Invited Review

Peroxisomal disorders: The single peroxisomal enzyme deficiencies

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Abstract

Peroxisomal disorders are a group of inherited diseases in man in which either peroxisome biogenesis or one or more peroxisomal functions are impaired. The peroxisomal disorders identified to date are usually classified in two groups including: (1) the disorders of peroxisome biogenesis, and (2) the single peroxisomal enzyme deficiencies. This review is focused on the second group of disorders, which currently includes ten different diseases in which the mutant gene affects a protein involved in one of the following peroxisomal functions: (1) ether phospholipid (plasmalogen) biosynthesis; (2) fatty acid beta-oxidation; (3) peroxisomal alpha-oxidation; (4) glyoxylate detoxification, and (5) H2O2 metabolism.

Keywords: Peroxisomes; Alpha-oxidation; Beta-oxidation; Zellweger syndrome; Refsum disease; Adrenoleukodystrophy; Hyperoxaluria; Plasmalogen

1. Introduction

The peroxisomal disorders (PDs) are usually subdivided into two groups, including: (i) the peroxisome biogenesis disorders (PBDs); and (ii) the single peroxisomal enzyme (transporter) deficiencies (PEDs).

The PBD group comprises Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD); infantile Refsum disease (IRD) and rhizomelic chondrodysplasia punctata (RCDP) type 1. After the recognition that mutations in the same gene can lead to either of the first three disease conditions, they are nowadays collectively called the Zellweger spectrum disorders (ZSDs), that constitute a triad of overlapping disorders with ZS being the most severe, followed by N-ALD and IRD. Common to all three are liver disease, variable neurodevelopmental delay, retinopathy, and perceptive deafness with onset in the first months of life. In addition, patients with ZS are severely hypotonic and weak from birth and have distinct facial features, peri-articular calcifications, severe brain dysfunction associated with neuronal migration disorders and die before 1 year of age. Patients with N-ALD have hypotonia, seizures, may have polymicrogyria, have progressive white matter disease, and usually die in late infancy. Patients with IRD may have external features reminiscent of ZS, but no neuronal migration disorder and no progressive white matter disease. The cognitive and motor development varies between severe global handicaps and moderate learning disabilities with deafness and visual impairment, due to retinopathy.

RCDP type 1 is clinically quite different from the Zellweger spectrum disorders (ZSDs) although there are some features shared by RCDP type 1 and the ZSDs including the cranial facial (broad low nasal bridge, epicanthus, high arched palate, dysplastic external ears, and micrognathia), and ocular abnormalities. Indeed, histopathology of the eyes of ZS and RCDP patients showed a number of important common features including abnormal corneal epithelium and endothelium, with mitochondrial proliferation. Furthermore, signs of ganglion atrophy and photoreceptor degeneration were found, which occurred earlier in ZS as compared to RCDP. These findings led Kretzer et al. to suggest a common etiological mechanism between ZS and RCDP already in 1981 [38]. This suggestion inspired Heymans et al. [33] to study whether RCDP is a peroxisomal disorder. RCDP type 1 is not only clinically different from the ZSDs but also its biochemistry, cell biology, and genetics are different. Indeed, the ZSDs are now known to be associated with mutations in a large number of different PEX genes, including PEX1,2,3,5,6,10,12,13,14,16,19 and 26 [74][96], whereas mutations in PEX7 are associated with RCDP type 1 [4,51], but not with any of the ZSDs.
The second group of peroxisomal disorders includes the single peroxisomal enzyme / transporter deficiencies (PEDs), which can be subdivided into distinct subgroups on the basis of the peroxisomal metabolic pathway affected. Table 1 lists the peroxisomal disorders (PBDs) identified so far and their classification into distinct subgroups as used throughout this review. Until recently, mevalonate kinase deficiency was also believed to be a peroxisomal disorder, but should no longer be considered a peroxisomal disorder because the earlier claim that mevalonate kinase is a peroxisomal enzyme has now been disproven [34].

In this review we will describe the clinical, biochemical, cell biological, and genetic aspects of each of the PEDs and will begin by giving a brief update on the function of peroxisomes in human beings and the enzymes and transporters involved.

2. Metabolic functions of peroxisomes in humans

Peroxisomes play an indispensable role in human physiology as concluded from the devastating consequences of a defect in the biogenesis of peroxisomes as observed in Zellweger patients. The metabolic functions attributed to peroxisomes are multiple and include both biosynthetic (ether phospholipid biosynthesis, bile acid synthesis, docosahexaenoic acid synthesis) and degradative (fatty acid alpha-oxidation, fatty acid beta-oxidation, glyoxylate detoxification, etcetera) pathways. Much of the knowledge on the metabolic functions of peroxisomes actually comes from studies on body fluids and tissues from Zellweger patients. The functions of peroxisomes, which are of key importance to the group of peroxisomal disorders are:

(1) ether phospholipid biosynthesis, (2) fatty acid beta-oxidation, (3) glyoxylate detoxification, and (4) fatty acid alpha-oxidation.

3. Ether phospholipid biosynthesis and its disorders

3.1. Ether phospholipid biosynthesis

Ether phospholipids are a special class of phospholipids, which differ from the regular diacyl phospholipids in having an ether-linkage rather than an ester-linkage at the sn-1 position of the glycerol backbone. Two groups of ether phospholipids can be distinguished with either an 1-O-alkyl or an 1-O-alk1-enyl linkage. The latter phospholipids (1-O-alk1-enyl-2-acyl phosphoglycerides) with an alpha-beta unsaturated ether-bond are also known by their trivial name plasmalogens. Biosynthesis of ether phospholipids involves the concerted action of enzymes localized in both peroxisomes and the endoplasmic reticulum. The ether bond, which is characteristic for ether phospholipids, is formed by the peroxisomal enzyme alkyl-DHAP synthase (encoded by AGPS) which turns the acyl-DHAP, generated from acyl-CoA and dihydroxyacetonephosphate (DHAP) by the peroxisomal enzyme dihydroxyacetonephosphate acyltransferase, into alkyl-DHAP (encoded by GNPAT), into alkyl-DHAP. After conversion of alkyl-DHAP into alkyl-G3P either in peroxisomes or the endoplasmic reticulum, the final steps of ether phospholipids synthesis are performed by endoplasmic reticulum enzymes (see Brites et al. [5] for review).

