Peroxisome biogenesis disorders

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1. Introduction

Peroxisome biogenesis disorders (PBD) are a group of conditions caused by a partial or generalized defect in peroxisome biogenesis. They encompass two phenotypic groups: 1. the Zellweger spectrum disorders (ZSD) including severe, intermediate and milder forms [previously known respectively as Zellweger syndrome (ZS), Neonatal Adrenoleukodystrophy (NALD) and Infantile Refsum Disease (IRD)] and 2. Rhizomelic Chondrodysplasia Punctata type 1 (RCDP1), as well as the variant phenotypes now being described for both groups. PBD represent the paradigm for metabolic malformation syndromes. In a little over 50 years, an intense, concerted multidisciplinary effort has led to elucidating the fundamental biochemical, pathophysiological and molecular bases for these disorders effectively paving the way for therapeutic interventions and trials.

Peroxisomes are membrane bound organelles derived from the endoplasmic reticulum, numbering up to several hundred per mammalian cell, mostly spherical in shape, up to 1\mu m in diameter. They are indispensable for normal life and highly conserved throughout evolution amongst most eukaryotes. Peroxisomes were first identified by biochemist Christian DeDuve in the 1960s, as cytoplasmic particles containing hydrogen peroxide-generating oxidases \[1\]. Later, the neurologist Hans Zellweger reported a group of children with similar craniofacial dysmorphism and malformations in the brain, eye, liver, and kidney \[2\]. These cases, initially described under the eponym of “cerebro-hepato-renal-syndrome”, were subsequently termed Zellweger syndrome (ZS) \[3\]. ZS is considered today the prototype for ZSD. The reported absence of morphologically identifiable peroxisomes in hepatocytes and renal tubular cells of affected individuals using the peroxisomal matrix enzyme marker, catalase, \[4\] demonstrated a connection between ZS and peroxisome dysfunction. However, the etiological connection was not recognized until the identification of multiple peroxisomal enzymes and their deficiencies in affected patients. Similar findings were later demonstrated in the intermediate and milder ZSD phenotypes and RCDP1, which were also being reported at that time \[5–7\].

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2. Peroxisome biochemistry

There are several hundred peroxisomes in all mammalian cells, each containing more than 50 matrix enzymes required for multiple vital metabolic pathways, including the catabolism and synthesis of important cellular lipids [reviewed in [8]]. The best characterized peroxosomal pathways include β-oxidation of very long chain fatty acids (VLCFA), α-oxidation of methyl-branched phytanic acid and the biosynthesis of ether phospholipid (plasmalogen) (see Table 1). The metabolites of these pathways are routinely screened when a peroxisome biogenesis defect is suspected.

Peroxisomal β-oxidation utilizes a variety of different fatty acid substrates in catabolic and synthetic processes: very long (≥ C22) straight chain saturated and unsaturated fatty acids (VLCFA), (2S) methyl-branched chain pristanic acid and (25S) di- and tri-hydroxycholestanolic acids (D/THCA). Docosahexanoic acid (DHA), a critical polyunsaturated omega-3 fatty acid, is synthesized in the peroxisome after β-oxidation of its precursor [9–11]. D/THCA are C27 intermediary bile acids that undergo one step of β-oxidation to form the primary C24 bile acids, cholic and chenodeoxycholic acid. Peroxisomal β-oxidation also participates in the regulation of other complex lipids, including pro-inflammatory molecules such as eicosanoids, leukotrienes, and prostaglandins [12–14] [reviewed in [8]]. Furthermore, peroxisomes are the site of β-oxidation of dicarboxylic acids [15]. The initial oxidation step is performed in peroxisomes by acyl-CoA oxidase 1 (ACOX1 for VLCFA, or ACOX2 for pristanic and D/THCA), generating hydrogen peroxide (hence the name, peroxisome) as a byproduct that is detoxified by peroxisomal catalase [16–19] [reviewed in [20]]. D-bifunctional protein (DBP or HSD17B4) catalyzes the second (hydration) and third (dehydration) step of peroxisomal β-oxidation [21–23]. The final step is catalyzed by thiolase (either 3-oxoacyl-CoA thiolase (ACAA1) or SCPX in humans) [reviewed in [8]]. The peroxisomal fatty acid oxidation system is generally not able to degrade fatty acids to completion, so C16 or shorter fatty acids are transported as an acylcarnitine derivative to mitochondria for complete oxidation [11, 24–27].

3-methyl branched fatty acids (predominantly phytanic acid), because of the position of the methyl group, require a preliminary α-oxidation step in order to undergo β-oxidation [28, 29]. This requires the peroxisomal enzymes, phytanyl-CoA hydroxylase (PHYH), phytanyl-CoA lyase, and pristanal dehydrogenase, to generate pristanic acid [30–33] [reviewed in [8]]. Both pristanic acid and D/THCA are generated as (R) and (S) enantiomers, which require conversion to the (S) form by peroxisomal alpha-methyl-CoA racemase (AMACR) before β-oxidation [34]. Phytanic acid is exclusively dietary in origin and obtained from meats and dairy products of ruminant animals and certain fatty fishes [35, 36]. It accumulates in patients with ZSD and RCDP1, as well as in patients with single enzyme defects in PHYH. For a detailed review, see [37].

