Peroxisomal disorders

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Key words: peroxisomes, inborn errors, errors of morphogenesis, Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease, rhizomelic chondrodysplasia punctata

Peroxisomes are subcellular organelles catalyzing a number of indispensable functions in cellular metabolism. The importance of peroxisomes is stressed by the existence of an expanding number of genetic diseases in which there is an impairment of one or more peroxisomal functions. The prototype of this group of diseases is the cerebro-hepato-renal syndrome of Zellweger (ZS), first described as a familial syndrome of multiple congenital defects in 1964. ZS is characterized by the presence of dysmorphias and polymalformative syndrome, severe neurologic abnormalities including neurosensory defects and hepato-intestinal dysfunction with failure to thrive and usually early death. Other peroxisomal disorders share some of these symptoms, but with varying degrees of organ involvement, severity of dysfunction and duration of survival. This paper provides an overview of the peroxisomal disorders including their clinical, biochemical and molecular characteristics with particular emphasis on the clinical presentation in neonates.

Clinical presentation

At least 21 clinically and biochemically markedly heterogeneous disorders of peroxisomal dysfunction have been identified, most of them involving neurological defects [1,2]. In the past the classification of these disorders became somewhat confusing as the amount of information rapidly expanded. Most of the new disorders were classified by reference to the three main original clinical descriptions, namely, Zellweger syndrome (ZS) [3], neonatal adrenoleukodystrophy (NALD) [4,5], and infantile Refsum disease (IRD) [6,7]. This is not surprising, as these three clinical conditions correspond to the three main early-infantile categories of clinical symptoms found in peroxisomal disorders, predominant polymalformative syndrome, as observed in classical ZS and in rhizomelic chondrodysplasia punctata (RCDP) [8], predominant neurological presentation, as in NALD, and predominant hepato-digestive symptoms, as in IRD. Given the diversity of clinical and biochemical abnormalities and the rapidly advancing knowledge on function and biology of the peroxisome, it is justified and simplest to regard the clinical diagnosis as a function of both the age of the patient (Table 1) [9] and the predominant features of recognition: congenital malformation and dysmorphism, neurologic dysfunction and hepatodigestive manifestations [10].

Two prototypes of neonatal presentation are ZS, which is the most severe condition, and RCDP. Their phenotypes are distinct from the other disorders and should not cause diagnostic difficulties when all the characteristic manifestations are present.

Zellweger syndrome

ZS is dominated by the typical craniofacial dysmorphism (high forehead, large anterior fontanel, hypoplasic supraorbital ridges, epicanthus, and deformed ear lobes) (Fig. 1) and profound neurologica

neonatal seizures, glaucoma, retinal degeneration, impaired hearing, and enlarged liver with impaired function. There is usually calcific stippling of the epiphyses and small renal cysts. Brain abnormalities in ZS include cortical dysplasia, a neuronal migration defect, and dysmyelination. Death usually occurs within the first year of life although a few patients survive beyond the first year of life and reach some developmental milestones. The presence of congenital malformations points to an abnormal process during pregnancy, as observed in other inborn errors or in several conditions of metabolic disturbance of the mother during pregnancy (Table 2). In ZS peroxisomes are deficient due to a genetic defect in one of the genes involved in peroxisome biogenesis. As a consequence many peroxisomal enzymes are not active and hepatic peroxisomes are not recognizable.

**Rhizomelic chondrodysplasia punctata**

RCDP is characterized by the presence of shortened proximal limbs, facial dysmorphia, cataracts, psychomotor retardation, coronal clefts of vertebral bodies, and stippled foci of calcification of the epiphyses during infancy, which may disappear after the age of 2 years [1,8]. The chondrodysplasia punctata is more widespread than in ZS and may involve extraskeletal tissues (Fig. 2). Some patients

![Figure 1](image-url)  **Figure 1.** Classical Zellweger syndrome: 2 weeks old, large fontanel, epicanthal folds, hypertelorism, prominent forehead.