3.2. Disorders of ether phospholipid biosynthesis

Two single enzyme deficiencies in the ether phospholipid biosynthetic pathway have been identified so far. These are: (i) DHAPAT deficiency, and (ii) alkyl-DHAP synthase deficiency.

3.2.1. DHAPAT deficiency (Rhizomelic chondrodysplasia punctata type 2)

DHAPAT deficiency was first described in 1992 [93] in a patient showing all the clinical signs and symptoms of RCDP, including: (i) cranial facial abnormalities at birth, including a high forehead, large fontanels, a low/broad nasal bridge, antverted nostrils, micrognathia, and a high-arched palate; (ii) severe hypotonia; (iii) cataract; (iv) dwarfism; (v) pronounced rhizomelic shortening, especially of the upper arms, and (vi) striking radiological abnormalities, consistent with RCDP, although there were no stippled calcifications of patellae and acetabulum. In order to substantiate the clinical diagnosis RCDP, erythrocyte plasmalogens levels were determined and found to be fully deficient. The levels of phytanic acid in plasma were normal, however. The significance of this finding was not immediately clear because phytanic acid is solely derived from exogenous (dietary) sources and may be completely normal despite a full block in phytanic acid oxidation as is the case in RCDP type 1 patients. The same applies to ZSD patients. Detailed studies in fibroblasts of the index patient revealed a novel type of RCDP. Indeed, in all RCDP patients, whose fibroblasts were studied at that time, four abnormalities were found including: (i) a (partial) deficiency of DHAPAT; (ii) a marked deficiency of alkyl-DHAP synthase;...
(iii) deficient alpha-oxidation of phytanic acid, and (iv) the presence of peroxisomal thiolase I (ACAA1/pTH1) in its 44 kDa precursor form with the 41 kDa mature form completely missing [94]. In fibroblasts from this patient, however, only a single abnormality was detected, i.e. a full deficiency of DHAPAT, which was soon found to be caused by mutations in the gene (GNPAT) coding for DHAPAT. Since this first report a number of additional patients have been reported. Barr and colleagues [1] reported the second patient with DHAPAT deficiency, who also had the severe phenotype. In 1994 Clayton and co-workers [8] reported on a 21-month-old boy and his 3½-year-old sister, who both had isolated DHAPAT deficiency. Both siblings had antverted noses, normal limbs, hypotonia, growth and developmental retardation, and feeding difficulties.

Eczema was present in a younger patient, whereas a cranial CT-scan showed mild cerebral atrophy. The older sister had seizures. The same patients plus an additional sibling in the same family were later reported by Sztriha and co-workers [80,81]. The third patient in the family had a comparable clinical presentation, again without rhizomelia. A similar, mild case of DHAPAT deficiency was reported first by Moser and co-workers [46] and later described in full detail by Elias et al. in 1998 [15]: the 6½-year-old girl displayed developmental delay, growth failure and cataract, but no rhizomelia and no cranial facial dysmorphism.

In all patients with established RCDP type 2 the activity of DHAPAT is severely deficient, which results in a virtually complete inability to synthesize ether phospholipids, including plasmalogens. This is reflected in deficient plasmalogens levels in all tissues investigated including erythrocytes. Importantly, in all patients studied thus far, erythrocyte plasmalogens levels are clearly deficient, which implies that erythrocyte plasmalogens analysis is a reliable initial biochemical test. Molecular studies have led to the identification of different mutations in the gene coding for DHAPAT [54].

3.2.2. AlkylDHAP synthase deficiency (rhizomelic chondrodysplasia Type 3)

In 1994, the first case of isolated alkyl-DHAP synthase deficiency (RCDP type 3) was described in a patient showing all the clinical characteristics of RCDP [91]. Also in this patient erythrocyte plasmalogens levels were completely deficient whereas plasma phytanic acid levels were normal. Subsequent studies in fibroblasts revealed the complete deficiency of alkyl-DHAP synthase with normal values for DHAPAT and phytanic acid alpha-oxidation and a normally processed peroxisomal thiolase. In literature only a single patient has been described since [10] although three additional patients have been identified by Dr. H.W. Moser, Baltimore, who all showed classical RCDP with early demise (Dr. H.W. Moser, personal communication). The patient described by de Vet et al. [10] was less affected and is still alive at >10 years, showing mild rhizomelia, generalized contractures, inability to sit or crawl, cataract, seizures and profound developmental delay. In the original patient described in Wanders et al. 1994 [91] alkyl-DHAP synthase was fully deficient, whereas DHAPAT, phytanic acid alpha-oxidation, and peroxisomal thiolase were normal. Also in the subsequent patient described by de Vet and colleagues [10] alkyl-DHAP synthase was deficient, but, in addition, DHAPAT activity was also low with a residual activity of about 15%. In order to resolve this peculiar finding, complementation studies were performed, which revealed that the two patients did belong to the same genetic complementation group representing alkyl-DHAP synthase deficiency.

Detailed studies by van den Bosch and co-workers [3] have shown that the deficiency of DHAPAT in the second case of alkyl-DHAP synthase deficiency is the secondary consequence of the fact that the alkyl-DHAP synthase protein is completely absent in this patient due to a homozygous 128-bp deletion which leads to a premature stop. On the other hand, studies in fibroblasts from the first patient with alkyl-DHAP synthase deficiency have shown that the protein is normally expressed and correctly localized in peroxisomes as established in fibroblasts from this patient [11]. The protein, however, is functionally inactive, due to a 1256G→A mutation, causing the substitution of the amino acid arginine at position 419 by a histidine [11]. Since DHAPAT is only stable if it forms a complex with alkyl-DHAP synthase within the peroxisome, these data explain why DHAPAT activity was found to be fully normal in fibroblasts from the first patient described by Wanders et al. [91], but deficient in patient 2 described by de Vet et al. [10]. Furthermore, these data resolve the puzzling situation in RCDP type 1 in which DHAPAT has long been known to be deficient, despite its identification as a typical PTS1 protein.

