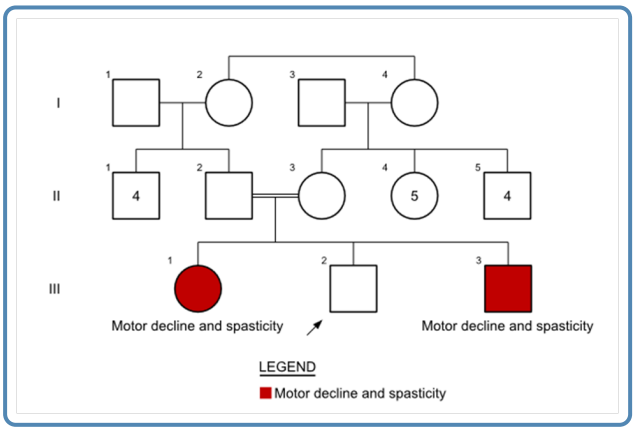


Figure 1 **Figure 1:** Pedigree of the family showing consanguinity and similar affected status of the elder sibling.

The parents were first cousins and were healthy and asymptomatic. There was no history of similar occurrence in the extended family. Paternal grandparents, who had passed away, had no neurological symptoms. The maternal grandparents were symptom free at the age of 70 years.

On examination, his growth parameters were within normal limits; weight: 10 kg (+0.33 Z), length: 75 cm (-0.32 Z), and head circumference: 46cm (-0.05 Z). The general physical examination was unremarkable. On neurological examination, the child was noted to be irritable and had appendicular hypertonia with axial hypotonia. Deep tendon reflexes were brisk in all four limbs. Bilateral plantar response was extensor. No fasciculations, myokymia or hyperekplexia were observed. Eye examination including fundus was unremarkable.

MRI brain was normal with no evidence of cerebral or cerebellar atrophy. In view of the progressive neurological symptoms in the child as well as his elder sibling with history of consanguinity, an autosomal recessive genetic condition was suspected and investigated for. Initial work up including hematological parameters, liver function tests, kidney function tests and serum creatine phosphokinase (CPK) were normal. Metabolic work up, including plasma lactate, homocysteine, acyl-carnitine, and amino acid profile by tandem mass spectrometry were also normal.

Whole exome sequencing revealed a homozygous pathogenic variant in the *SOD1* gene which is consistent with a diagnosis of autosomal recessive spastic tetraplegia and axial hypotonia, progressive type (**Table 1**).

This variant creates a shift in the reading frame starting at codon 112. The new reading frame introduces a premature stop codon 10 positions downstream. This variant has previously been reported as disease-causing (Andersen et al., 2019).

Discussion

The link between SOD1 variants and familial ALS is well-established, attributed to a gain of function in the *SOD1* gene. This leads to heightened oxidative activity, causing an overproduction of hydrogen peroxide. The mutated SOD1 also promotes increased protein–protein interaction, fostering aggregation, dimer destabilization, and oligomerization. These alterations contribute to abnormal axonal transport, microglia activation, heightened apoptosis, mitochondrial dysfunction, and oxidative stress, ultimately playing a critical role in motor dysfunction (de Souza et al., 2021; Kaur et al., 2016).

Biallelic truncating variants in *SOD1* result in spastic tetraplegia with axial hypotonia. There are very few cases reported in literature till now with the first case being reported in 2019 (Andersen et al., 2019). It is hypothesized that complete loss of function of SOD1 enzyme activity can produce an increased vulnerability to oxidative stress with mitochondrial dysfunction. It is characterized by onset of severe and progressive motor dysfunction in the first year of life. There is severe axial hypotonia combined with spastic tetraplegia, hyperekplexia, hypertonia, extensor plantar response and myokymia, reflecting upper motor neuron involvement. Cognitive development may be affected with absence of speech**.** Andersen et al. (2019) described the autosomal recessive *SOD1* gene-related disorder as a distinct entity, based on clinical differences (early onset spastic tetraplegia with axial hypotonia) and loss-of-function mechanism of gene malfunction. This was however challenged by de Souza et al. (2021), when they reported five

Table 1 Molecular genetic test results

Gene	Variant coordinates	Protein	Zygosity	Inheritance	Variant classification
SOD1	NM_000454.4: c.335dup (Human Genome Build GRCh37/hg19)	p.Cys112Trpfs*11	Homozygous	Autosomal recessive	Pathogenic (PS4, PVS1, PM2, PP5)

cases from two consanguineous families from Brazil, proposing it to be a varied spectrum of the same disorder. The patients reported by de Souza et al. who had the same variant as our patient, also exhibited features of lower motor neuron disease like fasciculations and fibrillations. They proposed that these patients may represent a very early infantile-onset ALS. However, there is insufficient evidence to suggest the same and long-term studies are required.

