Clinical, biochemical and genetic aspects of peroxisomal disorders – an expanding group of genetic diseases in humans

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Abstract

Zellweger syndrome (ZS) in its classic form is an autosomal recessive lethal disease characterized by the absence of morphologically recognizable peroxisomes. Detailed studies on ZS in the early 1980s have led to the discovery of a set of peroxisomal biomarkers in blood which has revolutionized our knowledge about peroxisomes and peroxisomal disorders, and formed the basis for the discovery of the group of peroxisomal diseases known at present. Peroxisomal disorders are classified into two distinct groups including the disorders of peroxisome biogenesis (group 1) and peroxisome function (group 2). The enzymatic and molecular basis of most peroxisomal disorders has been identified through the years and pre- and post-natal diagnostic methods have been established. This review describes the current state of knowledge with respect to peroxisomes and peroxisomal disorders with particular emphasis on the clinical biochemical and genetic aspects of these disorders.

Introduction

In 1964 Bowen, Lee, Zellweger and Lindenberg described a familial syndrome of multiple congenital defects in two pairs of siblings (three girls and one boy). Prenatal history and delivery were unremarkable. At birth, hypotonia and a number of congenital anomalies were noted including bilateral glaucoma with corneal opacities, bilateral epicanthal folds, abnormal ears, a high-arched palate, wide fontanelles, open metopic and lambdoid sutures, clitoris hypertrophy, camptodactyly and simian creases. Unaware of this publication Smith, Opitz and Inhorn in 1965 described ‘a syndrome of multiple developmental defects including polycystic kidneys and intrahepatic biliary dysgenesis in two sibs’, who presented with a large number of comparable defects including severe hypotonia, high forehead, shallow supraorbital ridges, camptodactyly, minor anomalies of the eyes, ears, palate and hands, and failure to thrive. Two years later, Passarge and McAdams described five sisters with similar clinical and pathological features and introduced the term cerebrohepatorenal syndrome. In 1969, Opitz et al. proposed the name Zellweger syndrome. In an editorial comment, McKusick suggested that the two designations proposed by Passarge and McAdams and Opitz et al. be combined, giving rise to the cerebrohepatorenal syndrome of Zellweger. In practice, the name Zellweger syndrome (ZS) is used most.

In the early 1980s, several patients were presented to the Department of Paediatrics of the University Hospital Amsterdam, the Netherlands, with all the signs and symptoms described for ZS. Confronted with the devastating clinical course of ZS in these patients with early death in most of them, it was obvious that prenatal diagnostic methods should become available as soon as possible. In the absence of any such method described in literature, we...
decided to perform a thorough literature search which might give a clue for future research aimed to develop a prenatal laboratory test. One of the first papers we stumbled across, was the – in retrospect – seminal publication by Goldfischer et al., who described the absence of morphologically distinguishable peroxisomes in hepatocytes and kidney cortex cells of ZS patients. Since at that time virtually nothing was known about peroxisomes, this observation went unnoticed. In fact, much more attention was paid to the mitochondrial abnormalities described in the same paper, as is clear from the title ‘Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome’. Indeed, Goldfischer et al. documented clear mitochondrial abnormalities characterized by a markedly reduced rate of oxygen uptake of mitochondria isolated from a brain biopsy of a ZS patient and a liver biopsy from another ZS patient with malate (plus glutamate) as substrate but not with ascorbate plus N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) as substrate. These findings led the authors to conclude that ‘the cytochrome portion of the electron transport chain is intact but that there is a defect in electron transport prior to the cytochromes’. Based on these results, ZS was considered to be a mitochondrial disorder. Subsequent studies by other investigators, however, revealed that the mitochondrial abnormalities at the level of the respiratory chain were remarkably variable among patients, ranging from near-normal togrossly impaired, which argued against ZS as a primary mitochondrial disorder.

Peroxisomes were first described as ‘spheric or oval bodies’ present in the cytoplasm of mouse proximal kidney tubules. Rouiller and Bernard identified similar organelles in rat hepatocytes and suggested that they were precursors (progenitors) of mitochondria, rather than distinct cell organelles sui generis. The addition of microbodies to the group of biochemically defined organelles is closely related to the development of cell fractionation techniques. Indeed, the conclusion that catalase, urate oxidase and α-amino acid oxidase are located in a distinct particle different from lysosomes, microsomes and mitochondria was reached on the basis of differential and isopycnic-gradient centrifugation studies by de Duve and Baudhuin. Importantly, earlier studies by de Duve and coworkers using the same technique had led to the identification of another subcellular organelle, the lysosome, for which Christian de Duve received the Nobel Prize in 1974. The concomitant occurrence of hydrogen peroxide-producing enzymes and catalase – which degrades H₂O₂ – in a single particle prompted de Duve and coworkers to introduce the name ‘peroxisome’. Combined morphological and biochemical investigations by Baudhuin et al. provided unequivocal evidence that microbodies are the morphological equivalent of peroxisomes.