In the few alkyl-DHAP synthase-deficient patients studied so far, erythrocyte plasmalogens levels were deficient like in RCDP type 1 and type 2 patients. This suggests that erythrocyte plasmalogens analysis is an excellent method to resolve whether a particular patient is affected by one of the peroxisomal forms of RCDP.

4. Peroxisomal fatty acid beta oxidation and its deficiencies

4.1. Peroxisomal fatty acid beta-oxidation

Peroxisomes contain a fatty acid beta-oxidation system which proceeds via the same principle set of four consecutive enzymatic reactions as in mitochondria, although the individual reactions of the beta-oxidation systems are catalyzed by distinct enzymes. The peroxisomal and mitochondrial beta-oxidation system serve different functions in human cells and catalyze the beta-oxidation of different fatty acids and fatty acid derivatives. Indeed, mitochondria catalyze the beta-oxidation of the bulk of FAs derived from the diet, including palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid, whereas peroxisomes catalyze the beta-oxidation of a different set of substrates. These include:

(1) Very-long-chain fatty acids (VLCFAs), notably hexacosanoic acid (C26:0): VLCFAs are in part derived from dietary sources but also formed by chain-elongation of long-chain fatty acids. In humans, most of the C26:0 is produced by chain elongation.

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(2) Pristanic acid (2,6,10,14-tetramethylpentadecanoic acid): pristanic acid cannot be synthesized endogenously in human beings and is solely derived from exogenous dietary sources either as pristanic acid itself, or via alpha-oxidation from its precursor phytanic acid (see Fatty acid alpha-oxidation).

(3) Di- and trihydroxycholestanolic acid (DHCA and THCA), which are intermediates in the formation of the primary bile acids cholic acid and chenodeoxycholic acid from cholesterol in the liver. After activation, DHCA and THCA undergo one cycle of beta-oxidation in peroxisomes to produce the CoA-esters of chenodeoxycholic acid and cholic acid, which are subsequently converted into the corresponding taurine or glycine conjugates within peroxisomes, followed by export from peroxisomes and final excretion into bile.

(4) Tetracosahexanoic acid (C24:6o-3). Peroxisomes play an important role in the formation of docosahexanoic acid (C22:6o-3) [75]. Indeed, recent studies have established that the formation of C22:6o-3 from linolenic acid (C18:3o-3) proceeds via subsequent steps of chain elongation and desaturation, but also involves the peroxisomal fatty acid oxidation system, since tetracosahexanoic acid is beta-oxidized in peroxisomes to produced C22:6o-3 (see [18]).

(5) Long-chain dicarboxylic acids. Recent studies have shown that at least a long-chain dicarboxylic acid like hexadecadioic acid, which is the dicarboxylic acid of palmitic acid, is oxidized exclusively in peroxisomes [22].

The actual beta-oxidation of all these different FAs in peroxisomes involves the participation of a number of different enzymes including two acyl-CoA oxidases, two multifunctional proteins with enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, and two different peroxisomal thiolases. The two acyl-CoA oxidases, called ACOX1 and ACOX2 serve different functions with ACOX1 (straight-chain acyl-CoA oxidase) preferentially reacting with the CoA-esters of straight-chain FAs, like C26:0, whereas the second acyl-CoA oxidase, i.e. ACOX2 (branched-chain acyl-CoA oxidase) catalyses the dehydrogenation of the CoA-esters of 2-methyl branched-chain FAs, like pristanoyl-CoA and the CoA-esters of the bile acid intermediates DHCA and THCA [88]. The enoyl-CoA esters of C26:0, pristanic acid, DHCA and THCA are all handled by a single bifunctional enzyme catalyzing the second (hydration) and third (dehydrogenation) steps of peroxisomal beta-oxidation. This enzyme with both enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activity forms and dehydrogenates d-3-hydroxyacyl-CoA esters rather than l-3-hydroxyacyl-CoA-esters and is the single enzyme involved in the oxidation of C26:0, pristanic acid as well as DHCA and THCA [12–14,35,36,56,57]. Different names have been given to this enzyme, including D-bifunctional protein (DBP), D-peroxisomal bifunctional enzyme (D-PBE), multifunctional enzyme II (MEFII), and multifunctional protein 2 (MFP2). Peroxisomes also contain a different bifunctional protein, which forms and dehydrogenates l-3-hydroxyacyl-CoA esters. Recent evidence suggests that this enzyme is, at least partially, involved in the peroxisomal oxidation of long-chain dicarboxylic acids [23].

Finally, human peroxisomes contain two peroxisomal thiolases, i.e. pTH1 and pTH2. Human pTH1 is the human equivalent of the clofibrate-inducible thiolase identified by Hashimoto and co-workers [45], whereas pTH2 is identical to the 58 kDa sterol carrier protein x (SCPx) with both thiolase activity and a sterol carrier protein domain [64]. It is clear now that pTH2 (SCPx) plays an indispensable role in the oxidation of 2-methyl branched-chain fatty acids whereas pTH1 and pTH2 are both involved in C26:0 oxidation. Fig. 2 depicts the involvement of the different enzymes in the oxidation of the different FAs and FA-derivatives.

