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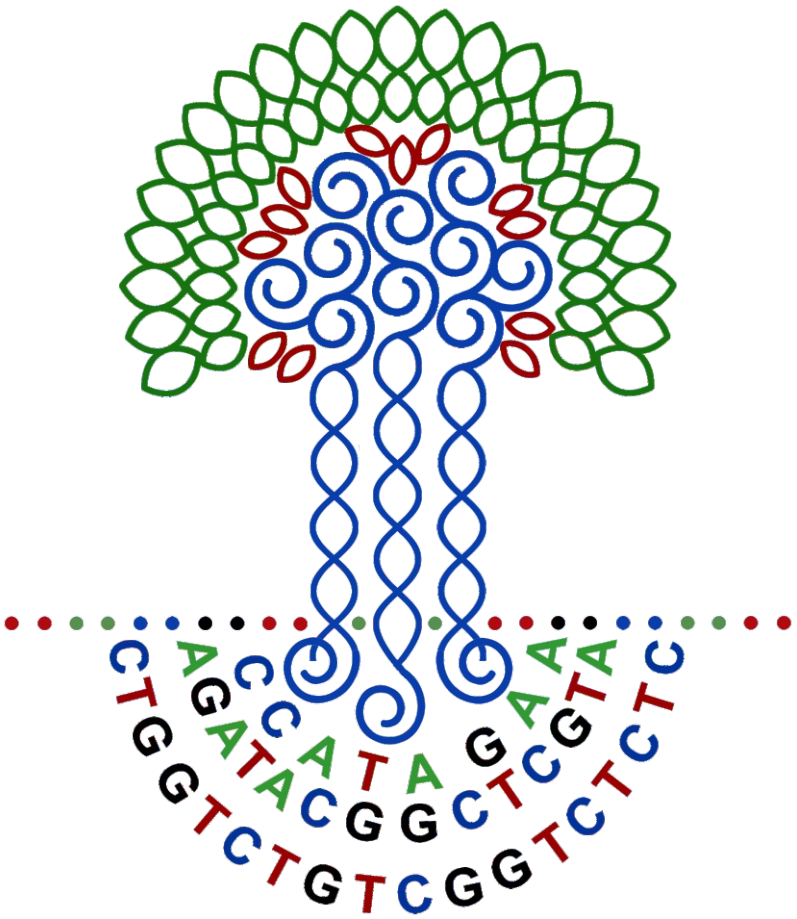

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PhotoQuiz - 67

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A four-year-old female child, born to third-degree consanguineous parents, presented with global developmental delay, nystagmus, truncal ataxia, and oculomotor apraxia. Magnetic resonance imaging (MRI) of the brain (images of T2 axial sections at the level of pons and midbrain provided) showed a pathognomonic finding. Identify the condition

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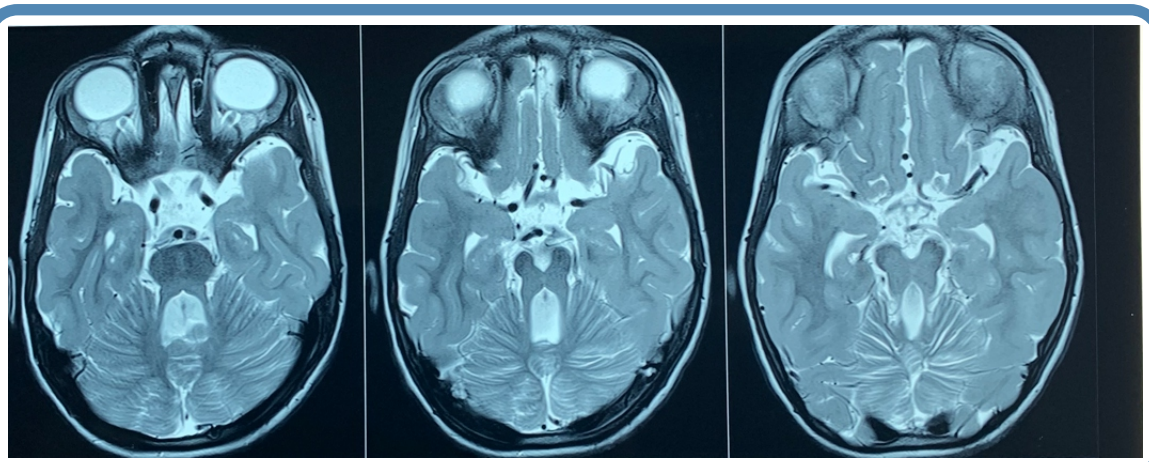
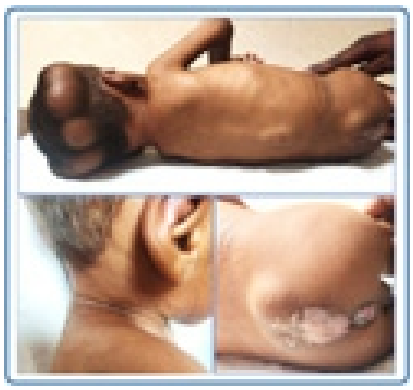
Or go to http://iamg.in/genetic_clinics/photoquiz_answers.php
to submit your answer.



Answer to PhotoQuiz 66

Hyaline Fibromatosis Syndrome (OMIM # 228600)



Hyaline fibromatosis syndrome (HFS) is characterized by hyaline deposits in the papillary dermis and other tissues. It can present at birth or in infancy with severe pain with movement, progressive joint contractures, and often with severe motor disability, thickened skin, and hyperpigmented macules/patches over bony prominences of the joints. Gingival hypertrophy, skin nodules, pearly papules of the face and neck, and perianal masses are common. Complications of protein-losing enteropathy and failure to thrive can be life threatening. Cognitive development is normal. Many children with the severe form (previously called infantile systemic hyalinosis) have a significant risk of morbidity or mortality in early childhood; some with a milder phenotype (previously called juvenile hyaline fibromatosis) survive into adulthood. The disorder is caused by biallelic variants in the ANTXR2 gene (OMIM * 608041) and is inherited in an autosomal recessive manner.

Correct responses to PhotoQuiz 66 are given on Page 6.

Novel Phenotypes, New Genes: Beyond Next Generation Sequencing

Editorial

I am feeling happy to present the first issue of 2025. An important reason is obvious in the articles in the GenExpress of this issue. The articles are from clinical scientists from India and reflect appropriate use of the vast clinical material available in India. India is a goldmine of rare phenotypes, and many novel phenotypes have been reported from India over the past few decades. Many are very rare disorders usually reported in single nuclear families, thus making identification of the causative gene very difficult in the previous era with the tedious technique of gene mapping using genome wide markers. With next generation sequencing many causative genes of novel phenotypes are being reported from India at an astonishing speed. Identification of lethal phenotypes has many additional challenges. Aggarwal et al. identified *SERPINA11* gene as a cause of a perinatally lethal disorder. Extensive phenotyping including histology and immunohistology, along with documentation of functional consequences of disrupting the gene have led to the identification and delineation of the fetal phenotype of a novel serpinopathy. This is the result not only of hard work but the joint collaborative work of medical geneticists with excellent clinical skills and basic scientists armed with techniques for functional evaluation. Other publications covered in the GenExpress have reported novel genes and novel variants supported by evidence of zebrafish model and computational studies respectively. This reflects that the medical geneticists in India are doing much more than next generation sequencing. The last article in the GenExpress by Srivastava et al. is the cherry on the cake. Long awaited gene therapies have come to clinics from research laboratories and from animal models to humans, but we were waiting for them to come to India. The success story of gene therapy in hemophilia A by a centre in India, will soon get replicated for other genetic diseases. This creates a lot of hope in the large population of rare genetic diseases in India. We, the clinicians who were waiting for more than three decades for all these, are excited.

The 9th Annual Conference of the Society for Indian Academy of Medical Genetics – IAMG-2024 held recently at Ahmedabad was an impressive exhibition of work done by bright young medical geneticists. The work presented in poster and oral presentations were in various advancing areas in medical geneticists. Awards and oration in the names of our teachers, namely Late Dr SS Agarwal and Late Dr IC Verma reminded us of their guidance and messages. They always stressed the need for collaboration between clinicians and basic scientists and this, over the decades, has trained clinicians competent in molecular genetics and basic sciences. The fruits are visible now. When the world is thinking of gene therapy as a practical therapy, India appears to be getting ready for the 'Make in India' gene therapy.

Happy new year!

Dr. Shubha Phadke
1st January, 2025

Clinical Vignette

Leber Congenital Amaurosis: Need for an Eye for Detail Beyond the Eye

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Abstract

A 10-year-old female child presented to our outpatient department with complaints of diminution of vision in both eyes since birth. On evaluation, she had reduced distant visual acuity in both eyes with no refractive correction and absent pupillary responses. Pendular nystagmus was present, and fundus examination revealed pigmentary nummular lesions at the level of retinal pigment epithelium (RPE) with sub-retinal flecks in the peripheral fundus. Electroretinogram revealed extinguished response and a clinical diagnosis of Leber congenital amaurosis (LCA) was made. On further evaluation she was found to have pallor with failure to thrive and short stature and investigations revealed anemia with deranged renal functions. Possibility of a ciliopathy was thought of due to eye and renal involvement. This was confirmed by whole exome sequencing performed after due counseling of the parents which revealed a pathogenic homozygous nonsense variant in the *IQCB1* gene (NM_001023570; c.1504C>T). Pathogenic variants in *IQCB1* are associated with Senior-Loken syndrome 5 and Leber congenital amaurosis with or without renal disease. Leber congenital amaurosis is a genetically heterogeneous condition. Pathogenic variants in some genes like *IQCB1* are associated with renal disease besides ocular involvement. Comprehensive evaluation by an ophthalmologist along with a clinical geneticist and nephrologist are essential in LCA patients as early intervention improves prognosis and clinical outcome.

Keywords: Leber congenital amaurosis, Senior-Loken syndrome 5, *IQCB1* mutation, chronic kidney disease.