Our literature search in the early 1980s revealed very little information on mammalian peroxisomes except for two publications. The first one was from Lazarow and de Duve, who described the presence of a fatty acid beta-oxidation system in peroxisomes. The significance of such a beta-oxidation system in mammalian cells next to that in mitochondria, however, was unclear. The other paper was by Hajra and coworkers, who reported that the enzyme dihydroxyacetone phosphate acyltransferase (DHAPAT), known to catalyse the first step in etherphospholipid synthesis, was localized in peroxisomes and not in mitochondria and microsomes as thought previously. This important finding was soon followed by the discovery that the second enzyme involved in etherphospholipid synthesis, i.e. alkylidihydroxyacetone phosphate synthase (ADHAPS), was also localized in peroxisomes. These two findings prompted us to study etherphospholipid metabolism in Zellweger patients. In mammals, the main end products of etherphospholipid biosynthesis are the plasmalogens (1-O-alk-1’-enyl-2-acylphosphoglycerides), which are characterized by the presence of an alpha-, beta-unsaturated ether bond at the sn-1 position of the glycerol backbone. Plasmalogen analysis in tissues and erythrocytes from Zellweger patients revealed a marked deficiency of this special type of phospholipids. This breakthrough finding, soon thereafter, paved the way to the development of prenatal diagnostic methods.

Parallel to the work done in Amsterdam, Moser and coworkers found that the plasma levels of very long-chain fatty acids (VLCFAs) in ZS patients were markedly elevated in contrast to the levels of the long-chain fatty acids, which were normal. These results immediately suggested that the accumulation of VLCFAs had to do with the presence of a beta-oxidation system in peroxisomes and that the peroxisomal and mitochondrial beta-oxidation systems might serve different physiological purposes, catalysing the oxidation of different sets of substrates, which turned out to be correct, as outlined below.
From Zellweger syndrome to a set of peroxisomal biomarkers in blood and the discovery of a whole group of peroxisomal disorders

The availability of two peroxisomal markers including the VLCFAs and plasmalogens, as described above, which could be measured in a simple blood sample opened the way to search for additional peroxisomal disorders. This search was greatly helped by the subsequent discovery of other peroxisomal abnormalities including elevated levels of phytanic acid, pristanic acid, di-and trihydroxycholestanolic acid and pipecolic acid in plasma. Importantly, the findings in ZS patients also elicited a renewed interest in peroxisomes and inspired biochemists, cell biologists and geneticists to work on peroxisomes, which has resulted in detailed knowledge about the metabolic role of peroxisomes and how these organelles are biosynthesized (see reference 15 for review). All this work has revolutionized our knowledge about peroxisomes and peroxisomal disorders. First, established diseases which were not known to have any relationship to peroxisomes were identified as peroxisomal disorders (PDs). This is true for infantile Refsum disease (IRD),16 neonatal adrenoleucodystrophy (NALD),17 X-linked adrenoleucodystrophy (X-ALD)18 and rhizomelic chondrodysplasia punctata (RCDP).19 Second, new PDs were identified including DHAPAT deficiency (RCDP type 2),20 alkyl-DHAP synthase deficiency (RCDP type 3),21 acyl-coenzyme A (CoA) oxidase deficiency,22 D-bifunctional protein deficiency,23,24 2-methylacyl-CoA racemase (AMACR) deficiency25 and sterol carrier protein x (SCPx) deficiency.26 Another major breakthrough which is of a more recent date is the identification of aberrant phenotypes not previously known to have a peroxisomal origin. In this respect, the identification of isolated cerebellar ataxia in patients with a clear defect in peroxisome biogenesis is worth mentioning.27,28

Our laboratory has played a key role in the identification of most of these disorders, of which 15 have been identified so far (Table 1). All this work has led to the identification of thousands of patients worldwide, with our centre as one of the reference centres for the clinical as well as pre- and post-natal laboratory diagnosis of PDs.