The enzymes described above are necessary and sufficient for the beta-oxidation of straight-chain fatty acids like C26:0 as well as 2-methyl branched-chain fatty acids with the methyl group in the (2S)-configuration. However, auxiliary enzymes are needed for the beta-oxidation of (2R)-methyl branched-chain FAs and unsaturated FAs. Oxidation of (2R)-methyl branched chain FAs requires the active participation of an enzyme, capable of converting (2R)- into (2S)-branched-chain acyl-CoAs. Studies, notably by Schmitz and Conzelmann and co-workers [61–63] have shown that peroxisomes contain 2-methylacyl-CoA racemase (AMACR), which can be converted to (2R)-methylacyl-CoAs into the corresponding (2S)-methylacyl-CoAs and vice versa. The importance of this enzyme for oxidation of 2-methyl branched-chain fatty acids is stressed by the findings in AMACR-deficient patients in which (2R)-pristanic acid accumulates as well as (2S)- di- and trihydroxycholestanolic acid [17,24,25,68]. The accumulation of the latter two species is explained by the fact that the 2-methyl group in DHCA and THCA, which are produced from cholesterol, has the (2S)-configuration (see Fig. 2).

4.2. The disorders of peroxisomal beta-oxidation

So far 5 different defects in the peroxisomal beta-oxidation system have been identified, which include: X-linked adrenoleukodystrophy, acyl-CoA oxidase deficiency, D-bifunctional protein deficiency, sterol carrier protein X (SCPx) deficiency, and 2-methylacyl-CoA racemase (AMACR) deficiency. These diseases will be described below. Biochemical abnormalities, associated with each of these disorders, are listed in Table 2 (see also Fig. 2).

4.2.1. X-linked adrenoleukodystrophy

The phenotype of X-linked adrenoleukodystrophy (X-ALD) varies widely with at least six phenotypic variants described. The classification of X-ALD is somewhat arbitrary and based on the age of onset and the organs principally involved. X-ALD is the most common single peroxisomal disorder with a minimum incidence of 1:21000 males in the USA [2] to 1:15000 males in France (Aubourg, cited in Kemp et al. [2001]) [37]. The two most frequent phenotypes are childhood cerebral...
4.2.1.1. Biochemistry and molecular basis of X-ALD. The biochemical hallmark of X-ALD is the accumulation of VLCFAs in plasma [47], fibroblasts [48], and other cell types (see [49] for review). Analysis of plasma VLCFAs has proven to be a powerful diagnostic method with very few, if any, false results. Indeed, the rate of C26:0 oxidation in X-ALD fibroblasts is 20 to 30% of control, compared with a virtually complete deficiency in fibroblasts from Zellweger patients lacking peroxisomes.

The X-ALD or ABCD1 gene was identified in 1993, using a positional cloning strategy [50]. The protein turned out to belong to the superfamily of ABC transmembrane transporter proteins and is called ALDP. ALDP is a so-called half ABC-transporter, which must dimerize to generate the functional ABC-transporter. Three additional mammalian peroxisomal half ABC-transporters have been identified, which are closely related by nucleic acid and protein sequence to ALDP. These are ALDR, PMP70 and PMP69, encoded by ABCD2, ABCD3, and ABCD4, respectively. The four transporters may form different heterodimeric combinations in the peroxisomal membrane.

According to Guimaraes et al. [31] ALDP preferentially forms homodimeric combinations. Although definitive experimental evidence is lacking, ALDP has been claimed to catalyse the transport of VLCFAs, probably in their coenzyme A ester form, across the peroxisomal membrane. This is inferred from experiments in the yeast S. cerevisiae, which contains two half ABC-transporters, called Pxa1p and Pxa2p, both showing high homology with ALDP [71–73] or [32,79]. The Pxa1p/Pxa2p heterodimer has been shown to transport long-chain acyl-CoA esters across the peroxisomal membrane. This conclusion has been disputed by McGuiness et al. [42,44], who showed that the oxidation of C24:0 is partially deficient in fibroblasts from patients with a defect in mitochondrial beta-oxidation at the level of carnitine palmitoyltransferase 1 (CPT1) and very-long-chain acyl-CoA dehydrogenase (VLCAD). Although the extent of the deficiency was only limited, these results led McGuiness et al. [41,43] to conclude that the reduced oxidation of C24:0 in X-ALD cells is due to mitochondrial abnormalities and that ALDP somehow facilitates the interaction between peroxisomes and mitochondria. Later studies, however, notably by Oezen et al. [53] showed that mitochondria, at least those isolated from Abd1 (+/−) mice, are indistinguishable from wild type mitochondria in all aspects studied, which includes normal rates of oxidative phosphorylation and normal P/O ratios.

Mutation analysis of the ABCD1 gene in X-ALD patients revealed many different, often private mutations. As of April 2006 the X-ALD mutation database (http://www.X-ALD.nl/) contains 866 mutations of which 526 (21%) are missense mutations, 196 (23%) are frame shifts, 84 (10%) are nonsense mutations, 32 (4%) are amino acid insertions or deletions, and 41 (5%) are large deletions comprising one or more exons. The majority of X-ALD kindreds have a unique mutation. A 10-fold search set in. The cranial CT-scan without contrast enhancement set in. The cranial CT-scan with contrast enhancement was performed in one of the siblings.

Footnote: (1) = plasma VLCFA-levels may not be elevated in all acylCoA oxidase deficient patients (see Rosewich et al. [60]; (2) = plasma VLCFA levels may not always be elevated in all DBP-deficient patients (see Ferdinandusse et al. [19]); *=plasma pristanic acid and phytanic acid are usually elevated but may be deceptively low to even normal as a consequence of the reduced dietary intake of pristanic acid and/or phytic acid. N=normal.