Introduction

Leber congenital amaurosis encompasses a spectrum of inherited conditions that cause subnormal vision. The chief manifestation is bilateral congenital blindness, with diminished or absent electroretinogram (ERG) before the age of six months. Wide clinical and genetic heterogeneity is known with more than 20 genes of LCA reported to date. Many children have systemic manifestations apart from blindness which may be subtle initially but are gradually progressive including nephronophthisis and chronic kidney disease (CKD). Renal involvement is reported with certain genetic subtypes of LCA (e.g., *IQCB1-*, *IFT140-*, and *CEP290*-associated LCA) as part of syndromes including Senior-Loken syndrome and Joubert syndrome (Braun et al., 2016; König et al., 2017). Thorough history taking and physical examination along with focussed biochemical and radiological investigations can help in timely identification, confirmation of molecular diagnosis and institution of supportive management in patients with this rare genetic disorder. We report a case of ten-year-old girl who presented with diminution of vision who was suspected to have LCA with juvenile nephronophthisis based on history and clinical examination. Whole exome sequencing (WES) showed a pathogenic variant in the *IQCB1 gene* which helped us clinch the diagnosis.

Figure 1 1a: Fundus photographs of both the eyes showing pigmented nummular lesions at the level of the retinal pigment epithelium (RPE)

Patient details:

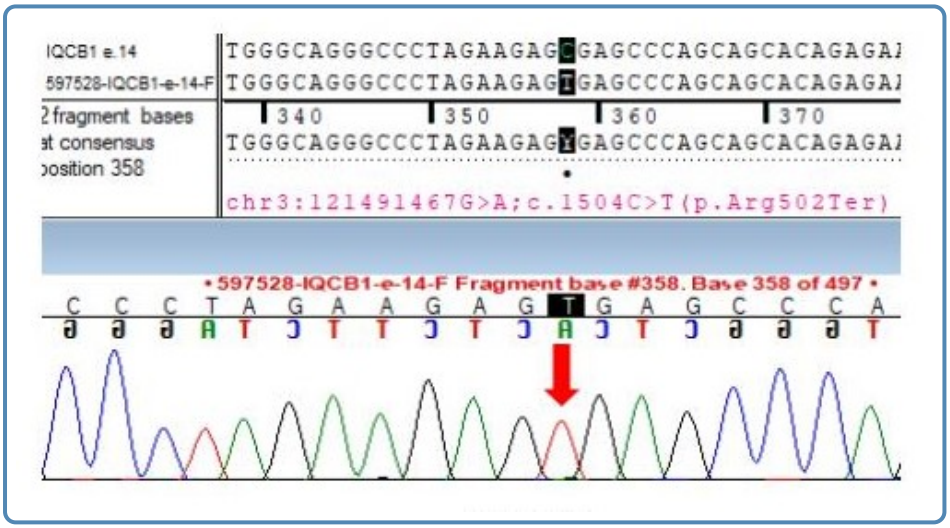
A ten-year-old female child, first born out of non-consanguineous marriage, was brought to the ophthalmology outpatient department of our institute with complaints of diminution of vision in both eyes since birth. The antenatal and birth history was unremarkable. She achieved developmental milestones as per age. On direct questioning parents confirmed that they had noticed polyuria and polydipsia since last 4-5 years along with poor weight and height gain as compared to her peers. There was no history suggestive of any other systemic involvement. The parents also reported similar eye complaints in their three-year-old daughter; the second daughter was unaffected as per the given history.

The ophthalmological findings of the proband at initial presentation are mentioned in **Table 1. Figure 1a** shows the significant eye findings seen in the proband.

Based on the above, the child was diagnosed to have Leber congenital amaurosis (LCA) and referred to the pediatric nephrology OPD for further evaluation. On examination there, the child was found to have weight of 22 kg and height of 118 cm (both <3 SD for age) and was normotensive for her age. Head to toe examination revealed eye signs as elucidated above along with pallor. The systemic examination was essentially normal. She had no focal neurological deficits, and her hearing examination was also normal. Investigations confirmed anemia (normocytic normochromic) and hypocalcemia along with deranged renal function with estimated glomerular filtration rate (eGFR) commensurate with chronic kidney disease (CKD) stage 3b. The investigation results are summarized in **Table 2**. The child was put on medical management for the same. Based on history, clinical examination findings and initial investigations, a diagnosis of a ciliopathy like Senior-Loken syndrome was suspected and opinion of the clinical geneticist was sought.

Parents were counseled and to confirm the diagnosis, exome sequencing was performed on genomic DNA with analysis of medically relevant genes listed in OMIM (https://www.omim.org/). A homozygous nonsense variation in exon 14 of the *IQCB1* gene (NM_001023570; chr3:g.121491467G>A; c.1504C>T) that results in a stop codon and premature truncation of the protein at codon 502 (p.Arg502Ter) was detected. This variant is classified as pathogenic as per the guidelines of the American College of Medical Genetics and Genomics (ACMG). The variant was further validated by Sanger sequencing (**Figure 1b**). Careful clinical association was done, and she was diagnosed as a case of Senior-Loken syndrome-5. This is a disorder characterized by nephronophthisis and Leber congenital amaurosis.

The elder of her other two siblings, a five-year-old girl, had no features of nephronophthisis or Leber congenital amaurosis. The younger one, a three-year-old girl, had features of amaurosis with depigmented fundus in both eyes. However, she had no features suggestive of renal involvement. Sanger



1b: Sanger sequence chromatogram showing the c.1504C>T variant in the *IQCB1* gene in the proband

sequencing confirmed the presence of the same variant in homozygous form in the youngest sibling, while the unaffected sibling and parents were confirmed to be asymptomatic heterozygotes. Parents were counseled about the autosomal recessive pattern of inheritance and the 25% risk of recurrence in each offspring. The importance of prenatal diagnosis in subsequent pregnancies was emphasized.

Discussion

We report a 10-year-old girl who presented with diminution of vision, whose ophthalmological evaluation showed all features of LCA including poor fixation at birth or within 6 months of age, nystagmus, amaurotic pupils, and extinguished ERG response before the age of 1 year. A thorough history however pointed towards other system involvement prompting a detailed multispecialty evaluation by a clinical geneticist and pediatric nephrologist which revealed features of nephronophthisis with CKD stage 3. Molecular confirmation of the clinical diagnosis was done by exome sequencing which demonstrated a homozygous nonsense variant in the *IQCB1* gene (NM_001023570; c.1504C>T) associated with Senior-Loken syndrome (SLNS). Leber congenital amaurosis is a clinically and genetically heterogenous condition. More than 20 genes are associated with this condition. LCA may be part of a 'ciliopathy syndrome' also known as NPHP-related ciliopathies (NPHP-RC) with associated multisystem involvement primarily affecting the nervous system, eye, renal and skeletal system like Joubert syndrome; renal system involvement like Senior-Loken syndrome; cerebellar vermis hypoplasia, oligophrenia, ataxia, coloboma, and hepatic fibrosis i.e., COACH syndrome; or be present as an isolated entity. Exact genotype-phenotype correlation has not been elucidated and with increasing confirmation of molecular diagnosis by next generation sequencing (NGS), varied phenotypes of described mutations continue to emerge (den Hollander et al., 2008). Mutations in the *NPHP1* gene have been reported to have predominantly renal manifestations with progression to CKD stage V by early adolescence though some patients may also have neurological and eye signs. In contrast, mutations in the *IQCB1* gene, which is also involved in ciliogenesis, manifest with LCA in early childhood. Renal symptoms are seen only in about half of the affected patients with relatively late progression to CKD stage V and few may also have neurological involvement (König et al., 2017; Kang et al., 2016; Wolf et al., 2024). In our patient, the eye signs appeared in early infancy and renal involvement was picked up only at 10 years of age by a focussed evaluation before the parents had noticed the same which has been reported earlier in patients with underlying mutations in the *IQCBI* gene (König et al., 2017). Diagnosis of nephronophthisis may be delayed as in early stages the patients have only mild symptoms which may go unnoticed and hence it is imperative that all LCA patients should be evaluated thoroughly for possible renal and other

Table 1 Ophthalmological examination of proband at initial presentation

	Right eye	Left eye
1. a. Visual Acuity b. Color vision c. Intraocular pressure	1/60 Unable to read any slides 16 mm of Hg	1/60 Unable to read any slides 18 mm of Hg
2. Anterior and posterior segment examination	a. Nystagmus + b. Absent pupillary response c. Pigmentary nummular lesions at the level of RPE with sub-retinal flecks in the peripheral fundus.	a. Nystagmus + b. Absent pupillary response c. Pigmentary nummular lesions at the level of RPE with sub-retinal flecks in the peripheral fundus.
3. Ophthalmological investigations a. Electroretinogram b. Fundus autofluorescence c. Optical Coherence Tomography (OCT) Macula	 Extinguished response Hypo-autofluorescence in the macular region Central macular thickness (CMT) 249 microns	 Extinguished response Hypo-autofluorescence in the macular region Central macular thickness (CMT) 266 microns

Table 2 Summary of Investigation Results of the Proband

Parameters	Value in Our Patient	Normal Range
Hemoglobin - 9.4 gm% Peripheral blood smear	9.4 gm% Normocytic normochromic anemia	12-13 mg/dL
Serum urea Serum creatinine	52.9 mg/dL 1.32 mg/dL	20-40 mg/dL 0.4-0.9 mg/dL
Calcium Organic phosphorus Alkaline phosphatase	5.8 mg/dL 5 mg/dL 655 IU/L	8.5-9.5 mg/dL 3-4.5 mg/dL 40-140 IU/L
Serum iron Total iron binding capacity (TIBC)	114 mcg/dL 390 mcg/dL	60-170 mcg/dL 240-450 mcg/dL
iParathormone 25-OH Vitamin D3	301 pg/mL 15 ng/mL	14-65 pg/mL 15-45 ng/mL
Ultrasound of kidneys, ureter and bladder (USG KUB)	Raised echogenicity of both kidneys; cortico-medullary differentiation normal. Kidney sizes: right kidney 7.3 cm, left kidney 7 cm	Normal kidney size for this age: 9.5-10 cm

system involvement.

Conclusion

Patients with LCA may have subtle extra-ocular manifestations. Thorough evaluation is essential in all patients as early intervention improves prognosis and clinical outcomes. Molecular confirmation of underlying genetic diagnosis helps in appropriate genetic counselling including prognostication and timely institution of supportive therapy of systemic involvement including CKD. It also aids in offering appropriate prenatal diagnosis to parents if planning further pregnancy which, is an important cog in the wheel of patient management in such cases.

