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An Overview of the Genetic Basis and Clinical Approach for Peroxisomal Disorders

Gayatri Nerakh

Department of Fetal Medicine and Medical Genetics,Fernandez Foundation, Hyderabad, India

Correspondence to: Dr Gayatri Nerakh. *Email:* maildrgayatri@gmail.com

Abstract

Peroxisomal disorders are a rare heterogeneous group of inherited inborn errors of metabolism. Most of the peroxisomal disorders manifest in neonatal, infantile, and childhood periods. There are certain intricacies in diagnosing peroxisomal disorders due to overlapping phenotypes, complex biochemical tests, clinical and genetic heterogeneity. The diagnosis of milder and atypical phenotypes is even more complicated.This article describes the genetics of peroxisomal disorders and provides guidelines for the diagnosis of these disorders in routine clinical practice.

Keywords: Peroxisomal disorders; Clinical approach

Introduction

Peroxisomes are single membrane cytoplasmic organelles with diverse dynamic functions. The word 'peroxisome' was coined by Christian de Duve. Peroxisomes are involved in multiple metabolic functions in the body (**Figure1**). They do not have their own DNA like mitochondria. Membrane proteins, matrix proteins, and peroxins are peroxisomal proteins that are responsible for maintaining the structure of peroxisomes, for

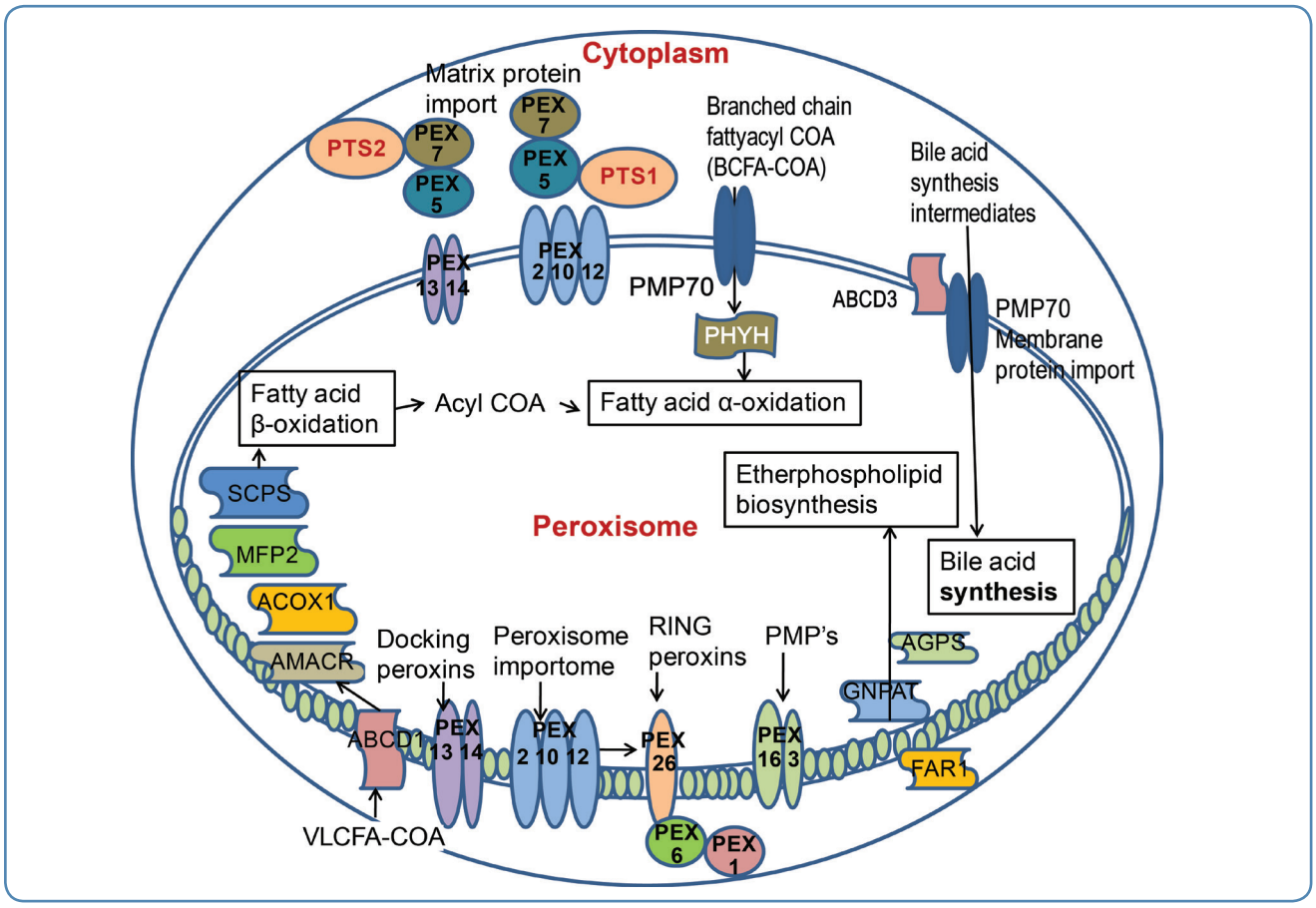

Figure 1 Functions of peroxisomes and the genes involved in peroxisomal disorders

peroxisomal division/assembly, and for performing various cellular biochemical functions.