The committing steps for ether phospholipid synthesis occur in the peroxisome to generate the alkylglycerol backbone and require the peroxisomal enzymes fatty acyl-CoA reductase (FAR1), dihydroxyacetonephosphate acyltransferase (GNPAT), and alkyl-dihydroxy-acetonephosphate synthase (AGPS). Further metabolism in the ER generates the mature ether phospholipids, the majority of which are plasmalogens and contain a fatty alcohol linked by a vinyl ether bond to the sn-1 position of the glycerol backbone and are enriched in DHA and arachidonic acid (AA) at the sn-2 position [reviewed in [38, 39]]. Plasmalogens are a unique class of membrane glycerophospholipids by virtue of their vinyl ether bond, which provides specialized and critical functions to cellular membranes [40–42]. These properties include unique physical membrane attributes [43], signal transduction through released DHA and AA, as well as the remaining lysophospholipid [44], and the ability to provide oxidant protection to other membrane lipids [reviewed in [38, 39]]. Plasmalogens are enriched in heart and skeletal muscles, kidney, brain, and erythrocytes, whereas liver has very low concentration of plasmalogens [45]. Plasmalogen deficiency causes the RCDP phenotype [46–48]. In ZSD, the phenotype is more complex due to multiple enzyme deficiencies.
| Biochemical pathways                                      | Enzymes\(^1\)                      | Features                                                                 | Relevance to disease                                      |
|----------------------------------------------------------|------------------------------------|-------------------------------------------------------------------------|----------------------------------------------------------|
| β-oxidation of Straight, very long chain (≥C22) fatty acids | ACOX1, DBP, ACAA1, [SCPX]          | Chain shortening of very long chain fatty acids (VLCFA), final step in the synthesis of docosohexanoic acid (DHA) | Tissue accumulation of VLCFA cause brain, nerve and adrenal damage. Deficiency of DHA affects brain function and vision |
| β-oxidation of Methyl-branched chain fatty acid (pristanic) and C27 bile acid precursors, di- and trihydroxycholestanolic acids (D/THCA) | ACOX2, DBP, SCPX                   | Chain shortening of pristanic acid and D/THCA utilizes a different oxidase than that used in straight chain shortening | Accumulation of pristanic acid affects brain and nerve. Increased D/THCA cause liver toxicity |
| β-oxidation of Dicarboxylic fatty acids                   | ACOX1, LBP, [DBP] ACAA1, [SCPX]    | Chain shortening of dicarboxylic acids utilizes L-bifunctional protein (LBP) in contrast to DBP used in straight chain shortening | Unknown                                                   |
| α-oxidation of Methyl-branched chain fatty acid (phytanic) | PHYH                               | Degradation of methyl-branched phytic acid requires an additional α-oxidation before entering the β-oxidation pathway as pristanic acid | Tissue accumulation of phytic acid causes retinal degeneration, cerebellar ataxia, peripheral neuropathy |
| Racemization of Pristanic and D/THCA acids from (R) to (S) enantiomers | AMACR                              | β-oxidation requires the (S) enantiomers of pristanoyl-CoA and C27-bile acyl-CoAs | Causes tissue accumulation of (R) forms of pristanic acid and D/THCA (and secondary elevation of phytic acid) |
| Biosynthesis of Ether phospholipids (plasmalogen)         | GNPAT, AGPS, FAR1, [FAR2]          | Initial steps of plasmalogen synthesis occur in the peroxisome           | Deficiency causes skeletal dysplasia, cataracts, profound growth and intellectual deficiency |
| Detoxification of Glyoxylate                              | AGXT                               | Prevents the conversion of glyoxylate into the toxic metabolite, oxalate  | Accumulation of oxalate results in calcium oxalate renal stones |
| Detoxification of Hydrogen Peroxide                       | CAT                                | Required for catabolism of hydrogen peroxide, produced as a by-product of oxidase enzymes | Increased oxidant damage                                  |
| Amino acid oxidation                                      | PIPOX, DAO                         | Regulation of lysine degradation through L-pipecolic acid, and D-serine and glycine levels | Pipecolic acid levels are used as a biomarker for peroxisome dysfunction, but the relationship to disease processes is unknown |

\(^1\)Redundant enzymes are in brackets []. Full enzyme names: ACOX1, acyl-CoA oxidase 1; ACOX2, acyl-CoA oxidase 2; DBP, D-bifunctional protein; LBP, L-bifunctional protein; ACAA1, acetyl-CoA acyltransferase 1 (peroxisomal thiolase); SCPX, sterol carrier protein-peroxisomal thiolase; PHYH, phytanyl-CoA hydroxylase; AMACR, alpha-methylacyl-CoA racemase; GNPAT, glyceronephosphate O-acyltransferase or dihydroxyacetonephosphate acyltransferase; AGPS, alkyl-glycerone phosphate synthase or alkyl-dihydroxy-acetonephosphate synthase); FAR1, fatty acyl-CoA reductase; FAR2, fatty acyl-CoA reductase 2; AGXT, alanine-glyoxylate aminotransferase; CAT, catalase; PIPOX, L-pipecolic acid oxidase; DAO, D-amino acid oxidase.
In addition, peroxisomes contain enzymes involved in the catabolism of D-amino acids (D-amino acid oxidase) and L-lysine (pipelicolic acid oxidase), polyamine oxidation, detoxification of glyoxylate (alanine-glyoxylate aminotransferase, AGXT), reactive oxygen species, and xenobiotics. [For a full catalog of peroxisomal enzymes see [8]]. Cholesterol levels tend to be low in PBD patients [49–51], although the underlying mechanism remains to be resolved [52]. Recent studies suggest that peroxisome dysfunction leads to chronic ER stress and consequent dysregulation of the sterol response pathway [53].

The catalog of peroxisomal enzymes continues to increase through the use of bioinformatic searches for proteins containing the conserved targeting signals [54–57], as well as proteomics and mass spectrometry techniques [58, 59]. Most peroxisomal enzymes are unique to peroxisomes, but some are shared with other cellular compartments, including mitochondria and cytosol. These multiple subcellular localizations may be due to the presence of multiple targeting signals within the same protein, as shown for 3-hydroxy-methylglutaryl-CoA lyase [60], alpha methyl-CoA racemase [61], malonyl-CoA decarboxylase [62] and others, or relate to properties of the oligomeric protein complex as in epoxide hydrolase [63], copper-zinc superoxide dismutase [64], and alanine glyoxylate aminotransferase [65].