Declaration of patient consent: The authors

certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/ have given his/ her/ their consent for his/ her/ their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

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Conflicts of interests: There are no conflicts of interests.

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Glass Syndrome Unveiled: A Unique Journey through Assisted Reproduction

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Abstract

Glass syndrome (OMIM #612313) is a rare disorder characterized by intellectual disability and distinct facial features, including down-slanting palpebral fissures, crowded teeth, cleft palate, and micrognathia. It was first described by Glass et al. in 1989 in a 16-year-old boy with an abnormal karyotype 46,XY,del(2)(q32.2q33.1) and thereafter found to be caused by disruption of the *SATB2* gene which is encompassed within this cytoregion. We report a 7-year-old Indian girl with Glass syndrome. The patient presented with unclear speech, intellectual disability, restlessness, and stubbornness. Unlike previous cases, she did not exhibit short stature or microcephaly. Her facial dysmorphic features were similar to those in previously reported cases, but she additionally had telecanthus and up-slanting rather than down-slanting palpebral fissures. Notably, this child was conceived through intrauterine insemination (IUI) with donor sperm. Multiplex ligation-dependent probe amplification (MLPA) identified a 2q33.1 deletion, confirmed by real-time polymerase chain reaction (PCR).

Keywords: Glass syndrome; Chromosome 2q32q33 deletion syndrome; *SATB2*-associated syndrome

Introduction

Glass syndrome (OMIM #612313) is marked by intellectual disability and distinct facial features such as down-slanting palpebral fissures, crowded teeth, cleft palate, and micrognathia. The condition arises from a heterozygous deletion in chromosome 2q33.1 or a chromosomal translocation involving 2q33.1 which disrupts the *SATB2* gene, or a heterozygous pathogenic variant in the *SATB2* gene (Zarate et al., 2022). The condition was first described in 1989 in a 16-year-old boy with an abnormal karyotype 46, XY, del (2) (q32.2q33.1) (Glass et al., 1989). We now present a case of Glass syndrome in an Asian Indian girl; this is the first case report of Glass syndrome from India to the best of our knowledge.

Case Presentation

A seven-year-old girl, born after intrauterine insemination (IUI) using donor sperm, presented with unclear speech, intellectual disability, stubbornness, and restlessness. She is in kindergarten, can write the English alphabet and numbers 1-20, but cannot verbalize them. The mother reported motor delay, walking unsupported at 2 years, and bi-syllable words at 9 months, with no further vocabulary development. Primary dentition appeared at 1 year and 3 months. The child had a surgically corrected cleft palate at 1 year and 7 months. She had pneumonia at 3 years, requiring 4 days of hospitalization. She indicated toilet needs by gesturing. The mother's antenatal history was uneventful, and antenatal ultrasounds showed no abnormalities. The girl was born at term via lower segment cesarean section (done in view of a nuchal cord), with a birth weight of 2.3 kg. She was admitted to the neonatal intensive care unit (NICU) for two days due to breathing difficulties and

readmitted at 6 days of life for jaundice and fever and was treated with phototherapy. There is no family history of a similar illness, but the mother was diagnosed to have lung carcinoma.

Figure 1 Images of the patient show a long, triangular face, a prominent beaked nose, up-slanting palpebral fissures, a small mouth, and a pointed chin.

On examination, her height (130 cm, z-score 0.94), weight (20 kg, z-score -1.34), and occipitofrontal circumference (51 cm) were age-appropriate. She exhibited a triangular, long, hypotonic face, telecanthus, up-slanting palpebral fissures, a prominent beaked nose with a wide nasal bridge, a broad nasal tip, a hanging columella, midface hypoplasia, smooth philtrum, repaired cleft palate, dental caries, small uvula, small mouth, hypoplastic mandible, and pointed chin. Arachnodactyly was also noted. Her facial features are shown in **Figure 1**. The rest of the systemic examination was normal.

Blood investigations, including hemoglobin (12.6 g/dL), thyroid-stimulating hormone (1.358 µIU/ml), aspartate transaminase (24 U/L), alkaline phosphatase (301 U/L), calcium (10.01 mg/dL), phosphorus (5.3 mg/dL), and creatinine (0.33 mg/dL), were within normal limits. The electroencephalogram was normal. Ultrasonography of the abdomen and pelvis showed no significant abnormalities.

Informed consent was obtained for genetic testing. Multiplex ligation-dependent probe amplification (MLPA) using SALSA MLPA P245-B1 Microdeletion Syndromes-1A probe mix (MRC-Holland, Amsterdam, Netherlands) revealed a heterozygous deletion of exon 3 of *SATB2* at chromosomal position 2q33.1 (**Figure 2a**). This deletion was validated by real-time polymerase chain reaction (PCR), which showed a relative gene expression reduction (0.6) in the patient compared to the mother (1.2) (**Figure 3**). *ACTB* (beta-actin) served as the housekeeping gene, and reactions were duplicated for real-time PCR. The mother's MLPA was normal (**Figure 2b**). Chromosomal microarray analysis (CMA) (315K and 750K array) did not show any abnormalities. Exon array could not be performed due to financial constraints, and the biological father could not be tested due to confidential paternity.

Discussion

Glass syndrome, also known as chromosome 2q32q33 deletion syndrome or *SATB2*-associated syndrome (SAS) (Zarate et al., 2022), involves a protein that can activate or repress gene expression (Döcker et al., 2014). A gene associated with significantly decreased expression in mutant *SATB2* cases is *UPF3B* (Leoyklang et al., 2013), leading to overlapping clinical features with mental retardation X-linked syndromic 14 (MRXS14) (OMIM #300676) caused by hemizygous variants in *UPF3B*. Common features include a long thin face and long fingers and feet, as observed in our patient. However, speech abnormalities, cleft palate, and behavioral issues pointed more toward Glass syndrome. Unlike previous reports (Glass et al., 1989), our patient did not have short stature or microcephaly. She had similar dysmorphic features except for telecanthus and up-slanting palpebral fissures (Döcker et al., 2014).

The microdeletion detected at chromosome 2q33.1 by the SALSA MLPA probe mix 245-B1 corresponds to exon 3 in the *SATB2* gene [transcript ENST00000417098 (GRCh37)]. This is an intragenic deletion. There is another report of a child conceived by intra-cytoplasmatic sperm injection, diagnosed with SAS due to duplication of the region (Kaiser et al., 2015).

The current management plan includes speech and behavioral therapy, annual ophthalmological examination, and evaluation of nutritional status, growth, and developmental progress at each visit.

This child, conceived via IUI using donor sperm, raises the possibility of ART leading to *SATB2*-related disorders, warranting further research. CMA could not locate the chromosomal coordinates due to low single nucleotide polymorphism (SNP) and copy number variation (CNV) probe density in the deletion region. Karyotyping usually misses microdeletions less

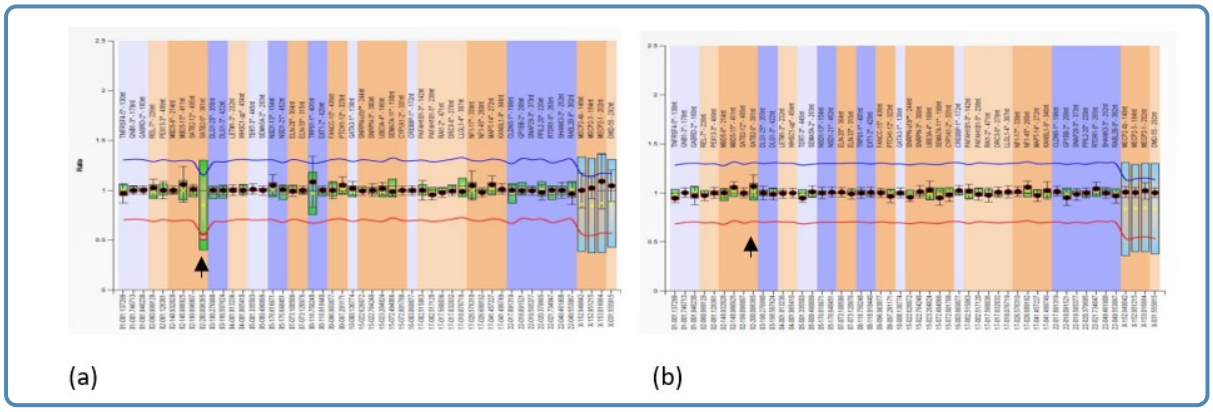

Figure 2 **a**. MLPA ratio chart showing heterozygous 2q33.1 deletion involving exon 3 of the *SATB2* gene in the proband, marked with a black arrow. **b.** Mother's MLPA chart shows normal results.

than 5Mb, making MLPA more useful in such cases. RNA expression studies would help understand the functionality of *SATB2*-associated proteins.

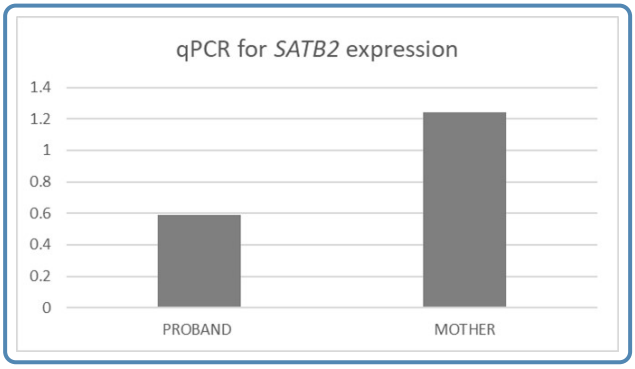

Figure 3 A bar chart representing relative quantification values obtained from real-time PCR in the proband and the mother.

Genetic counseling was challenging as the child was born through assisted reproductive technology (ART) with donor sperm. The father inquired about prenatal detection techniques. Despite improvement after a year of developmental therapy, the diagnosis was hard for the parents to accept. This case highlights the potential of preimplantation genetic testing (PGT) in detecting genetic disorders before ART, although it raises ethical dilemmas and anxiety. PGT can produce complex genetic data, complicating decision-making without a genetic disorder history. Genetic counseling should be recommended before ART, and counselors must disclose that ART outcomes are not always unaffected conceptuses. The mother requires evaluation for lung cancer and is not considering further pregnancies.