The main functions of peroxisomes are α and β oxidation of fatty acids, synthesis of plasmalogen, cholesterol and bile acids, detoxification of glyoxylate and hydrogen peroxide, and catabolism of lysine.

Peroxisomal disorders are a broad spectrum of disorders with phenotypic and genotypic variability. Most of them present in the neonatal and pediatric age groups.They are classified into two subgroups. The first group is peroxisome biogenesis disorders (PBDs) which occur due to defective peroxisome assembly. The second group is peroxisomal dysfunction due to single peroxisome enzyme deficiencies.The most typical disorder under peroxisome biogenesis disorders is the 'classical' Zellweger syndrome, a severe form of the Zellweger spectrum disorders (ZSDs). The most common disorder of peroxisomal dysfunction is X-linked adrenoleukodystrophy (X-ALD).

Classification of peroxisomal disorders

The classification of peroxisomal disorders is outlined in **Tables 1 and 2** (Takashima et al., 2019; Wanders, 2017).

Genetics of peroxisomal disorders

Most of the peroxisomal disorders have an autosomal recessive pattern of inheritance, with few exceptions such as X-linked adrenoleukodystrophy. The phenotypic severity of the ZSDs is based on the type of pathogenic variants in PEX genes. Mutations in the PEX1 gene account for 60% and mutations in *PEX6* account for 10-15% of cases of ZSDs. As ZSDs are a rare group of disorders, the exact genotype and phenotype correlations have not been established but loss-of-function (LoF) variants like truncating variants have been found to lead to a more severe phenotype than missense variants. Biallelic pathogenic variants in the *PEX7* gene are known to cause severe rhizomelic chondrodysplasia punctata type 1, but some variants in *PEX7* can cause the less severe peroxisome biogenesis disorder 9B with absence of chondrodysplasia and rhizomelia, which includes the adult-onset Refsum disease phenotype of cataracts, retinitis pigmentosa, hearing loss, ataxia, neuropathy and ichthyosis, and the more recently described phenotype of cataract with neurodevelopmental disability (Masih et al., 2021).

Some of the peroxisomal disorders are now being identified to be autosomal dominant. *PEX6* is associated with autosomal recessive PBD 4A and

Table 1 Classification of peroxisomal biogenesis disorders

Disorder	Gene	Protein/function	Peroxisomes and their structure in IHC
i. Group A(Zellweger spectrum disorders)			
1. Severe phenotype	PEX1 PEX2 PEX 5 PEX 2,10,12, PEX 13,14 PEX 3,16,19 PEX 6,26	RING peroxins Peroxisome importome PTS1-linked signaling Peroxisome importome Docking peroxins Peroxisomal membrane proteins RING peroxins	Absent
2. Intermediate phenotype			Absent
3. Milder phenotype			Absent
ii. Group B			
1. Rhizomelic chondrodysplasia punctate type 1	*PEX7*	PTS2-linked signaling	Enlarged
2. Rhizomelic chondrodysplasia punctata type 5	*PEX5*	PTS1-linked signaling	NA

NA – Not available

Table 2 Disorders of peroxisome function (Single enzyme deficiency)

Disorder	Gene	Protein/function	Peroxisomes and their structure in IHC
i. Etherphospholipid biosynthesis			
1. Rhizomelic chondrodysplasia type2 (Dihydroxyacetonephosphate acyltransferase deficiency)	*GNPAT*	Plasmalogens synthesis	NA
2. Rhizomelic chondrodysplasia type 3 (Alkyldihydroxyacetone phosphate synthase deficiency)	*AGPS*		
3. Rhizomelic chondrodysplasia type 4 (Fatty acyl-CoA reductase 1 deficiency)	*FAR1*		
ii.Fatty acid β-oxidation			
1. X-linked adrenoleukodystrophy	*ABCD1*	VLCFA transporter	Normal
2. Acyl COA oxidase deficiency	*ACOX1*	Peroxisomal β-oxidation	Enlarged
3. D-Bifunctional protein deficiency	*HSD17B4*		Enlarged
4. Sterol carrier protein X deficiency	*SCP2*	Peroxisomal β-oxidation	NA
5. Alphamethylacyl COA racemase (AMACR) deficiency	*AMACR*		NA
iii.Fatty acid α-oxidation			
1.Refsum disease (Phytanoyl-CoA hydroxylase deficiency)	PHYH	Peroxisomal α-oxidation	NA
iv.Disorders of Glyoxylate cycle			
1.Hyperoxaluria type 1 (Alanine-glyoxylate aminotransferase deficiency)	*AGXT*	Peroxisomal glyoxylate cycle	Small
2.Glycolate oxidase deficiency	*HAO1*	Glyoxylate metabolism	NA
v. Bile acid synthesis			
1.Bile acid-CoA: amino acid N-acyltransferase deficiency	*BAAT*	Peroxisomal acyl-CoA acyltransferase	NA
2.Acyl-CoA oxidase 2 deficiency	*ACOX2*	Branched-chain acyl CoA oxidase	NA
3.PMP70 deficiency	*ABCD3*	Peroxisomal membrane protein	NA
vi. H2O2 Metabolism			
1.Acatalasemia (Catalase deficiency)	*CAT*	Antioxidant	Normal
vii. Lysine catabolism			
1.L-lysine oxidation		L-pipecolic acid degradation	NA

Abbreviations: IHC-Immunohistochemistry, NA-Not available

4B, but in a few patients with a heterozygous variant in the *PEX6* gene, allelic expression imbalance leading to an overrepresentation of a mutant allele and ZSD phenotype has been reported (Falkenberg et al., 2017). Likewise, biallelic pathogenic variants in the *FAR1* gene lead to the autosomal recessive peroxisomal fatty acyl-CoA reductase 1 disorder, which is characterized by severe psychomotor retardation during infancy followed by childhood spasticity, but a few patients with a heterozygous pathogenic variant in *FAR1* have been reported to have cataracts and spastic paraparesis.

