

## 25.1 Introduction

### ■ Functions of Peroxisomes in Humans

Peroxisomes play an indispensable role in human metabolism as exemplified by the devastating consequences of the absence of peroxisomes in Zellweger patients. The most important functions of peroxisomes so far identified include: (1) the  $\beta$ -oxidation of a range of fatty acids and fatty acid derivatives, (2) the biosynthesis of a special group of phospholipids called ether-phospholipids, (3) the  $\alpha$ -oxidation of 3-methyl-branched fatty acids like phytanic acid, and (4) the biosynthesis of isoprenoids. Other functions of peroxisomes, not covered here, include: (5) the detoxification of glyoxylate via the peroxisomal enzyme alanine glyoxylate aminotransferase (AGT) (see Chap. 26), (6) the oxidation of L-pipecolic acid, a metabolite of L-lysine, via L-pipecolate oxidase, (7) the oxidation of glutaryl-CoA via glutaryl-CoA oxidase, (8) fatty acid chain elongation, and (9) breakdown of hydrogen peroxide via catalase, among other functions.

### ● Peroxisomal Fatty Acid $\beta$ -Oxidation

The most important substrates handled by the peroxisomal fatty acid oxidation system from the perspective of peroxisomal disorders are: (1) *very-long-chain fatty acids* (VLCFA), notably hexacosanoic acid (C26:0), (2) *pristanic acid* (2,6,10,14-tetramethylpentadecanoic acid), as derived from dietary sources either directly or indirectly from phytanic acid and (3) *di- and trihydroxycholestanoic acid* (DHCA and THCA). The latter two compounds are intermediates in the formation of the primary bile acids cholate and chenodeoxycholate from cholesterol in the liver.

Another major function of the peroxisomal  $\beta$ -oxidation system concerns the “biosynthesis” of polyunsaturated fatty acids including docosahexaenoic acid (C22:6 $\omega$ 3). Recent studies have clearly established that the formation of docosahexaenoic acid (C22:6 $\omega$ 3) from linolenic acid (C18:3 $\omega$ 3) involves the active participation of the peroxisomal  $\beta$ -oxidation system at the level of the conversion of C24:6 $\omega$ 3 to C22:6 $\omega$ 3 (see Fig. 25.1).

With respect to the enzymes involved in the  $\beta$ -oxidation of all these compounds, it was long thought that only a single set of enzymes including acyl-CoA oxidase, L-bifunctional protein with enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activity and peroxisomal thiolase would catalyze the  $\beta$ -oxidation of all these compounds. It now turns out, however, that peroxisomes harbor *two* acyl-CoA oxidases, one for straight-chain fatty acids like C26:0 and one for branched-chain fatty acids (pristanic acid, DHCA and THCA), *two* bifunctional enzymes and *two* peroxisomal thiolases (see Fig. 25.2). This will be discussed in more detail under paragraph 25.2.

### ● *Etherphospholipid Biosynthesis*

Ether phospholipids are a special class of phospholipids which differ from the regular, more well-known diacyl phospholipids in one major aspect which is the occurrence of an ether linkage rather than an ester linkage at the *sn*-1 position of the glycerol backbone. Two groups of etherphospholipids can be distinguished with either an 1-*O*-alkyl or an 1-*O*-alk-1'-enyl-linkage. The latter phospholipids (1-*O*-alk-1'-enyl-2-acyl-phosphoglycerides) with an  $\alpha,\beta$ -unsaturated ether-bond are also known by their trivial name *plasmalogens*. Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-glycerophosphocholine) is the best known etherphospholipid. The alcohol moiety in plasmalogens is usually of the ethanolamine- or choline-type. Figure 25.3 depicts the enzymology of the etherphospholipid biosynthetic pathway with part of the enzymes localised in peroxisomes and another part in the endoplasmic reticulum.

### ● *Fatty Acid $\alpha$ -Oxidation*

3-Methyl-branched fatty acids need to undergo oxidative decarboxylation via a process called  $\alpha$ -oxidation to release the terminal carboxylgroup as CO<sub>2</sub>. One of the most important physiological 3-methyl-branched fatty acids is phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), long known to accumulate in Refsum disease as well as in other disorders.

The mechanism involved in the oxidative decarboxylation of 3-methyl fatty acids like phytanic acid has long remained mysterious but has now been resolved (see [1]).

Figure 25.4 depicts the enzymology of the pathway. Current evidence holds that the complete pathway from phytanoyl-CoA to pristanic acid, pristanoyl-CoA and then on to 4,8-dimethylnonanoyl-CoA is intraperoxisomal (Fig. 25.4).

## ■ Biogenesis of Peroxisomes

Peroxisomal proteins have been found to be synthesised on *free* polyribosomes. This applies to both peroxisomal matrix as well as membrane proteins. These and other findings led Lazarow and Fujiki (2) to formulate a model for peroxisome biogenesis which is generally accepted. The principal features of this model are:

1. Peroxisomal matrix and membrane proteins are synthesised on free polyribosomes; 2. The newly synthesised proteins are posttranslationally imported from the cytosol into pre-existing peroxisomes; 3. Import of new polypeptides expands the peroxisomal compartment making them grow until they reach a critical size which results either in division of peroxisomes into two daughter peroxisomes or in budding from the peroxisomal reticulum followed by subsequent growth.

The fact that proteins destined for peroxisomes are synthesised on free polyribosomes implies that these proteins must possess specific signals to direct them to peroxisomes. Two of such Peroxisome Targeting Signals (PTS) have been identified. The first PTS involves a carboxyterminal tripeptide of the sequence serine-lysine-leucine (SKL) or a variant thereof and many peroxisomal proteins contain such a PTS1.

The second peroxisome targeting signal (PTS2) has been found in far less peroxisomal proteins and involves a stretch of 9 amino acids of which amino acids 1, 2, 8 and 9 are essential. The following consensus has been found for the PTS2-motif: (R/K)-(L/V/I)-XXXXX-(H/Q)-L/A) in which X may be any amino acid. In mammals, the PTS2 has only been identified in peroxisomal thiolase 1, phytanoyl-CoA hydroxylase and alkyl-DHAP synthase. Peroxisomal membrane proteins lack either a PTS1 or PTS2 signal which implies the existence of additional signals. The identification of the PTS1 and PTS2 targeting signals was soon followed by the discovery of a growing list of so-called PEX-genes coding for proteins playing an indispensable role in peroxisome biogenesis. These genes were first identified in different yeast species. In the last few years, however, most of the corresponding human homologues have been identified too which has allowed molecular analysis in patients affected by a disorder of peroxisome biogenesis (PBD) (see later).

The proteins encoded by the various PEX-genes are called peroxins and virtually all of them are localised in the peroxisomal membrane playing a role in the complicated machinery required for the transmembrane transport of peroxisomal proteins. Pex5p and Pex7p are exceptions since they are largely cytosolic proteins. Pex5p is the receptor which picks up proteins containing a PTS1-motif and directs them to the peroxisome whereas Pex7p does the same for PTS2-proteins. Figure 25.5 depicts the current model for peroxisome biogenesis as favoured by Gould and Valle [3].

## ■ The Peroxisomal Disorders

### ● *The Disorders of Peroxisome Biogenesis*

Zellweger syndrome (ZS) is generally considered to be the prototype of the group of peroxisomal disorders. ZS belongs to the “multiple congenital anomaly” disease category and is dominated by: 1. The typical craniofacial dysmorphism including a high forehead, large anterior fontanel, hypoplastic supraorbital ridges, epicanthal folds, and deformed ear lobes and 2. Profound neurological abnormalities. ZS children show severe psychomotor retardation, profound hypotonia, neonatal seizures, glaucoma, retinal degeneration and impaired hearing. There is usually calcified stippling of the epiphyses and small renal cysts. Brain abnormalities in Zellweger syndrome include cortical dysplasia, and neuronal heterotopia but also regressive changes. There is dysmyelination rather than demyelination. In Zellweger syndrome peroxisome biogenesis is defective due to mutations in one of the many PEX-genes.

Apart from Zellweger syndrome with its neonatal onset and early fatal course, the disorders of peroxisome biogenesis also include neonatal adrenoleukodystrophy (NALD) with a less progressive course and leukodystrophy, and infantile Refsum disease (IRD) with the least severe course. Since the clinical features between Zellweger syndrome, NALD and IRD are strongly overlapping it is often difficult to assign a particular patient to one of these clinical subtypes.

When the group of patients suffering from a disorder of peroxisome biogenesis is taken together, roughly 2 profiles can be distinguished which should both warrant detailed biochemical investigations.