<table>
<thead>
<tr>
<th>Clinical symptoms of peroxisomal disorders related to age</th>
<th>Disorder</th>
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<tr>
<td><strong>Neonatal period</strong></td>
<td>ZS, ZS-like syndrome, NALD</td>
</tr>
<tr>
<td>Hypotonia, areactivity</td>
<td>Hyperpippecolic acidemia*</td>
</tr>
<tr>
<td>Encephalopathy, seizures</td>
<td>Acyl-CoA oxidase deficiency (pseudo-NALD)</td>
</tr>
<tr>
<td>Craniofacial dysmorphism</td>
<td>Bifunctional protein deficiency</td>
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<tr>
<td>Dysmorphic features</td>
<td>Peroxisomal thiolase deficiency (pseudo-ZS)</td>
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<td></td>
<td>Unclassified peroxisomal β-oxidation disorders</td>
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<tr>
<td></td>
<td>Mevalonic aciduria</td>
</tr>
<tr>
<td>Skeletal abnormalities (calcific stippling, shortened proximal limbs)</td>
<td>RCDP type I–III</td>
</tr>
<tr>
<td><strong>First 6 months of life</strong></td>
<td>IRD, pseudo-IRD</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>Milder or atypical forms of ZS, NALD and RCDP</td>
</tr>
<tr>
<td>Digestive problems, malabsorption</td>
<td>Hyperpippecolic acidemia*</td>
</tr>
<tr>
<td>Hypocholesteremia, Vit. E deficiency</td>
<td>Mevalonic aciduria</td>
</tr>
<tr>
<td>Hepatomegaly, prolonged jaundice</td>
<td></td>
</tr>
<tr>
<td>Visual abnormalities (retinopathy, cataracts, optic nerve dysplasia)</td>
<td></td>
</tr>
</tbody>
</table>

ZS, Zellweger syndrome; NALD, neonatal adrenoleukodystrophy; RCDP, rhizomelic chondrodysplasia punctata. *Enzyme defect not established definitively.
have ichthyosis. The clinical heterogeneity of RCDP is best exemplified by patients with the classical peroxisomal defects observed in RCDP but no or little rhizomelic shortening of the extremities and only moderate mental retardation [11,12]. Conversely, some patients with the typical clinical phenotype of RCDP have only a single enzyme deficiency [13,14]. Classical RCDP and its variants must be distinguished from other forms of chondrodysplasia punctata such as Conradi–Hünermann syndrome [15] and the X-linked dominant and recessive forms of chondrodysplasia punctata.

Neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD)

ZS, NALD, and IRD are now generally accepted as representing a continuous spectrum of disease severity; ZS is at the severe end of this spectrum and IRD represents the mildest syndromic phenotype [1]. At birth, the predominant symptom is often a severe hypotonia with no reactivity (Fig. 3), which can be mistaken for several pathological conditions including congenital neuromuscular disorders, disorders of the central and autonomic nervous system, and malformation syndromes (Table 3). NALD appears clinically as a milder form of ZS with often more prominent cerebral

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**Table 2. Metabolic processes inducing congenital malformations**

**Inborn errors affecting the fetus**
- Peroxisomal disorders (ZS, RCDP, mevalonic aciduria)
- Inborn errors of cholesterol biosynthesis (Smith–Lemli–Opitz, desmosterolosis)
- Lysosomal storage disorders
- Respiratory chain defects
- Pyruvate dehydrogenase deficiency
- Glutaric aciduria type II (multiple acyl-CoA dehydrogenase deficiency)
- Carnitine palmitoyl-transferase-II deficiency
- 3-Hydroxy-isobutyryl-CoA-deacylase deficiency
- Nonketotic hyperglycinemia
- Congenital defects of glycosylation
- Inborn errors of collagen
- Hypophosphatasia
- Hypoparathyroidism
- Leprechaunism

**Metabolic disturbances of the mother**
- Phenylketonuria
- Alcohol, drugs
- Diabetes
- Vitamin deficiencies (riboflavin, folate)

*Associated with intrauterine growth retardation.*

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Figure 2. Rhizomelic chondrodysplasia punctata: Rhizomelia with shortening of proximal limbs, abnormal calcific stippling of epiphyses and extraskeletal tissues.

Figure 3. Zellweger syndrome: 2 weeks old, severe hypotonia.
demyelination but absent or milder craniofacial dysmorphia. Chondrodysplasia and renal cysts are absent.

Although IRD patients share some clinical features with ZS, they differ from ZS with respect to age of onset, initial symptoms, degree of dysfunction, and survival. Little or no facial dysmorphia is noted and early developmental milestones are usually normal before psychomotor retardation begins between the age of 1–3 years. This is followed by a complete arrest and autistic behaviour associated with chorioretinopathy and sensorineural hearing loss [10].