Peroxisome biogenesis disorders also exhibit mosaicism in a few patients. In type 1 mosaicism, normal peroxisomal activity is revealed in fibroblasts with abnormal biochemical profiles. In type 2 mosaicism, with the same genotype,there is a difference in peroxisome morphology in different tissues.

Clinical features of peroxisomal disorders

1.Peroxisome biogenesis disorders (PBDs)

These are autosomal recessive genetic disorders with an incidence of approximately 1:30,000 to 1:50,000 newborns.The Zellweger spectrum of disorders and rhizomelic chondrodysplasia punctata (RCDP) spectrum are included under this category.

i. Zellweger spectrum of disorders (ZSDs)

The phenotype of ZSDs usually ranges from severe form to intermediate and milder forms with the typical presentation. Patients with atypical presentation lacking the classical signs and symptoms of ZSDs have also been described. Dysmorphic features in severe ZSDs include the high forehead, large anterior fontanelle, hypoplastic supraorbital ridges, epicanthal folds, corneal clouding, cataract, and broad nasal bridge (**Figure 2**). Prognosis is guarded with early neonatal or infantile death. Most of the intermediate forms of ZSDs have late childhood deaths. Clues to the diagnosis of Zellweger spectrum disorders in different age groups with the differential diagnosis are depicted in **Figure 3**. The neonatal phenotype of X-linked adrenoleukodystrophy due to contiguous deletion of the ABCD1 gene which mimics peroxisome biogenesis disorders has also been described.

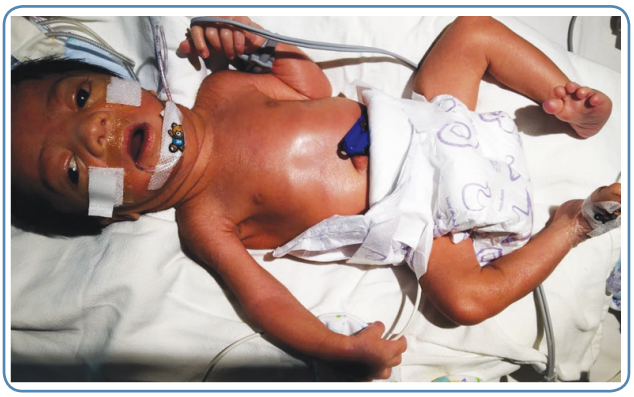

Figure 2 Dysmorphic features in a neonate with the severe Zellweger spectrum disorder

ii.Rhizomelic chondrodysplasia punctata spectrum (RCDP 1 and 5)

The phenotype of RCDP spectrum usually ranges from the severe form who present with midfacial hypoplasia, rhizomelic shortening at birth, to the intermediate form who present in childhood with joint contractures and spastic quadriparesis, and the milder forms who present with mild rhizomelic shortening. Severe phenotype can present either in the prenatal period or in the neonatal period.

2. Disorders of peroxisome function

i. Etherphospholipid biosynthesis disorders

RCDP types 2, 3, 4 are included under this category. The clinical features are similar to RCDP 1, 5 and they are differentiated by their genetic etiology. *FAR1*-related disorder is referred to as RCDP4 by some authors, but this disorder lacks the classical skeletal features of RCDP.

ii. Disorders with impaired fatty acid β-oxidation

X-linked adrenoleukodystrophy (X-ALD): X-ALD is an X-linked recessive disorder. It does not have any clinical features at birth. It can manifest with three different phenotypes. Different forms of X-ALD, their specific clinical features, and their age of presentation are depicted in **Figure 4**. Some female carriers can present with adrenomyeloneuropathy.

Alpha methylacyl-CoA racemase (AMACR) deficiency, acyl-CoA oxidase 1 (ACOX1) deficiency, D-bifunctional protein (DBP) deficiency, and sterol carrier protein X deficiency (SCPx) are the other disorders included under impaired fatty acid β-oxidation (Arora et al; 2020). SCPx deficiency has been described in one adult patient with