Conflict of interests: None

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GeNeViSTA

Fatty Acid Oxidation Disorders: An Update

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Abstract

Fatty acid oxidation disorders (FAODs) are inborn errors of metabolism caused by genetic variants which lead to disruption of the beta oxidation of fatty acids. The underlying pathogenetic mechanism is either a defect in an enzyme of the beta oxidation cycle or a defect in carnitine transport, which results in energy deficiency. FAODs have a wide spectrum of clinical presentations with variable age of onset of symptoms. The most common presentation is an acute Reye syndrome-like condition associated with hypoketotic hypoglycemia and liver dysfunction, which can lead to seizures, coma, and death. Newborn metabolic screening and availability of molecular genetic tests have significantly added to early and accurate diagnosis of these disorders. However, symptomatology can be non-specific and of sudden onset precipitated by intercurrent illness and may sometimes be overlooked in the absence of a high index of suspicion. Hence, they are associated with significant mortality and morbidity. Early diagnosis and management are crucial to improve the outcomes in affected patients. This paper provides an overview of the etiopathogenesis, clinical presentations, diagnosis and management of fatty acid oxidation defects, including current diagnostic modalities and recent therapeutic advances.

Keywords: Fatty acid oxidation disorders; carnitine shuttle; mitochondrial fatty acid β-oxidation; hypoketotic hypoglycemia

Introduction

Fatty acid oxidation defects (FAODs) are a group of genetic metabolic disorders which are inherited in an autosomal recessive manner. There is a disruption of the carnitine shuttle or the mitochondrial beta oxidation pathway due to the reduced or absent function of the transporter proteins or enzymes, leading to deficient energy production and accumulation of the intermediate metabolites upstream to the block or defect. The spectrum of clinical presentations ranges from recurrent acute hypoglycemic episodes with hepatic dysfunction in neonates to myopathy and cardiomyopathy in older children and adults. They are potentially fatal, and a high index of suspicion is needed to quickly identify the patients with fatty acid oxidation defects in order to manage the metabolic decompensations promptly.

Epidemiology

Numerous newborn screening (NBS) programs have been conducted amongst different populations through which the incidence and/or prevalence of all fatty acid oxidation defects have been reported. Marsden et al. observed that, in general, the combined incidence of all FAODs ranges from 0.9 to 15.2 per 100,000 (Marsden et al., 2021). The incidence of all FAODs is lower in Asian populations than in non-Asian populations, ranging from 0.9 to 4.9 per 100,000. However, as there are no population screening programs in India, the true incidence of fatty acid oxidation defects in the Indian population is not known at present.

Pathophysiology

The energy requirements of our body are met from metabolism of carbohydrates (40-60%), lipids (30-40%) and to a lesser extent from proteins (10-15%). The primary fuel for the brain is glucose which is derived from carbohydrates. Fatty acids are the primary fuel for the heart, skeletal muscle and liver (McGuinness et al., 2023). They are also the major fuel source during periods of prolonged fasting beyond 6 hours. The hepatic glycogen

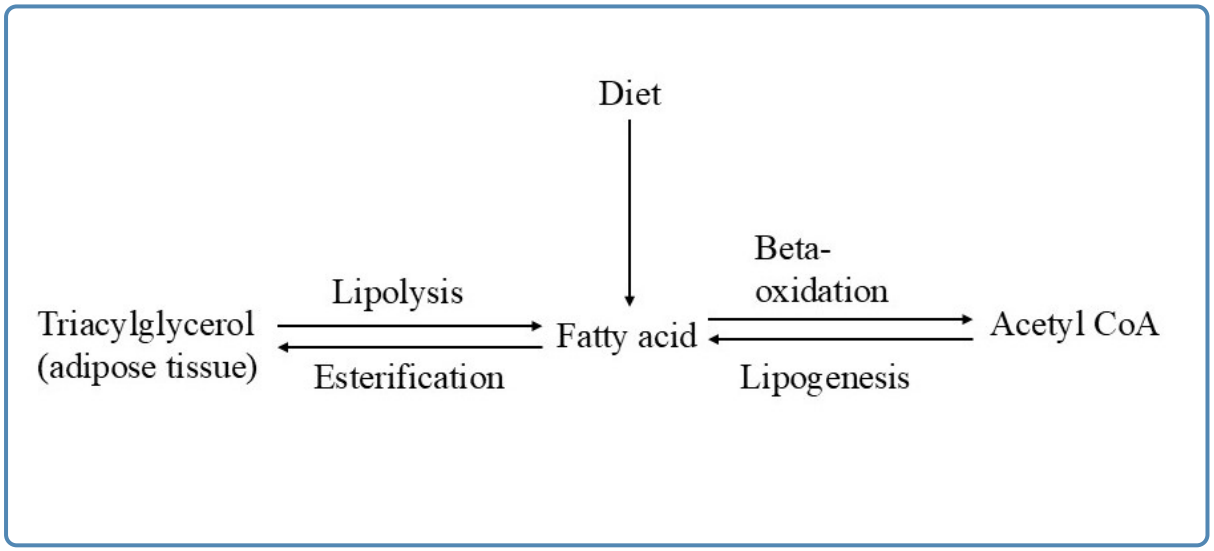

Figure 1 Overview of fatty acid metabolism

stores are completely exhausted after 12 to 18 hours of fasting (Bender & Mayes, 2018). The lipolysis of the stored triacylglycerols, the body's main fuel reserve, in the adipose tissue releases free fatty acids which are taken up by the liver and further oxidized by beta oxidation to form acetyl coenzyme A. Acetyl CoA, thus formed, may enter the citric acid cycle to be oxidized to carbon dioxide and water, may enter the cholesterol synthesis pathway or may be used to synthesize ketone bodies. Fatty acids are the main metabolic fuels during inter-prandial periods and during periods of metabolic stress like infections and exercise (Houten & Wanders, 2010).

Fatty acids in our body are derived from diet (predominantly, long chain fatty acids), from the adipose tissue (stored as triacylglycerols), and from lipogenesis (from carbohydrates and amino acids) as shown in **Figure 1**. Fatty acids can be classified into four types according to the length of their aliphatic carbon chain:

i. Short chain fatty acids (SCFA) are fatty acids with aliphatic tails of 2-6 carbon atoms.

ii. Medium chain fatty acids (MCFA) are fatty acids with aliphatic tails of 6-12 carbon atoms.

iii. Long chain fatty acids (LCFA) are fatty acids with aliphatic tails of 14-20 carbon atoms.

iv. Very long chain fatty acids (VLCFA) with aliphatic tails of 22 or more carbon atoms.

The oxidation of fatty acids is a biochemical process through which the fatty acids are broken down into smaller molecules during which energy is generated. There are different types of fatty acid oxidation occurring in different cellular organelles namely, alpha oxidation, beta oxidation and omega oxidation. Alpha oxidation helps in the degradation of phytanic acid, the by-product of green vegetables consumed in the diet. It occurs in the peroxisomes to release carbon dioxide. Beta oxidation of fatty acids occurs both in the mitochondria and the peroxisomes. Beta oxidation of short chain, medium and long chain fatty acids occurs in the mitochondria whereas the beta oxidation of very long chain fatty acids occurs in the peroxisomes of the cells. Omega oxidation is an alternative pathway to beta oxidation occurring in the endoplasmic reticulum, to degrade large fatty acid molecules which would be otherwise toxic to the body in higher concentrations. These water insoluble fatty acids are hydroxylated to water soluble dicarboxylic acids which are then excreted in the urine.

Fatty acids are transported in the bloodstream bound to albumin and lipoproteins (Kerner & Hoppel, 2000). The transport of short chain and medium chain fatty acids across the plasma membrane occurs by passive diffusion and long chain fatty acids are transported via specific fatty acid transporter proteins (Guerra et al., 2022). After entry into the cytosol, the long chain fatty acids are activated to their specific acyl

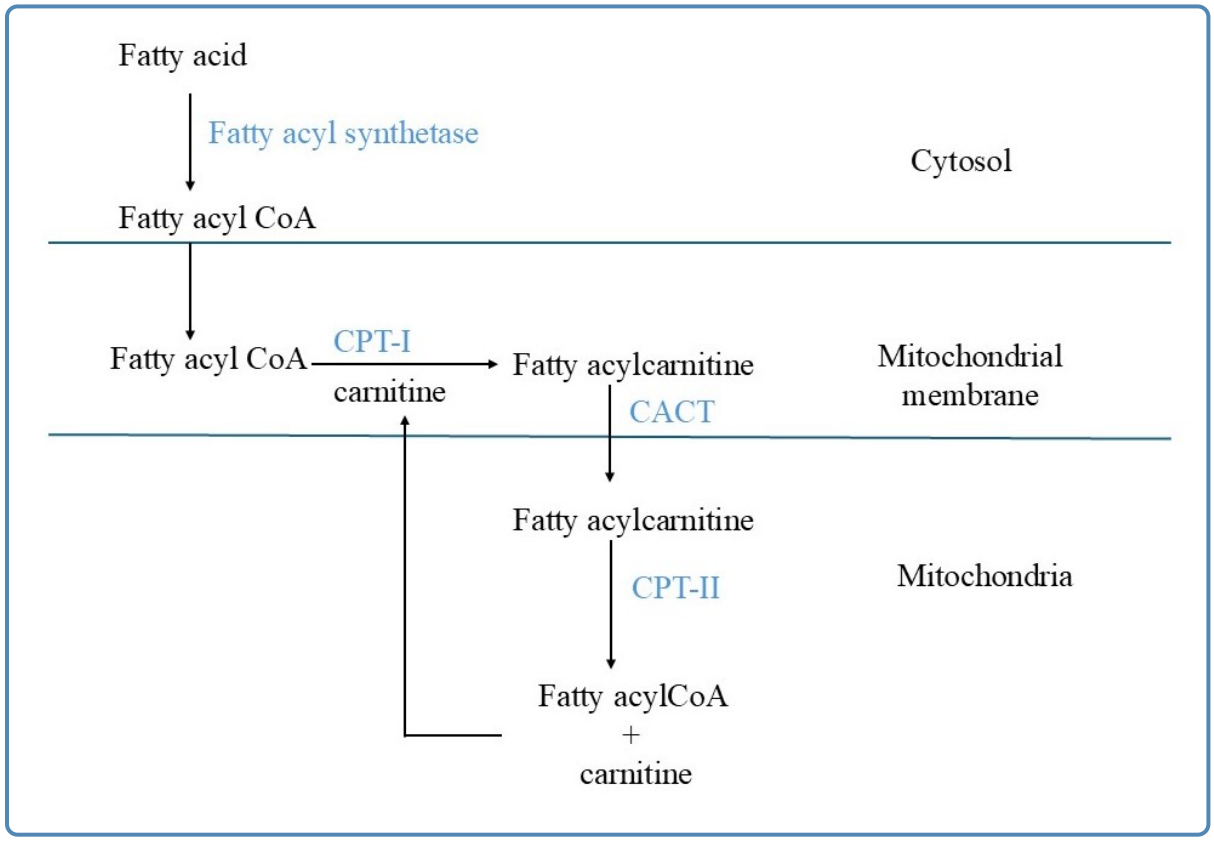

Figure 2 Schematic representation of the carnitine shuttle. CPT-I: Carnitine palmitoyl transferase-I; CPT-II: Carnitine palmitoyl transferase-II; CACT: Carnitine acylcarnitine translocase