Regarding parental carriers who are heterozygous for the variant c.335dupG (p.Cys112Trpfs*11) in the *SOD1* gene, it is not possible to comment about the future risk of them developing a neurodegenerative disease, but they are not expected to develop any symptoms of ALS due to the very nature of the variant (loss of function). However, a long-term clinical follow-up would be necessary to detect any neurological manifestations related to ALS or any other type of neurodegeneration.

The final word is yet awaited regarding the molecular mechanism of the disease as the pathophysiology continues to unfold. However, it is clear that monoallelic as well as biallelic variants in the *SOD1* gene should be studied carefully, keeping in mind the clinical features as well as the mechanism of gene dysfunction created by the variant before making a confirmed diagnosis.

Conflict of interests: None

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Midgut Volvulus in Trisomy 21: Unveiled on Fetal Autopsy

Somya Srivastava, Shubha R Phadke

Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Science, Lucknow, India

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

Abstract

Midgut volvulus is a rare developmental anomaly with a largely unknown etiology that is challenging to diagnose antenatally in the second trimester. The objective of this case report is to describe a rare case of midgut volvulus associated with trisomy 21. Amniocentesis was done in a 19-week fetus with severe ventriculomegaly, hypoplastic cerebellum, echogenic bowel, and growth restriction to rule out chromosomal anomalies. The couple opted to terminate the pregnancy in view of the severity of the anomalies. The fetal autopsy revealed a characteristic whirlpool appearance of midgut volvulus, and the karyotype was suggestive of trisomy 21. This is the second reported case of midgut volvulus associated with trisomy 21 where ultrasound was not specific for this anomaly and the gut loops were coiled in a distinctive whirlpool pattern detected incidentally on fetal autopsy.

Keywords: Midgut volvulus, trisomy 21, fetal autopsy, whirlpool appearance, ultrasound, amniocentesis

Introduction

Down syndrome is the most common numerical chromosomal abnormality with an incidence of 1 in 850 live births and is characterized by intellectual disability, dysmorphism and congenital malformations (Al-Nbaheen et al., 2018). Anomalies of the gastrointestinal tract make up 5-7% of all congenital malformations and these are due to atresia or stenosis (Morris et al., 2014). In the present case report, we describe a rare case of midgut volvulus without atresia which was incidentally discovered in a fetus with trisomy 21.

Patient Details

A 29-year-old second gravida (G2A1) was referred at 19 weeks 1 day (19 weeks 6 days as per the first trimester dating scan done at 6 weeks 1 day) of gestation in view of ventriculomegaly and short long bones in the fetus. The couple were married non consanguineously, and the pregnancy was conceived spontaneously after one previous spontaneous abortion in the first trimester. In the present pregnancy, the first trimester was uneventful. Aneuploidy screening was not done. On ultrasonographic evaluation, diameters of atria of both lateral ventricles of the brain were more than 15 mm, suggestive of severe ventriculomegaly along with hypoplastic cerebellum (cerebellar diameter - 17.2 mm; -2.2 SD for 19 weeks of gestation). The bowel was slightly echogenic. Growth parameters and measurements of all long bones fell below -3 SD (corresponding to 16 weeks of gestation) suggestive of potential growth restriction in the fetus. No other soft marker or malformation was noted. A chromosomal etiology was suspected, and the couple opted for amniocentesis for fetal karyotype, after counselling. In view of severity of the anomalies and poor prognosis for growth and survival, the couple decided to terminate the pregnancy and submitted the fetus for autopsy.

On external examination, the fetus was female with subcutaneous edema below the chin and lower jaw extending to the rest of the body. Slight retrognathia and low set ears were noted in the face with intact lips and palate (**Figure 1a and b**). Both upper and lower limbs were devoid of any abnormality with no anorectal malformation. The cord had three vessels. The anthropometric measurements and fetal weight fell below the 5th centile for gestational age of 19 weeks and were indicative of growth restriction in the fetus. Internal examination of the brain confirmed the presence of ventriculomegaly and hypoplastic