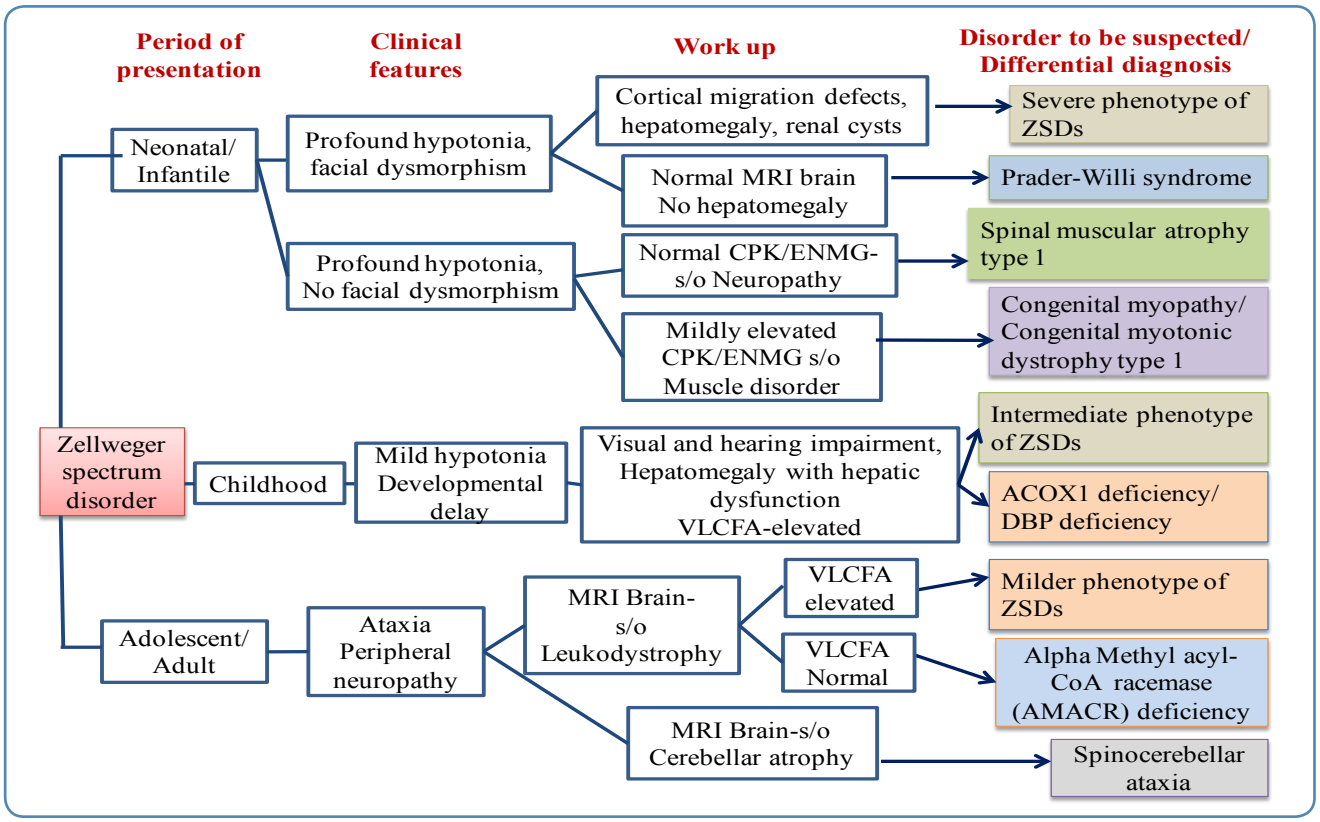

Figure 3 Clues to diagnosis of Zellweger spectrum disorders in different age groups with differential diagnosis

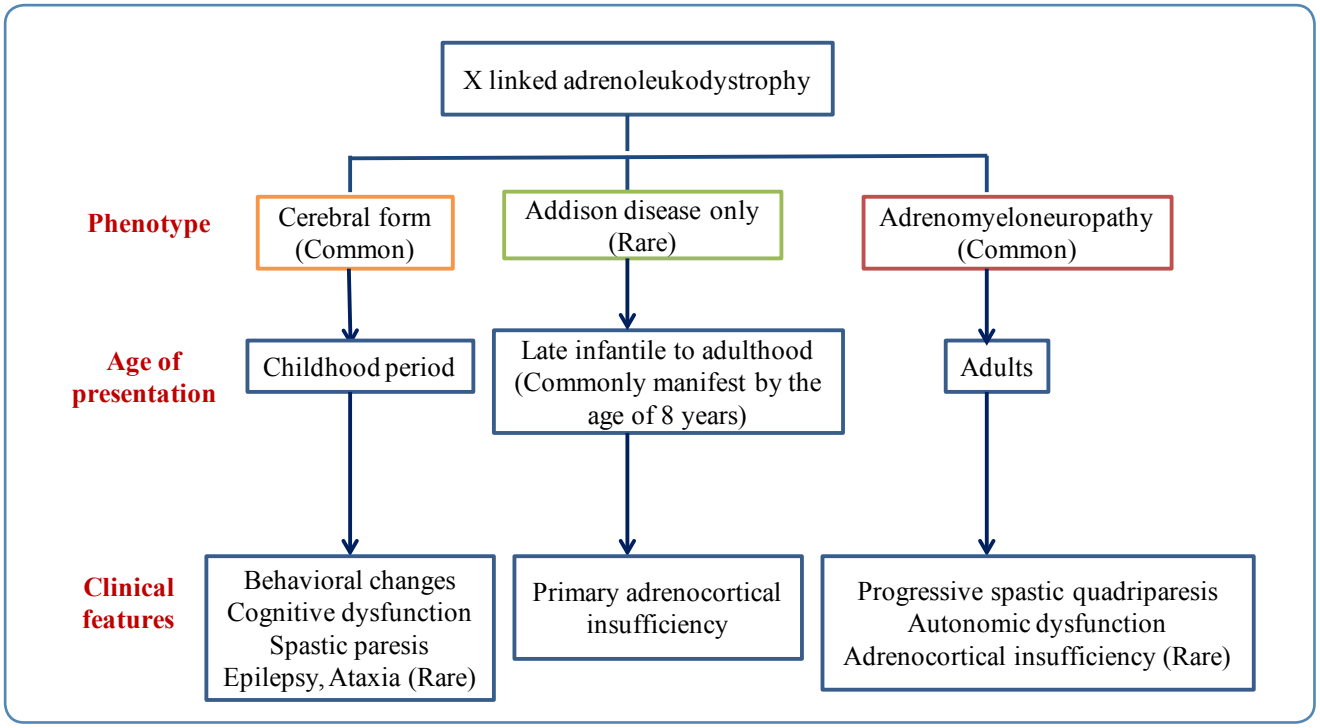

Figure 4 Different phenotypes, age of onset, and clinical features of X-linked adrenoleukodystrophy

dystonia, cerebellar signs, and motor neuropathy (Ferdinandusse et al., 2006).