Table 2

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<tr>
<th>Metabolite abnormalities in each of the single peroxisomal beta-oxidation deficiencies</th>
<th>VLCFA</th>
<th>Pristanic acid</th>
<th>Phytanic acid</th>
<th>DHCA/THCA</th>
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<td>X-Linked adrenoleukodystrophy</td>
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<td>Acyl-CoA oxidase deficiency</td>
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<td>D-bifunctional protein deficiency</td>
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<td>Sterol carrier protein X (SCPX) deficiency</td>
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<td>2-methylacyl-CoA racemase (AMACR) deficiency</td>
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showing bilateral enhancing lesions in the centrum semiovale and a generally hypodense cerebral white matter. A second patient was described by Wanders et al. [92]. This patient, when examined at the age of 3 years and 10 months, showed generalized hypotonia, failure to thrive, deafness, hepatomegaly, psychomotor retardation, absent reflexes, and frequent convulsions. At autopsy, multiple abnormalities were found including atrophy of skeletal muscles, brain stem ganglions, and cerebral nerves. Furthermore, in the brain and adrenals large PAS-positive, sudanophilic astrocytes, and macrophages were found. The liver was fibrotic. Using complementation analysis two further patients were identified by Suzuki et al. in 1994 [39,78]. Both patients showed profound hypotonia, dysmorphic features, including hypertelorism, epicantthus, long nasal bridge, low-set ears, and polydactyly in the neonatal period and there was no hepatomegaly or calcific stippling. In the first patients there were focal seizures of the left arm at 4 months of age. There was head control at 5 months and crawling at 12 months. At 32 months of age this patient could walk without assistance. Since then his motor ability has regressed. At 41 months of age he could not sit, but he did respond to members of the family by smiling or crying. The other patient described by Suzuki [78] is the younger sister of the first patient and clinical manifestations were quite similar. She could control her head at 6 months of age, crawled at 18 months and walked with support at 22 months, followed by regression. Both patients were alive at four and 7 years when reported in 1994. Again, using complementation analysis, Watkins et al. [98] identified three additional patients, followed by a more recent report by Kurian et al. [39], who described a patient with facial dysmorphia, bifacial neurodevelopmental delay, sensori-neural hearing loss and hepatomegaly bringing the total number of patients to nine. In general, patients with acyl-CoA oxidase deficiency show moderate neonatal hypotonia, frequent seizures with the onset typically between 2 and 4 months of age but less severe when compared with D-bifunctional protein deficiency and usually controlled with medication. Most patients do not show cranial facial dysmoria, although this is not true for all patients. Many of the patients do show some development, followed by regression. Renal and skeletal abnormalities and liver disease are usually not observed in acyl-CoA oxidase deficiency. The average age of death is much later than in patients with D-bifunctional protein deficiency.

4.2.2.1. Biochemistry and molecular basis of acyl-CoA oxidase deficiency. Since acyl-CoA oxidase-1 is only involved in the beta-oxidation of VLCFAs but not in pristanic acid and di- and trihydroxycholestanolic acid (Fig. 1), the only abnormality observed in plasma from patients is elevated VLCFAs (Table 2, Fig. 2b). Studies in fibroblasts have shown that the oxidation of C26:0 is markedly deficient with normal pristanic acid beta-oxidation. Immunofluorescence microscopy analysis of peroxisomes usually reveals enlarged peroxisomes, which are reduced in number. Molecular analysis has so far been performed in only a few patients [29,59,76].

4.2.3. D-bifunctional protein deficiency

D-bifunctional protein deficiency (DBP deficiency) is one of the more recently identified peroxisomal disorders and is now known to be relatively frequent among the group of peroxisomal beta-oxidation deficiencies, second to X-ALD. After its first identification by Suzuki et al. [77], and van Grunsven et al. [83] many patients have been identified. The availability of methods to diagnose DBP-deficiency has led to detailed studies in previously published patients, affected by an isolated defect in the peroxisomal beta-oxidation system of unknown etiology [6,9,16,40,52,84,87,95]. Furthermore, several published patients in whom doubt had arisen about the final diagnosis could now be reinvestigated. One of these patients was described by Watkins et al. in 1989 [97]. Interestingly, at that time it was believed that this patient had a deficiency of the other peroxisomal bifunctional enzyme, called L-bifunctional.
Fig. 2. Schematic illustration of the peroxisomal metabolites which accumulate as result of a deficiency of ALDP (a), acyl-CoA oxidase (b), D-bifunctional protein (c), sterol-carrier protein X (SCPx) (d), and 2-methylacyl-CoA racemase (AMACR) (e).
protein. A second patient who was reinvestigated was described already earlier by Goldfischer et al. in 1986 [30] as pseudo-zellweger syndrome indicating that the clinical signs and symptoms of this patient were similar to those described for classical Zellweger syndrome. This patient was originally believed to have peroxisomal 3-ketoacyl-CoA thiolase deficiency, but upon reinvestigation we found that the true defect in this patient is again at the level of D-bifunctional protein [26]. A number of other patients described in literature with an unknown defect in peroxisomal beta-oxidation, all turned out to suffer from DBP deficiency as well [21].

D-bifunctional protein is a protein with two catalytic activities including a 2-enoyl-CoA hydratase unit and a (3R)-hydroxyacyl-CoA dehydrogenase unit. As a consequence three different types of DBP deficiency can be distinguished depending on which activity is deficient: (i) type 1 deficient patients have a deficiency of both the hydratase and dehydrogenase activities of DBP (in fibroblasts of virtually all type 1 deficient patients no DBP protein can be detected by immunoblotting and upon immunofluorescence using an antibody raised against DBP); (ii) type 2 deficient patients, have an isolated deficiency of the hydratase unit; and (iii) type 3 deficient patients, have an isolated deficiency of the dehydrogenase unit. This classification can be made on the basis of enzyme activity measurements in combination with mutation analysis.