These include:

1. A neonatal profile, essentially involving: a. severe muscular hypotonia, b. seizures, c. hepatic dysfunction including mixed hyperbilirubinemic jaundice, d. dysmorphic signs.
2. An infantile profile, essentially involving: a. retinopathy often leading to early blindness, b. sensorineural deafness, c. hepatic dysfunction that may involve symptoms of portal hypertension, d. mental deficiency, e. (often) failure to thrive, f. dysmorphic signs.

Bony changes in the neonatal and infantile group, related to the impairment in etherphospholipid synthesis, involve a large fontanel which only closes after the first birthday, osteopenia of long bones, and, in approximately half of the cases, calcified stippling in the epiphyseal and periarticular regions of large joints, especially the patellar region. Cerebral abnormalities in the neonatal and infantile groups essentially comprise two sets of abnormalities: (1) neocortical dysgenesis, due to neuronal migration failure arising during the embryo-fetal period; (2) metabolic changes involving the process of myelination, and in some cases leading to later demyelination.

### ● *The Peroxisomal $\beta$ -Oxidation Disorders*

Four defined disorders of peroxisomal  $\beta$ -oxidation have been identified including: (1) X-linked adrenoleukodystrophy/adrenomyeloneuropathy, (2) acyl-CoA oxidase deficiency (3) D-bifunctional protein deficiency and (4) peroxisomal thiolase deficiency. The clinical presentation of the latter 3 disorders resembles that of the disorders of peroxisome biogenesis whereas the clinical presentation of X-linked ALD/AMN patients is completely different which explains why it is better to discuss this entity separately under d.

*Acyl-CoA oxidase deficiency* was first described in 1988 by Poll-The et al. [4] under the name pseudo-neonatal adrenoleukodystrophy since the 2 patients described had the typical signs and symptoms described for neonatal adrenoleukodystrophy by Kelley et al. [5]. However, peroxisomes were present in the patients' liver albeit of enlarged size. In addition THCA and DHCA were completely normal.

*D-bifunctional protein deficiency (D-BP)*, the second peroxisomal  $\beta$ -oxidation disorder, was only delineated recently [6–9]. All patients identified so far (>40: see [10]) show severe clinical abnormalities including hypotonia, craniofacial dysmorphism, neonatal seizures, hepatomegaly, and developmental delay. Most patients with D-BP deficiency die in the first year of life. A remarkable observation is that patients with D-BP deficiency show disordered neuronal migration as in ZS.

*Peroxisomal thiolase deficiency*, originally described as pseudo-Zellweger syndrome, has so far been described in only a single patient and remains ill-defined [11].

### ● *Rhizomelic Chondrodysplasia Punctata (RCDP) Type 1, 2 and 3*

The third group of disorders that can be treated as a relatively distinct entity includes rhizomelic chondrodysplasia and its variants. The classic prototype of this disorder involves severe growth failure, proximal shortening of extremities, contractures, spasticity, mental deficiency and cataracts. The clinical picture is highly specific and a presumptive diagnosis of RCDP can be made on clinical grounds. The finding of a deficiency of erythrocyte plasmalogens confirms the diagnosis. Furthermore, plasma phytanic acid levels are usually increased due to the fact that phytanic acid  $\alpha$ -oxidation is deficient in RCDP next to the deficient activities of DHAPAT and alkyl-DHAP synthase (see Fig. 25.3), and the aberrant molecular weight of peroxisomal thiolase (44 kDa instead of 41 kDa).

Patients with the clinical picture of classical RCDP but with isolated deficiencies of DHAPAT and alkyl-DHAP synthase have also been described (see [10] for review). In these patients phytanic acid is normal, which would suggest that distinction between classical RCDP and the two variants

can best be made on the basis of plasma phytanic acid levels. This may lead to erroneous conclusions, however, the reason being that phytanic acid levels may be normal in young patients affected by classical RCDP since phytanic acid is derived from dietary sources only. Furthermore, some patients have been described with a milder form of classical RCDP [12], with normal plasma phytanic acid levels despite a deficiency of phytanic acid  $\alpha$ -oxidation in fibroblasts as part of the classical tetrad of four abnormalities. Milder phenotypes, showing atypical skeletal dysplasia, mental retardation and, in most cases, cataracts are known [13]. Such cases have been found both in the group of multiple peroxisomal abnormalities as well as with isolated DHAPAT deficiency [14].

- *X-linked Adrenoleukodystrophy (X-ALD)/Adrenomyeloneuropathy (AMN)*

X-linked adrenoleukodystrophy/adrenomyeloneuropathy comprises a group of disorders with cerebral, spinal long-tract and endocrine symptoms in affected males, and to some extent also in female heterozygotes. A pattern of leukodystrophy arises in affected boys between 7 and 14 years of age and usually leads to a vegetative state within 3 years after clinical onset. A pattern of spastic paraparesis, sphincter insufficiencies and a mixed demyelinating and axonal peripheral polyneuropathy is seen in adult males and symptomatic heterozygotes. Other patterns are also known. Despite the large variability in clinical expression, all patients affected seem to suffer from defects in the same gene, which encodes a peroxisomal membrane protein belonging to the ABC-superfamily of transporters (which also includes CFTR) but with unknown function [15]. Biochemical diagnosis is based on measurement of VLCFA in plasma, which is reliable except in some exceptional cases. Additional studies in fibroblasts, including immunological analysis of the ALD protein and mutation analysis, should be performed.

- *Refsum Disease*

Characteristic manifestations of the disease include retinitis pigmentosa, cerebellar ataxia, chronic polyneuropathy, and an elevated CSF protein level. Less constant features include sensorineural hearing loss, anosmia, ichthyosis, skeletal malformations, and cardiac abnormalities. The clinical picture of Refsum disease is often that of a slowly developing, progressive peripheral neuropathy manifested by severe motor weakness and muscular wasting, especially of the lower extremities. Onset has occasionally been detected in early childhood but not until the fifth decade in others. Most patients have clear-cut manifestations before 20 years of age. Patients with Refsum disease may die suddenly probably from cardiac arrhythmias [16].

### ● *$\alpha$ -Methylacyl-CoA Racemase Deficiency*

We recently identified a new peroxisomal disorder in two patients suffering from an adult-onset sensorimotor neuropathy [17]. The enzyme involved catalyzes the interconversion of (2R)- and-(2S)-stereoisomers of  $\alpha$ -methyl-branched-chain fatty acids like pristanic acid, THCA and DHCA whereas this enzyme is not involved in VLCFA  $\beta$ -oxidation, which explains the accumulation of THCA, DHCA and pristanic acid but not C26:0 in these patients. The clinical picture has some resemblance to that of Refsum disease with sensorimotor neuropathy.

## 25.2 Nomenclature

No.	Disorder	Protein/synonym	Tissue	Chromosome	MIM
25.1	Zellweger syndrome (ZS)	Peroxisins	Generalized	Multiple loci	214100 214110
25.2	Neonatal adrenoleukodystrophy (NALD)	Peroxisins	Generalized	Multiple loci	202370
25.3	Infantile Refsum disease (IRD)	Peroxisins	Generalized	Multiple loci	266510
25.4	Hyperpipecolic acidemia (HPA) <sup>a</sup>	Peroxisins	Generalized	Multiple loci	239400
25.5	(Rhizomelic) chondrodysplasia punctata (RCDP) type 1	Pex7p	Generalized	6q22-q24	215100
25.6	(Rhizomelic) chondrodysplasia punctata (RCDP) type 2 (acyltransferase (DHAPAT) deficiency)	DHAPAT	Generalized	1q42.1-42.3	222765
25.7	(Rhizomelic) chondrodysplasia punctata (RCDP) type 3 (alkyl-DHAP synthase deficiency)	Alkyl-DHAP synthase	Generalized	2q31	600121
25.8	X-linked adrenoleukodystrophy (XALD)	ALD protein	Generalized	Xq28	300100
25.9	Acyl-CoA oxidase deficiency (pseudo-neonatal adrenoleukodystrophy)	Straight-chain acyl-CoA oxidase (SCOX)	Generalized	17q25	264470
25.10	D-Bifunctional enzyme deficiency (D-BP deficiency)	D-bifunctional protein (DBP)	Generalized	5q2	261515
25.11	Peroxisomal 3-ketothiolase deficiency (pseudo-Zellweger syndrome)	Peroxisomal thiolase 1 (pTh1)	Generalized	3p23-p22	261510
25.12	$\alpha$ -Methylacyl-CoA racemase deficiency	AMACR	Generalized	5q13.2-q11.1	604489
25.13	Refsum disease (adult form) (phytanoyl-CoA hydroxylase deficiency)	Phytanoyl-CoA hydroxylase	Generalized	10pter-p11.2	266500

<sup>a</sup> Hyperpipecolic acidemia (HPA) will probably turn out to be heterogeneous. The four patients with HPA described in literature, have now been found to be affected by a peroxisome biogenesis defect whereas in other patients in literature have been described in which peroxisome biogenesis is normal, the accumulation of pipecolic acid being secondary to a defect in pipecolic acid metabolism which remains to be identified (see ref. [4] for discussion).