CoA esters by fatty acyl CoA synthetase, before their uptake into the mitochondrial matrix. The short and medium chain fatty acids diffuse passively across the mitochondrial membrane and are activated inside the mitochondrial matrix. The mitochondrial membrane is not permeable to long chain fatty acids. Hence, the carnitine shuttle is an obligatory pre-requisite for their uptake into the mitochondrial matrix (Houten & Wanders, 2010; Kerner & Hoppel, 2000). The carnitine shuttle needs proper functioning of three distinct membrane-bound proteins namely carnitine palmitoyl transferase-I (CPT-I), carnitine acylcarnitine translocase (CACT), and carnitine palmitoyl transferase- II (CPT-II). Carnitine palmitoyl transferase I (CPT-I) is located at the outer mitochondrial membrane and catalyzes the formation of acylcarnitine by addition of L-carnitine to the fatty acyl CoA esters. This is the rate-limiting step of beta oxidation and is regulated by the energy status of the cell. Malonyl CoA, formed during lipogenesis, is the inhibitor of this enzyme. The acylcarnitine, thus formed, is transported across the inner mitochondrial membrane in exchange for carnitine by CACT. Inside the mitochondrial matrix, CPT-II is located bound to the inner mitochondrial membrane and converts the acylcarnitine to fatty acyl CoA ester by releasing the carnitine, as shown in **Figure 2**. This carnitine is again recycled by its addition into the carnitine pool. The transport of carnitine across the plasma membrane into the cytosol is by organic cation/carnitine transporter 2 (OCTN2) (Tamai et al., 1998).

Inside the mitochondrial matrix, the activated acyl CoA esters are degraded into acetyl CoA, a two-carbon intermediate metabolite, through a cyclic process known as beta oxidation. Each cycle of beta oxidation comprises of four sequential steps through which one unit of acetyl CoA is formed, thus shortening the length of the fatty acyl CoA chain by two carbons (Houten & Wanders, 2010). The even chain fatty acids are completely broken down to form acetyl CoA molecules. The

odd chain fatty acids are broken down to finally form propionyl CoA (three-carbon molecule) which is an intermediate product in the tricarboxylic acid cycle. The acetyl CoA formed in the skeletal muscle and the cardiac muscle enters the citric acid cycle for adenosine triphosphate (ATP) production, and that formed in the liver tissue is also supplied for the synthesis of ketone bodies.

There are four substrate-specific acyl CoA dehydrogenase enzymes specific to the chain length of fatty acyl CoA on which they act (Sim et al., 2002). However, there is some overlap between the substrates of each enzyme. The short chain acyl CoA dehydrogenase (SCAD) acts on substrates of chain length between C4 and C6, the medium chain acyl CoA dehydrogenase (MCAD) acts on substrates of chain length between C6 and C12, and the very long chain acyl CoA dehydrogenase (VLCAD) acts on substrates of chain length between C14 and C24. After the first step of dehydrogenation, hydrogenation of trans-2-enoyl CoA is carried out by the action of 2-enoyl CoA hydratase, which produces 3-hydroxyacyl CoA. 3-hydroxyacyl CoA is dehydrogenated by the action of 3-hydroxyacyl CoA dehydrogenase. The medium and short chain 3-hydroxyacyl CoA (chain length between C4 to C10) are dehydrogenated by medium/short chain 3-hydroxyacyl CoA dehydrogenase (M/SCHAD). The long chain 3-hydroxyacyl CoA dehydrogenase (LCHAD), which is present at the alpha subunit of the mitochondrial trifunctional protein (MTP) reduces the long chain hydroxy acyl CoA substrates (chain length between C12 to C16) (Guerra et al., 2022). The fourth and final step is the cleavage of 3-hydroxyacyl CoA to yield a molecule of acetyl CoA, catalyzed by 3-ketoacyl CoA thiolase. The medium chain 3-ketoacyl CoA thiolase (MCKT) acts on substrates of length between C4 and C12 whereas the long chain 3-ketoacyl CoA thiolase (LCKT) acts on long chain substrates. The long chain enoyl CoA hydratase and long chain 3-hydroxy acyl CoA dehydrogenase and the long chain 3-ketoacyl CoA thiolase are a part of the mitochondrial trifunctional protein.

Classification

Fatty acid oxidation defects can be broadly classified as carnitine shuttle defects, beta oxidation defects, and defects of electron transfer.

Carnitine shuttle defects are subclassified, based on the defect in the transporter protein involved, as:

- Carnitine transporter deficiency (CTD)/ Systemic primary carnitine deficiency
- Carnitine palmitoyl transferase 1A (CPT1A) deficiency
- Carnitine-acylcarnitine translocase (CACT) deficiency
- Carnitine palmitoyl transferase II (CPT II) deficiency

Beta-oxidation defects can be subclassified, on the basis of the defect in the oxidation of specific acyl CoA chain, as:

- Disorders of long chain fatty beta oxidation -
    1. Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency
    2. Long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency
    3. Mitochondrial trifunctional protein deficiency (MTPD1 & MTPD2)
- Disorders of medium chain fatty acid beta oxidation -
    1. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency
- Disorders of short chain fatty acid beta oxidation -
    1. Short-chain acyl-CoA dehydrogenase (SCAD) deficiency
    2. 3-hydroxyacyl-CoA dehydrogenase (HADH) deficiency

Defects of electron transfer includes:

- Multiple acyl- CoA dehydrogenase (MAD) deficiency (also known as Glutaric aciduria II)

The pathological features seen in this group may be due to the intracellular accumulation of the fatty acids and/or their metabolites upstream to the defect/ block in the cycle and also due to metabolic decompensation secondary to deficient ATP production. Lack of energy supply disrupts the pathways of gluconeogenesis, ketogenesis and urea cycle leading the manifestations of the same in the affected individual.

Table 1 Genetic Basis of Fatty Acid Oxidation Defects

Fatty Acid Oxidation defect	Gene Associated	Cytogenetic Locus
Carnitine Shuttle Defects		
Carnitine uptake defect / Systemic primary carnitine deficiency	*SLC22A5*	5q31.1
Carnitine palmitoyl transferase 1A deficiency	*CPT1A*	11q13.3
Carnitine-acylcarnitine translocase deficiency	*SLC25A20*	3p21.31
Carnitine palmitoyl transferase II deficiency	*CPT2*	1p32.3
Beta-Oxidation Defects		
Very long-chain acyl-CoA dehydrogenase deficiency	*ACADVL*	17p13.1
Medium-chain acyl-CoA dehydrogenase deficiency	*ACADM*	1p31.1
Short-chain acyl-CoA dehydrogenase deficiency	*ACADS*	12q24.31
Mitochondrial trifunctional protein deficiency 1 & Mitochondrial trifunctional protein deficiency 2	*HADHA & HADHB*	2p23.3
Long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency	*HADHA*	2p23.3
3-hydroxyacyl-CoA dehydrogenase deficiency	*HADH*	4q25
Electron Transfer Defects		
Multiple acyl-CoA dehydrogenase deficiency	*ETFA, ETFB, ETFDH*	15q24.2-24.3, 19q13.41, 4q32.1

Genetic Basis

All the defects in fatty acid oxidation were identified to be inherited in an autosomal recessive pattern and the presence of bi-allelic pathogenic variants (homozygous or compound heterozygous) in the associated gene is required for the manifestation of the disease in an individual. Heterozygous carriers are usually unaffected clinically. However, there are some exceptions to this. Heterozygous mothers carrying affected fetuses of long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD)/trifunctional protein (TFP)/CPT-1A deficiency have been observed to be at risk of developing acute fatty liver of pregnancy (AFLP) and HELLP syndrome (hemolysis, elevated liver enzymes and low platelets) (Spiekerkoetter et al., 2003; Karall et al., 2015; Innes et al., 2000). **Table 1** sums up the genetic basis of the FAODs.

Clinical Manifestations

During fetal life, the main source of energy is from the continuous supply of glucose to the growing fetus from the maternal circulation. After birth, the neonate derives energy from the beta oxidation of fatty acids. These fatty acids are obtained from breast milk which predominantly contains long chain fatty acids. Hence, individuals with long-chain fatty acid oxidation defects present in the neonatal period. After weaning, during infancy, most of the fatty acids in the diet are medium-chain fatty acids. Hence, the symptoms of medium-chain fatty acid oxidation defects develop in late infancy after dietary transition from breastfeeding. Metabolic decompensations are precipitated by prolonged fasting, exercise, intercurrent illnesses and the patient may appear relatively well in the periods between these episodes (Morris & Spiekerkoetter, 2016).

Affected individuals may have broadly three main clinical presentations – hepatic manifestations, cardiac manifestations, and myopathy. Hepatic manifestations are due to hepatic dysfunction leading to Reye-like symptoms with acute hypoglycemic episodes with dullness of activity, lethargy, vomiting, seizures, encephalopathy and coma. This hypoglycemia is not associated with ketone body production and is of hypoketotic type. During the acute

episode, the patient may have hepatomegaly with elevated transaminases and hyperammonemia. Liver biopsy, if done at this time, has been reported to show microvesicular lipid deposition, similar to Reye syndrome. In some patients, chronic liver dysfunction has also been seen.