iii. Disorders with impaired fatty acids α-oxidation

Refsum Disease (RD): RD is caused due to phytanoyl-CoA hydroxylase deficiency which is the first enzyme involved in the α-oxidation of fatty acids. Patients with RD usually present in late childhood. Clinical features of this disorder are represented in Figure-5. The entire spectrum of clinical manifestations is not seen in all cases.

iv. Disorders of the Glyoxylate cycle

Primary Hyperoxaluria type 1: Symptoms of this disorder manifest from infancy to adulthood but majority of them present in childhood or early adolescence. They present with recurrent nephrolithiasis due to deposition of calcium oxalate and nephrocalcinosis. Death in these cases is due to end-stage renal disease and renal failure.

v. Bile acid synthesis defects

Acyl-CoA oxidase 2 (ACOX2) deficiency, peroxisomal membrane protein 70 (ABCD3) deficiency, and bile acid-CoA: amino acid N-acyltransferase (BAAT) deficiency are included under this category. ACOX2 deficiency and ABCD3 deficiency present in childhood. Both these disorders have similar clinical features with predominant involvement of the liver (**Figure 5**). BAAT deficiency presents with itching and steatorrhea.

vi. H2O2 Metabolism

Acatalasemia/Hypocatalasemia: It is caused by either complete or partial loss of catalase activity in erythrocytes.This disorder is usually asymptomatic. In rare cases, it may be associated with oral ulcerations or gangrene, or diabetes mellitus.

The age of onset, clinical features, and features that should lead one to suspect the disorders of peroxisomal dysfunction are outlined in **Figure 5**.

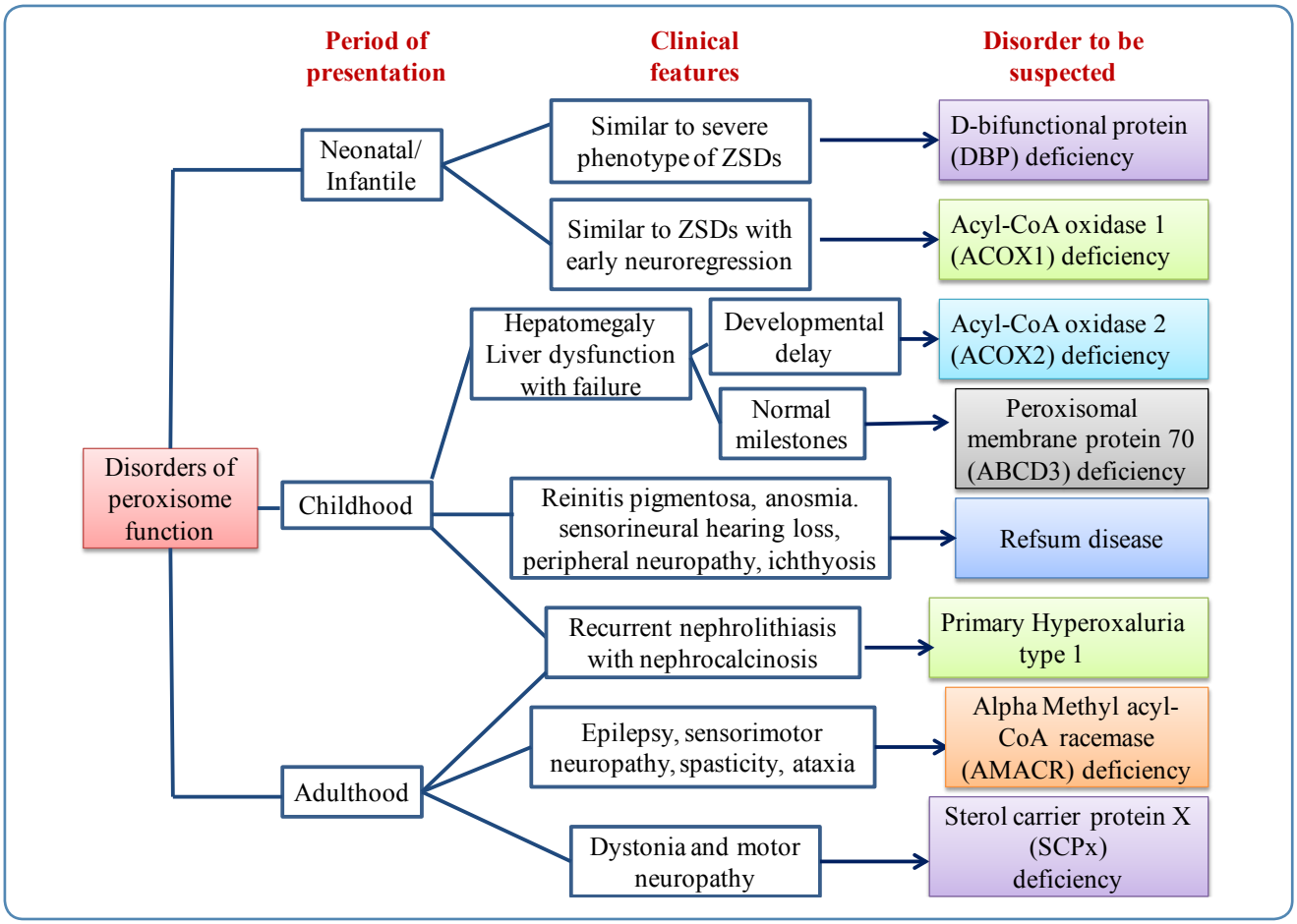

Figure 5 Flow chart showing clues to the diagnosis of disorders of peroxisomal function (single enzyme deficiency disorders)