Metabolic functions of peroxisomes

Peroxisomes catalyse a number of essential metabolic functions, of which four have been directly linked to peroxisomal diseases: (1) fatty acid beta-oxidation; (2) etherphospholipid biosynthesis; (3) fatty acid alpha-oxidation; and (4) glyoxylate detoxification. These will be discussed only briefly here. For a detailed review see Wanders and Waterham.15

Peroxisomal beta-oxidation

Peroxisomes oxidize their own set of fatty acids (FAs) including the VLCFA C26:0, the branched-chain fatty acid pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) and di-and trihydroxycholestanolic acid. The last two acids are synthesized from cholesterol in the liver and are converted into cholic acid and Chenodeoxycholic acid by beta-oxidation (Figure 1). The enzymes involved in peroxisomal beta-oxidation have been identified through the years and include two acyl-CoA oxidases, two bifunctional proteins with enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities and two thiolas, which are all different from their mitochondrial counterparts. Since peroxisomes lack a citric acid cycle and respiratory chain, full oxidation of fatty acids as first handled by peroxisomes requires mitochondria for further processing into CO₂ and H₂O (Figure 1).29,30

Etherphospholipid biosynthesis

Peroxisomes play an essential role in the synthesis of etherphospholipids with plasmalogens as main end products in mammals (see Figure 1) since the first part of the biosynthetic pathway formed by the two enzymes DHAPAT and ADHAPS occurs solely in peroxisomes (see Figure 1).31,32

Fatty acid alpha-oxidation

Some FAs, notably those which contain a methyl group at the 3-position, cannot be beta-oxidized but instead require alpha-oxidation first to remove the terminal carboxyl group as CO₂ to generate a 2-methyl FA which can be degraded by beta-oxidation. The structure of the pathway and the enzymes involved have been identified in recent years.33

Glyoxylate detoxification

Glyoxylate is a toxic metabolite which has to be degraded rapidly. This occurs in peroxisomes as mediated by the peroxisomal enzyme alanine glyoxylate aminotransferase (AGT). In the case of a deficiency of AGT, as in hyperoxaluria type 1,
### TABLE 1 The peroxisomal disorders

<table>
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<tr>
<th>Disorder abbreviation</th>
<th>MIM number</th>
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ARCD1, adenosine triphosphate (ATP)-binding cassette, subfamily D (ALD), member 1; ACOX, acyl-CoA oxidase; ADHAPS, alkylhydroxyacetone phosphate synthase; AGPS, alkylglycerone phosphate synthase; AGT, alanine glyoxylate aminotransferase; AGXT, alanine glyoxylate aminotransferase; AMACR, alpha-methylacyl-CoA racemase; ARD, adult Refsum disease; BAAT, bile acid-CoA, amino acid N-acyltransferase; CRD, classic Refsum disease; DBP, D-bifunctional protein; GNPAT, glyceronephosphate O-acyltransferase; HSD17B4, hydroxysteroid (17-beta) dehydrogenase 4; IRD, infantile Refsum disease; MIM, Mendelian Inheritance in Man; NALD, neonatal adrenoleucodystrophy; PBD, peroxisome biogenesis disorder; PEX, peroxisomal biogenesis factor; RCDP-1, rhizomelic chondrodysplasia punctata type 1; RCDP-2, rhizomelic chondrodysplasia punctata type 2; RCDP-3, rhizomelic chondrodysplasia punctata type 3; SCPx, sterol carrier protein X; PH-1, hyperoxaluria type 1; X-ALD, X-linked adrenoleucodystrophy; ZSD, Zellweger spectrum disorder.
glyoxylate accumulates and gives rise to glycolate and oxalate, which precipitates as calcium oxalate with devastating consequences, as observed in hyperoxaluria patients.34

Peroxisome biogenesis

In recent years much has been learned about the biogenesis of peroxisomes and many of the key players in peroxisome biogenesis have been identified, which has paved the way to the discovery of the molecular basis of most of the peroxisome biogenesis disorders. The biogenesis of peroxisomes resembles that of mitochondria in several respects, although there are differences as well. A common feature of the biogenesis of peroxisomes and mitochondria is that both organelles acquire their proteins through the specific uptake of proteins from the cytosol by virtue of specific receptors recognizing certain target sequences in the different proteins ultimately destined for the peroxisome or mitochondrion, respectively. In the case of peroxisomes, peroxisomal membrane proteins (PMPs) are imported first, with peroxisomal biogenesis factor 19 (PEX19) as cycling receptor, followed by the uptake of the peroxisomal matrix proteins via two different cycling receptors, i.e. peroxisomal biogenesis factor 7 (PEX7) and peroxisomal biogenesis factor 5 (PEX5), which recognize distinct groups of proteins containing either a peroxisomal targeting signal 2 (PTS2; in the case of PEX7) or peroxisomal targeting signal 1 (PTS1; in the case of PEX5) sequence as targeting signal. It was originally thought that peroxisomes are autonomous organelles like mitochondria, which means that they cannot form de novo but only from pre-existing organelles, but this is no longer true for peroxisomes (Figure 2). Resolution of the principal features of peroxisome biogenesis and the identification of the genes involved, called PEX genes, has allowed the identification of the molecular defect in virtually all patients with a defect in peroxisome biogenesis, as recently described by us in > 600 patients.35