Recently, we reported the clinical, biochemical, and genetic characteristics of a large cohort of DBP-deficient patients. Enzyme activity measurements in combination with molecular data revealed that 27% of the patients were DBP type 1 deficient, 28% type 2 deficient, and 45% type 3 deficient. No evident differences were found between the clinical signs and symptoms of patients affected by type 1, 2 or 3 deficiency. Virtually all patients presented with neonatal hypotonia (98%), and seizures (93%) within the first months of life. 19 patients (15%) were reported to have had infantile spasms at any time. Failure to thrive was observed in 43% of the patients. Visual system failure, including nystagmus, strabismus or failure to fixate objects at 2 months was reported in 45% of patients. Almost none of the patients acquired any psychomotor development. External dysmorphism was present in 68% of patients and resembled that of patients with Zellweger syndrome. Frequently described abnormalities were: high forehead, high arched palate and large fontanel, long philtrum, epicantal folds, hypertelorism, microcephaly, shallow supra-orbital ridges, retrognathia, and low set ears. Kaplan–Meier survival analysis revealed that all type 1 deficient patients died within the first 14 months of life (mean age of death: 6.9 months), whereas the mean age of death for type 2 and type 3 deficiency was 10.7 months and 17.6 months. The major cause of death was pneumonia for almost all patients. Only five of the patients showed prolonged survival (>5 years), one of whom was a type 2 patient and the other 4 type 3 patients.

4.2.3.1. Biochemistry and molecular basis of DBP-deficiency. D-bifunctional protein holds a central position in the peroxisomal fatty acid beta-oxidation pathway and is involved in the beta-oxidation of C26:0, pristanic acid, as well as di- and trihydroxycholestanolic acid. This implies that, in principle, patients with DBP-deficiency would show the accumulation of C26:0, pristanic acid, and di- and trihydroxycholestanolic acid in plasma (Fig. 2c). It is now clear, however, that this full set of peroxisomal metabolite abnormalities is not seen in all DBP-deficient patients. Indeed, in 50 of the 53 patients in whom plasma C26:0 levels and the C26:0/C22:0 ratio could be analyzed, definite abnormalities were found. In the remaining three patients a normal profile of VLCFAs was found whereas phytic acid and pristanic acid levels were also normal as well as the bile acid intermediates THCA and DHCA. In contrast, a clearly abnormal VLCFA profile was observed in fibroblasts from these three patients, which points to a discrepancy between results in plasma as compared to fibroblasts. In the 50 patients with abnormal plasma VLCFAs, phytic and pristanic acid levels varied considerably, which follows logically from the fact that both compounds originate from dietary sources only. As first reported by van Grunsven et al. [82] DBP-deficient patients may show no accumulation of THCA and DHCA. In the large cohort study by Ferdinandsusse et al. [20] 12 patients (26%) had no accumulation of THCA and DHCA. Docosahexaenoic acid (DHA) and arachidonic acid (AA) were determined in only a small number of patients. In 43% reduced levels of DHA and AA were found in plasma whereas in erythrocytes only a single patient had a marginally decreased DHA level. Plasmalogens in erythrocytes were normal in all DBP-deficient patients studied. Interestingly, biochemical analysis showed that there is a good correlation between several biochemical parameters and the survival of DBP-deficient patients with C26:0 beta-oxidation in cultured skin fibroblast being the best predictive marker in terms of life expectancy. The fact that three patients were identified without any biochemical abnormalities in plasma indicates that D-bifunctional protein deficiency cannot be excluded on the basis of measurement of peroxisomal parameters in plasma only. Molecular analysis of the HSD17B4 gene has revealed a large number of often private mutations with only one mutation being more frequent [27,83].

4.2.4. Sterol carrier protein X (SCPs) deficiency
Sterol carrier protein X (SCPx) deficiency has so far been described in a single patient who was only studied in detail at 45 years of age, following a 20-year history of dystonic head tremor and spasmodic torticollis. At 29 years of age, hypergonadotrophic hypogonadism and azoospermia were diagnosed. Cranial MRI revealed a number of abnormalities including bilateral hyper intense signals in the thalamus, butterfly-like lesions in the ponds, as well as lesions in the occipital region without gadolinium enhancement. Neurological examination revealed hypomia, pathological saccadic eye movements, and a slight hypoacusis. Ophthalmological investigations revealed no abnormalities. Nerve conduction studies of the lower extremities showed a predominantly motor and slight sensory neuropathy with conduction blocks in the tibial nerves, reduced motor action potentials in the left peroneal nerve, and reduced amplitude of the left sural nerve.
The combination of adult-onset sensory motor neuropathy and the grey and white matter abnormalities observed upon MRI, raised the possibility of AMACR deficiency despite the absence of retinitis pigmentosa, which is a frequent finding both in AMACR deficiency as well as in Refsum disease. Detailed laboratory investigations revealed slightly abnormal di- and trihydroxycholestanolic acid levels, whereas pristanic acid was clearly elevated (>10-fold the upper limit of normal). Plasma VLCFAs were completely normal. Taken together, these abnormalities clearly pointed to a defect in the peroxisomal metabolism of 2-methyl branched-chain fatty acids.

4.2.4.1. Biochemistry and molecular basis of SCPx deficiency. Studies in fibroblasts from the index patient revealed normal oxidation of C26:0 whereas the beta-oxidation of pristanic acid was markedly deficient with residual activity amounting to <10% of mean control. These results pointed to either branched-chain acyl-CoA oxidase, 2-methylacyl-CoA racemase, or sterol carrier protein X (SCPx) as deficient enzyme (see Fig. 1). Subsequent enzyme activity measurement showed normal activities of branched acyl-CoA oxidase as well as 2-methyl acyl-CoA racemase. In contrast, the activity of SCPx was fully deficient. Immunoblot analysis showed the absence of both the full-length 58-kDa SCPx as well as the 46-kDa thiolase domain of SCPx in fibroblasts of the patient. In contrast, the 13-kDa SCP2 protein was normally present. Molecular analysis revealed a homozygous 1-nucleotide insertion, 545_546insA leading to a frame shift and premature stop codon (I184fsX7).

As shown in Fig. 2d pristanic acid and the bile acids intermediates di- and trihydroxycholestanolic acid are the expected peroxisomal metabolites to accumulate in SCPx deficiency.