### Defects of peroxisomal β-oxidation:
Acyl-CoA oxidase deficiency, D-bifunctional protein deficiency, peroxisomal thiolase deficiency, 2-methylacyl-CoA-racemase deficiency

Interestingly, the clinical presentation of the first three disorders resembles that of the ZS/NALD-spectrum. This is especially true for patients with D-bifunctional protein deficiency since most patients identified so far (>40) show severe clinical abnormalities including hypotonia, craniofacial dysmorphia and neonatal seizures [2,16]. Acyl-CoA oxidase deficiency was first described in 1988 [17] in siblings with all the signs and symptoms of NALD. Peroxisomal thiolase deficiency has only been described in a single patient with a ZS-like presentation [18]. In contrast to all other patients with disorders of peroxisomal β-oxidation, those with racemase deficiency do not present early in life, but instead develop a late-onset neuropathy [19].

### Mevalonic aciduria

Mevalonate kinase deficiency is the only known disorder involving the peroxisomal component of isoprenoid synthesis. Interestingly, apart from the classical form of mevalonate kinase deficiency, which is associated with profound developmental delay, facial dysmorphia and cataracts [20], this deficiency has also been observed in hyperimmunoglobulinaemia D and periodic fever syndrome [21,22].

### Unusual presentations

Several unusual presentations of peroxisomal disorders (mostly biogenesis defects) have recently come to attention including a patient presenting with eye abnormalities characteristic of Leber congenital amaurosis [23], an infant with a presentation similar to spinal muscular atrophy (Werdnig–Hoffmann) [10,24], a patient with an atypical form of Refsum disease characterized by psychomotor retardation and bone abnormalities [25] and individuals with peroxisome biogenesis disorders.

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<table>
<thead>
<tr>
<th>Table 3. Differential diagnosis in the neonatal period</th>
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<tbody>
<tr>
<td><strong>Disorders with hypotonia</strong></td>
</tr>
<tr>
<td><em>Neuromuscular disorders</em></td>
</tr>
<tr>
<td>● Werdnig–Hoffmann disease</td>
</tr>
<tr>
<td>● Congenital myopathies</td>
</tr>
<tr>
<td>● Congenital muscular dystrophies</td>
</tr>
<tr>
<td>● Congenital myasthenia</td>
</tr>
<tr>
<td>● Congenital polynuenceopathy</td>
</tr>
<tr>
<td>● Myotonic dystrophy</td>
</tr>
<tr>
<td><em>Familial dysautonomia</em></td>
</tr>
<tr>
<td><em>Central nervous system disorders</em></td>
</tr>
<tr>
<td>● Pelizaeus–Merzbacher disease</td>
</tr>
<tr>
<td>● Prader–Willi syndrome</td>
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<tr>
<td>● Lowe syndrome</td>
</tr>
<tr>
<td>● Chromosomal aberrations</td>
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</table>

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presenting at an older age with less severe manifestations and a more gradual progression [26,27].

X-linked adrenoleukodystrophy/adrenomyeloneuropathy and Refsum disease

These are peroxisomal disorders with onset in the school-age period and will not be discussed here.

Metabolic defects

Peroxisomes are ubiquitous components of eukaryotic cells and can range in abundance from hundreds to thousands per cell in mammals. The name peroxisome derives from the presence of catalase, which converts hydrogen peroxide into oxygen and water. The single peroxisome membrane is impermeable to protons and small metabolites, creating an enzymatically and chemically unique microenvironment within the cell [28]. Peroxisomal enzymes catalyze a number of essential metabolic functions, most of which involve the metabolism of lipids (Fig. 4) [29]. Only the most relevant metabolic functions will be discussed below. From a biochemical viewpoint, peroxisomal disorders can be divided into two groups reflecting the extent of peroxisomal dysfunction. Group 1 is characterized by multiple defects (also called peroxisome biogenesis disorders) whereas group 2 contains single peroxisomal protein/enzyme deficiencies (Table 4) [30]. This biochemical classification is not very useful for the clinician, because there is a poor correlation between clinical and biochemical phenotype. Similar clinical phenotypes may correspond to different biochemical lesions, as illustrated by RCDP, which is associated with either four biochemical abnormalities [1] or with an isolated enzyme defect (Table 4) [13,14]. Conversely, the same biochemical defect(s), or even the same genetic complementation group, can be associated with very dissimilar clinical phenotypes, as illustrated by classic ZS and IRD [10,26].