Diagnosis of peroxisomal disorders

i. Biochemical workup

For most peroxisomal disorders, biochemical workup involves metabolite assay in plasma and/or red blood cells(RBCs). Very long-chain fatty acids (VLCFA), docosahexaenoic acid, phytanic acid, and plasmalogen are the biochemical parameters measured by gas chromatography/mass spectrometry (GCMS). VLCFAs are measured by analyzing the concentration of C26:0, the ratio of C24:0 to C22:0, and the ratio of C26:0 to C22:0. In ZSDs,VLCFA, phytanic acid, pristanic acid, docosahexaenoic acid, pipecolic acid, and bile acids are elevated in the plasma, and plasmalogens are decreased in the RBCs. An increase in phytanic acid with decreased pipecolic acid in plasma and decreased plasmalogen in RBC is suggestive of RCDP. In Refsum disease, phytanic acid levels are increased but plasmalogen and pipecolic acid levels are normal. In X-ALD, VLCFA is markedly elevated with normal levels of other biochemical substances (Wanders et al) 2018.

Table 3 shows the list of various biochemical substances measured in blood and their levels in different peroxisomal disorders.

Table 3 Biochemical workup for suspected peroxisomal disorders

In plasma							
	Zellweger Spectrum Disorders			**RCDP**	**X-ALD**	**RD**	**Fatty acid B-oxidation**
	Severe	**Intermediate**	**Mild**				
VLCFA (C26:0 & C26:1 Ratios of C24, C22 & C26/C22)	Markedly increased	Markedly increased	Increased	Normal	Markedly increased	Normal	Normal except increase in DBP deficiency & ACOX1 deficiency
Phytanic Acid	Markedly increased	Markedly increased	Increased	Markedly increased	Normal	Markedly increased	Normal except increase in DBP deficiency
Pristanic acid	Normal to increased	Normal to increased	Normal to increased	Normal	Normal	Normal	Normal except increase in AMACR & DBP deficiency
Pipecolic acid	Markedly increased	Markedly increased	Increased	Markedly increased	Normal	Normal	Normal
Bile acids	Markedly increased	Markedly increased	Increased	Normal	Normal	Normal	Normal except increase in AMACR & DBP deficiency
In RBC							
Plasmalogen	Markedly decreased	Markedly decreased	Decreased	Markedly decreased	Normal	Normal	Normal

Abbreviations: DBP-D-Bifunctional protein, RCDP-Rhizomelic chondrodysplasia punctata, ALD-Adrenoleukodystrophy, RD-Refsum disease, VLCFA-Very long-chain fatty acids, AMACR-Alphamethyl acyl COA racemase deficiency

X-linked adrenoleukodystrophy is included in neonatal screening programs in several countries (Turk et al., 2020). Recently changes in phospholipid metabolites are found to be reliable biomarkers to indicate neuroinflammation in mice models and X-ALD patients. Further studies need to be done in a large cohort of X-ALD patients to use these metabolites as early biomarkers for neuroinflammation (Kettwig et al., 2021).

In primary hyperoxaluria type 1, the diagnosis is made by the presence of high urinary oxalate excretion and for glycolate oxidase deficiency the diagnosis is by documenting high urinary glycolate levels.

ii. Radiological features

Skeletal abnormalities

Chondrodysplasia punctata at the knee and/or ankle joints and along the vertebrae in early childhood and rhizomelic shortening are noted in skeletal radiographs in cases with RCDP and Zellweger syndrome (**Figure 6**). In addition, vertebral cleftsare seen on the skeletal survey in RCDP.

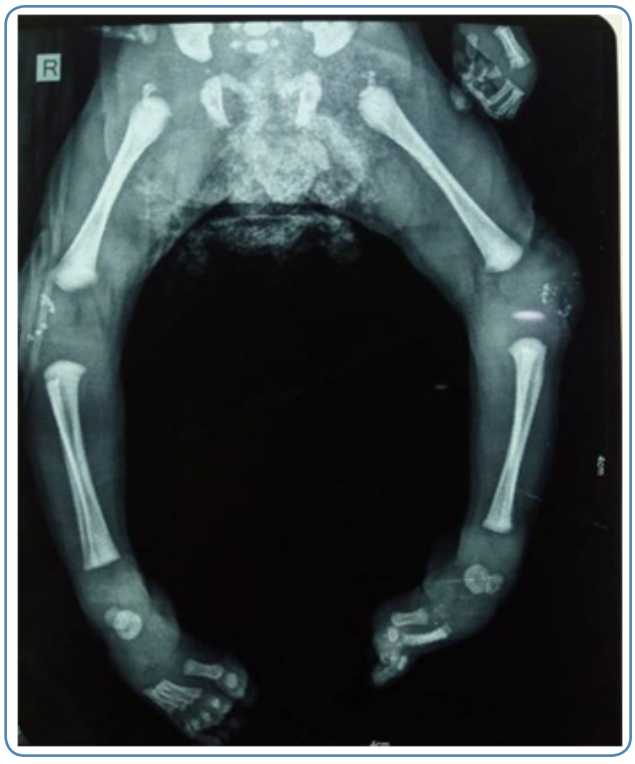

Figure 6 Skeletal radiograph showing chondrodysplasia punctata at the knee joint in a child with rhizomelic chondrodysplasia punctata