Cardiac manifestations present with symptoms related to hypertrophic cardiomyopathy which may gradually progress to heart failure. The individual may also develop pericardial effusion, arrythmias and sudden death may occur due to the arrhythmias. Cardiac manifestations are predominantly seen with accumulation of long chain fatty acids in the cardiac muscle as they interfere with the ion channels and cause arrythmia.

Some individuals may present with muscle weakness, fatigue and muscle pain (myalgia). There may also be episodes of rhabdomyolysis with variable severity characterized by elevated serum creatine phosphokinase (CPK), myoglobinuria and acute renal failure (Morris & Spiekerkoetter, 2016).

A proportion of infant deaths certified as sudden infant death syndrome (SIDS) have been later attributed to fatty acid oxidation disorders (identified either postmortem or retrospectively after the diagnosis of an affected sibling). Majority of the cases have been linked to long chain fatty acid oxidation defects like MTP deficiency and LCHAD deficiency (Boles et al., 1998; Mathur et al., 1999).

Clinical manifestations of specific fatty acid oxidation defects

Carnitine Transporter Deficiency (CTD)

CTD, also known as 'Carnitine Uptake Defect' or 'Systemic Primary Carnitine Deficiency', occurs due to the abnormality in the transporter protein of carnitine in the cell membrane, 'organic cationic transporter 2 (OCTN2)' which is encoded by *SLC22A5* gene on 5q (Wang et al., 2001). There is increased renal loss of carnitine leading to low plasma concentrations of carnitine. The most common presentation is with cardiac failure (due to progressive cardiomyopathy) although there may be accompanying myopathy manifesting as proximal muscle weakness. (Stanley et al., 2006). The median age of onset of cardiac symptoms is three years. Routine 2D echocardiography done at the time of diagnosis may detect asymptomatic cardiac findings. Majority of individuals identified to have primary carnitine deficiency by newborn screening remain asymptomatic even without treatment (Crefcoeur et al., 2023).

Carnitine palmitoyl transferase I deficiency

Three isoforms of CPT-1 have been identified (Morris & Spiekerkoetter, 2016). CPT-1A is present in the liver and kidney. CPT-1B is present in the cardiac and skeletal muscles. CPT-1C is present in the brain. Only deficiency of CPT-1A has been described in literature, to date (Jones et al., 2020). As CPT-1A is present only in the liver and kidneys, the long-chain fatty acid uptake into the mitochondrial matrix of the hepatocytes is affected and these individuals present with hepatic manifestations of non-ketotic hypoglycemia and coma. The usual age of manifestations is between 6- 12 months of life. In rare instances, CPT- 1A deficiency has been observed to cause renal tubular acidosis along with hepatic dysfunction. There is no cardiac or skeletal muscle involvement seen in this defect.

Carnitine-acylcarnitine translocase (CACT) deficiency

This is a rare defect occurring due to bi-allelic variants in the *SLC25A20* gene. It is associated with high mortality. The age of presentation is during the neonatal period, typically in the first days of life. The newborn presents with hypoketotic hypoglycemia, hyperammonemia, encephalopathy features, hepatopathy and myopathy with poor head control. A notable feature is the presence of cardiac arrhythmias which may contribute to sudden death in these patients.

Carnitine palmitoyl transferase II (CPT-II) deficiency

There are three phenotypic forms of CPT-II deficiency depending on the level of residual enzyme activity in the tissues, i.e., severe neonatal form, intermediate form and the milder adult form (Nyhan et al., 2020). The neonatal form presents as life-threatening coma, cardiomyopathy and hypotonia. This is associated with high mortality in the newborn. It occurs due to *CPT2* variations that result in almost negligible enzyme activity. Affected individuals may have associated congenital brain and renal malformations which may have been detected in the prenatal scans. In the antenatal period, there may be oligohydramnios with polycystic kidneys in the prenatal scan. After birth, the neonate may have palpable, enlarged kidneys with associated renal failure and hyperkalemia. The brain may have dysplastic and cystic changes which may be detected in neurosonography. In some neonates, non-specific dysmorphic features

in the form of microcephaly, high sloping forehead, bulbous nose, flat occiput and low-set ears, long tapering fingers, widely spaced nipples and contractures of knees, elbows and small joints of the hands have been described. The intermediate form presents during infancy with fasting hypoketotic hypoglycemia. The adult-onset form is milder and presents in the second or third decade of life with low exercise tolerance/ rhabdomyolysis which is triggered by prolonged fasting or exercise. It presents as myopathy with proximal muscle weakness and fatigue. The adult-onset myopathic form is the most common phenotype of CPT2 deficiency (Wieser et al., 2019). The intermediate and adult-onset forms usually occur due to missense variations in both alleles of the *CPT2* gene that result in residual or partial enzyme activity.

Acyl-CoA dehydrogenase deficiency

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common type of FAOD and is also the least severe form. About 30 – 50% of affected individuals may remain asymptomatic, which has become evident after the advent of newborn screening. Early identification through newborn screening would help prevent metabolic decompensations. MCADD has an exclusively hepatic type of presentation with episodic illness with normal intervals between the episodes. Hepatomegaly may be present during the acute episode. Onset of symptoms is around one to two years of age, when the nocturnal feeds are stopped, and the hypoglycemic episodes occur in the mornings after prolonged overnight fasting. Fasting tolerance of the individual improves with increasing body mass; hence, the frequency of attacks decreases with age.

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is diagnosed in a vast majority of asymptomatic individuals through newborn screening or family members of affected probands. In previous studies, the clinical findings reported were developmental delay, hypotonia, seizures and failure to thrive (Pederson et al., 2008). However, asymptomatic patients have also been reported. The clinical features do not correlate with the SCAD enzyme activity. Hence, SCAD deficiency is now considered as a biochemical phenotype.

3-hydroxyacyl-CoA dehydrogenase deficiency

Long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency is the more common form. LCHAD enzyme is a component of a mitochondrial trifunctional protein, which has an octameric structure with 4 alpha and 4 beta subunits. The 4 alpha subunits are encoded by *HADHA* gene, and the 4 beta subunits are encoded by *HADHB* gene. Patients with LCHAD deficiency may have an isolated LCHAD deficiency or deficient activity of all three component enzymes. Isolated LCHAD deficiency is due to specific variation in *HADHA* gene. Phenotypes of affected individuals may be variable, ranging from a mild form (resembling MCAD deficiency) to severe disease (resembling VLCAD deficiency). The age of onset of symptoms is usually in infancy. Along with the usual presentations of FAODs, there may be additional manifestations in these individuals in the form of pigmentary retinopathy (Lawlor & Kalina, 1997) and peripheral neuropathy (Grünert et al., 2021). Pigmentary retinopathy may occur in about 70% of patients with LCHAD deficiency presenting with decreased color vision, nyctalopia and field defect in the center of field of view. Peripheral neuropathy is more common in deficiency of MTPD than LCHAD deficiency, with prevalence of 70% and 50% respectively (Grünert et al., 2021). It occurs later in life with onset during adolescence, manifesting as slow, progressive sensorimotor neuropathy with absent deep tendon reflexes. It may be associated with limb-girdle myopathy with recurrent episodes of myoglobinuria. Heterozygous mothers carrying a homozygous affected fetus with LCHAD deficiency have been found to have an increased risk of developing acute fatty liver of pregnancy (AFLP) and hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome. About 20% of at-risk pregnancies may have one of these complications.

Short-chain 3-hydroxyacyl CoA dehydrogenase (SCHAD) deficiency, also known as 3-hydroxyacyl-CoA dehydrogenase (HADH) deficiency, is a very rare condition. In SCHAD deficiency, unlike the other subtypes of FAODs, hypoglycemia is associated with hyperinsulinism.

Multiple Acyl–CoA Dehydrogenase (MAD) Deficiency

This is also known as Glutaric aciduria type II. It occurs due to defect in electron transport in the mitochondrial membrane from acyl CoA to ubiquinone (CoQ10). This requires the activity of electron transport flavoprotein (ETF) present in the mitochondrial matrix and the electron transport flavoprotein: ubiquinone oxidoreductase (ETF- QO)

which is present on the inner mitochondrial membrane. ETF accepts hydrogen from FAD linked dehydrogenases. This includes acyl CoA dehydrogenases of beta oxidation as well enzymes of branched chain amino acid metabolism and choline metabolism. The electron from reduced ETF is transferred to ETF-QO and further to coenzyme Q. ETF has alpha and beta subunits encoded by *ETFA* and *ETFB*, respectively. ETF-QO is encoded by *ETFDH.* Most cases are attributed to pathogenic variants in *ETFDH*.

The clinical spectrum of MAD deficiency can be broadly divided into three types. Type I / II (neonatal onset with and without congenital anomalies, respectively) which present in neonatal period with severe metabolic decompensation (hypoglycemia, metabolic acidosis and hyperammonemia) and have high mortality. Congenital anomalies if present may include facial dysmorphism (high forehead, depressed nasal bridge, low set ears), polycystic kidneys (may present with Potter sequence) and hypospadias/chordee in male individuals. Type III is the late onset form (most common presentation) and can present from infancy to adulthood. Affected individuals present with chronic musculoskeletal symptoms of muscle pain/weakness and exercise intolerance (Grünert et al., 2014). About 20% have episodic metabolic decompensation after stressors.

Investigations

The biochemical features of FAODs are due to deficient energy production (because of reduced production of acetyl CoA and ketone bodies) and changes secondary to disruption of other metabolic pathways due to lack of ATP, such as the gluconeogenesis and urea cycle pathways. There is accumulation of free fatty acids and the respective fatty acyl CoA intermediates upstream to the enzyme block. This results in the formation of dicarboxylic acid and hydroxy dicarboxylic acids from the omega oxidation pathway. There is also conversion of fatty acyl CoA esters to their corresponding acylglycines and acylcarnitines which bind to the carnitine and get excreted in the urine leading to secondary depletion of carnitine. The measurement and analysis of these metabolites in the patients thus helps in the diagnosis of fatty acid disorders.