Peroxisomes start their life in the endoplasmic reticulum, but may also form from pre-existing peroxisomes. The peroxin peroxisomal biogenesis factor 3 (PEX3) is essential for this initial phase of peroxisome biogenesis in which a pre-peroxisome is generated. The peroxin peroxisomal biogenesis
factor 16 (PEX16) also plays a role – albeit unresolved yet – in this initial phase of peroxisome biogenesis. Subsequently, the PMPs are inserted into this pre-peroxisomal vesicle with a key role for the cytosolic cycling receptor, PEX19. The next phase is the import of matrix proteins mediated by PEX5 for PTS1 proteins and PEX7 for PTS2 proteins.

Next, peroxisomes undergo proliferation and division. The division of peroxisomes involves three distinct sequential steps: elongation of peroxisomes, membrane constriction and, finally, fission of peroxisomes. The proteins dynamine-like protein 1 (DLP1), mammalian fission 1 (hFIS1) and mammalian mitochondrial fission factor (Mff) are involved in this process. Remarkably, the last three proteins play a role not only in the fission of peroxisomes but also in the fission of mitochondria. With respect to the elongation of peroxisomes, different PEX11 proteins are involved.

The peroxisomal disorders

The group of peroxisomal disorders is generally subdivided into two distinct groups with the disorders of peroxisome biogenesis in subgroup 1 and the disorders of peroxisome function in subgroup 2 (see Table 1).

Peroxisome biogenesis disorders

The PBD group is also subclassified into two groups, A and B. In group A disorders, both the PTS1 and PTS2 pathways of peroxisome biogenesis are impaired (group A), whereas in the disorders of group B only the PTS2 pathway is deficient (group B). This subclassification is important because the PBDs involved are clinically very different and also require different laboratory methods for identification.

Zellweger spectrum disorders

Peroxisome biogenesis disorders group A comprises three different disorders: ZS, neonatal adrenoleucodystrophy (NALD) and IRD. Since the identification of phenotypes in between ZS and IRD and ZS and NALD, the name Zellweger spectrum disorders (ZSDs) has been introduced. Clinical signs and symptoms of ZSD patients vary markedly, ranging from the full constellation of abnormalities seen in ZS patients (craniofacial, neurological,
skeletal, ocular and hepatological) to the milder presentations observed inNALD and especially IRD patients. Because of this diversity in clinical signs and symptoms we advocate performing peroxisomal biomarker profiling in blood samples of any patient with a (variable) combination of neurodevelopmental delay, retinopathy, perceptive deafness, plus or minus liver disease. In the meantime, however, new milder phenotypes have been identified, including isolated cerebellar ataxia, lacking these features.  

Laboratory diagnosis

The laboratory diagnosis of patients suspected to suffer from a ZSD starts with the analysis of known peroxisomal biomarkers with VLCFA analysis as a first-line test. If abnormal, the other parameters should be tested, followed by detailed studies in fibroblasts, which includes complementation analysis. Already in 1987 we established that ZS is a genetically heterogeneous disease through identification of four complementation groups. In subsequent years, additional complementation groups have been identified and now total 12 distinct groups.  

The underlying gene defect in each of these complementation groups has been determined, as shown in Table 2. We recently published our combined results of complementation analysis followed by molecular analysis of the relevant PEX gene in >600 ZSD patients (Table 2). Inspection of Table 2 shows that about two-thirds of our cohort of ZSD patients carry mutations in PEX1, whereas mutations in any of the other PEX genes are far less frequent.

**Treatment**

As a result of the multiplicity and severity of deficits, only supportive and symptomatic care is recommended for patients with classic ZS. For patients with the somewhat milder variants, considerable success has been achieved with multidisciplinary early intervention including physical and occupational therapy, hearing aids, alternative communication, nutrition and support for the parents. Although most patients continue to function in the profoundly or severely retarded range, some make significant gains in self-help skills, and several are now in a stable condition in their teens or even early 20s. In the past, several strategies have been tried to correct the biochemical abnormalities: (1) oral administration of cholic acid and chenodeoxycholic acid; (2) administration of the ethyl ester of docosahexaenoic acid; and (3) the oral administration of batyl alcohol in order to restore plasmalogen levels. Unfortunately, reports remain anecdotal and no firm conclusions can be drawn at present about the efficacy of any of the three strategies.