### 25.3 Metabolic Pathways

Figure 25.2 shows the organisation of the peroxisomal  $\beta$ -oxidation machinery involved in the oxidation of very-long-chain fatty acids like C26:0, pristanic acid and DHCA and THCA. Peroxisomes contain *two* acyl-CoA oxidases, *two* bifunctional proteins and *two* thiolases. Current knowledge holds (see [10] for review) that the oxidation of the CoA-esters of C26:0 on the one hand and pristanic acid and DHCA and THCA on the other hand is catalyzed by the two different acyl-CoA oxidases, respectively (see Fig. 25.2).

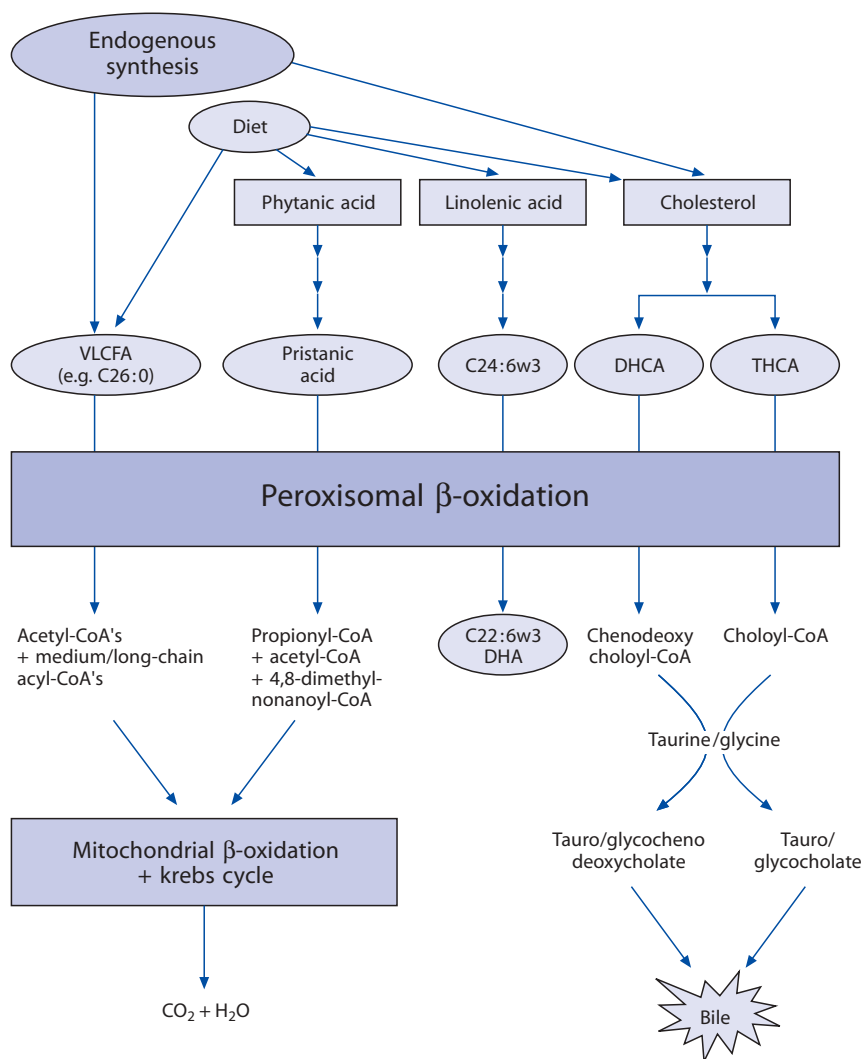
In line with this notion, pristanic acid, DHCA and THCA are completely normal in patients with straight-chain acyl-CoA oxidase (SCOX/ACOX1 = enzyme 1 in Fig. 25.2) deficiency.

The situation with respect to the subsequent steps in peroxisomal  $\beta$ -oxidation has long remained an enigma but recent data have resolved this. It turns out that it is *not* the originally discovered bifunctional protein which forms and dehydrogenates L-3-hydroxyacyl-CoA esters but the more recently discovered D-bifunctional protein which forms and dehydrogenates D-3-hydroxyacyl-CoAs which is involved in the  $\beta$ -oxidation of C26:0, pristanic acid and the two cholestanoic acids DHCA and THCA (Fig. 25.2).

The role of the two thiolases in peroxisomal  $\beta$ -oxidation has also been largely resolved, especially with respect to the role of peroxisomal thiolase 2 which is the suggested name for the 58 kDa protein with sterol-carrier-protein (SCP) and thiolase activity (SCPx). Studies in mutant mice have shown that this enzyme plays an indispensable role in the oxidation fatty acids with an  $\alpha$ -methyl side chain as in pristanic acid, DHCA and THCA. Most likely, pTH1 and pTH2 are both involved in C26:0  $\beta$ -oxidation as concluded from the fact that C26:0  $\beta$ -oxidation is normal in both pTH1-deficient fibroblasts as well as in pTH2-(SCPx)-deficient fibroblasts (see [1]).

Figures 25.3 and 25.4 depict the enzymology of the etherphospholipid biosynthesis and phytanic acid  $\alpha$ -oxidation systems, respectively (see legends for detailed information).





**Fig. 25.1.** Schematic representation of the role of the peroxisomal  $\beta$ -oxidation system in the oxidation of C26:0, pristanic acid, di- and trihydroxycholestanoic acid and C24:6