Shared enzymes and the movement of substrates and products through various cellular compartments position peroxisomes to be versatile organelles that dynamically adapt their number and metabolic functions in response to cellular needs. This is observed in yeast when grown on fatty acid media, by concomitant transcriptional up-regulation of peroxisomal β-oxidation enzymes and peroxisome proliferation. Rodent peroxisomes also proliferate secondary to increased β-oxidation activity and this is controlled by PPARα [66]. PPARα belongs to a transcription factor superfamily that binds to, and is activated by, a broad range of fatty acids and is a key regulator of peroxisomal and mitochondrial β-oxidation of fatty acids. Nevertheless, PPARα agonists have thus far not been associated with peroxisome proliferation in humans [67]. However, a metabolic control of peroxisome abundance in humans is expected considering the reduced number and enlargement of peroxisomes observed in fibroblast cultures from both ZSD patients and patients with single enzyme defects in β-oxidation pathways (ACOX1 and DBP) [68]. Human peroxisome numbers can also be increased through a PPARα independent mechanism that may rely on PEX11 activation [reviewed in [69]]. Significantly, the PPARγ co-activator (PGC)-1α, a regulator of cellular energy metabolism and mitochondrial biogenesis, was shown also to stimulate peroxisome biogenesis by upregulating PEX11, as well as other PEX proteins [70].

3. Peroxisome biogenesis

Peroxisome biogenesis encompasses the different processes required to assemble and maintain functional peroxisomes including matrix protein import, synthesis of new organelles, and fission of existing organelles. The coordinated activity of 16 PEX proteins, or peroxins, encoded by their corresponding PEX genes, is required for this process in mammals. There is evidence that peroxisome movement and assembly utilizes the cytoskeleton [71][reviewed in [72]]. Current models of eukaryotic peroxisome biogenesis have been recently reviewed [69, 73–75] and briefly are summarized below with emphasis on mammalian systems (see Fig. 1 for diagram of matrix protein import). Peroxisomal enzymes are synthesized on cytosolic ribosomes and contain one of two peroxisomal targeting signals (PTS) required for their import into the peroxisome [reviewed in [76]]. PTS1, the most common, is a C-terminal tripeptide, -Ser-Lys-Leu or consensus variant thereof [77–79]. PTS2 is a N-terminal nonapeptide found in at least 3 mammalian enzymes: 3-oxoacyl-CoA thiolase (ACAA1), PhyH, and AGPS [54, 80–84]. PTS1 proteins are recognized by the cytosolic receptor, PEX5, which also acts as a chaperone for PTS1 proteins [85–87]. The crystal structure of PEX5 demonstrating the PTS1
Fig. 1. Peroxisome matrix protein import. (1) PTS receptor binding. PEX5 and PEX7 cytosolic receptors bind their cognate ligands (PTS1 and PTS2 enzymes, respectively) in the cytosol. PEX5 has two isoforms that differ by alternative splicing. The longer isoform, PEX5L, binds both PTS1 enzymes and PEX7 and delivers them to the peroxisome membrane. (2) Docking. The receptor-ligand complex docks at the peroxisome membrane by binding PEX13 and PEX14. (3) Matrix enzyme translocation. PEX5, together with PEX14, forms a dynamic membrane pore through which the ligands are transported into the peroxisome matrix. (4) Receptor recycling. PEX2, PEX10, and PEX12 mono-ubiquitinate PEX5, allowing its removal from the membrane. The PEX1-PEX6 AAA-ATPase heterohexamer (anchored to the peroxisome membrane by PEX26) uses the energy from ATP hydrolysis to remove PEX5-Ub from the peroxisome membrane for another round of import. PEX7 is recycled to the cytosol after PEX5 in an ATP independent manner. Note that defects in PEX7 prevent import of PTS2 enzymes, but do not disrupt the PEX5/PTS1 import pathway.

binding site has been solved [88]. There are two isomers of PEX5: PEX5S and PEX5L, resulting from differential splicing of the primary transcript of the PEX5 gene [89]. The PTS2 sequence forms a structural motif that is recognized by the cytosolic receptor PEX7 [90]. Comparative homology modeling has identified the PEX7-PTS2- binding groove [54]. In mammals, PEX7 needs to interact with PEX5L for targeting to the peroxisomal membrane [89, 91]. PEX5L and PEX7 receptors form cytosolic complexes with their ligands and efficiently ferry them to the peroxisome membrane [91]. The receptor/cargo complex binds at the peroxisome membrane to the PEX13 and PEX14 docking complex [92–96]. The cargo proteins are subsequently translocated into the matrix, possibly through a transient pore, which assembles at the peroxisome membrane to enable matrix protein translocation, before it is disassembled afterwards and its components recycled for further rounds of protein import [97, 98]. Direct evidence for the existence of a transient import pore was shown by electrophysiological studies, which demonstrated that a Pex5p-Pex14p sub-complex of S. cerevisiae harbors pore forming activity [99]. Through this transient pore, which can widen to 9 nm, peroxisomes can import
folded, oligomeric and cofactor bound proteins [99–101]. An alternate emerging model postulates that the peroxisomal import machinery displays a preference for monomeric proteins. In this model: (i) newly synthesized peroxisomal proteins are folded by cytosolic chaperones and released as soluble monomers; (ii) PEX5 binding to these monomers (PTS1) blocks their oligomerization; and finally, (iii) these monomeric cargoes are translocated by a single PEX5 molecule into the matrix of the organelle where oligomerization occurs [102].

After translocation, the cargo dissociates from the PTS-receptors and is released into the peroxisomal lumen [103]. The PTS receptors are released from the peroxisomal membrane for another import cycle or directed to the proteasome for their degradation [104–106]. Translocation of the ligands into the peroxisomal matrix is also associated with the integral membrane complex: PEX2, PEX10, and PEX12. PEX2/10/12 are ubiquitin ligase (E3)-like proteins containing RING (really interesting new gene) domains and are involved in facilitating the mono- or polyubiquitination based PTS receptor recycling and degradation [107]. In humans, PEX2 mono-ubiquitination of PEX5 occurs on a conserved cysteine residue in the N-terminal region, and is mediated by redundant ubiquitin-conjugating (E2)-enzymes (UbcH5a, UbcH5b and UbcH5c) [108, 109]. This mono-ubiquitination could provide a handle for PEX5 recycling back to the cytosol [110, 111]. If the monoubiquitination dependent recycling pathway is impaired, the PTS-receptors enter a polyubiquitination-dependent alternative pathway where PEX10/12 poly-ubiquitinate PEX5 on lysine residues, targeting it for degradation by the 26S proteasome [111, 112]. In wild type cells there is very little receptor turnover by degradation [112, 113]. For a complete review of PEX5 ubiquitination and dislocation, see [114, 115].