**Rhizomelic chondrodysplasia punctata**

Peroxisome biogenesis disorders group B contains only a single representative, i.e. RCDP. RCDP is clinically characterized by a disproportionately short stature primarily affecting the proximal parts of the extremities, typical facial appearance, congenital contractures, characteristic ocular involvement and severe mental and growth retardation. Radiological studies usually reveal shortening, metaphyseal cupping and disturbed ossification of humeri and/or femora, together with epiphyseal and extra-epiphyseal calcifications. The gene defective in RCDP type 1 is the PEX7 gene, which codes for PEX7, which is the cyclic receptor involved in the recognition of PTS2 proteins in the cytosol followed by the delivery to the peroxisome and uptake into the peroxisome (see Figure 2). Typical PTS2 proteins are ADHAPS, involved in etherphospholipid biosynthesis, and phytanoyl-CoA hydroxylase (PHYH/PHAX), involved in fatty acid alpha oxidation, which explains why RCDP patients have low plasmalogen levels in erythrocytes and high plasma phytanic acid levels.
Laboratory diagnosis

If a patient is suspected of suffering from RCDP, plasmalogen analysis is the first-line test to be performed since in virtually all patients, at least in our hands, plasmalogens in erythrocytes are grossly deficient. Analysis of phytanic acid is also helpful, although it should be remembered that phytanic acid levels may vary from normal to markedly elevated as phytanic acid is derived from dietary sources only. Subsequently, studies in fibroblasts are warranted to discriminate between RCDP type 1, 2 and 3 (see below) followed by analysis of the relevant genes, including PEX7, GNPAT or AGPS (see Table 1).

Treatment

No realistic options for therapy have been documented in literature so far.

Disorders of peroxisome function

The disorders of peroxisome function can best be subclassified according to the metabolic function which is actually lost. This includes peroxisomal fatty acid beta-oxidation, etherphospholipid synthesis, fatty acid alpha-oxidation, glyoxylate metabolism, bile acid conjugation and $\text{H}_2\text{O}_2$ metabolism.

The disorders of peroxisome fatty acid beta-oxidation

At present, five different disorders of peroxisomal beta-oxidation can be distinguished: (1) X-linked adrenoleucodystrophy; (2) acyl-CoA oxidase deficiency; (3) D-bifunctional protein deficiency; (4) SCPx deficiency; and (5) alpha-methylacyl-CoA racemase (AMACR) deficiency.

X-linked adrenoleucodystrophy

X-linked adrenoleucodystrophy has a widely variable phenotypic presentation with at least six different phenotypic variants described. The classification of X-ALD is somewhat arbitrary and based on the age at onset and the organs principally involved. X-ALD is the most common single peroxisomal disorder with a minimum incidence of 1:21 000 males in the USA\textsuperscript{38} to 1 : 15 000 males in France. The two most frequent phenotypes are childhood cerebral ALD (CCALD) and adrenomyeloneuropathy (AMN). Onset of CCALD is between 3 and 10 years of age, with progressive behavioural, cognitive and neurological deterioration, often leading to total disability within 3 years. The cerebral phenotype is not only observed in childhood, but may also present later in life in adolescence (adolescent cerebral ALD; ACALD) or adulthood (adult cerebral ALD). There is a marked difference between the cerebral phenotypes, on the one hand, and AMN, on the other, since the cerebral phenotypes show an inflammatory reaction in the cerebral white matter which resembles, but can be distinguished from, what is observed in multiple sclerosis. In contrast with CCALD, the inflammatory response is absent or mild in AMN, which has a much later age of onset (28 ± 9 years) and a much lower rate of progression. It is important to mention that approximately 40–50% of women heterozygous for X-ALD develop AMN-like symptoms in middle age or later. Cerebral involvement and adrenocortical insufficiency, however, are rare.

Laboratory diagnosis

Plasma VLCFA analysis is the first line of testing in patients suspected of suffering from one of the X-ALD phenotypes and has been proven to be an exceptionally robust biomarker for X-ALD, especially when the algorithm developed by Moser et al.\textsuperscript{38} is used. If abnormal, molecular analysis of the adenosine triphosphate (ATP)-binding cassette, subfamily D (ALD), member 1 (ABCD1) gene is warranted, which has so far revealed > 600 different mutations (see: http://www.x-ald.nl).

Treatment

It is crucially important to provide adrenal steroid hormone therapy for every ALD patient with adrenocortical insufficiency. Almost all affected boys and 60% of men with AMN have impaired adrenal reserve.\textsuperscript{38} Consequently, all patients diagnosed should undergo an adrenocorticotropic hormone (ACTH) stimulation test.