Neuroimaging findings

In peroxisomal disorders, specific findings in magnetic resonance imaging (MRI) of the brain are seen only in Zellweger syndrome (**Figure 7**) and X-ALD. In other peroxisomal disorders, MRI brain findings may provide clues to the diagnosis. MRI brain findings in peroxisomal disorders are listed in **Table 4**. Typical findings of the MRI brain may not be found in the initial stages of the disease in peroxisomal disorders as they evolve gradually during the disease course. In such a scenario biochemical workup and genetic evaluation would help in the diagnosis.

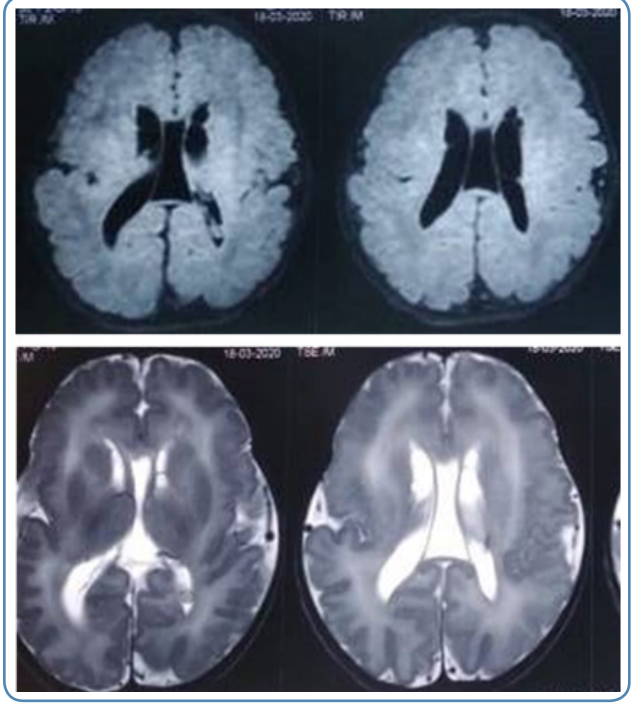

Figure 7 MRI brain findings in Zellweger syndrome
6A.T2 weighted MRI brain axial view showing germinolytic cysts
6B. T1 weighted MRI brain axial view showing diffuse polymicrogyria

Genetic evaluation

Peroxisomal disorders are rare inherited metabolic disorders. The diagnosis of a specific peroxisomal disorder can be made based on clinical features, biochemical workup, skeletal survey, MRI brain, and molecular genetic testing by whole/clinical-exome sequencing. Biochemical analysis is required to corroborate the genetic diagnosis in some cases especially in those with variants of uncertain significance.The genomics-first approach

Table 4 MRI brain findings in peroxisomal disorders

Peroxisomal disorder		Findings in MRI Brain
Zellweger Spectrum disorders (ZSDs)	Severe phenotype	Cortical migration abnormalities like perisylvian or diffuse polymicrogyria, germinolytic cysts (Figure 6) with or without myelination abnormality
	Intermediate and milder phenotype	Myelination abnormalities; demyelination initially starts in the cerebellum first and later on involves the entire cerebral region
Rhizomelic chondrodysplasia punctata (Severe type)		Ventriculomegaly, progressive cerebellar atrophy, delayed myelination of the supratentorial white matter, white matter signal abnormalities in the parieto-occipital region
Rhizomelic chondrodysplasia punctata (Milder type)		Normal
Cerebral type X-ALD		T2 weighted hyperintensities in parieto-occipital white matter; the frontal, parietal regions are involved rarely
Alpha Methylacyl-CoA racemase (AMACR) deficiency		Cerebral atrophy and T2-weighted hyperintensities are noted in the deep white matter of both hemispheres, thalami, midbrain, and pons.
Acyl CoA oxidase 1 deficiency and D-bifunctional protein deficiency		Cerebellar atrophy and periventricular white matter hyperintensities (Arora et al., 2020)
Sterol carrier protein X deficiency		Bilateral T2 weighted hyperintense signals in the thalamus and pons
Refsum disease		No specific MRI findings (Poll-The et al., 2012)

would be helpful in cases with milder and atypical phenotypes.

A diagnostic flowchart that can be used for peroxisomal disorders is given in **Figure 8**.

Management

Surveillance of most of the peroxisomal disorders is by regular monitoring of liver functions, renal and adrenal functions, and annual hearing and ophthalmologic evaluation. In addition, MRI brain is recommended in suspected cases of ZSDs, X-ALD, and also in ACOX1, AMACR, and DBP deficiency.

There is no complete cure available for peroxisomal disorders at present. Supportive therapies like adequate nutrition, physiotherapy, and occupational therapy are to be given. Symptomatic management includes antiepileptic therapy for seizures, and gastrostomy tube feeding for those with feeding difficulty.