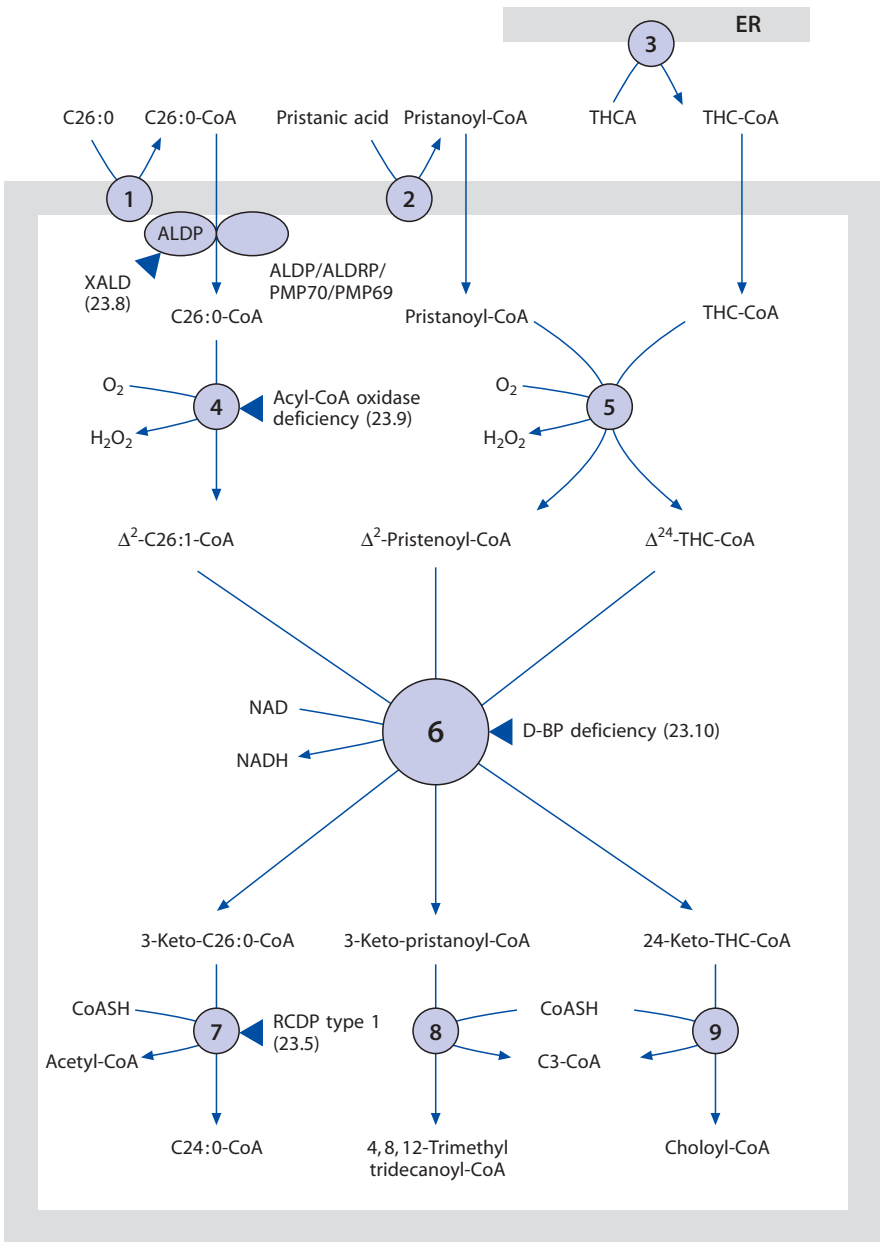
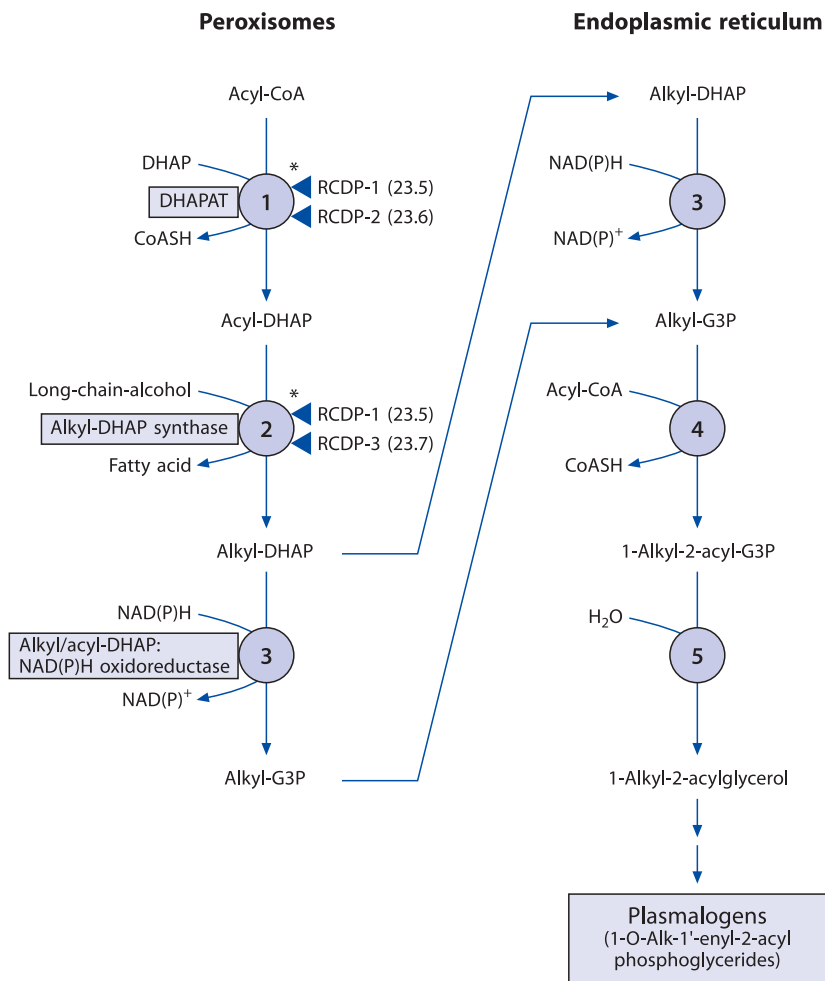
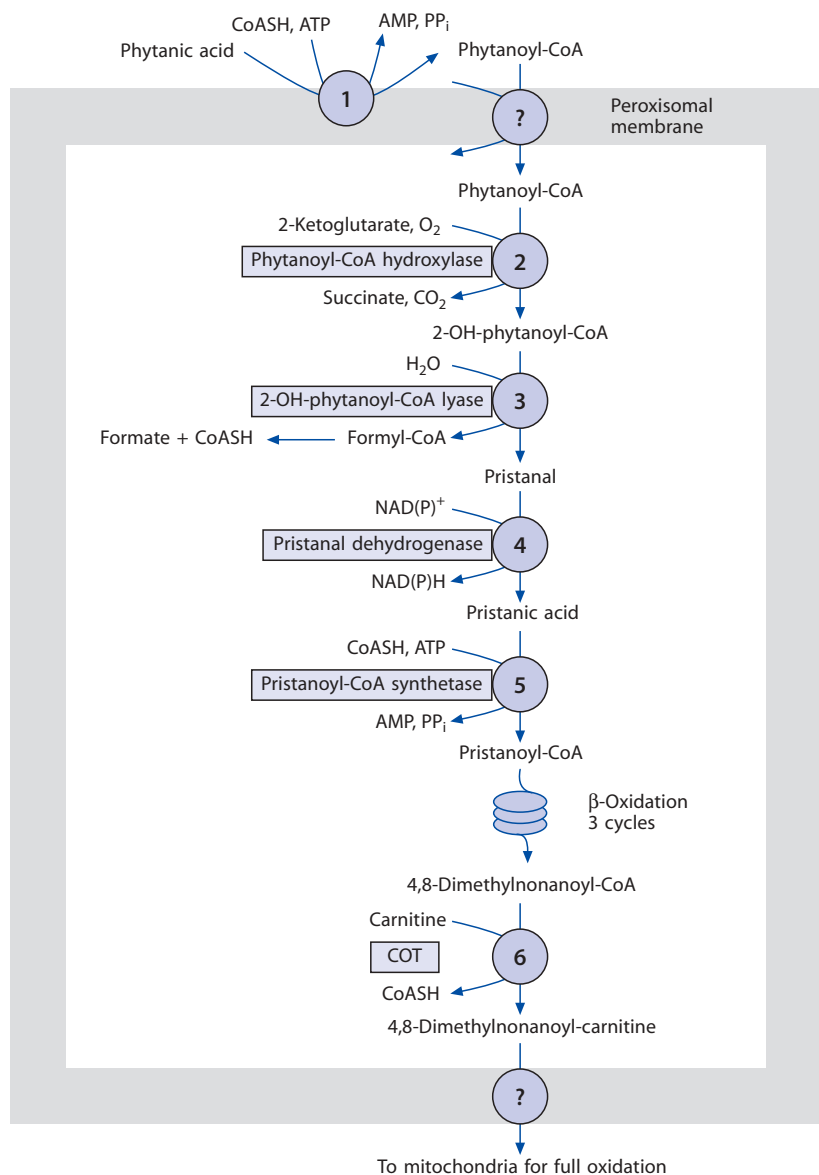


Fig. 25.2

**Fig. 25.3.** Pathway for the synthesis of ether-linked phospholipids and especially plasmalogens (1-O-alk-1'-enyl-2-acylphosphoglycerides) which are the primary end product of etherphospholipid biosynthesis in humans. Biosynthesis starts in the peroxisome with the production of dihydroxyacetonephosphate (DHAP) to acyl-DHAP via the peroxisomal enzyme dihydroxyacetonephosphate acyltransferase (DHAPAT) followed by the introduction of the typical ether-bond by the enzyme alkyl-DHAP synthase. Both these enzymes are strictly peroxisomal. The third step is catalyzed by the enzyme alkyl/acyl-DHAP:NAD(P)H oxidoreductase which has a *bimodal* distribution in both peroxisomes and endoplasmic reticulum. The product alkylglycerol-3-phosphate (alkylG3P) then undergoes acylation to form 1-alkyl-2-acyl-G3P followed by hydrolysis to produce 1-alkyl-2-acyl-glycerol which can then be converted into the different 1-alkyl-2-acyl-phosphoglycerides and 1-O-alk-1'-enyl-2-acyl-phosphoglycerides (plasmalogens). The asterisk in the figure indicates that the enzyme is also deficient in the disorders of peroxisome biogenesis