The ‘exportomer’ complex of PEX1, PEX6, and PEX26 is involved in the recycling of the PTS1-receptor, PEX5, and presumably PEX7, back to the cytosol for additional rounds of import [116]. PEX1 and PEX6 are members of the AAA protein family (ATPases Associated with various cellular Activities) that contain one or two AAA cassettes responsible for ATP-binding and ATP hydrolysis. PEX1 and PEX6 each contain two ATPase domains (D1 and D2), and are therefore members of the double-ring ATPase family [reviewed in [117]]. These proteins typically form hexameric rings that change conformation during the ATPase cycle, providing the motion and force required for the variety of cellular functions they perform [118–120]. ATP binding is required for the interaction of PEX1 and PEX6 [121], and association with the peroxisomal membrane occurs by interaction of the N-terminal region of PEX6 with PEX26 [122]. PEX5 export, but not import, is an ATP driven process, with the energy of ATP hydrolysis providing the conformational change required in the PEX1/6 complex to pull monoubiquitinated PEX5 out of the peroxisome membrane [123]. Recently, cryo-electron microscopy studies have shown that yeast Pex1/Pex6 ATPases form a unique double-ring structure in which the two proteins alternate around the ring [124–126]. These data shed light on the mechanism and function of this ATPase complex and suggest a role in peroxisomal protein import analogous to that of p97 in ER-associated protein degradation, in which the ATPase extracts poly-ubiquitinated proteins from the ER membrane [discussed in [117, 127]].

Peroxisomes can form de novo or by growth and division of pre-existing peroxisomes. PEX3, PEX16, and PEX19 are required for these processes and for correct localization of peroxisome membrane proteins (PMPs) [128–136]. In the de novo pathway, PEX16 is first incorporated into the ER, followed by PEX3 and other PMPs [137]. This leads to the formation of specialized ‘peroxisome-like’ domains, or pre-peroxisomal vesicles, which can detach from the ER to form peroxisomes [137, 138] [reviewed in [139]]. During peroxisome growth, PEX19, a cytosolic protein, recognizes newly synthesized PMPs via their internal targeting signal motifs and binds them [140]. The complex then docks to PEX3 and PEX16, located in the peroxisomal membrane [141, 142]. In this pathway, PEX19-PEX3-PEX16 mediates import for most PMPs, including metabolite transporters and PEX proteins [143][ reviewed in [144]]. Peroxisome fission requires the integral membrane protein PEX11β and shares several components of its division machinery with mitochondria. These include dynamin-like proteins (DLPs), Fis1,
Mff and ganglioside-induced differentiation-associated protein 1 (GDAP1) [145–148]. PEX11β is required for membrane elongation, and binds to Fis1, which recruits DLPs [reviewed in [69]]. PEX11β is constitutively expressed in all tissues, whereas its isoform PEX11α is inducible [149, 150], and PEX11γ is constitutively expressed in liver [151]. PEX11γ is involved in peroxisome membrane protrusion [152] and may function in the de novo pathway to mediate the import of peroxisomal matrix proteins into the pre-peroxisomal vesicles that are derived from the ER [153]. The relative contribution of the ER-derived versus fission pathway to the total peroxisome population in vivo, and the detailed mechanisms of ER entry and exit of PMPs are controversially discussed. For a detailed review, see [154–156].

Peroxisome turnover, termed pexophagy, is by general mechanisms related to autophagy of cellular contents, as well as mechanisms selective for peroxisomes [reviewed in [157, 158]]. Deficiencies in various PEX proteins increase pexophagy, including PEX3 [159] and PEX14 [160]. Studies in yeast show that deficiencies in exportomer components Pex1, Pex6, or Pex15 (the yeast ortholog of PEX26) increase pexophagy more than other peroxin deficiencies [161]. The constitutive turnover of peroxisomes is estimated to be around 30% per day [162].

4. Clinical disorders

Biallelic pathogenic mutations in any one of 14 PEX genes cause disorders of peroxisome assembly. These are collectively and classically known as peroxisome biogenesis disorders. No human disease has yet been associated with defects in PEX11α and PEX11γ. Defects in PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX11β, PEX12, PEX13, PEX14, PEX16, PEX19 and PEX26 genes cause Zellweger spectrum disorder (ZSD) including Zellweger syndrome (ZS), Neonatal Adrenoleukodystrophy (NALD), and Infantile Refsum Disease (IRD). These historically distinct phenotypes are now considered different presentations within the same clinical and biochemical spectrum with ZS being the most severe presentation, NALD intermediate and IRD milder [4, 163, 164]. The term ZSD is preferred, given the overlap in individual clinical presentations, and the atypical phenotypes currently being identified by new sequencing technologies. In general, disease severity correlates with patient age at onset of symptoms and the predicted consequence of the mutation on peroxin function, capacity for matrix protein import, residual enzyme functions, and peroxisome numbers. At the cellular level, defects causing ZSD lead to decreased numbers of enlarged peroxisomes that show reduced import of matrix enzymes [165]. These structures are termed peroxisome ‘ghosts’ [165, 166]. Defects in PEX7 cause the distinct phenotype of RCDP1 [167]. Since only PTS2 protein import is disrupted in RCDP1, peroxisome number and morphology are normal and there is deficiency of only a subset of matrix enzymes. A phenotypic spectrum of severity is also present in RCDP1 and correlates to residual plasmalogen levels [46–48]. The overall estimated birth incidence for ZSD is 1/50,000 [168] and 1/100, 000 for RCDP1 [169]. The prevalence of both ZSD and RCDP1 varies among different populations [170–173]. These disorders are autosomal recessive and panethnic, however founder mutations account for the high frequency of the PEXI-c.[2528G>A] allele (p.[Gly843Asp], or p.G843D), the PEX1-c.[2097_2098insT] allele (p.[Ile700Tyrfs42*]) [174, 175], and the PEX7-c.[875T>A] allele (p.[Leu292*]) [172, 176, 177] in individuals of Northern European heritage. Other founder alleles include PEX10-c.[815-815delCT] in the Japanese population [171], PEX6-c.[802_815del] in French Canadians [170], and PEX12-c.[−26G>A; 102A>T] in individuals of Persian Jewish descent [178] (The PEX12-c.[−26G>A] cis mutation is provided by N. Braverman, personal communication).