Dietary restriction of phytanic acid helps to some extent in patients with Refsum disease. Pyridoxine supplementation has been tried in hyperoxaluria type 1 but has limited success. Good hydration, lithotripsy, and surgical intervention would be helpful to some extent in hyperoxaluria type 1 but there is a high chance of recurrence of renal stones. In cases with adrenocortical insufficiency, corticosteroid replacement therapy is recommended. In X-linked ALD, Lorenzo's oil has been tried but has limited success as it does not prevent the progression of neurological symptoms. If done in the early stages, allogeneic hematopoietic stem cell transplantation (HSCT) is shown to either prevent progression or reverse demyelination. Lenti-D gene therapy tried for patients in the early stages of cerebral type of X-ALD showed beneficial results in phase III clinical trial (Eichler et al., 2017). In 2018, Lenti-D™ was heralded as a breakthrough therapy by the United States Food and Drug Administration (US FDA) for treating the cerebral type of X-ALD, as it is found to

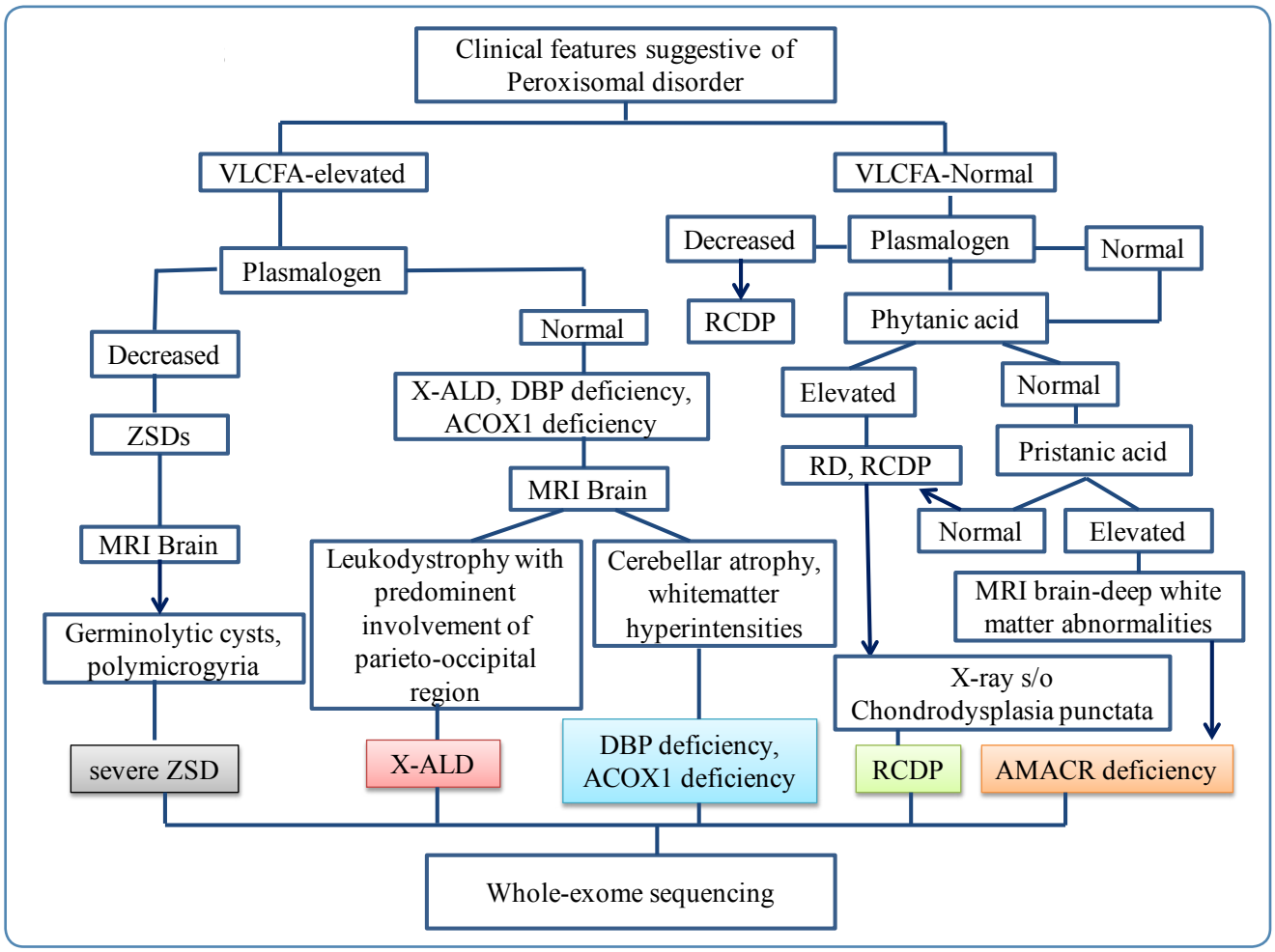

Figure 8 Diagnostic flow chart for peroxisomal disorders

provide significant improvement when compared to other available therapies.

Conclusion

Though most of the peroxisomal disorders can be recognized based on specific clinical clues and diagnostic workup, the milder and atypical phenotypes need a structured clinical and diagnostic approach to establish the exact diagnosis. There is no complete cure for these disorders except for supportive treatment and symptomatic management by a multidisciplinary team. Exact molecular diagnosis, therefore, helps in appropriate genetic counseling and definitive prenatal testing, and also helps the couples to make informed reproductive choices.

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