**Fig. 25.2.** Enzymology of the peroxisomal fatty acid  $\beta$ -oxidation system. Human peroxisomes contain two acyl-CoA oxidases, *one* specific for straight-chain fatty acids like C26:0, therefore called straight-chain acyl-CoA oxidase (SCOX or ACOX1) and a *second* one, catalysing the dehydrogenation of  $\alpha$ -methyl branched-chain fatty acids like pristanoyl-CoA and di- and trihydroxycholestanoil-CoA. The latter enzyme is called branched-chain acyl-CoA oxidase (BCOX or ACOX2). The enoyl-CoA esters of C26:0, pristanic acid and DHCA and THCA are all handled by a single bifunctional enzyme harboring both enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activity (see text under 25.3). The newly identified bifunctional protein which forms and dehydrogenates D-3-hydroxy-acyl-CoA esters rather than L-3-hydroxyacyl-CoAs, is the single enzyme involved in the oxidation of C26:0, pristanic acid and DHCA and THCA. With respect to the two peroxisomal thiolases, pTH1 and pTH2 (=SCPx), it is clear now that pTH2 is involved in the oxidation of  $\alpha$ -methyl fatty acids. Current evidence holds that pTH1 and pTH2 are both involved in C26:0  $\beta$ -oxidation which explains why a deficiency of either pTH1 or pTH2 is *not* associated with a defect in C26:0  $\beta$ -oxidation. The obligatory involvement of the X-linked adrenoleukodystrophy protein (ALDP) in C26:0  $\beta$ -oxidation is shown. ALDP is a so-called half ABC-transporter which can either form homo- or heterodimers. The protein is localized in the peroxisomal membrane and probably transports C26:0-CoA esters across the peroxisomal membrane. The asterisk (\*) denotes that this enzyme is also deficient in the disorders of peroxisome biogenesis



**Fig. 25.4.** Structure and enzymology of the peroxisomal phytanic acid  $\alpha$ -oxidation system. Phytanic acid first undergoes activation to phytanoyl-CoA, probably by the long-chain acyl-CoA synthetase (LACS) present at the outer aspect of the peroxisomal membrane and then enters the peroxisomal matrix, probably via metabolite carrier protein. Once inside peroxisomes, phytanoyl-CoA undergoes hydroxylation by the enzyme phytanoyl-CoA hydroxylase deficient in adult Refsum disease. The same enzyme is also deficient in RCDP type 1 (but not in type 2 and 3) since the hydroxylase is a PTS2-protein (see text). 2-Hydroxyphytanoyl-CoA then undergoes cleavage to pristanal followed by its subsequent oxidation to pristanic acid. After activation to its CoA-esters, pristanoyl-CoA undergoes 3 cycles of  $\beta$ -oxidation in the peroxisome to produce 4,8-dimethylnonanoyl-CoA which leaves the peroxisome in the form of a carnitine ester to undergo full oxidation in mitochondria.

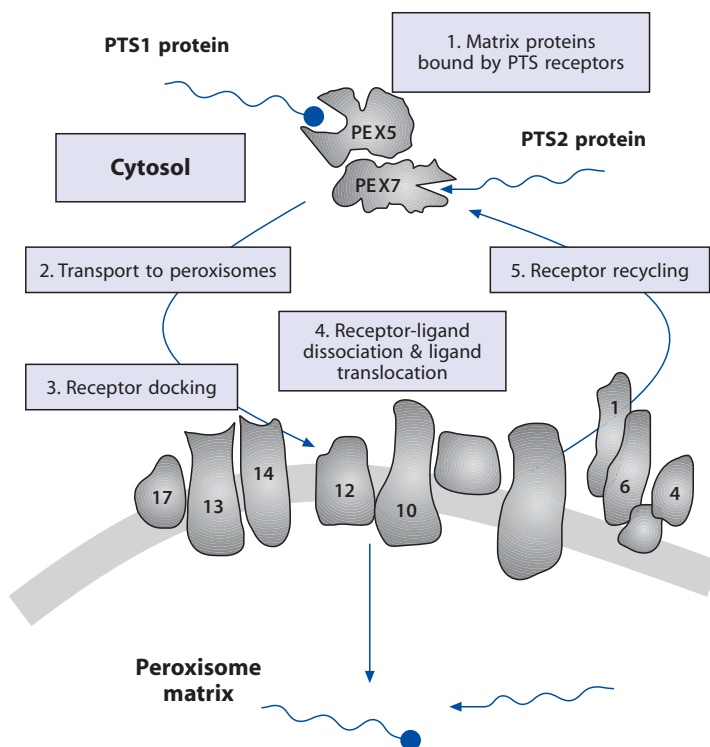


Fig. 25.5. Current model for peroxisome biogenesis [3]. See text

## 25.4 Signs and Symptoms

**Table 25.1.** ZS (25.1), NALD (25.2) and IRD (25.3) (>1000 patients), acyl-CoA oxidase deficiency (25.9) (2 patients). D-BP deficiency (25.10) (>40 patients), 3-ketothiolase deficiency (25.11) (1 patient)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Cataracts	±	±	±
	Sensorineural deafness		+	+
	Jaundice	±	±	--
	Liver enlargement	±	±	±
	Muscle hypotonia	±	±	±
	Fontanel enlarged	+	+	±
	Failure to thrive	±	±	±
	Mental deficiency		+	+
	Dysmorphic features	+	+	+
	X-ray: epiphyseal and periarticular calcific stippling	±	±	-
	X-ray: osteopenia		+	+
	Ultrasonography: renal cysts	±	±	-
	MRI: cerebral white matter involvement	±	±	±
	MRI: cerebral neocortical dysplasia	±	±	±
	ERG extinguished		+	+
	BAEP diminished response		+	+
Routine laboratory	ASAT/ALAT (P)	↑	↑	↑
	Bilirubin (P)	n-↑	n-↑	-
	Cholesterol (P)		↓-n	↓-n
Special laboratory	VLCHA (S)	↑	↑	↑
	Bile acids intermediates (P)	↑-n	↑-n	↑-n
	Pipelic acid (S, U)	n-↑	↑-n	↑-n
	Dicarboxylic acids (U)	n-↑	n-↑	n-↑
	Phytanic acid (S)	n-↑	↑-n	↑-n
	Pristanic acid (S)	n-↑	↑-n	↑-n
	Plasmalogens (RBC)	↓-n	↓-n	↓-n
	Docosahexaenoic acid (S, RBC)	↓	↓-n	↓-n
	Fat-soluble vitamins (S)	↓-n	↓	↓
	Adrenocortical reserve		↓	↓
GI	Diarrhoea	-	±	±
Eye	Diminished visual acuity		+	+
Inner ear	Sensorineural deafness		+	+
Liver	Hepatomegaly	±	±	±
	Portal hypertension	-	±	±
CNS	Jaundice	±	±	-
	Seizures	±	±	±
	Mental deficiency		+	+
	Delayed motor milestones		+	+
	Spastic pareses	-	-	±
Neuromuscular system	Hypotonia	±	±	±
	Clubfeet	±	±	±

ERG, Electroretinogram; BAEP, brainstem acoustic evoked potential; GI, Gastro-intestinal tracts; CNS, Central nervous system.

**Table 25.2.** Rhizomelic chondrodysplasia punctata type 1 (Pex7-deficiency) (25.5), type 2 (DHAPAT deficiency) (25.6) and type 3 (alkyl-DHAP synthase deficiency) (25.7): classical forms (80/10/10 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Shortening of long bones, disproportionately affecting humeri and femora	+	+	+
	Joint contractures	+	+	+
	Cataracts	+	+	+
	Microcephaly		±	+
	Severe mental deficiency		+	+
	Growth disturbance	±	+	+
X-ray	Epiphyseal and periarticular calcific stippling	+	+	±
	Metaphyseal and epiphyseal dysplasia	+	+	+
	Coronal clefts of thoracic and lumbar vertebral bodies	±	±	±
Special laboratory	VLCFA (S)	n	n	n
	Bile acids intermediates (P)	n	n	n
	Phytanic acid (S)	n-↑	n-↑	n-↑
	Pristanic acid (S)	↓-n	↓-n	↓-n
	Plasmalogens (RBC)	↓	↓	↓
Eye	Cataracts, blindness	+	+	+
Inner ear	Sensorineural deafness		±	±
CNS	Severe mental deficiency		+	+
	Spastic pareses		±	±
	Epilepsy		±	±
Skeletal system	Shortening of long bones	+	+	+
	Severe growth disturbance	±	+	+
	Contractures	+	+	+
	Skeletal dysplasia	+	+	+
Skin	Ichthyosis		±	±
Respiratory system	Increased rate of infections: pneumonia, otitis		+	+