4.2.5. 2-Methyl-acylCoA racemase (AMACR) deficiency

AMACR deficiency was first described in 2000 by Ferdinandusse et al. [17] in three patients, two whom suffered from adult-onset sensory motor neuropathy. One patient also had pigmentary retinopathy, reminiscent of Refsum disease, as well as epilepsy, migraine and depression, whereas the second patient had pyramidal signs. The third patient was a child without neuropathy but it should be noted that this child also had Niemann–Pick type C. Interestingly, Setchell et al. [67] reported two siblings with racemase deficiency but a completely different phenotype. Both patients displayed fulminant liver disease, starting at early age. The index patient presented at 2 weeks of age with coagulopathy, vitamin D and E deficiency and mild cholestasis. Plasma analysis revealed elevated levels of the bile-acid intermediates di- and trihydroxycholestanolic acid (DHCA and THCA). Furthermore, plasma VLCFAs, phytanic acid, and red blood cell plasmalogens were all normal, whereas pristanic acid was markedly elevated (10-fold). These data suggested AMACR deficiency, which was confirmed in fibroblasts [85]. Remarkably, a previous child in the family had died earlier at 5.5 months of age from an intracranial bleed secondary to vitamin K deficiency and his liver was harvested for orthotopic transplantation (OLT) and placed into a 28-month-old child with end-stage liver disease. Studies in the recipient revealed marked elevation of both (25R)-THCA and (25R)-DHCA in urine indicating that the sibling, whose liver was transplanted, had also been affected by AMACR deficiency. This finding prompted institution of a therapy involving ursodeoxycholic acid. The recipient has remained in good health 8 years after transplantation while still receiving ursodeoxycholic acid, at least when reported in 2003 [66]. Interestingly, the patients described by Setchell et al. [65] were homozygous for the same mutation as described by Ferdinandusse et al. [17] in the two patients with adult-onset sensory motor neuropathy. The mutation which changes a serine at position 52 by a proline leads to a complete loss of enzyme activity as established by expression studies in E. coli [17]. Clarke et al. [7] have described an additional patient with tremor, retinitis pigmentosa, and deep white matter MRI changes, who subsequently developed encephalopathy with left hemiparesis and an unchanged MRI. Taken together, these data suggest that AMACR deficiency can have two wildly different phenotypic presentations, one characterized by early onset liver failure, which may resolve, but may also lead to early death, as described by Setchell et al. [69], whereas the other presentation is dominated by a late onset sensory neuropathy plus additional features.

4.2.5.1. Biochemistry and molecular basis of racemase deficiency. AMACR plays a crucial role in the degradation of FAs with a methyl group in the (2R)-configuration such as (2R)-pristanic acid, (25R)-THCA, and (25R)-DHCA (Fig. 1). In all patients with AMACR deficiency reported so far, pristanic acid, THCA and DHCA have been found to accumulate, which follows logically from the role of AMACR in the oxidation of pristanic acid as well as di- and trihydroxycholestanolic acid, but not C26:0 (see Fig. 2e). It has been established that racemase-deficient patients accumulate exclusively the (25R)-isomer of free and taurine-conjugated DHCA and THCA, whereas in patients with cholestatic liver disease for instance, both (25R)- and (25S)-isomers accumulate [24,70].

Interestingly, AMACR is a protein equipped with both a mitochondrial and peroxisomal targeting signal, which explains its presence in both mitochondria and peroxisomes. Mitochondrial AMACR plays a similar crucial role in the degradation of (2R)-FAs just like peroxisomal AMACR. One of the (2R)-FAs, the oxidation of which is dependent on mitochondrial AMACR activity, is (2R, 6R)-dimethylheptanoic acid, which is produced from pristanic acid by beta-oxidation (Fig. 3). Pristanic acid is a mixture of two diastereomers including (2R, 6R, 10R) and (2S, 6R, 10R) pristanic acid. Oxidation of the (2R)- but not the (2S)-form is deficient in case of AMACR deficiency. Hence, one would expect accumulation of (2R, 6R)-dimethylheptanoic acid in patients with racemase deficiency. However, no specific fatty acid abnormalities resulting for the deficiency of mitochondrial AMACR in AMACR deficient patients have been documented in literature.

Mutation analysis has been performed in all patients reported so far. Remarkably, four of the patients were homozygous for a point mutation (154T>C), which leads to a single amino acid substitution (S52P).
5. Peroxisomal alpha-oxidation and its disorders

5.1. Fatty acid alpha-oxidation

Fatty acids with a methyl group at the 3-position cannot be beta-oxidized because the 3-methyl group simply blocks beta-oxidation. Fortunately, there are other mechanisms by which such FAs can be degraded including alpha- and omega-oxidation. Alpha-oxidation involves the oxidative decarboxylation of 3-methyl FAs to produce 2-methyl FAs, which can be degraded by beta-oxidation. In higher eukaryotes, including humans, peroxisomes are the sole site of alpha-oxidation. The enzymatic machinery required for FA alpha-oxidation, has been resolved in recent years and requires the concerted action of 3 enzymes including: (1) phytanoyl-CoA hydroxylase; (2) 2-hydroxyphytanoyl-CoA lyase, and (3) pristanal-dehydrogenase as described in detail elsewhere in this volume. The actual decarboxylation is catalyzed by the enzyme 2-hydroxyphytanoyl-CoA lyase, which splits 2-hydroxyphytanoyl-CoA, the product of the first enzyme of the pathway, i.e. phytanoyl-CoA hydroxylase, into formyl-CoA and pristanal. Subsequently, pristanal is oxidized into pristanic acid via a peroxisomal aldehyde dehydrogenase, which has not been identified yet. Pristanic acid, which now has its first methyl-group at the 2-position, can then be degraded by beta-oxidation. The first two enzymes, including phytanoyl-CoA hydroxylase and 2-hydroxyphytanoyl-CoA lyase have been identified in different species. Human phytanoyl-CoA hydroxylase is a typical PTS2 protein which explains why it is not only deficient in Refsum disease but also in rhizomelic chondrodysplasia punctata type 1 (RCDP type 1), which is caused by mutations in the PEX7 gene encoding the PTS2 receptor. Human 2-hydroxyphytanoyl-CoA lyase is a typical PTS1 protein [28].