Allogeneic haematopoietic stem cell transplantation (HCT) is the only treatment that can arrest or even reverse cerebral demyelination provided the procedure is performed at an early stage of the disease and this point is absolutely crucial (see reference 40 for the latest information). Although more than 200 X-ALD patients have now been transplanted successfully\textsuperscript{41} and 20 years' follow-up of treated patients has confirmed the beneficial effect of allogeneic HCT in X-ALD definitively, the procedure remains associated with serious limitations. Therefore, human stem cell (HSC) gene therapy will certainly be an alternative to allogeneic HCT in the near future.
Two X-ALD patients have recently been successfully treated using this strategy.\textsuperscript{42}

**Acyl-CoA oxidase deficiency**

All patients identified so far had psychomotor retardation, but did acquire limited skills, including the ability to sit and stand up unsupported, with voluntary control of hand function and limited speech. In most patients (83%), however, there was loss of motor achievements with a mean age at regression of 28 months. Brain imaging [magnetic resonance (MRI) and/or computerized tomography (CT)] revealed cerebral and/or cerebellar white-matter abnormalities in all patients investigated (12 out of 12). Three of these patients showed neocortical dysplasia. Other abnormalities include hypotonia (97%), seizures (91%), visual system failure (78%), impaired hearing (77%), facial dysmorphia (50%), hepatomegaly (50%) and failure to thrive (37%). Interestingly, two adult patients with acyl-CoA oxidase deficiency have recently been described.\textsuperscript{43}

**Laboratory diagnosis**

Elevated plasma VLCFA levels have been found in all patients described in literature with proven acyl-CoA oxidase deficiency except for one. Indeed, Rosewich \textit{et al.}\textsuperscript{44} described a true case of acyl-CoA oxidase deficiency with normal plasma VLCFAs. Acyl-CoA oxidase deficiency in this patient was suspected based on the characteristic MRI findings. The molecular basis of acyl-CoA oxidase deficiency has been worked out and has revealed marked genetic heterogeneity with often private mutations.\textsuperscript{45}

**Treatment**

No treatment options have been described for acyl-CoA oxidase deficiency.

**D-Bifunctional protein deficiency**

D-Bifunctional protein deficiency was first described independently by Suzuki \textit{et al.}\textsuperscript{24} and our own group.\textsuperscript{24} In 2006, we reported on the clinical and biochemical spectrum of DBP deficiency in 126 patients. The clinical presentation of DBP deficiency is dominated by neonatal hypotonia (98%) and seizures (93%) within the first months of life. Failure to thrive was observed in 43% of the patients. Visual system failure, including nystagmus, strabismus and/or failure to fixate objects at 2 months, was also frequent (54%). Almost none of the patients acquired any psychomotor developments and the few patients who did showed progressive loss of motor achievements subsequently. External dysmorphism was a frequent finding (58%) and resembled that of patients with ZS as characterized by high forehead, high-arched palate, large fontanelles, long philtrum, epicanthal folds, hypertelorism, macrocephaly, shallow supraorbital ridges, retrognathia and low-set ears.

D-Bifunctional protein deficiency is subdivided into three different types depending on whether it is the complete DBP which is missing (type I) or only the hydratase (type II) or 3-hydroxyacyl-CoA dehydrogenase component (type III) which is deficient. Most patients die before 2 years of age.

**Laboratory diagnosis**

The deficient activity of DBP leads to a number of biochemical abnormalities including elevated plasma levels of VLCFAs, phytanic acid, pristanic acid and the bile acid intermediates di- and trihydroxycholestanolic acid, although not in all patients. First-line testing should include VLCFA analysis, which is abnormal in virtually all patients. Subsequent detailed studies in fibroblasts need to be done to determine whether or not there is a full deficiency of DBP or a defect in one of the two components of DBP only. Finally, molecular analysis has to be done to pinpoint the molecular defect, which has revealed a multitude of often private mutations with only one frequent mutation in the hydroxysteroid (17-β) dehydrogenase 4 (\textit{HSD17B4}) gene (c.46G→A; allele frequency 24%).

**Treatment**

No realistic therapeutic measures for DBP deficiency have been described.

**Peroxisomal sterol carrier protein X deficiency**

This defect has only been described in a single patient so far. The patient involved is a 45-year-old white man with a 28-year history of dystonic head tremor and spasmodic torticollis. He had noticed a starter for the first time at 7 years of age. At 17 years of age he developed spasmodic torticollis to the left side with dystonic head tremor in stressful situations. On check-up at 29 years of age, brain MRI showed bilateral hyperintense signals in the thalamus, butterfly-like lesions in the pons and some lesions in the occipital region. Neurological examination revealed hyposmia, pathological saccadic eye
movements and a slight hyperacusis. There were signs of slight cerebellar ataxia with a left-sided intention tremor, balance and gait impairment, and a slight left-sided rebound phenomenon.