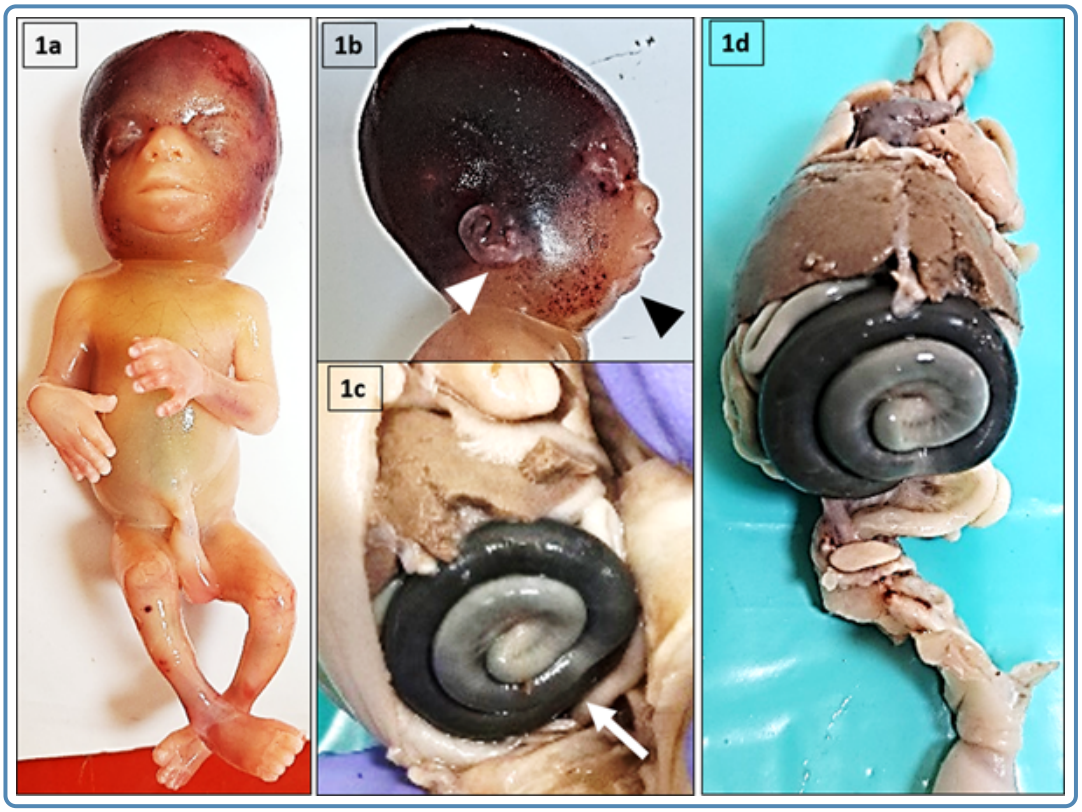

Figure 1 **Images of fetal autopsy. 1a:** Front profile of the fetus. **1b:** Side profile of the face with white pointer towards the low set ears and the black pointer showing the retrognathia. **1c:** White arrow pointing towards the characteristic whirlpool coiling of the midgut in situ. **1d:** Internal examination of the fetal organs with the midgut volvulus.

cerebellum. On opening the abdomen, loops of dilated gut were coiled in a characteristic whirlpool pattern suggestive of volvulus (**Figure 1c and d**). No evidence of atresia or stenosis were found on examination of the midgut. No malformation was noted in the esophagus, trachea, lungs, and heart with an intact diaphragm. Remainder of the abdomen and pelvic organs were unremarkable with normal histopathology. Fetal karyotype revealed free trisomy 21.

Discussion

Association of midgut volvulus with trisomy 21 is extremely rare. Midgut volvulus is seen in 3.9/10,000 live births and can be picked up antenatally in the third trimester by various ultrasound findings of which dilated stomach and bowel, polyhydramnios and whirlpool sign are the most frequent (Schulman et al., 1993; Shen et al., 2022). Review of videos of prenatal ultrasonography done in our case did not reveal anything other than echogenic bowel around the umbilicus suggesting that at early gestation the whirlpool sign may not be obvious on ultrasonography. Echogenic bowel has been reported in 11.4% cases at a median gestational age of 31 weeks (Shen et al., 2022).

The most common cause of midgut volvulus is intestinal malrotation. Other rare associations include intestinal atresia and duplication, cystic fibrosis, and segmental mesenteric defect (Shen et al., 2022). Only a single case series with 11 cases reported one fetus with trisomy 21 (Yang et al., 2022).

This is the second reported case of midgut volvulus seen with trisomy 21. Such findings can be missed in the second trimester anomaly scan. In our case it was detected incidentally at fetal autopsy, reiterating the importance of doing a systematic fetal autopsy in uncovering rare developmental anomalies.

Acknowledgements: The authors are thankful

to the patient and her family for their consent.

Conflict of interest: None

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Clinical Vignette

Tyrosine Hydroxylase Deficiency: Report of a Novel Phenotype

Sunil Taneja

Sushrut Medical Centre Pvt Ltd, Kanpur, India.

Correspondence to: Dr Sunil Taneja *Email:* tanejasunil17@gmail.com

Abstract

Dopamine-responsive dystonia is a rare disorder. The clinical diagnosis is usually made based on the response to levodopa. It is a conglomerate of three enzymatic deficiencies: guanosine triphosphate cyclohydrolase 1, sepiapterin reductase and tyrosine hydroxylase. All of these have some classical presentations and variability in the time of onset and severity, but dystonia and diurnal variation of symptoms have been reported in almost all patients to date. Here, we report the case of a male child, born to consanguineous parents and having symptoms since early infancy, who had only generalized hypotonia with no diurnal variation in symptoms. There was a dramatic response to levodopa therapy. Genetic evaluation revealed a homozygous known pathogenic variant c.698G>A in the *TH* gene (NM_199292.3), confirming the diagnosis of tyrosine hydroxylase deficiency.