4.1. ZSD

Severe ZSD (Zellweger syndrome) is a classic malformation syndrome described by its characteristic features as cerebro-hepato-renal syndrome [reviewed in [144, 179, 180]]. Patients present as
newborns with severe hypotonia, seizures, and characteristic craniofacial dysmorphisms with high forehead, large anterior fontanel, hypertelorism, epicanthic folds, high arched palate and micrognathia. Brain MRI shows microgyria, pachgyria and heterotopia consequent to neuronal migration defects [reviewed in [181]]. Generalized brain atrophy and subependymal germinolytic cysts [182] have also been described. The pattern of medial pachgyria and lateral (perisylvian) polymicrogyria is a distinguishing feature [183]. Eye disease includes cataracts, congenital glaucoma, retinal dystrophy and optic atrophy. Sensorineural deafness is present. The liver is enlarged and hepatic functions show elevated transaminases, cholestasis and coagulopathy. Cortical renal cysts are observed on ultrasound, but are not usually clinically significant. Chondrodysplasia punctata especially in the knees and hips, can be seen on skeletal x-rays. Cardiovascular malformations and pulmonary hypoplasia have also been reported [184]. Fetuses with severe ZSD, as early as 13 and 21 weeks’ gestation, show brain dysplasia, renal cysts, and calcific stippling of cartilage [185]. Fetal ultrasound examinations have detected ventriculomegaly in the 3rd trimester and follow-up MRI at 35 weeks gestation showed gyral malformations, renal cysts and hepatosplenomegaly [186]. Thus, the most severe degree of peroxisome dysfunction affects prenatal organ and tissue development. In contrast, hepatic fibrosis is a late event that is not present in the fetus, but can be severe postnatally [184]. These infants generally do not survive beyond the first year of life [180].

The intermediate and milder (previously termed NALD and IRD) group of patients present after the newborn period with more varied symptomatology (see Table 2 for a list of ZSD features by age of onset). Hypotonia, failure to thrive, developmental delays, sensory deficits, amelogenesis imperfecta and history of neonatal cholestasis are usually present. The characteristic Zellweger facies is attenuated or absent. Subtle neuronal migration defects have been described in some intermediate patients, but are absent in the milder forms of ZSD [187, 188] [reviewed in [181]]. Overall, this group is

| Clinical features of ZSD by severity and age of onset |
|------------------------------------------------------|
| **Features**                                           | **Neonate** | **1-6 mos** | **6 mos-4 yrs** | **>4 yrs** |
| Neuronal migration defects                           | S           | I, M        | I, M             | I, M       |
| Chondrodysplasia punctata                            | S           | I, M        | I, M             | I, M       |
| Renal cortical cysts                                 | S           | I, M        | I, M             | I, M       |
| Respiratory compromise                               | S           | I, M        | I, M             | I, M       |
| Craniofacial dysmorphism                             | S, I, M     | I, M        | I, M             | I, M       |
| Jaundice                                             | S, I, M     | I           | I, M             | I, M       |
| Hepatomegaly, liver dysfunction, cholestasis          | S           | I, M        | I, M             | I, M       |
| Hypotonia, failure to thrive                         | S, I        | I, M        | I, M             | M          |
| Sensorineural hearing loss                            | S           | I, M        | I, M             | M          |
| Cataracts                                            | S           | I, M        | I, M             | M          |
| Retinal degeneration                                 | S           | I, M        | I, M             | M          |
| Psychomotor retardation                              | S           | I, M        | I, M             | M          |
| Seizures                                             | S           | I           | I, M             | I, M       |
| Leukodystrophy                                      | I           | I           | I, M             | M          |
| Adrenal insufficiency                                | I, M        | I           | I, M             | M          |
| Osteopenia                                           | I           | I           | I, M             | M          |
| Calcium oxalate renal stones                         | I, M        | I, M        | I, M             | M          |
| Peripheral neuropathy                                | M           | M           | M                | M          |
| Cerebellar ataxia, atrophy                           | M           | M           | M                | M          |
| Amelogenesis Imperfecta                              | I, M        | I, M        | I, M             | M          |

Abbreviation for clinical severity classes: S, severe; I, intermediate; M, mild.
clinically distinguished from severe ZSD by the absence of congenital malformations, and is instead a disorder marked by progressive peroxisome dysfunction over time. Most individuals have progressive sensorineural hearing loss and visual loss due to retinal degeneration, leading to deafness and blindness. Other complications include seizures, adrenal insufficiency, leukodystrophy, and osteopenia, the latter leading to pathological fractures [189]. Nephrolithiasis from hyperoxaluria due to deficiency of AGXT has been reported [190]. Liver dysfunction can quiesce, or less frequently, worsen [191]. The prediction of which children will develop these complications and when, as well as the timeline of their progression remains unknown. However, children with the intermediate phenotype generally develop more complications at earlier times, almost all develop adrenal insufficiency, and some will develop leukodystrophy. Lifespan is usually shortened, and many of these individuals do not survive past late childhood. Most individuals with milder ZSD have better cognitive functions, fewer and less severe symptoms of disease and can survive through adulthood [191].