**Laboratory diagnosis**

Analysis of the peroxisome metabolites in plasma of the patient revealed no abnormalities except for trace amounts of the bile acid intermediates 3α, 7α-dihydroxy-5β-cholestanolic acid (DHCA) and 3α, 7α, 12α-trihydroxy-5β-cholestanolic acid (THCA). Interestingly, more marked abnormalities were found in the patient’s urine including large amounts of bile alcohols (see Ferdinandusse et al. for further details).

**Treatment**

As a result of the elevated pristanic acid levels, the single patient with SCPx deficiency identified so far began a phytanic acid-restricted diet, which led to decreased pristanic acid levels at a marginally elevated level. Since the beginning of the diet, no progression of symptoms has been observed.

### 2-Methylacyl-CoA racemase deficiency

2-Methylacyl-CoA racemase deficiency was first described in 2000 in two patients whose clinical presentation was dominated by a late-onset sensory motor neuropathy. Subsequently, this defect has been found in a few additional patients. Interestingly, Setchell et al. described a completely different phenotype of AMACR deficiency dominated by severe liver abnormalities early in life. Recently, Kapina et al. described yet another clinical presentation of AMACR deficiency, characterized by stroke-like episodes and recurrent rhabdomyolysis.

**Laboratory diagnosis**

Patients identified so far have shown clear peroxisomal abnormalities in plasma characterized by elevated pristanic acid and di- and trihydroxycholestanoic acid, but normal VLCFA levels. Urine analysis has also revealed clear abnormalities showing large amounts of abnormal bile alcohols.

**Treatment**

No therapeutic efforts have been tried in AMACR deficiency so far.

### Disorders of etherphospholipid biosynthesis

#### Rhizomelic chondrodysplasia punctata type 2 and type 3

In 1992, we reported a patient with all the signs and symptoms described for RCDP, but with a different biochemical profile both in blood as well as in fibroblasts. The finding that erythrocyte plasmalogen levels were fully deficient while plasma phytanic acid was normal suggested that we were dealing with a different peroxisomal form of RCDP. Detailed studies in fibroblasts followed up by molecular studies a few years later revealed that DHAPAT deficiency in this patient was due to mutations in the encoding gene GNPAT. Two years later, we identified another peroxisomal form of RCDP, again in a patient with all the signs and symptoms of RCDP, but in whom a full deficiency of alkyl-DHAP synthase (ADHAPS) was identified as caused by mutations in the encoding gene AGPS. RCDP type 1 due to mutations in PEX7 has remained the most frequent form of RCDP with type 2 and type 3 described in only 10 and 5 patients, respectively.

**Laboratory diagnosis**

First-line testing of RCDP type 2 and type 3 includes plasmalogen analysis in erythrocytes. In all patients we have identified through the years erythrocyte plasmalogen levels have been found to be very much deficient (< 10%). Subsequent studies in fibroblasts followed up by molecular studies are needed to pinpoint the true enzymatic and molecular defect.

### Fatty acid alpha-oxidation

#### Refsum disease

Refsum disease was first described in the 1940s. The full constellation of cardinal features, as described by Refsum, which includes retinitis pigmentosa (RP), cerebellar ataxia and chronic polyneuropathy, is rarely seen in individual Refsum patients. Indeed, detailed studies by Wierzbicki et al. have shown that RP is an early clinical sign present in all Refsum patients, with ataxia and polyneuropathy, which develop later, observed in only around 70% and 50% of patients respectively. Interestingly, virtually every individual ultimately diagnosed as having Refsum disease experiences visual symptoms first. Night blindness and loss of visual capacity, especially when combined
with anosmia, should lead to prompt analysis of plasma phytanic acid in order to start therapy. Furthermore, electroretinography (ERG) should be done, which shows either reduction or a complete loss of rod and cone responses.

**Laboratory diagnosis**

First-line testing for Refsum disease includes phytanic acid analysis in plasma, which is abnormal in all Refsum patients identified so far. Subsequent work in fibroblasts, followed up by molecular studies, has to be done to pinpoint the enzymatic and molecular defect.

**Treatment**

No curative therapy currently exists for Refsum disease. Plasma phytanic acid concentrations can be reduced by 50–70%, typically to about 100–300 µmol/l, by restricting dietary intake of phytanic acid or eliminating phytanic acid by plasmapheresis or lipid apheresis. This reduction in plasma phytanic acid successfully resolves symptoms of ichthyosis, sensory neuropathy and ataxia in approximately that order. However, it remains uncertain whether or not treatment affects the progression of the retinitis pigmentosa, anosmia and deafness. Sudden weight loss should be avoided in order to prevent mobilization of phytanic acid into plasma. Post-operative care requires parenteral nutrition.