**Table 25.3.** Rhizomelic chondrodysplasia punctata type 1 (Pex7-deficiency) (25.5) and type 2 (DHAPAT deficiency) (25.6): atypical forms (5–10 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Joint contractures		±	±
	Cataracts		±	±
	Mental deficiency		+	+
	Growth disturbance		+	+
X-Ray	Epiphyseal and periarticular calcific stippling		±	±
	Metaphyseal and epiphyseal dysplasia		±	±
Special laboratory	VLCFA (P)		n	n
	Bile acids intermediates (S)		n	n
	Phytanic acid (S)		n-↑	n-↑
	Pristanic acid (S)		↓-n	↓-n
	Plasmalogens (RBC)		↓	↓
Eye	Cataracts		±	±
CNS	Mental deficiency		+	+
Skeletal system	Growth disturbance		+	+
	Contractures		±	±
	Skeletal dysplasia		+	+

**Table 25.4.** X-linked adrenoleukodystrophy (25.8) (>2000 patients)

System	Symptoms/markers	Neonatal male	Infancy male	Childhood male	Adolescence male	Adult male	Adult female
Characteristic clinical findings	Visual loss			±	±	±	±
	Perceptive hearing loss			±	±	±	-
	Spastic pareses			±	±	±	±
	Epilepsy			±	±	±	±
	Dementia			±	±	±	±
	Sphincter problems			±	±	±	±
	Sexual impotence				±	±	-
	Addison syndrome			±	±	±	±
	Electrolyte changes			±	±	±	±
	MRI: signs of leukodystrophy			±	±	±	±
	EEG, VEP, BAER abnormalities			±	±	±	±
	Peripheral nerve involvement			±	±	±	±
Special laboratory	VLCFA (S)	↑	↑	↑	↑	↑	n-↑
	Diminished adrenocortical reserve			±	±	±	±
	Endocrine gonadal failure			±	±	±	



**Table 25.5.** *α*-Methylacyl-CoA racemase deficiency (25.12) (3 patients)

System	Symptoms/markers	Childhood	Adulthood
Characteristic clinical findings	Motor retardation	+	(+)
	Sensorimotor neuropathy		±
	Convulsions		(+)
	Spastic paraparesis		(+)
Routine laboratory	ASAT, ALAT (P)	↑	
	Alkaline phosphatase (P)	↑	
	Phosphate (U)	↑	
	Amino acids (U)	↑	
Special laboratory	Phytanic acid (P)	↑	(↑)
	Pristanic acid (P)	↑	↑
	C <sub>27</sub> -bile acid (P)	↑	↑
	C <sub>26:0</sub> fatty acid (P)	n	n
GI	Diarrhoea	+	–

**Table 25.6.** Refsum disease (25.13) (>200 patients)

System	Symptoms/markers	Infancy male	Childhood male	Adolescence male	Adult male
Characteristic clinical findings	Loss of night vision	±	±	±	±
	Progressive retinitis pigmentosa	±	±	±	±
	Anosmia	±	±	±	±
	Deafness	±	±	±	±
	Paresis/atrophy	±	±	±	±
	Sensory disturbances	±	±	±	±
	Skeletal malformations		±	±	±
	Cardiac abnormalities	±	±	±	±
	Ataxia	±	±	±	±
Special laboratory	Phytanic acid (P)	↑	↑	↑	↑
	Pristanic acid (P)	↓	↓	↓	↓

## 25.5 Reference Values (Plasma)

Substance	Level (μmol/l)
Hexacosanoic acid (C26:0)	0.79 (mean value) 0.45–1.32 (5–95% interval)
Trihydroxycholestanoic acid (THCA)	<0.05
Dihydroxycholestanoic acid (DHCA)	<0.05
Phytanic acid	<10
Pristanic acid	<3
Plasmalogens (RBC)	16:0 6.9–11.9% 18:0 10.6–24.9%

## 25.6 Pathological Values

Type of peroxisomal disorder		Plasma				Erythrocytes Plasmalogens
		C26:0	THCA	PHYT	PRIS	
25.1	Zellweger syndrome	↑↑	↑	n-↑ <sup>a</sup>	n-↑ <sup>a</sup>	↓
25.2–	Neonatal adrenoleukodystrophy, infantile Refsum	↑	↑	n-↑ <sup>a</sup>	n-↑ <sup>a</sup>	n
25.4	disease					
25.5	Rhizomelic chondrodysplasia punctata, type 1	n	n	n-↑ <sup>a</sup>	↓	↓
25.6	Rhizomelic chondrodysplasia punctata, type 2	n	n	n	n	↓
25.7	Rhizomelic chondrodysplasia punctata, type 3	n	n	n	n	↓
25.8	X-linked adrenoleukodystrophy	↑	n	n	n	n
25.9	Acyl-CoA oxidase deficiency	↑	n	n	n	n
25.10	D-Bifunctional protein deficiency	↑	n-↑	n-↑ <sup>a</sup>	n-↑ <sup>a</sup>	n
25.11	Peroxisomal thiolase deficiency	↑	↑	?	?	n
25.12	$\alpha$ -Methylacyl-CoA racemase deficiency	n	↑	n-↑	↑	n
25.16	Refsum disease	n	n	↑	↓	n

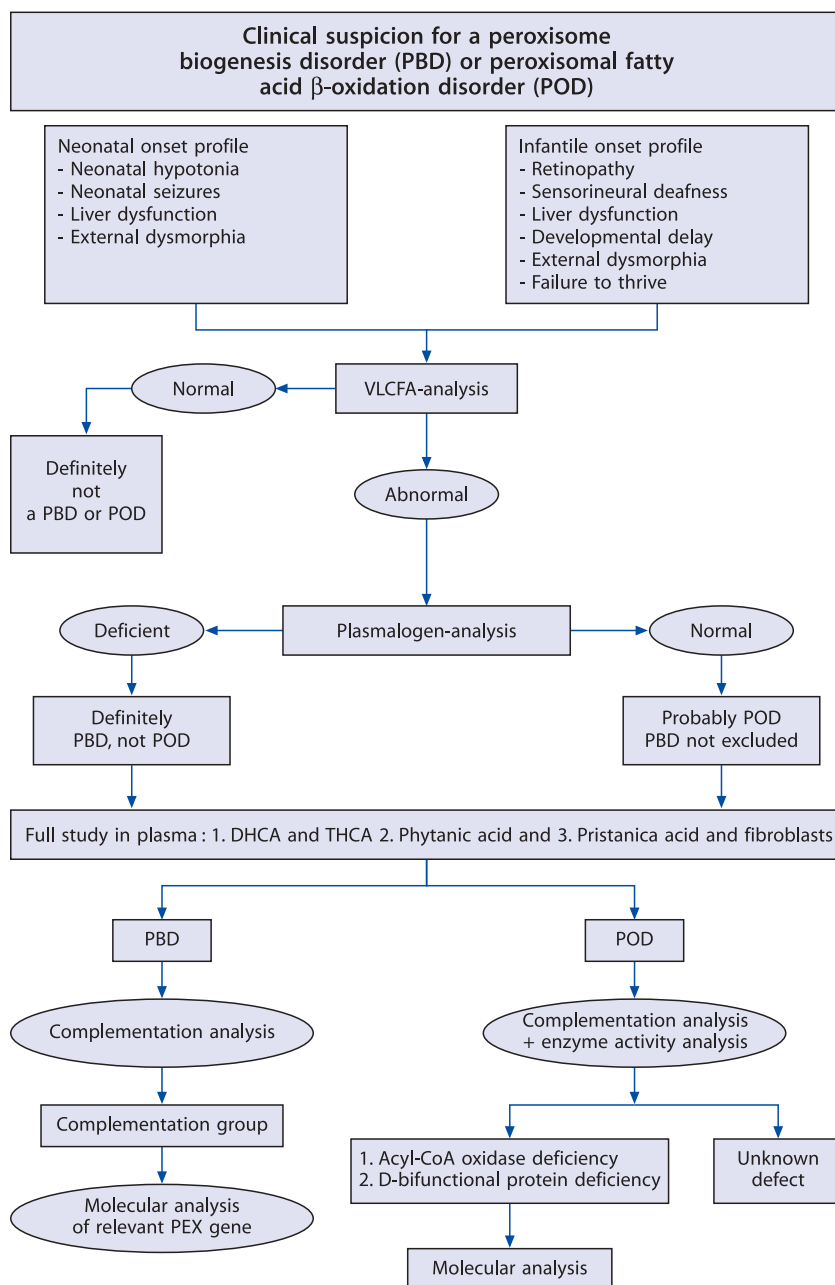
<sup>a</sup> Phytanic acid and pristanic acid may be normal despite a block in their oxidation since both fatty acids are only derived from exogenous, dietary sources.