β-oxidation of fatty acids and bile acid synthesis

Peroxisomes catalyze the β-oxidation of a distinct set of fatty acid derivatives, including very-long-chain fatty acids (VLCFAs) such as C24:0 or C26:0 and branched-chain fatty acids such as pristanic acid and di-/trihydroxycholestanolic acid. With
respect to peroxisomal disorders, the most important substrates are: 1. VLCFAs; 2. pristanic acid, as derived mainly from dietary phytanic acid by α-oxidation; and 3. di- and trihydroxycholestanolic acid (DHCA and THCA). After activation to their coenzyme A esters the straight chain fatty acids and branched chain fatty acids are degraded by two different sets of enzymes including two acyl-CoA oxidases, D-bifunctional protein and two peroxisomal thiolases (Fig. 4) [2]. DHCA and THCA are produced from cholesterol in the liver and undergo β-oxidation in the peroxisome to produce the CoA-esters of chenodeoxycholic and cholic acid, respectively. This implies that ‘bile acid synthesis’ represents a degradative mechanism rather than a true biosynthetic process.

**Ether-phospholipid (plasmalogen) biosynthesis**

The two enzyme activities responsible for the introduction of the characteristic ether linkage in ether-linked phospholipids (i.e. dihydroxyacetonephosphate acyltransferase [DHAPAT]) and alkyl-dihydroxyacetonephosphate synthase [alkyl-DHAP synthase]) are both located in peroxisomes. Remarkably, the functional role of plasmalogens, which are particularly abundant in nervous tissue, has remained enigmatic until now. However, the identification of isolated deficiencies of DHAPAT and alkyl-DHAP synthase in patients with severe RCDP [13,14] clearly shows that ether-phospholipids are of central importance in humans.

**Phytanic acid α-oxidation**

The degradation of this branched chain fatty acid – exclusively of dietary origin – requires first an activation to phytanoyl-CoA, which is followed by a three-step cycle of α-oxidation ending in pristanic acid [31].

**Isoprenoid (cholesterol) biosynthesis**

There is growing evidence that the first part of the isoprenoid biosynthetic pathway from acetyl-CoA...
to farnesylpyrophosphate is peroxisomal [2]. This implies that mevalonate kinase deficiency is a peroxisomal disorder.

**L-pipecolic acid oxidation**

Lysine is normally degraded via the saccharopine pathway, but may also be degraded via the L-pipecolic acid pathway which may especially be important in brain. L-pipecolic acid is produced from L-Lysine in two enzymatic steps and then oxidized by L-pipecolate oxidase, a peroxisomal enzyme in humans [2].

**Biosynthesis of polyunsaturated fatty acids such as docosahexaenoic acid (DHA)**

The last step involved in DHA (C22:6ω3) formation occurs strictly in peroxisomes [2]. This explains the deficiency of DHA in patients with ZS as originally found by Martinez [32].

**Genetics of peroxisome biogenesis disorders (PBDs)**

Individuals with PBDs synthesize peroxisomal proteins normally but display defects in the import of peroxisomal enzymes into the lumen of the organelle. Peroxisomal proteins are encoded by nuclear genes, synthesized on free cytosolic ribosomes and imported posttranscriptionally [33]. Newly synthesized peroxisomal proteins contain specific peroxisomal targeting signals (short amino acid sequences) that direct them to and into the peroxisomes. For the matrix proteins, these include the peroxisome targeting signal 1 (PTS1), which is used by more than 90% of the known matrix proteins. A few matrix proteins are targeted by a distinct signal, PTS2. The targeting signals for the integral peroxisomal membrane proteins (PMP) are poorly understood [34]. Complementation studies have established that the ZS/NALD/IRD-spectrum is genetically heterogeneous with at least 12 distinct complementation groups (CGs) [1,35]. Virtually all these patients are defective both in PTS1 and PTS2 protein import [1], while patients with classic RCDP, in contrast, have a defect of the PTS2 receptor [36–38]. Genetic studies mainly in yeast have identified numerous PEX genes and proteins (peroxins) required for peroxisome biogenesis [39]. Subsequently, gene identification strategies have determined the molecular basis in all known PBD CGs other than CG8 (Table 5) [1,35]. A founder effect appears to account for the large number of PBD patients in CG1 (ZS/NALD/IRD-spectrum) and in CG11 (RCDP).