Keywords: Neurotransmitter deficiency, dystonia, atypical

Introduction

Dopamine-responsive dystonia (DRD) is a rare disorder. It has a prevalence of 0.5 per million population (Malek et al., 2015). The first case reports were from Segawa et al. in 1976, when it was labelled as hereditary progressive dystonia or Segawa's disease. The term dopamine-responsive dystonia (DRD) was introduced in 1988 by Nygaard. Based on genetic studies, causative variants have been identified in three genes namely *GCH1* (OMIM*600225) encoding guanosine triphosphate cyclohydrolase 1, *SPR* (OMIM*182125) encoding sepiapterin reductase, and *TH* (OMIM*191290) encoding tyrosine hydroxylase. Patients with dopamine-responsive dystonia may have variants in any one of these three genes; however, as majority of them respond to levodopa, they are all labelled as DRD (Randby et al., 2018). Each of these have specific phenotypes although variations are present. Their clinical and genetic heterogeneity makes them a diagnostic challenge, as is highlighted by this case of a child with tyrosine hydroxylase deficiency that is discussed here.

Patient Details

A male child was seen at the age of three years. He was born to first-degree consanguineous parents. The child was delivered per vaginum. There were no perinatal issues. He was the second sib. The elder sib was a four-years-old female child with normal development. On examination, the child was completely hypotonic. There was no head control. The limbs and trunk were totally hypotonic. However, there were no contractures. Deep tendon reflexes were not elicitable. The child was alert, able to understand oral commands and respond with monosyllables. There was no diurnal variation and no dystonic posturing. The parents said that right from the beginning he used to move his limbs less and never achieved any motor milestones. He had been investigated extensively. His metabolic profile including gas chromatography/mass spectrometry (GCMS) of the urine and tandem mass spectrometry (TMS) of the blood were normal. Electroencephalogram (EEG) and magnetic resonance imaging (MRI) of the brain did not show any abnormality. Nerve conduction study (NCS) and electromyography (EMG) were noncontributory. He was empirically started on 25 mg of levodopa. Due to the COVID19 epidemic, he was lost to follow up. After three years he came walking with his father to the outpatient clinic. His father explained that as soon as the medicine was started, he started lifting his head and sitting by the third day. As he had not started walking by the fifth day, he increased the

dose himself to 50 mg and after this the child started walking. There was however now a diurnal pattern. By evening the child would tire out and once the next dose was given, he would again be normal. Now he is eight years old, attends primary school, speaks only bisyllables, and scribbles but has not yet started writing words.

Whole exome sequencing test was done. A homozygous c.698G>A missense variant was detected in exon 5 of the *TH* gene (NM_199292.3), that results in the amino acid substitution of histidine for arginine at codon 233 (p.Arg233His). This variant nests within the biopterin-dependent aromatic amino acid hydroxylase domain of the TH protein coding gene, which is a functional domain. This variant has not been reported in the 1000 genomes databases and has a minor allele frequency (MAF) of 0.01%, 0.01%, and 0.009% in the gnomAD (v3.1), gnomAD (v2.1) and TOPMed databases respectively. Various in-silico prediction tools like MutationTaster2, PolyPhen-2 (HumDiv), SIFT etc. predict this variant to be 'damaging'. This variant has previously been reported in patients affected with tyrosine hydroxylase deficiency and is reported as 'pathogenic' in the ClinVar database [Variation ID:12327; *TH* (NM_000360.4): c.605G>A; p.(Arg202His)]. Homozygous or compound heterozygous pathogenic variants in the *TH* gene result in Segawa disease (OMIM#605407), and the diagnosis of *TH* gene-related autosomal recessive Segawa disease due to tyrosine hydroxylase deficiency was thus established in the child.

Discussion

Tyrosine hydroxylase deficiency has two presentations. In type A, which presents after infancy there is hypotonia, rigidity, and dystonia with diurnal variation. In type B, the onset is in infancy with encephalopathy, hypotonia, hypokinesia, dystonia, tremors, myoclonus, oculogyric crisis, dystonic crisis, and dysautonomia (Goswami et al., 2017; Willemsen et al., 2010). The patient in our case report had an onset in infancy but had no other symptoms other than hypotonia. Absence of wasting and lack of any contractures which develop in all myopathies over a period of time, were absent in this child; this was considered as a pointer for neurotransmitter deficiency. A similar presentation has been reported in some DRD patients with GCH1 deficiency (Eye et al., 2019), but has not been previously reported with TH deficiency.

In addition to genetic testing, analysis of cerebrospinal fluid (CSF) is also important for the diagnosis of TH deficiency. In TH deficiency, homovanillic acid levels (HVA) are low and levels of 5-hydroxyindolic acetic acid (5-HIAA), neopterin and biopterin are normal. The ratio of HVA to 5-HIAA is less than 1 (normal range is 1-3.7). These tests could not be done in our patient.