## 25.7 Loading Tests

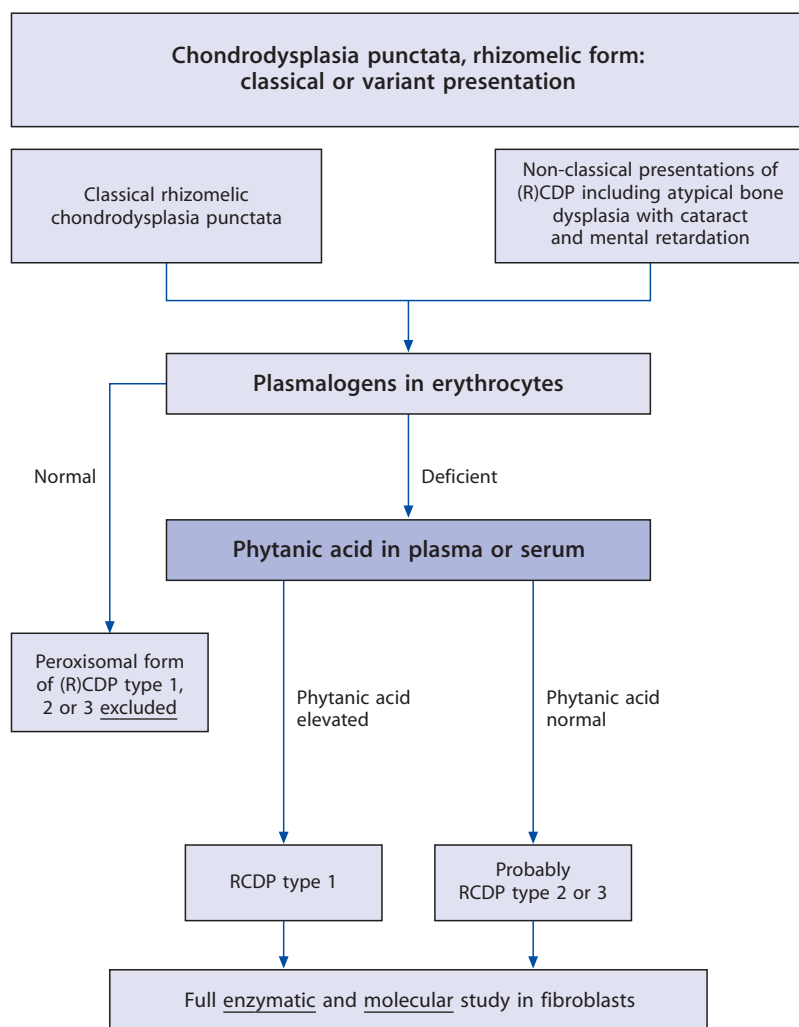
No loading tests have been described for any of the peroxisomal disorders.

## 25.8 Diagnostic Flow Charts

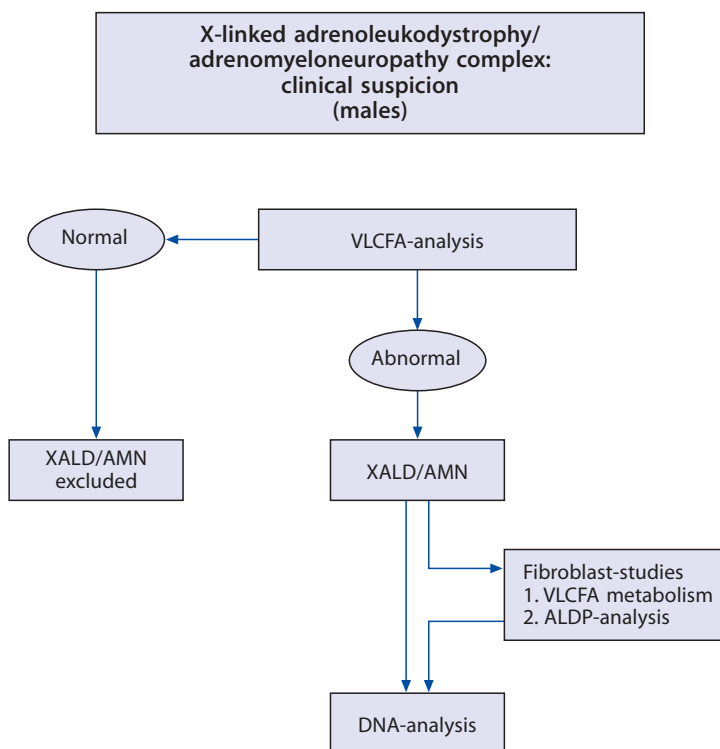
From a diagnostic point of view the group of peroxisomal disorders can be subdivided into 4 distinct categories including: (1) the peroxisome biogenesis disorders (PBDs) and the peroxisomal  $\beta$ -oxidation disorders (PODs), (2) the (non)rhizomelic chondrodysplasia punctata (RCDP) group, (3) the X-linked adrenoleukodystrophy/adrenomyeloneuropathy complex and (4) the remaining disorders which share very little with the disorders under groups 1, 2 and 3 and should be treated individually.



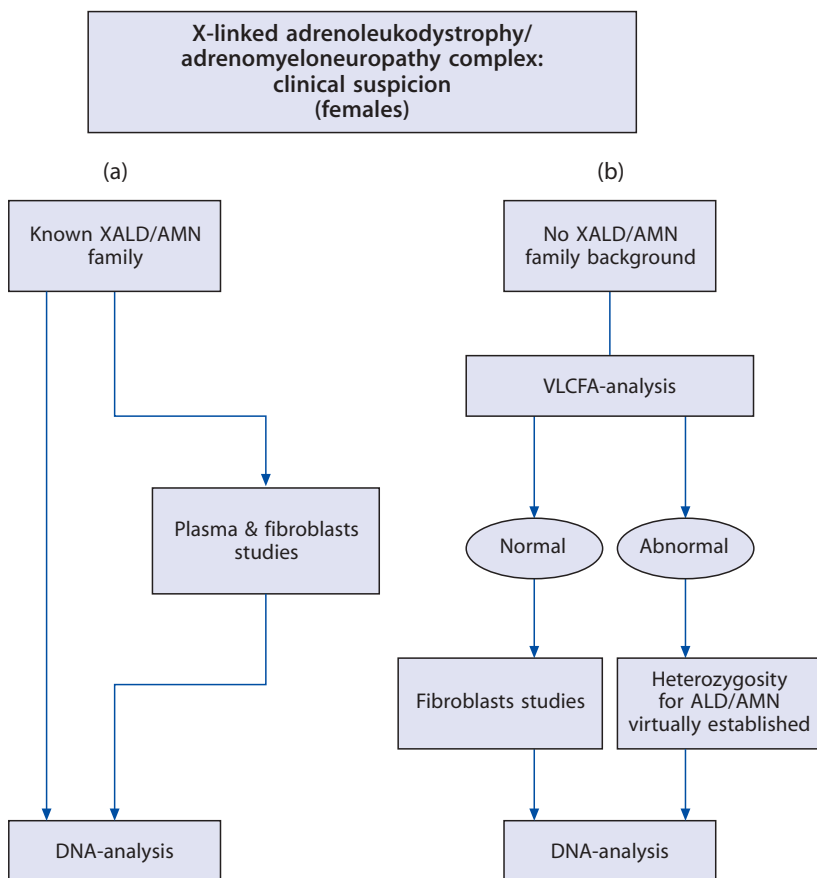
**Fig. 25.6.** Flow chart for the differential diagnosis of patients suffering from a peroxisome biogenesis disorder (PBD) or a peroxisomal  $\beta$ -oxidation disorder (POD)



**Fig. 25.7.** Flow chart for the differential diagnosis of rhizomelic chondrodysplasia punctata (RCDP) type 1, 2 or 3



**Fig. 25.8.** Flow chart for the diagnosis of X-linked adrenoleukodystrophy/adrenomyeloneuropathy (males)



**Fig. 25.9.** Flow chart for the diagnosis of X-linked adrenoleukodystrophy/adrenomyeloneuropathy (females)

#### ■ Peroxisome Biogenesis Disorders (PBDs) and the Peroxisomal $\beta$ -Oxidation Disorders (PODs) (Fig. 25.6)

When a patient is suspected to suffer from a PDB or POD, the first analysis to be made is analysis of very-long-chain fatty acids. If abnormal, it is clear that the patient is either affected by a PDB or POD. If normal, a PDB or POD is excluded. This conclusion is based on our analyses in >500 established patients in which VLCFA analysis has been found to be 100% reliable. Usually, if VLCFAs have been found to be abnormal, we immediately ask for fibroblasts since a detailed analysis in fibroblasts is *absolutely* essential to ascertain with full certainty whether peroxisome biogenesis is primarily affected or whether one is dealing with a peroxisomal  $\beta$ -oxidation disorder.