Routine blood investigations might show hypoglycemia, hyperammonemia, elevated transaminases, elevated creatine phosphokinase with hyperuricemia, and absence of ketones in the urine. Though hypoketotic hypoglycemia is a hallmark, ketone bodies may be synthesized in FAODs, especially in medium chain fatty acid oxidation defects and also when there is presence of residual enzyme activity. But the concentration of ketone bodies is lower than expected for the degree of hypoglycemia. Studies report that up to 29% of MCAD deficiency patients have ketonuria (Ruiz-Sala et al., 2021). The presence of hypoglycemia with the inappropriately low ketone bodies should raise a strong suspicion of FAOD. There may be associated metabolic acidosis. Specific disease-related metabolites should be quantitatively assessed. Plasma carnitine levels are low in primary carnitine transporter defects and in other subtypes, the levels are secondarily reduced due to renal loss of carnitine in the urine. Analysis of acylcarnitine levels should be analyzed through tandem mass spectrometry (TMS) in plasma or whole dried blood spot on filter paper. A clue to the enzyme deficiency can be obtained depending on specific acylcarnitines which are detected to be elevated. Measurement of urinary organic acids and acylglycines through gas chromatography mass spectrometry (GCMS) is also helpful in the diagnosis. **Table 2** shows the plasma and urine metabolites that are elevated in the various FAODs.

Confirmation of the diagnosis in the presence of clinical manifestations and specific metabolite abnormalities is by the identification of homozygous or compound heterozygous variants in the specific gene through molecular testing. Next-generation sequencing (NGS)-based methods such as multigene panel tests or whole-exome sequencing (WES) are preferred as these disorders have overlapping phenotypes.

Newborn screening

Metabolic decompensations are associated with high mortality, and this can be prevented by early initiation of dietary management and supportive care during times of acute stress. Screening for FAOD can be done through tandem mass spectrometry (TMS) for acylcarnitine profile for early identification of asymptomatic newborns in dried blood spot (DBS) samples. The screening test is not a determinant of disease status and positive results can arise due to other non-specific conditions. The presumptively positive newborn

Table 2 Metabolic Assays for Fatty Acid Oxidation Defects

Disorder	Plasma Carnitine	Plasma Acylcarnitines Elevated	Urinary Organic Acids & Acylglycines Elevated
CPT1A deficiency	High	• C16 (palmitoyl-) • C18:1 (oleyl-)	Variable elevation of C6-C12 saturated and unsaturated straight chain dicarboxylic acids
CACT deficiency	Low	• C14:2 (tetradecadienoyl-) • C14 (myristoyl-) • C16 (palmitoyl-) • C18:2 (linoleyl-) • C18:1 (oleyl-) • C18 (stearoyl-)	Variable elevation of C6-C12 saturated and unsaturated straight chain dicarboxylic acids
CPT II deficiency	Low	• C14:2 (tetradecadienoyl-) • C14 (myristoyl-) • C16 (palmitoyl-) • C18:2 (linoleyl-) • C18:1 (oleyl-) • C18 (stearoyl-)	Variable elevation of C6-C12 saturated and unsaturated straight chain dicarboxylic acids
VLCAD deficiency	Normal/low	• C14:2 (tetradecadienoyl-) • C14 (myristoyl-) • C16 (palmitoyl-) • C18:2 (linoleyl-) • C18:1 (oleyl-) • C18 (stearoyl-)	Variable elevation of C6-C12 saturated and unsaturated straight chain dicarboxylic acids
MCAD deficiency	Normal	• C6 (hexanoyl-) • C8 (octanoyl-) • C10:1 (decanoyl-) • C10 (decanoyl-)	Elevation of suberic (C8) and adipic (C6) acid; urinary acylglycines – hexanoylglycine (C6), octanoylglycine (C8), suberylglycine (C8), and phenylpropionylglycine (C9)
SCAD deficiency	-	• C4 (butyryl-/isobutyryl-)	Elevation of ethylmalonic and methylsuccinic acid; butyrylglycine (C4)
LCHAD deficiency	Normal/low	• C12-OH (2-hydroxy dodecanoyl-) • C14:2 (tetradecadienoyl-) • C14 (myristoyl-) • C14-OH (3-hydroxy tetradecanoyl-) • C16 (palmitoyl-) • C16-OH (3-hydroxy hexadecanoyl-) • C18:2 (linoleyl-) • C18:1 (oleyl-) • C18 (stearoyl-) • C18:1-OH (3-hydroxy oleyl-) • C18-OH (3-hydroxy oleyl-)	3-hydroxybutyric and 3-hydroxyglutaric acid may be elevated or normal
HADH deficiency	-	• C4-OH (3-hydroxy butyryl-) • C10-OH (3-hydroxy decanoyl-)	-

MTP deficiency	Normal/low	• C12-OH (2-hydroxy dodecanoyl-) • C14:2 (tetradecadienoyl-) • C14 (myristoyl-) • C16 (palmitoyl-) • C16-OH (3-hydroxy hexadecanoyl-) • C18:2 (linoleyl-) • C18:1 (oleyl-) • C18 (stearoyl-) • C18:1-OH (3-hydroxy oleyl-) • C18-OH (3-hydroxy oleyl-)	Elevation of 3-hydroxydicarboxylic acid
MAD deficiency	-	• C4 (butyryl-/isobutyryl-) • C5 (isovaleryl-) • C6 (hexanoyl-) • C5DC (glutarylcarnitine) • C8 (octanoyl-) • C10 (decanoyl-) • C12 (dodecanoyl-) • C14:1 (tetradecenoyl-) • C16 (palmitoyl-) • C18:1 (oleyl-)	Elevation of isobutyryl-, isovaleryl-, and 2-methyl-butyryl-glycine, along with glutaric aciduria and dicarboxylic aciduria

CPT1A: Carnitine palmitoyl transferase 1A; CACT: Carnitine-acylcarnitine translocase; CPT II: Carnitine palmitoyl transferase II; VLCAD: Very long-chain acyl-CoA dehydrogenase; MCAD: Medium-chain acyl-CoA dehydrogenase; SCAD: Short-chain acyl-CoA dehydrogenase; LCHAD: Long-chain 3-hydroxy acyl-CoA dehydrogenase; HADH: 3-hydroxyacyl-CoA dehydrogenase; MTP: Mitochondrial trifunctional protein; MAD: Multiple acyl-CoA dehydrogenase

screening should be followed up with confirmatory diagnostic molecular testing. The American College of Medical Genetics and Genomics (ACMG) has recommended screening for CTD, VLCAD, LCHAD, TFP and MCAD deficiency. However, newborn screening has some drawbacks. Patients identified to have SCAD deficiency were found to remain asymptomatic in spite of severe enzyme deficiency. Normal newborns of mothers with CTD deficiency were falsely identified to be affected.

Management

The mainstay of treatment is through dietary management by removing lipids from the diet or through replacement of accumulating lipids with those that can bypass the block.

General measures: Common elements of the nutritional management of all fatty acid oxidation defects are listed as follows (Rohr, 2015):

i. Aggressive treatment during increased metabolic needs: During intercurrent illness when appetite is reduced, carbohydrate rich fluids should be provided via oral or enteral route every 3-4 hours. If the patient is unable to take orally, or when fasting for surgery, intravenous fluids with 10% dextrose should be given at 10-12 mg/kg/min to maintain plasma glucose > 100 mg/dl. Insulin may be given if hyperglycemia develops. Appropriate electrolyte correction needs to be done. If there is acute hyperammonemia, it is treated with sodium benzoate or sodium phenylacetate. Hemodialysis may be done based on the requirement. The goals are to prevent hypoglycemia, suppress lipolysis, and suppress fatty acid oxidation.

ii. Avoidance of fasting: The guidelines given by the Genetic Metabolic Dietitians International are given in Table 3 (Shirley, 2020). Frequent feeds have to be given to prevent fasting. In older children, snacks should be given in the intervals between regular meals. Cornstarch supplementation 1.5 – 2 g/kg should be given at bedtime.

iii. Carnitine supplementation: Levocarnitine supplementation is important especially in carnitine transporter defects (CTD). In these disorders, levocarnitine has been shown to improve the cardiac and skeletal muscle function to almost near normal within a few months of initiating treatment. The oral

Table 3 Avoidance of fasting in fatty acid oxidation defects

Age	Allowed period of fasting
0-4 months	To be fed every 3 hours with no more than 4 hours of fasting between two meals
5-12 months	An additional hour of fasting is allowed per month of age up to a maximum of 8 hours
>1 year	Avoid overnight fasting for more than 10-12 hours

levocarnitine dose is 100 mg/kg per day. The role of carnitine supplementation in disorders with secondary carnitine deficiency is controversial as the blocks in the enzyme steps do not directly involve carnitine. The proposed hypothesis for supplementing carnitine is that it might help remove the accumulated toxic metabolites, i.e., acyl CoA intermediates by combining with them to form acylcarnitine which are then excreted in the urine.

Specific management:

a. Medium chain acyl CoA dehydrogenase (MCAD) deficiency: Breastmilk or standard infant formula is appropriate to meet the nutritional needs of the infant. Medium chain triglycerides (MCT) should be avoided. Excess consumption of coconut oil and infant formulas containing high MCT (such as premature infant formulas) should be avoided.

b. Long chain fatty acid oxidation disorders: A fat-restricted diet is advised as majority of the fatty acids in the diet are long-chain fatty acids. Breastmilk also has high fat, predominantly containing long-chain fatty acids. The diet should be supplemented with MCT (medium chain triglycerides) which act as substrate for beta oxidation. Breastfeeding may be continued in milder disease or asymptomatic infants, while in severely symptomatic infants, breastfeeding may be discontinued.

For mild to moderate VLCADD, breastfeeding can be continued along with MCT supplemented formula; 20% energy needs are met by long-chain fat and 20% from MCT. For severe VLCADD, breastfeeding should be stopped and MCT formula should be started. Diet should be planned such that 10% of the energy needs are obtained from long-chain + 30% from MCT. After infancy, MCT can be supplemented as an oil or powder prescribed by a physician. The MCT may be mixed in non-fat milk and should be drunk at regular intervals or may be mixed with food. MCT supplementation is associated with the reversal of cardiomyopathy in CACT deficiency and VLCAD deficiency.

After the infant formula has been discontinued, the essential fatty acids must be supplemented along with supplementation of fat-soluble vitamins to avoid deficiency. Docosahexaenoic acid (DHA) is supplemented at a dose of 60 mg/day for infants < 20 kg, 100mg/day for children > 20 kg, and 100-200mg/day for adults.