As per review of literature, various genetic mutations are reported in the *TH* gene, but the commonest are the c.698G>A and c.707T>C variants (Eye et al., 2019; Dong et al., 2020). Our patient also had the homozygous c.698G>A variant but had an atypical presentation of early infantile onset pure hypotonia. This case highlights the variability of phenotypic presentation, in spite of similar mutation, in TH deficiency.

Conflict of interest: None

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GeNeXpreSS

High Throughput Functional Assay Platforms to Screen Multiple Variants

Ch Neeraja, Ashwin Dalal

Diagnostics Division, Centre for DNA Fingerprinting ad Diagnostics, Hyderabad, India

Correspondence to: Dr Ch Neeraja *Email:* cneeraja22@gmail.com

Technological advances have led to the development of high-throughput sequencing platforms enabling human genome sequencing to be used in clinical practice. Several genomic variants are being identified across individuals of diverse populations, using high-throughput sequencing studies. Inability to ascertain clinical relevance to the identified pool of genetic variants continues to be a critical roadblock towards the development of precision medicine.

Variant Interpretation: Functional Assays to the Rescue (Starita et al., 2017)

Next generation sequencing has revolutionized the field of genetic diagnostics. However, ability to sequence large parts of the human genome has thrown up new challenges too. Notably, many of the variants are categorized as Variants of Unknown Significance (VUS) based on the ACMG/AMP criteria. Functional analysis of variants is quite challenging owing to the limitations of screening multiple variants using biochemical methods or computational predictions. Multiplexed assays for variant effects (MAVEs) is a powerful method to screen thousands of variants in a single experiment. The result of MAVE is a variant effect map which reveals the functional relevance of single variants in the genetic element. MAVEs are a family of methods that includes Deep Mutational Scanning (DMS) experiments to study protein sequence-function relationships and Massively Parallel Reporter Assays (MPRA) on gene regulatory sequences. The authors have reviewed various high throughput functional assays and have highlighted their utility in reclassification of variants of uncertain significance.

Massively parallel reporter assays for characterization of de novo promoter variants (Koesterich J et al., 2023)

Autism spectrum disorder (ASD) is a heritable and complex neurodevelopmental disorder that includes both common and rare/de novo variants (DNVs) in the coding and non-coding genome. Koesterich and team studied the DNVs of non-coding regions, in view of the limited insight regarding variations in the promoter and enhancer regulatory regions. Defects in neural progenitor cells (NPCs) have been implicated in ASD and similar neurodevelopmental disorders. In the present study, the transcriptional impacts of DNVs were examined in NPCs. NPCs are also tractable for the lentivirus-based massively parallel reporter assay (lentiMPRA), which can simultaneously test thousands of sequences in a single experiment. These sequences included both wildtype (reference) and mutated (alternate) forms upstream of a minimal promoter and reporter gene, so that changes in the expression of the reporter gene can be detected for each sequence variant. Using this approach, 3600 promoter DNVs were characterized in ASD cases and sibling controls. A subset of 165 high confidence DNVs (HcDNVs) was identified. These HcDNVs were enriched with transcriptionally related genomic annotations including transcription factor binding and epigenetic markers of active transcription, suggesting their role in gene regulation.

Homology-directed repair (HDR) reporter assay to evaluate *BRCA1* variants (Nagy G et al., 2023)

Identification of variants which are functionally abnormal in tumor suppressor proteins is critical

for cancer surveillance, prognosis, and treatment options. *BRCA1* is an essential gene owing to its tumor suppression activity by regulating the repair of DNA double strand breaks *via* the homology directed repair (HDR) mechanism. Knowledge on the impact of pathogenic variants in ''actionable'' genes (e.g., *BRCA1* and breast cancer) provides evidence for medical management. Multiplexed functional assay includes testing of hundreds of protein variants simultaneously and determines their functional impact. MAVE studies reveal functional importance of residues in the *BRCA1* coiled-coil and serine cluster domains. Libraries of *BRCA1* mutated at single amino acid residues from 1280–1576 were generated and function of these variants was analysed in the homology-directed repair (HDR) reporter assay. Nagy and team have employed a HDR Reporter assay to evaluate over 300 missense and nonsense *BRCA1* variants between amino acid residues 1280 and 1576, which encompasses the coiled-coil and serine cluster domains. It was inferred that the functionally abnormal variants tended to cluster in residues known to interact with *PALB2*, which is critical for homology-directed repair. Multiplexed results were confirmed by singleton assay and by ClinVar database variant interpretations.