In the meantime plasmalogen levels should be measured in erythrocytes, using the method described by Bjorkhem et al. [18] or another established method like the one described by Vreken et al. [19]. If plasmalogens are decreased, a peroxisome biogenesis defect is established. If plasmalogens are normal, however, it probably is a POD but it may also be a peroxisome biogenesis defect simply because in milder PDB forms, notably infantile Refsum disease patients, plasmalogens may be completely normal.

In addition to the measurement of plasmalogens, we measure the levels of the peroxisomal metabolites di- and trihydroxy/cholestanoic acid, phytanic acid and pristanic acid which may also help to reach to correct diagnosis (see Fig. 25.6).

If a PDB has been established, complementation analysis needs to be done followed by analysis of the relevant PEX-gene. This is done in only few laboratories in the world including our own and that of Moser, Gould and Valle.

If a POD has been established, the nature of the true enzymatic defect needs to be determined using direct enzyme assays for acyl-CoA oxidase, D-bifunctional protein [8] and the peroxisomal thiolases [20] followed by molecular analyses. If no abnormalities are found, complementation analysis is warranted (see [10]).

#### ■ Rhizomelic Chondrodysplasia Punctata (RCDP), Classical and Variant Phenotypes (Fig. 25.7)

If a patient is suspected to suffer from RCDP, either the classical or variant phenotype, erythrocyte plasmalogens should be determined immediately using the simple method described by Bjorkhem et al. [18] or any other established method including the one described by Vreken et al. [19]. The finding of deficient plasmalogens implies that one is dealing with a peroxisomal form of RCDP, either type 1, 2 or 3. This can be resolved by detailed studies in fibroblasts which involves a series of enzymatic studies including determination of DHAPAT and alkylDHAP synthase activities, immunoblot analysis, phytanic acid  $\alpha$ -oxidation, and phytanoyl-CoA hydroxylase activity measurement, de novo plasmalogens biosynthesis, a.o. Finally, mutation analysis needs to be done to identify the true molecular basis (see [10] for review).

#### ■ X-linked Adrenoleukodystrophy/Adrenomyeloneuropathy Complex (Figs. 25.8 and 25.9)

Two diagnostic flow charts need to be considered depending whether one is dealing with a male or female patient.

(a) If a *male* patient is suspected to suffer from some form of XALD/AMN, very-long-chain fatty acids should be analyzed which is unequivocal

in *all* cases with one or two possible exceptions reported in literature. If abnormal, XALD/AMN is virtually established. This should be followed up by DNA analyses in lymphocytes prepared from the same sample. We usually do fibroblast studies too to ascertain that C26:0  $\beta$ -oxidation is, indeed, deficient in the patient's cells. Furthermore, this allows us to do immunofluorescence studies to establish whether the ALD-protein is present or not which is the case in about 75% of XALD/AMN-patients. If ALDP is absent, this can be of great help in carrier detection (see below).

(b) *Females*: if a female is suspected to suffer from XALD/AMN in its heterozygous form, *two* situations must be distinguished. The simplest scenario is when the lady is from a *known* XALD/AMN family. In that case, VLCFA analysis can be done but much better is to do DNA analysis right away. If the lady has no family background of XALD/AMN, we suggest to perform the complete diagnostic program including: (1) VLCFA analysis in plasma/serum, (2) VLCFA- and ALDP-immunofluorescence analysis in fibroblasts and ultimately (3) DNA-analysis.

## 25.9 Specimen Collection

Very-long-chain fatty acids (VLCFA), trihydroxycholestanoic acid (THCA), phytanic acid and pristanic acid can all be measured in plasma/serum ( $\geq 1$  ml). From the same blood sample erythrocytes, platelets and/or leukocytes can be prepared for determination of plasmalogens and dihydroxyacetone-phosphate acyltransferase (DHAPAT), respectively.

Test	Preconditions	Material	Handling	Pitfalls
VLCFA		Random serum/ plasma	Room tempera- ture	1. On ketogenic diet: may be $\uparrow$ 2. Hemolytic sample: $\uparrow$
THCA		Random serum/ plasma	Room tempera- ture	None
Phytanic acid		Random serum/ plasma	Room tempera- ture	None
Pristanic acid		Random serum/ plasma	Room tempera- ture	None
Plasmalogens		Erythrocytes from EDTA-blood	Room tempera- ture	None
DHAPAT		Platelets/leuko- cytes from EDTA-blood	Room tempera- ture	



## 25.10 Prenatal Diagnosis

Prenatal diagnostic methods have been developed for all peroxisomal disorders and the feasibility of the methods has been established through the years. An especially powerful and reliable technique is immunoblot-analysis of peroxisomal acyl-CoA oxidase and peroxisomal thiolase, which is used for the prenatal diagnosis of the disorders of peroxisome biogenesis, rhizomelic chondrodysplasia punctata, acyl-CoA oxidase deficiency and thiolase deficiency. Results are especially good in chorionic villous tissue, thus eliminating the risk of material overgrowth which may occur upon culturing cells.

Type of peroxisomal disorder	Material	Type of analysis
25.1– 25.3 Disorders of peroxisome biogenesis	CVB	DHAPAT-activity Immunoblot analysis
	CVF/AF	Catalase immunofluorescence C26:0 levels Pristanic acid $\beta$ -oxidation
25.5– 25.7 Rhizomelic chondrodysplasia punctata type 1–3	CVB	Plasmalogen level DHAPAT/alkylDHAP synthase
	CVF/AF	De novo plasmalogen synthesis DHAPAT/alkyl-DHAP synthase
25.8 X-linked adrenoleukodystrophy	CVB	Mutation analysis
	CVF/AF	C26:0 levels, C26:0 $\beta$ -oxidation, ALDP analysis
25.9 Acyl-CoA oxidase deficiency	CVB/CVF/AF	Acyl-CoA oxidase activity
25.10 D-Bifunctional protein delay	CVB/CVF/AF	D-bifunctional enzyme activity
25.16 Refsum disease	CVB/CVF/AF	Phytanoyl-CoA hydroxylase activity

## 25.11 Initial Treatment

In Refsum disease, reduction of plasma phytanic acid levels by a low-phytate diet (especially prohibition of ruminant meat and fats), with plasmapheresis, has been successful in arresting the progress of the peripheral neuropathy. The stored phytanic acid is exclusively of exogenous origin.

In X-linked adrenoleukodystrophy, the usefulness of bone marrow transplantation has been encouraging in clinically mildly affected patients with the childhood form of X-linked ALD. Adrenocortical insufficiency should be treated with steroid-hormone substitution.

For patients with abnormal peroxisome biogenesis, the possibilities for treatment are very poor. Supplementation of docosahexaenoic acid is now being tested in patients with the milder forms of disorders of peroxisome biogenesis.

### 23.12 Molecular Analysis

In the past few years much knowledge has become available on the molecular basis of the various peroxisomal disorders as summarised below:

- Peroxisome biogenesis disorders: after the identification of extensive genetic heterogeneity among patients affected by a PBD by means of complementation studies, many of the underlying genes have now been identified and molecular diagnosis is now possible in the vast majority of cases (see [3]).
- Rhizomelic chondrodysplasia punctata: the three genes underlying RCDP types 1, 2 and 3 have been identified and molecular analysis is now possible for all 3 forms (see [10]).
- Peroxisomal fatty acid  $\beta$ -oxidation deficiencies: Acyl-CoA oxidase deficiency and D-bifunctional protein deficiency have both been resolved at the molecular level [10] and many often private mutations have been identified. The same is true for Refsum disease and 2-methylacyl-CoA racemase deficiency [16].

### 25.13 Summary

The peroxisomal disorders represent a heterogeneous group of disorders, with neurological involvement in most of them. Identification of patients is important, especially since methods for prenatal diagnosis have been developed. If acatalasemia, hyperoxaluria type 1, glutaryl-CoA oxidase deficiency and mevalonate kinase deficiency are excluded, the remaining disorders can be placed into three groups, based on similarities in the clinical presentation. This includes the disorders of peroxisome biogenesis and peroxisomal  $\beta$ -oxidation, rhizomelic chondrodysplasia punctata and related disorders, and the X-linked adrenoleukodystrophy complex. Appropriate diagnostic flow charts as described in this chapter lead the way to correct identification of patients.

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