These individuals have a high risk of exercise-induced rhabdomyolysis. The occurrence of rhabdomyolysis and improvement of exercise tolerance can be achieved through additional MCT supplementation 0.15-0.2g/kg mixed with glucose solution prior to exercise and 3:1 carbohydrate: protein snack after the exercise. As the individual is advised for fat restriction, it may be beneficial for them to be taking higher protein intake through lean meat rather than carbohydrates alone as it would help maintain the body composition.

For LCHAD-associated retinopathy, adherence to the strict diet plan along with MCT supplementation decreases the levels of hydroxy acyl carnitines and hydroxy fatty acids. This would help slow down the progression of vision loss. There is no specific treatment to prevent the progression of retinopathy.

c. Multiple acyl CoA dehydrogenase deficiency: High dose riboflavin supplementation at 100-300mg/day should be tried in all patients to assess for riboflavin responsiveness. Supplementation of levocarnitine at 50 – 100mg/kg/day in three divided doses and coenzyme Q10 supplementation of 60- 240mg/day in 2 divided doses are recommended.

Recent advances

Triheptanoin, sold under the brand name 'Dojolvi' was approved by the US Food and Drug Administration (FDA) in 2020 for the treatment of pediatric and adult patients with confirmed disorders of long-chain fatty acid oxidation (Shirley, 2020). It is a triglyceride of three 7-carbon fatty acids. It serves as an alternative fuel source and thus suppresses lipolysis and accumulation of toxic metabolites. Each triheptanoin molecule is hydrolyzed in the small intestine into 3 molecules of heptanoic acids. The heptanoic acid enters the beta oxidation pathway to produce acetyl CoA and propionyl CoA. The propionyl CoA formed enters the citric acid cycle. As per the randomized controlled trial conducted by Gillingham and coworkers (Gillingham et al., 2017), the group receiving triheptanoin was found to have an improvement in the left ventricular ejection fraction (LVEF) by 7.4%, though no difference was found in the occurrence of rhabdomyolysis between the group receiving triheptanoin and those on dietary control with MCT supplementation.

Glycerol phenylbutyrate, sold under the trade name 'Ravicti' is an FDA-approved drug to treat urea cycle disorders. This has been shown to bind as a substrate to the MCAD enzyme and has been hypothesized to have a stabilizing effect on MCAD associated with the p.Lys304Glu (K304E) missense mutation. Phase 2 clinical trial in MCAD patients homozygous for K304E is ongoing (https://go.drugbank.com/conditions/DBCOND0008334).

Conclusion

Most of the individuals affected by FAODs, especially the defects of long chain and very long chain fatty acids had significant mortality in the past. With the advent of newborn screening, earlier diagnosis and prompt treatment have improved patient outcomes. It has also identified a large number of patients who might never have developed symptoms. Follow up and monitoring is needed for asymptomatic patients as well along with measures to prevent episodes of metabolic decompensation (especially with MCAD deficiency).

Conflict of Interests: None

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GeNeXpreSS

Next Generation Sequencing, Functional Genomics and Gene Therapy: Highlights from Indian Genetics Research

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SERPINA11 related novel serpinopathy – A perinatal lethal disorder (Aggarwal et al., 2024)

Fetal autopsy describing a perinatal lethal phenotype of two fetuses by Aggarwal et al. and subsequent identification of the *SERPINA11* was published in 2024. The highlight was detailed phenotyping and functional studies performed to describe this novel serpinopathy by the authors. Western blot and immunofluorescence were performed to check the expression of the protein in various mouse tissue. The authors demonstrated decreased immunofluorescence in the affected fetal lung as compared to the healthy fetus. These functional studies thereby confirm the effect of *SERPINA11* causing abnormality of serine proteases leading to extracellular matrix disruption and deranged fetal development. A case of fetal hydrops having a blended phenotype with a truncating *SERPINA11* pathogenic variant was reported from another Indian fetus subsequently (Beck et al., 2024). However, further reports of similar cases would be required to establish the gene-disease relationship.

Biallelic variants in CCN2 underlie an autosomal recessive kyphomelic dysplasia (Singh et al., 2024)

An autosomal recessive form of kyphomelic dyplasia with cleft palate and bowing of long bones in humans being caused by *CCN2* was identified by Singh S, et al in 2024.Earlier *Ccn2* knockout mice were known to show twisted limbs, short and kinked sterna and other skeletal defects. Functional studies of the biallelic variants were performed by CRISPR-Cas9 gene editing in zebrafish which showed altered body curvature, impaired cartilage formation in craniofacial region and either bent or missing tails. This is a novel gene-disease relationship for kyphomelic dysplasia which is a heterogenous entity. The autosomal recessive condition is described in two unrelated consanguineous families who had total six affected offspring of which three had died before the study and three of them underwent exome analysis. Two novel homozygous variants in *CCN2* as possible candidates that segregated with the phenotype in the families were picked up: a missense variant c.443G>A;p.(Cys148Tyr) in exon 3 and a frameshift variant, c.779_786del; p.(Pro260LeufsTer7) in exon 5. Parental segregation was done, to confirm the autosomal recessive inheritance. *CCN2* is crucial for proliferation and differentiation of chondrocytes. Hence *CCN2* was determined as one of the causes of kyphomelic dysplasia. Further, F0 knockout zebra fish also showed skeletal involvement supporting this observation.

Functional Characterization of Thyroid Peroxidase Missense Variants Causing Thyroid Dyshormonogenesis in Asian Indian Population (Sarma et al., 2024)

The study by Sarma et al. involves in silico and functional characterization of the novel variants found in the *TPO* gene which codes for thyroid peroxidase enzyme which is one of the crucial enzymes involved in thyroid hormone biosynthesis. Biallelic pathogenic variants cause thyroid dyshormonogenesis (TDH) 2A. Exome sequencing was conducted initially for TDH followed by Sanger validation and computational studies. Functional studies were done by immunofluorescence and enzyme assay. There were nine bi-allelic variants detected out of which eight were novel. Nine unrelated families with a

total number of 12 patients were studied. Of these, seven families were consanguineous, and segregation analysis fulfilled the autosomal recessive inheritance patterns. All the variants are private. The six missense variants were shown to affect the protein structure on computational analysis. Further in-vitro analysis showed reduced enzyme levels when compared to the wild type. In addition, two novel nonsense variants were also identified. Thus, this study revealed that the novel missense variants were pathogenic, based on the functional analysis. Deletions, insertions, duplications and splice site variants have also been described previously which were not found in this series of patients.

Lentiviral Gene Therapy with CD34+ Hematopoietic Cells for Hemophilia A (Srivastava et al., 2024)

As an attempt to cure severe hemophilia A without factor VIII inhibitors by gene therapy, Srivastava et al. conducted a single centre study in five patients at Christian Medical College, Vellore. They transduced autologous hematopoietic stem cells (HSCs) with a lentiviral vector (CD68-LV-ET3). The patients received myeloablative conditioning before being transplanted with transduced cells without and with transduction enhancer (group 1 and 2 respectively). Safety and efficacy were assessed with regards to toxic effects and factor VIII activity and annualized bleeding rate respectively. Results showed severe neutropenia lasting for 7-11 days and severe thrombocytopenia of 1-7 days as expected. Cumulative follow up was for 81 months and annualized bleeding rate was zero. The authors conclude gene therapy for hemophilia A with use of lentiviral vector transduced autologous HSCs resulted in stable factor VIII expression and the activity correlated to the vector copy number in peripheral blood.

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Dr Shyam S Agarwal Oration Awardee for the Year 2024: Dr David P Bick

Dr David P Bick, MD, Principal Clinician for the Newborn Genomes Programme at Genomics England, United Kingdom, is a world-renowned expert in the field of medical genetics. He has made immense contributions in the field of genomic medicine. Prior to his work in England, he was the Chief Medical Officer at the HudsonAlpha Institute for Biotechnology in the United States. He also served as Professor in the Department of Pediatrics and Director of the Clinical Sequencing Laboratory at the Medical College of Wisconsin, and as Medical Director of the Genetics Clinic at Children's Hospital of Wisconsin, where he established one of the first genomic medicine clinics in the United States.

9th Annual Conference of the Society for Indian Academy of Medical Genetics - IAMG-2024

The 9th Annual Conference of the Society for Indian Academy of Medical Genetics (IAMG-2024) was held from December 5th to 8th at the Belvedere Golf Country Club, Adani Shantigram, Ahmedabad, Gujarat. The event was hosted by the Sandip Bhavini Research Institute, a non-profit academic organization affiliated with Gujarat University, dedicated to advancing core research, study design, data assimilation, documentation, publication, and staying current with global technologies and trends.

Reflecting the contemporary landscape of multiomics, the conference theme was *"CLINI-OMICS: Navigating the Diagnostic Maze through the OMICS Compass."* With the rapid advancement of sequencing technologies and their increasing cost efficiency, diagnostic testing has become more accessible than ever. However, this progress also brings a surge of uncertain findings that require further resolution. From December 6th to 8th, the conference featured didactic lectures by eminent national and international experts, showcasing the latest research in medical genomics and the evolving field of multiomics. We were honored by the presence of 18 international faculty members from the USA, UK, Australia, and the UAE, alongside 55 national experts. The coveted Dr SS Agarwal oration was delivered by Dr David Bick, UK. The event attracted 300 participants from across the country, who actively engaged in interactive sessions, attended thought-provoking lectures, and networked with peers from around the world. On December 5th, as part of the pre-conference sessions, four workshops were conducted: *Deciphering the DECIPHER (Deep Genotyping), Single Cell Genomics, EYE or AI (Deep Phenotyping), and Long Read Technologies.* Dr Neerja Gupta was presented the Dr I C Verma Outstanding Researcher Award and Dr Purvi Majethia won the Dr S S Agarwal Young Scientist award for year 2024. There were high quality oral and poster presentations. The best platform presentation award was bagged by Dr Udhaya Kotecha. The Best poster award instituted in memory of Dr Alka Ekbote was awarded to Dr Neeraja Chilukoti. The 2nd and 3rd best poster awards were presented to Dr Shruti Bajaj and Dr Hariswar PT respectively. The success of IAMG-2024 highlights the growing collaboration within the global clinical genomics and genetic research communities, paving the way for innovative, inclusive, and accessible healthcare solutions in the future.

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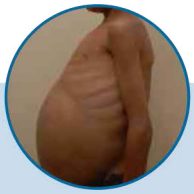

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