**Diagnostic tests**

According to our experience, biochemical investigation for peroxisomal functions should be

### Table 5. Genetic defects in peroxisome biogenesis disorders

<table>
<thead>
<tr>
<th>CG</th>
<th>% patients</th>
<th>Mutated gene</th>
<th>Characteristics of gene product</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG1</td>
<td>54</td>
<td><strong>PEX1</strong></td>
<td>143 kDa AAA-ATPase</td>
</tr>
<tr>
<td>CG2</td>
<td>1</td>
<td><strong>PEX5</strong></td>
<td>68 kDa PTS1 receptor</td>
</tr>
<tr>
<td>CG3</td>
<td>4</td>
<td><strong>PEX12</strong></td>
<td>41 kDa PMP, C-terminal zinc-binding</td>
</tr>
<tr>
<td>CG4</td>
<td>12</td>
<td><strong>PEX6</strong></td>
<td>104 kDa AAA-ATPase</td>
</tr>
<tr>
<td>CG7</td>
<td>2</td>
<td><strong>PEX10</strong></td>
<td>37 kDa PMP, C-terminal zinc-binding</td>
</tr>
<tr>
<td>CG8</td>
<td>5</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>CG9</td>
<td>&lt;1</td>
<td><strong>PEX16</strong></td>
<td>39 kDa PMP required for PMP import</td>
</tr>
<tr>
<td>CG10</td>
<td>1</td>
<td><strong>PEX2</strong></td>
<td>35 kDa PMP, C-terminal zinc-binding</td>
</tr>
<tr>
<td>CG11</td>
<td>19</td>
<td><strong>PEX7</strong></td>
<td>36 kDa PTS2 receptor</td>
</tr>
<tr>
<td>CG12</td>
<td>1</td>
<td><strong>PEX3</strong></td>
<td>42 kDa PMP, required for PMP import</td>
</tr>
<tr>
<td>CG13</td>
<td>&lt;1</td>
<td><strong>PEX13</strong></td>
<td>44 kDa PMP, C-terminal SH3 domain</td>
</tr>
<tr>
<td>CG14</td>
<td>&lt;1</td>
<td><strong>PEX19</strong></td>
<td>33 kDa, putative PMP-receptor</td>
</tr>
</tbody>
</table>

**PEX**, genes encoding peroxins (proteins involved in peroxisomal import and biogenesis); **CG**, complementation group; **PTS**, peroxisome targeting signal; **PMP**, peroxisomal membrane protein.
considered in patients showing one or more of the symptoms listed in Table 1. Not all peroxisomal disorder patients may fulfill these criteria and others may display features so far not considered as indicative.

Urinary pipecolic acid, medium- and long-chain dicarboxylic aciduria, hyperoxaluria and mevalonic aciduria can be detected by general metabolic screening. Most of the peroxisomal disorders with neurologic involvement are associated with an accumulation of VLCFA and/or impaired bio-synthesis of ether-phospholipids (plasmalogens), suggesting that assays of plasma VLCFA and plasmalogens in erythrocytes should be used as primary tests. If either result is abnormal, a peroxisomal disorder should be substantiated by further analysis in plasma, fibroblasts and/or liver. A variety of diagnostic assays considered ideal for the workup of patients with a suspected peroxisomal disorder is listed in Table 6. It must be stressed that not all peroxisomal disorders can be detected by just measuring plasma VLCFAs. Extensive peroxisomal investigations are necessary even when the clinical phenotype is typical, since some disorders may be associated with very atypical biochemical phenotypes [10,30].

Prenatal diagnosis

Almost all peroxisomal disorders can be identified either by enzyme assays using (cultured) chorion villous samples or amniocytes or by analysis of VLCFAs or bile-acid intermediates in amniotic fluid. If the molecular defect has been identified in the index patient, DNA analysis can be performed [1,2].

<table>
<thead>
<tr>
<th>Material</th>
<th>Type of assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>VLCFAs, C26:0/C22:0 and C26:1/C22:1 ratios; phytanic and pristanic acids, pristanic/phytanic acid ratio; THCA and DHCA, THCA/CA and DHCA/CDCA ratios; pipecolic acid, plasmalogens, and PUFAs including DHA</td>
</tr>
<tr>
<td>Urine</td>
<td>Organic acids, pipecolic acid</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>Plasmalogens, PUFAs including DHA</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Plasmalogen biosynthesis, DHAPAT, alkyl-DHAP synthase; particle bound catalase; VLCFAs, β-oxidation, phytanic acid α-oxidation; immunoblot β-oxidation proteins</td>
</tr>
<tr>
<td>Liver</td>
<td>Cyto-/immunochemical localization of peroxisomal proteins, trilamellar inclusions, insoluble lipid</td>
</tr>
</tbody>
</table>

Table 6. Diagnostic tests in peroxisomal disorders

VLCFAs, very long chain fatty acids; THCA, trihydroxycholestanolic acid; DHCA dihydroxycholestanolic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DHAPAT, dihydroxyacetone phosphate acyltransferase; DHAP, dihydroxyacetone phosphate; PUFAs, polyunsaturated fatty acids; DHA, docosahexaenoic acid.

References

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