Leukodystrophy was classically described in intermediate ZSD patients in late infancy and early childhood. It manifests with active demyelination in the cerebrum, midbrain and cerebellum and consequent psychomotor regression [192–194]. The boundaries of the demyelinated areas are usually sharply demarcated and there is reactive gliosis; many cases have been associated with perivascular accumulation of lymphocytes as in X-linked adrenoleukodystrophy [187]. In some cases, mild initial symptoms are later followed by severe CNS demyelination and death of the patient [187, 192, 194]. However, leukodystrophy can occur at any age, remain stable, or progress [191, 195]. Limited pathological studies in brain from intermediate patients have shown reduced neuron number and cerebellar atrophy without active demyelination. There can be dysplastic olives and irregular, heterotopic, clumps of Purkinje cells [187, 196]. In the milder forms of ZSD, where patients can survive into adulthood, brain MRI has also revealed white matter abnormalities, although it can be normal. A study including neuroimaging findings in 19 such patients demonstrated high proportion of white matter abnormalities restricted to the cerebellar hilus of the dentate nucleus and/or the peridentate region. Interestingly, at the time of diagnosis, 17 of these patients had a blood metabolite profile typical of a peroxisomal disorder; but at later time points the concentration of many originally accumulating metabolites had declined and, in some patients, even a complete normalization was observed [191]. Histopathological analysis of the brain from an milder patient showed hypoplasia of the cerebellar granular layer and ectopic location of purkinje cells in the molecular layer without active demyelination [197].

Retinal degeneration is a prominent feature of all ZSD patients and can manifest as early as the first year of life in the intermediate and milder spectrum [198]. Cataracts can also present [180, 199]. Clinical case descriptions have included Leopard spot retinal pigmentation [200], congenital amaurosis [201, 202], Usher syndrome [203, 204], as sensorineural hearing loss is typically also present, and retinitis pigmentosa with a predominant macular dystrophic component [201, 202, 205, 206]. ERG recordings typically show severely reduced or extinguished cone and rod responses [198, 206, 207]. The course and progression of the retinal degeneration is unknown, but in general the progression is slower in the milder phenotypes. Histological reports showed degeneration of photoreceptor outer segments and reduced inner segments, and extensive loss of nerve fiber and ganglion cell layers [208]. Areas of photoreceptor degeneration were associated with pigmentary changes. The retinal pigment epithelium can show patchy hypertrophy, nodular hyperplasia, and atrophy. Pigmented cells can be found in the subretinal space and in other layers in the retina. Macrophages may contain pigment and degenerated photoreceptors. Lamellar lipid inclusions were observed in most layers of the retina and within the optic nerve by electron microscopy [208]. These inclusions have also been observed in brain, adrenal glands, and liver [197, 200]. Recently, peroxisomes were shown to cluster at the base of photoreceptor outer segments, suggesting that peroxisomes are intimately involved in maintaining outer segments [203]. Cochlear histology has shown degeneration of the sensory epithelium, with preservation of ganglion cells and nerve fibers [197].
4.2. Atypical ZSD presentations

Recently, atypical and milder phenotypes have been reported that may relate to specific mutations in specific \textit{PEX} genes. These children and adults with normal or near normal intellect, slow disease course and prolonged survival, illustrate that the phenotypic spectrum of these disorders is much broader than initially thought [153, 178, 188, 199, 204, 205, 209–219]. Table 3 summarizes the pertinent findings reported in this novel group of patients. These patients are arbitrarily classified according to major presenting symptomology: those with prominent sensory deficits (visual and hearing loss), and those with cerebellar dysfunction, peripheral neuropathy or relapsing encephalopathy. These individuals inspire fundamental reflections on PBDs and highlight some of the molecular, biochemical and cellular mechanisms by which these variant phenotypes arise.

The underlying molecular mechanisms of these atypical presentations often involve hypomorphic \textit{PEX} alleles encoding peroxins with residual protein function. This can include bi-allelic hypomorphic alleles, as well as a mono-allelic, hypomorphic mutation in trans with a severe, usually loss of function mutation [220]. Mutations in specific domains (ring finger) of \textit{PEX2}, \textit{PEX10} and \textit{PEX12} can lead to specific phenotypes of cerebellar dysfunction and peripheral neuropathy [discussed in [211]]. In addition, an adult with milder disease was identified with two null mutations in \textit{PEX11B}. It was suggested that functional redundancy of \textit{PEX11\alpha} and \textit{PEX11\gamma} supported the absence of \textit{PEX11\beta} [153]. In most of these cases, routine peroxisome metabolite testing in blood (VLCFA, plasmalogen and phytanic acid levels) showed only subtle dysfunction or none at all [153, 188, 209, 210, 220, 221] (Table 3). In this context, if a PBD is strongly suspected, additional peroxisome functions should be investigated. Piopecolic acid, D/THCA and pristanic acid levels, as well as fibroblast enzymatic testing, morphological analysis of cellular peroxisomes and catalase distribution has targeted PBD in difficult cases [222, 223].

Morphological analyses of peroxisomes in fibroblast cultures in milder cases have shown more peroxisomes with residual function, mosaicism, and temperature sensitivity, phenomena that likely reflect a partially functional peroxin [220]. Mosaicism refers to the observation of a heterogeneous cell population (by both immunohistochemistry and electron microscopy techniques) in fibroblast cultures and in hepatocytes from liver cell biopsies, in which some cells contain functional peroxisomes that import matrix proteins, while adjacent cells contain peroxisomes (ghosts) that show minimal or no import of matrix proteins [224–226]. Temperature sensitivity refers to improvement in peroxisome formation and biochemical function in ZSD fibroblasts cultured at 30\degree C rather than 37\degree C [227–229]. It has been suggested that temperature sensitivity is a consequence of an imbalance of folding and unfolding kinetics of mutant proteins, resulting in an increase in the correctly folded protein as temperature decreases [230]. The etiology for peroxisome mosaicism is not known, but could reflect microenvironment or cell cycle differences that enable recovery of a partially functional peroxin, or a partially functional import system. Taken together, these data imply residual peroxisome functions in body tissues that are dependent on a threshold of temperature, microenvironment and other unknown factors which can improve function. In fibroblast cultures from some patients with atypical phenotypes and normal peroxisomal morphology, increasing the culture temperature to 40\degree C unmasks the underlying peroxisome dysfunction [178]. Finally, peroxisome morphology can provide a clue to the underlying gene defect. For example, fibroblast cultures from patients with defects in \textit{DLP1} or \textit{Mff} exhibit elongated, constricted peroxisomes that are unable to divide (i.e. “pears on a string” phenotype) [231, 232]. Fibroblasts from a \textit{PEX11\beta} patient have enlarged, elongated peroxisomes, indicating a defect in peroxisome constriction and/or division [153]. Cells from patients with milder variants in \textit{PEX16} show a reduced number of enlarged, but import competent peroxisomes [199]. Thus far, peroxisomes in patient cell lines with \textit{PEX7} defects (RCDP1) have normal morphology and do not show a temperature response.
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4.3. Other disorders that affect peroxisome biogenesis