**Disorders of glyoxylate metabolism**

**Hyperoxaluria type 1**

Only a single form of hyperoxaluria is due to a deficient activity of a peroxisomal enzyme (alanine glyoxylate aminotransferase). This form of primary hyperoxaluria is called type I (PH-I). The phenotypic variability of PH-I is large, ranging from severe early-onset oxalosis and early death to adult presentations that resemble idiopathic kidney stone disease. PH-I often goes undetected for years until severe, irreversible kidney damage has occurred. In general, PH-I has a bad prognosis, especially in its severe form, unless liver (plus kidney) transplantation is performed. PH-I should be considered in all cases of familial stone disease and renal failure of unknown cause.

**Laboratory diagnosis**

Urinary oxalate analysis is the method of choice to check for PH-I. In PH-I patients overproduction of oxalate in the liver results in very high urinary oxalate excretion, typically amounting to >1 mmol/1.73 m² per day. A raised urinary glycolate level is strongly suggestive of PH-I, but glycolate may also be entirely normal in PH-I patients. Although measurement of AGT enzyme activity in a liver biopsy specimen has long been the gold standard for the diagnosis of PH-I, direct molecular analysis of the alanine glyoxylate aminotransferase (AGXT) gene is rapidly becoming the first choice for diagnosis. This is because in a significant proportion of PH-I patients AGT enzyme activity is not deficient, at least not in vitro in a liver homogenate, since the enzyme is targeted to mitochondria rather than to peroxisomes.

**Treatment**

Overall, the treatment of PH-I greatly depends on the degree of renal function. Conservative measures should be initiated as soon as possible with the goal of preserving renal function. The following measures apply to all types of PH-I: (1) high fluid intake has been proven to be effective in kidney stone diseases including PH-I; (2) alkalinization of the urine with alkali citrate can reduce urinary calcium oxalate saturation by forming complexes with calcium, consequently decreasing calcium oxalate precipitation; and (3) dialysis, both peritoneal dialysis and haemodialysis, has been used either alone or in combination in order to maximize oxalate removal. In all patients with PH-I, pyridoxine should be tried. Indeed, one-third of patients with PH-I respond to pharmacological doses of pyridoxine. Pyridoxine 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 pyridoxine-resistant forms of the disease. In the latter group of patients, liver transplantation and/or combined liver–kidney transplantation is the only option left. Pre-emptive isolated liver transplantation may be an option in selected patients, but in most cases the liver is replaced only after sufficient kidney damage has occurred, thus opting for combined liver–kidney transplantation. It has been used successfully with excellent outcome even in small infants. A sequential procedure (first liver transplantation, then dialysis until sufficient oxalate has been cleared from the body, followed by kidney transplantation) may be
Disorder of bile acid conjugation

Bile acid-CoA, amino acid N-acyltransferase deficiency

Bile acid-CoA, amino acid N-acyltransferase deficiency was first identified by Sutchell et al.54 followed by subsequent cases described by Carlton et al.55 Indeed, Carlton et al. described the identification of patients from the Amish community with bona fide mutations in the BAAT gene. Patients were homozygous for a c.226A→G mutation (M76V) and showed increased serum bile acids, which were virtually fully unconjugated. Clinical features of these patients included fat malabsorption, failure to thrive, coagulopathy, pruritus and chronic upper respiratory infection. They did not have jaundice and had normal serum γ-glutamyltransferase (gamma-GT) concentrations. In 2007, Heubi et al.56 reported six additional patients with BAAT deficiency.

Laboratory diagnosis

Laboratory diagnosis of BAAT deficiency involves analysis of bile acids in plasma and urine, preferably by tandem mass spectrometry, characterized by the virtually complete absence of glycine and taurine bile acid conjugates in body fluids. Subsequent molecular testing needs to be done to establish BAAT deficiency with certainty.

Treatment

In patients with BAAT deficiency, symptoms such as fat malabsorption, failure to thrive and coagulopathy reportedly respond to treatment with ursodeoxycholic acid.55

Prenatal diagnosis

Without going into any detail it is sufficient to state that prenatal diagnosis is now possible in all peroxisomal diseases identified so far. With the identification of the molecular defect in virtually all peroxisomal disorders, especially the disorders of peroxisome biogenesis in recent years, methods have now shifted from biochemical and cell biological methods to molecular methods. These methods all can be applied to chorionic villous biopsy specimens, thus allowing early detection of affected fetuses.

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