Comprehensive functional characterization of *SGCB* coding variants predicts pathogenicity in limb–girdle muscular dystrophy type R4/2E (Li C et al., 2023)

Limb-girdle muscular dystrophy (LGMD) type R4/2E is caused by mutations in β-sarcoglycan (*SGCB*), which is a key component of the dystrophin-associated protein complex. In muscle cells, the dystrophin-associated protein complex localizes to the membrane and connects the intracellular cytoskeleton to the extracellular matrix, allowing for coordinated force production in muscle. The sarcoglycan subcomplex (SGC) is composed of 4 single-pass transmembrane proteins: α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, and δ-sarcoglycan. Biallelic loss-of-function mutations in any subunit can lead to LGMD. More than 50% of patients clinically diagnosed with a myopathy carry a variant of unknown significance in a myopathy gene, often leaving them without a genetic diagnosis. To provide functional evidence for the pathogenicity of missense variants, Li and team performed deep mutational scanning of *SGCB* and assessed SGC cell surface localization for all 6,340 possible amino acid changes. Lentiviral expression of YFP-SGCB-HA-WT plasmid construct in ADG-HEK cells displayed strong cell surface expression. ADG-HEK cells transduced with presumptive pathogenic variants had a significant decrease in cell surface expression of SGCB. Single amino acid saturation mutagenesis was employed to generate libraries comprising every possible missense, synonymous, and nonsense variant. Variant functional scores were bimodally distributed and perfectly predicted pathogenicity of known variants. Variants with less severe functional scores more often appeared in patients with slower disease progression, implying a relationship between variant function and disease severity.

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The Boon Becomes the Bane: The Ballad of the Blue-eyed Boy

Remya Rajkumar, Sankar VH

Genetic Clinic, Department of Pediatrics, SAT Hospital, Thiruvananthapuram, India

Correspondence to: Dr Remya Rajkumar *Email:* remyarajkumar@gmail.com

Beauty lies in the eyes of the beholder. However, beauty can also literally be due to a person's eyes, especially the eye colour. In South India, the population generally has black-coloured irises and hence, a different hue, especially blue or green, is a matter of great pride and wonder. However, even something as innocuous as a different colour of the iris can herald an underlying genetic syndrome which might affect future generations to come, as illustrated by this report.

The proband, a 3-month-old male child, the first child of a non-consanguineous marriage, was born at term with a birth weight of 2.9 kg. At birth he was noticed to have a white forelock of hair, a depigmented patch on the forehead and brilliant blue irises of both eyes. He was admitted in the neonatal intensive care unit (NICU) for 3 days in view of mild respiratory distress which resolved. He did not pass his preliminary otoacoustic emissions (OAE) test, and brainstem evoked response audiometry (BERA) and auditory steady-state response (ASSR) tests which were done further were suggestive of bilateral profound sensorineural hearing loss. Since his features were phenotypically suggestive of Waardenburg syndrome, genetic testing was planned for confirmation of the diagnosis. Clinical exome sequencing unveiled a heterozygous nonsense variant c.667C>T (p.Arg223Ter) in exon 5 of the *PAX3* gene (NM_181457), which is classified as a 'pathogenic' variant as per the American College of Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/AMP)criteria, confirming the diagnosis of Waardenburg syndrome type 1. This variant is predicted to cause loss of normal protein function through protein truncation. Loss-of-function variants in *PAX3* are known to be pathogenic (Wildhardt et al., 2013). Parental segregation analysis showed that the father had the same heterozygous mutation, and the mother did not have it.

Pedigree analysis of the family (**Figure 1**) reflects the high degree of variability in the clinical phenotype caused by the mutation in the family. The first known affected member in the family was the proband's great grandmother with history of premature greying of hair and blue eyes. She had seven children of whom two children were unaffected and four had blue eyes with early greying of hair. The proband's grandfather had only premature greying. It was interesting to note that the grandfather had premature greying, the father additionally had blue eyes and the proband had all the classical features of Waardenburg Syndrome. Since many family members had blue eyes without any other features it was considered a matter of pride within the family; they considered themselves to be unique. However, it was only after the birth of the proband with a white forelock and hearing loss, that the family realized that there were probable repercussions to bear along with the beauty of blue eyes! The child is on hearing aids at present and cochlear implant is being planned. The parents and rest of the family are now concerned about blue-eyed children and genetic counselling has been advised to all members of the family.

Waardenburg syndrome (WS) is a group of genetic conditions that can cause hearing loss and changes in coloring (pigmentation) of the hair, skin, and eyes. The hearing loss in WS1, observed in approximately 60% of affected individuals, is congenital, typically non-progressive, either unilateral or bilateral, and sensorineural. Most commonly, hearing loss in WS1 is bilateral and profound (>100 dB). Majority of individuals with WS1 have either a white forelock or early greying of the scalp hair before the age of 30 years. The classic white forelock observed in approximately 45% of individuals is the most common hair pigmentation anomaly seen in WS1.