A few patients were reported with mutations in the shared mitochondrial and peroxisome fission proteins, DLP1 [231], Mff [232], and GDAP1 [148]. These individuals displayed clinical evidence of combined mitochondrial and peroxisome dysfunction. Mitochondrial and peroxisomal morphology indicated fission dysfunction. The patient with DLP1 deficiency presented with affected brain development including an abnormal gyral pattern in both frontal lobes associated with dysmyelination, microcephaly, optic atrophy. She died a few weeks after birth. The patients with Mff deficiency showed delayed psychomotor development, microcephaly, pale optic discs, and mild hypertonia. Brain MRI revealed an abnormality in the globus pallidus. The eldest of the 2 patients (brothers) was 4.5 years old at the time of publication. GDAP1 mutations affect peroxisome and mitochondrial dynamics and cause the peripheral neuropathy Charcot-Marie-Tooth disease (CMT) [148, 233]. Interestingly, peroxisome metabolic functions (as measured by biomarkers indicative of classic peroxisomal disorders) were not altered in the tested Mff patient. The DLP1 patient showed only a slight elevation of VLCFA. This minimal or nonexistent biochemical evidence of peroxisomal dysfunction highlights the importance of using alternate methods in patient diagnosis (discussed previously). It is likely that defects in other proteins involved in peroxisome assembly and maintenance that can result in a clinical disease remain to be identified.

The increased use of next-generation sequencing technologies has led to the identification of many of these atypical patients [see Table 3] [153, 178, 188, 199, 205, 209, 211, 212, 216–219] and will continue to do so. However, analysis of peroxisome biochemical and cellular functions remains necessary to prove a peroxisome etiology, especially in cases where there are novel PEX gene variations, novel phenotypes, and defects identified in other pathways involved in peroxisome assembly and maintenance. All this will lead an improved understanding of peroxisome biology in various tissues over time and thus enable more precise genotype and phenotype correlations.

4.4. RCDP

The majority of patients with RCDP have defects in PEX7 (RCDP1) [48, 167, 177]. The 3 well-characterized mammalian PTS2 enzymes transported by PEX7 are peroxisomal thiolase (ACAA1), phytanoyl CoA hydroxylase (PHYH), and AGPS [81–84]. ACAA1 is a redundant enzyme, and its functions are assumed by SCPX, which has thiolase activity and is transported by PEX5 [235]. Thus β-oxidation defects have not been detected and VLCFA levels are normal in RCDP1 plasma and fibroblasts. Defects in PHYH result in accumulation of phytanic acid over time, causing the distinct phenotype of Adult Refsum Disease (ARD) [83]. In RCDP1 patients, who typically do not survive to adulthood, the phenotypic features of ARD are absent. In contrast, certain defects in PEX7 result in normal plasmalogen synthesis but retain reduced phytanic acid α-oxidation, resulting in an ARD phenotype [48, 234].

RCDP can also result from defects in the peroxisomal enzymes required for plasmalogen synthesis: GNPAT (RCDP2), and AGPS (RCDP3) [236–243]. RCDP1, 2 and 3 share identical phenotypes and phenotype severity depends on the degree of residual plasmalogen levels [46–48, 236]. Plasmalogen deficiency is thus considered the primary pathophysiological etiology. Newly discovered causes of RCDP phenotypes are PEX5L mutations in the PEX7 binding domain region (RCDP5), which causes a loss of PEX7 transport to the peroxisome, but retains all other PEX5 functions [244]. As expected, the clinical and biochemical phenotype in the two patients reported was identical to classical RCDP1. In addition, mutations in the FAR1 gene (RCDP4), which supplies the fatty alcohol substrate to AGPS, also result in plasmalogen deficiency but the RCDP phenotype was attenuated, perhaps due to overlapping functions of the FAR2 enzyme [245].
RCDP is clinically characterized by proximal shortening of the humerus and to a lesser degree the femur (rhizomelia), punctate calcifications in epiphyseal cartilage (chondrodysplasia punctata), with progressive metaphyseal abnormalities, coronal clefts of the vertebral bodies, and congenital cataracts [246–248]. Typically, there is severe postnatal growth and intellectual deficiency [249]. Up to 52% of patients have congenital heart malformations [250, 251]. Cleft palate, renal malformations, and ichthyosis have also been reported [249]. Life span is broad although most affected children do not survive the first decade of life. A proportion die in the neonatal period and this might be related to primary cardiac abnormalities or pulmonary hypoplasia [252]. Neuroimaging studies show various abnormalities including ventriculomegaly without increase in intracranial pressure, multilevel cervical canal stenosis [253, 254], hypomyelination [46, 255], other supratentorial (involving the parieto-occipital regions) white matter signal anomalies [46, 256, 257], and progressive cerebellar atrophy. Head growth deceleration is seen in all patients with severe RCDP, possibly reflecting myelin or neuronal loss [46]. The striking neuronal migration defects seen in ZSD are not typically observed in RCDP. Pathological studies of RCDP brain show a general decrease in neuron number and white matter, dyplastic olives and progressive cerebellar degeneration [247, 258–260]. Although most reported RCDP patients have a severe, classical phenotype, milder phenotypes are now being recognized in which affected individuals have congenital cataracts and epiphyseal dysplasia, but lack rhizomelia and have less severe intellectual and growth deficiency [48]. In these patients, MRI can be normal or show slowly progressive cerebellar atrophy. The severity of the cerebellar atrophy, as with all RCDP features, correlates directly to the degree of plasmalogen deficiency and not to the phytanic acid elevation [46, 48].