5.2. The disorders of fatty acid alpha-oxidation

5.2.1. Refsum disease

Refsum disease usually presents in late childhood with progressive deterioration of night vision, the occurrence of a progressive retinitis pigmentosa, and anosmia, later in life followed by deafness, ataxia, polyneuropathy, ichthyosis and cardiac arrhythmias. Short metacarpals and metatarsals are also found in some 30% of Refsum disease patients. Furthermore, psychiatric disturbances have been reported in a subset of patients. It is quite clear now that only few patients develop the full spectrum of clinical signs and symptoms originally defined by Refsum [58]. Indeed, a study of 16 Refsum disease patients with full clinical information for a period for up to 60 years revealed that all 16 patients developed retinitis pigmentosa, 15 out of 16 developed anosmia, whereas neuropathy, deafness and ataxia was only found in 11, 10 and 8 patients, respectively [99]. Finally, ichthyosis was only present in 4 of the 16 patients studied. Refsum disease is one of the few peroxisomal disorders for which a therapy has been developed which is based on the dietary restriction of phytic acid. The average human daily diet intake of phytic acid in western countries amounts to 50 to 100 mg. Restriction of common phytic acid containing foods, such as butter, cheeses, lamb, beef and certain fish, helps to lower plasma phytic acid levels, which may stop the progression of the peripheral neuropathy with improved muscle strength and regression of ichthyosis and correction of non-specific abnormalities of electrocardiogram.

5.2.2. Biochemistry and molecular basis of Refsum disease

Plasma phytic acid is generally considered to be an excellent marker for Refsum disease since all Refsum disease patients have so far been found to have markedly elevated plasma phytic acid levels, even if they are on therapy. Although this may be true for patients with classical Refsum disease caused by mutations in the phytanoyl-CoA hydroxylase gene, the situation may be different in those patients in which PEX7 is mutated. Indeed, in these patients plasma phytic acid levels are generally much lower, and recent studies by Horn et al. (manuscript submitted for publication) have shown that levels can actually be completely normal. If plasma phytic acid levels are elevated additional studies, preferably in fibroblasts, should be done to pinpoint the underlying defect. In all Refsum disease patients studied to date whether mutated in PAHX or PEX7, discrimination between the two forms of Refsum disease can be made by performing detailed studies in fibroblasts, especially by analyzing other PTS2 proteins, like peroxisomal thiolase and alkyl-DHAP synthase, using immunoblot analysis, enzyme activity measurements, and immuno-fluorescence microscopy analysis, followed by molecular analysis of the two different genes [90].

6. Glyoxylate detoxification and its disorders

6.1. Glyoxylate detoxification

In humans, peroxisomes play a crucial role in the detoxification of glyoxylate as exemplified by the devastating consequences of a deficiency of the peroxisomal enzyme alanine glyoxylate aminotransferase (AGT) in hyperoxaluria type 1. AGT catalyses the transamination of glyoxylate to glycine with alanine as the preferred amino group donor. It is not exactly known from which precursors glyoxylate is formed. If glyoxylate is not immediately detoxified by AGT, either due to its deficient activity in peroxisomes or its mislocalization to mitochondria as described below, glyoxylate will accumulate in peroxisomes and will either be reduced to glycoclate or oxidized to oxalate. Glycolate is water soluble and can be excreted via the urine. This is not true, however, for oxalate, which precipitates as calcium oxalate. Deposition of calcium oxalate within the kidney parenchymatous tissue (nephrocalcinosis) or the renal pelvis/urinary tract (urolithiasis) continues until eventually renal function is impaired causing subsequent sequelae, like uremia and systemic oxalosis. The failure to clear oxalate from the body leads to its further deposition in almost all areas of the body, affecting multiple tissues in addition to the kidneys and urinary tract, including: (a) the myocardium with heart block, myocarditis, and cardioemolic stroke; (b) the nerves (peripheral neuropathy); (c) bone (bone pain, multiple fractures,
PH1 is one of the few peroxisomal diseases for which some therapeutic options are available. Treatment of PH1 is directed towards: (a) decreasing the production of oxalate by inhibiting oxalate synthesis, and (b) increasing the solubility of oxalate at a given urinary concentration of oxalate. Most of the efforts have concentrated on ways to increase the solubility of calcium oxalate with high fluid intake and alkalinization of the urine as the main stays. Indeed, excessive volume is necessary to help excrete the enormous amounts of endogenously reproduced oxalate. Haemodialysis can remove large quantities of oxalate and its precursors. Importantly, a substantial percentage of PH1 patients respond to pyridoxin. Indeed, pyridoxin at the usual daily dose of 1000 mg/m² body surface area can bring about a substantial reduction in the production and excretion of oxalate, except in patients with a pyridoxin-resistant form of the disease. The efficacy of pyridoxin is probably direct related to the extent to which AGT is deficient. Indeed, if there is some residual activity, high levels of pyridoxal phosphate, which is the obligatory cofactor in the AGT enzyme reaction, may allow residual enzyme activity operate optimally. In this way, flux through AGT may be stimulated substantially, leading to a reduced production of oxalate. Recent studies have shown a clear association between homozygosity for the two mistargeting mutations Gly170Arg and Phe152Ile, and pyridoxin responsiveness, the reason being that pyridoxin is able to interfere with the mistargeting of AGT to the mitochondria thereby increasing the residual activity in peroxisomes from 5 to 10%. In our experience, all patients homozygous for the Gly170Arg substitution with a preserved renal function at the time of diagnosis, were able to preserve renal function throughout the follow-up period when treated with pyridoxin, high fluid intake, and potassium citrate [86]. In case of pyridoxin insensitivity combined liver / kidney transplantation is warranted.

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References

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