In this family reported here, multiple

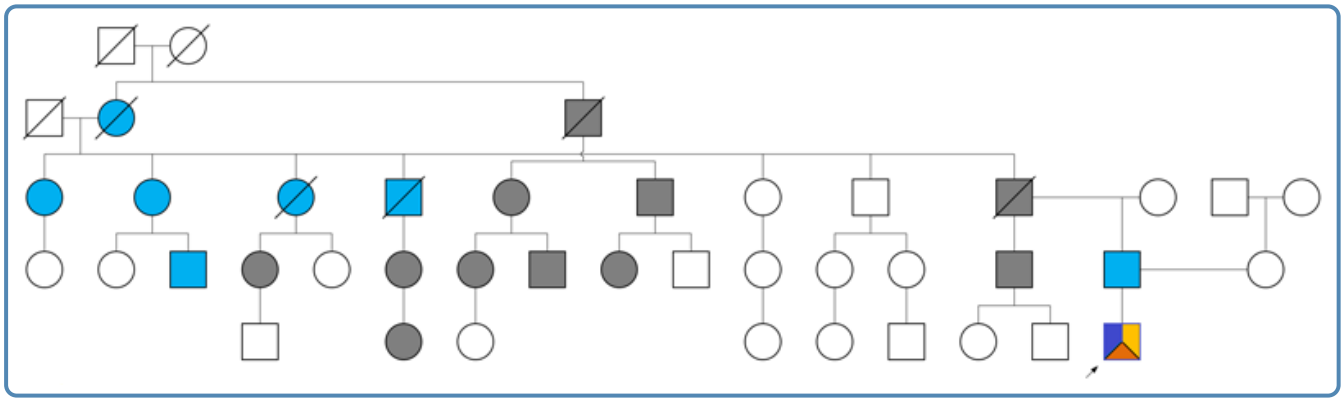


Figure 1 Five-generation pedigree of the family showing multiple affected members of the family with variable expressivity of the disorder.
Light blue shading represents affected members with blue irises and premature greying.
Grey shading represents affected members with only premature greying of hair.
Dark blue shading represents blue irises.
Yellow shading represents white forelock of hair.
Orange shading represents sensorineural hearing loss.

generations of the family had individuals with blue irises with or without other classical features of Waardenburg Syndrome. However, until the proband was born, there was no member of the family with a white forelock or hearing loss and hence, the members of the family were never seen from a genetic angle for many generations. This case illustrates the variable expressivity of a novel mutation in Waardenburg Syndrome type 1 and its implications in a family over multiple generations.

Waardenburg syndrome is notorious for variable expression and hence, can go undetected in multiple generations of a family if hearing loss or significant hypopigmentation is not present. Therefore, even if a person with blue eyes/ heterochromia irises comes to a clinic for an unrelated issue, it would be beneficial to ask a family history of premature greying, hearing loss, and/or hypopigmentation. It can be useful in picking up a hidden syndrome with more ominous symptoms.

Obituary

In memory of

Professor Ishwar Chander Verma,

FRCP (London), FAMS (India), FAAP (USA)
25th December 1936 to 8th February 2024

Professor I C Verma is hailed as the "Father of Genetics" in India and received the Padma Shri award in 2023 amongst multiple other awards through his career. Many of us have had the privilege to be associated with Dr I C Verma whilst many others have indirectly benefitted from his groundbreaking work in genetics in India. He has contributed extensively towards the development of genetics in India including genetics research at a time when little was known about the subject and its implications in clinical care. He worked to develop affordable tests, as well as education and training of scientists and doctors, and thereby the above bestowed title is most befitting.

Professor Verma had his schooling in East Africa, came to Mumbai for premedical training followed by MBBS at Amritsar Medical College. He received the PN Chuttani Gold Medal for standing first in Clinical Medicine. His residency training was at Dar es Salaam, Tanzania. He obtained MRCP London in 1966, as well as DCH at Glasgow University. He is the first student to be bestowed MNAMS from the National Academy of Medical Sciences (NAMS) by examination. He received genetics training in Zurich, London, Edinburgh, Manchester, Boston and NIH, USA. Dr Verma is credited with setting up two state-of-the-art genetic centres in India – the first at the All India Institute of Medical Sciences (AIIMS), Delhi where he served for 30 years. He then established the genetic centre at Sir Ganga Ram Hospital (SGRH) in 1997 and was associated with it till the very end.

Dr Verma had multiple sterling qualities which are worth noting for those who did not work under his tutelage, especially his ability to delve for new opportunities for diagnosis and treatment that could serve patients with genetic disorders in India. He was always impatient to implement the latest in technology, believing that diagnostic testing must be available in India for our patients. It was under his chairmanship that the first ever compassionate access program for patients with lysosomal storage disorders was initiated. More than 300 patients have received treatment under this and additionally many genetic specialists enhanced their knowledge and skills for treatment of patients with genetic disorders. His extensive experience made him the most important resource person for the formation and implementation of the National Policy for Rare Diseases (NPRD) in India. His humility made it easy for anyone to access him and seek his opinion, guidance, and blessings – patients, young doctors, scientists, entrepreneurs, and students. He would always provide advice that the person kept close to his/ her heart and followed towards success and achievement. He was instrumental in the initiation of the initial parent groups – the Down syndrome society, Fragile X society, Lysosomal Storage Disorders Support Society (LSDSS), and many more.

Dr Verma believed in being updated and read extensively. He was buying till now the newest editions of genetic books and would always read his most favourite journals - the New England Journal of Medicine, The Lancet, and the British Medical Journal (BMJ). He enjoyed sharing the latest information and knowledge with his colleagues.

Despite his busy schedule, he never forgot the exceptional non-genetic talents and abilities of his colleagues and students and made it a point to appreciate and encourage them with his gentle, charming smile. Dr Verma, our 'Sir' always, has left a void in the institution of genetics in India. We are indebted to him to carry forward his legacy of passion for the subject, a vision beyond the ordinary and a quest to keep the light of genetics burning bright always.

We will miss him immensely. We pray for him for everlasting peace. Om Shanti!

Ratna Dua Puri

Dr Ratna Dua Puri is Chairperson and Senior Consultant at the Institute of Medical Genetics & Genomics, Sir Ganga Ram Hospital, New Delhi.

Rare Disease Day Related Events at Different Centres Across India

1st February 2024: The All India Institute of Medical Sciences (AIIMS), New Delhi conducted a CME titled 'Empowering Healthcare Professionals with Essential Insights for Diagnosing and Care of Rare Genetic Disorders' under the aegis of the Centre of Excellence (CoE) for Rare Diseases of the Ministry of Health and Family Welfare, Government of India, in association with the Society for Indian Academy of Medical Genetics (SIAMG) and the DBT Mission Program on Pediatric Rare Genetic Disorders.

3rd February 2024: The Department of Medical Genetics, Nizam's Institute of Medical Sciences (NIMS), Hyderabad in association with the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad organized an awareness program and CME titled 'Management of Genetic Disorders: A Primer for Health Care Professionals' under the aegis of SIAMG and the DBT Mission Program on Pediatric Rare Genetic Disorders (PRaGeD)

22nd February 2024: The Institute of Medical Genetics and Genomics at Sir Ganga Ram Hospital, New Delhi commemorated Rare Disease Day with a symbolic walk through the hospital, with doctors, patients, and their families joining hands in solidarity, carrying banners that read "I care for rare". The event culminated with the release of balloons as a beacon of hope and a reminder that even in the face of challenges, there is a community ready to support and care!

GeneEvent


24th February 2024: The Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow in collaboration with the Academy of Pediatrics, Uttar Pradesh and Lucknow Academy of Pediatrics organized a Rare Disease Day event. The morning half was devoted to patients of spinal muscular atrophy, osteogenesis imperfecta, achondroplasia, and lysosomal storage disorders (LSDs) and their families. Adult patients with LSDs on enzyme replacement therapy shared their stories of triumph and struggle. The afternoon session was a CME on 'Inborn Errors of Metabolism' and was dedicated to Late Dr I C Verma.


24th February 2024: The Nodal Centre for Rare Diseases, JK Lone Hospital, Jaipur in collaboration with Rare Disease India Foundation (RDIF) celebrated Rare Disease Day at the Rajasthan International Centre, Jaipur. The event was an awareness program with poster release on the theme of 'Aware to Rare' and was well attended by around 120 doctors and healthcare personnel.

25th February 2024: Awareness Marathon on the theme 'One Nation, One Day Together for Rare' was organized by the Department of Medical Genetics, JSS Medical College, Mysuru in association with the Organization for Rare Diseases India (ORDI).

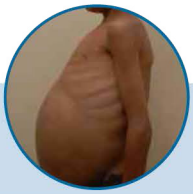
National Symposium on Rare Diseases

On the occasion of Rare Disease Day, the Ministry of Health & Family Welfare (MoHFW), Government of India, in collaboration with the World Health Organization (WHO), India organized a two-day multi-stakeholder consultation titled **'National Symposium on Rare Diseases: Challenges, Opportunities and Way Forward'** on 29th February-1st March 2024 at Radisson Blu Palace, Udaipur. The two-day event captured the progress made under the purview of the National Policy for Rare Diseases (NPRD). Participation of Ms. L. S Changsan (Additional Secretary, Department of Health and Family Welfare, MoHFW), Mr Rajiv Manjhi (Joint Secretary, MoHFW) and Dr L. Swasticharan (Additional Deputy Director General of Health Services, MoHFW), reiterated the huge will of the government to take forward the cause of rare diseases.

Down Syndrome Day Celebrations

The Genetic Clinic, Dwarka, New Delhi, in association with the Society for Indian Academy of Medical Genetics (SIAMG) celebrated Down syndrome day on 20th March 2024. The program was attended by children with Down syndrome and their parents. A Pediatric Cardiologist, who is the mother of a boy with Down syndrome, gave a talk on parenting tips for children with Down syndrome. Parents discussed their journey and the challenges faced by them and the means to overcome them. The need for creating awareness in society for supporting individuals with Down syndrome to help them lead a dignified and respectful life and to provide job opportunities for them, was also discussed.

Are you suspecting a **Lysosomal Storage Disorder (LSD)** in your patient?



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