5. Diagnosis

Clinical suspicion of PBD requires confirmation by determining peroxisome functions (Table 4). The fundamental problem in ZSD is the inability to generate and maintain functional peroxisomes [4, 5]. Consequently, the typical biochemical signature of ZSD is concomitant dysfunction of multiple peroxisomal pathways. This is detected in blood, urine and cells by accumulation of VLCFA, phytanic, pristanic and piperolic acids, D/THCA and deficiency of plasmalogens, docosahexanoic and primary bile acids (cholic and chenodeoxycholic acids) [diagnosis reviewed in [261]]. In RCDP1, the PEX7 defect impairs only PTS2 protein import, causing reduced plasmalogens and increased phytanic acid levels. Around 10–15% of patients with ZSD phenotypes have single enzyme defects in fatty acid β-oxidation (ACOX1, DBP, SCPX, AMACR), and a similar number of patients with RCDP phenotypes have single enzyme defects in plasmalogen synthesis (GNPAT, AGPS, FAR1). Thus, it is critical to evaluate several peroxisome functions to tease out true PBD from phenocopies due to single enzyme defects. The fact that patients with single enzyme defects in the β-oxidation pathway have phenotypes identical to ZSD [213, 218, 262, 263] implies that β-oxidation defects contribute in large part to the pathology of ZSD. Similarly, defects in plasmalogen synthesis are directly responsible for the RCDP phenotype [46–48, 236]. Metabolite testing is usually followed by enzymatic confirmation of PBD in fibroblast cultures and/or molecular confirmation of the PEX gene defect.

PEX gene sequencing was previously limited due to the challenge of multiple genes involved [174, 264]. However, with the advent of next generation sequencing, molecular diagnosis has become cost efficient, comprehensive, and has enabled additional genotype-phenotype correlations in atypical patients. Over the past decade, studies on more than 800 patients show that defects in PEX1 represent around 60% of all ZSD alleles [174, 264, 265]. In these reports, 12–30% of ZSD alleles are PEX1-p.G843D, and 5–20% are PEX1-p.[Ile700Tyrfs42*]. About 50% of all RCDP alleles are PEX7-p.[Leu292*]. The high frequency of these alleles is due to a founder effect in persons of Northern
Table 4
Diagnostic laboratory studies in PBD

| Test                          | Metabolite/gene                          | PBD          | Note                                                                                                                                 |
|-------------------------------|------------------------------------------|--------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Plasma VLCFA                  | Increased C26:0, C26:1 and C24/C22, C26/C22 ratio | ZSD          | False positives in ketogenic diet, non-fasting or hemolyzed samples, normal in RCDP                                              |
| Plasma phytanic and pristanic acid | Increased                               | ZSD, RCDP1,5 | Phytanic acid is normal in newborns and accumulates only through dietary intake; pristanic acid is normal in RCDP               |
| RBC plasmalogens              | Reduced                                  | ZSD, RCDP1,5 | Greatest reduction in RCDP                                                                                                         |
| Urine/plasma bile acid        | Increased D/THCA                          | ZSD          | Adjunct to VLCFA and plasmalogen testing                                                                                         |
| Plasma/urine pipecolic acid   | Increased                                | ZSD          | Adjunct to VLCFA and plasmalogen testing                                                                                         |
| Cultured fibroblasts          | Confirm abnormal metabolites by enzymatic assays, evaluate peroxisome morphology and catalase distribution | ZSD, RCDP1,5 | Allows more complete characterization of peroxisome functions, number and morphology in difficult cases. Validates disease due to novel PEX alleles |
| Molecular genetic testing+,*  | PEX 1, 2, 3, 5, 6, 10, 11β, 12, 13, 14, 16, 19, 26 | ZSD          | Defects in PEX1, 2, 6, 10, 12, 26 account for ∼80% of ZSD174                                                                 |
|                               | PEX 7                                     | RCDP1        | Four common alleles in exons 7 and 9, account for ∼70% of cases48                                                                 |

*Several PEX mutations are more common due to founder effects in persons of Northern European origin: PEX1-p.[Gly843Asp], PEX1-p.[Ile700Tyrfs42*], PEX7-p.[Leu292*]. Genetic testing allows for carrier detection, preimplantation genetic diagnosis, improved prenatal diagnoses and can also provide prognostic information. It is required for diagnosis when the clinical and biochemical phenotypes do not fit the classic criteria.174,48 Superscript numbers correspond to reference list.

European extraction [175, 176]. PEX1-p.G843D is a missense protein with residual functions; the presence of at least one allele predicts an intermediate phenotype, and homozygosity generally results in milder phenotypes [227, 266–268]. The large variations in phenotype severity observed amongst PEX1-p.G843D homozygotes (unrelated, as well as intra-familial) suggest the influence of modifier genes [191, 205].

PEX1-p.[Ile700Tyrfs42*] and PEX7-p.[Leu292*], are null alleles and predict a severe phenotype when homozygous [48, 177, 269]. Overall, bi-allelic mutations in PEX1, 6, 26, 10, 12, and 2 contribute to the majority of ZSD alleles [174]. When previously unknown or uncharacterized variants are identified, functional biochemical studies remain a necessary tool to determine causality and pathogenicity [reviewed in [144, 223]].

In addition to diagnoses of affected individuals, early diagnoses of relative asymptomatic infants will be possible with the advent of newborn screening. The combination of liquid chromatography and tandem mass spectrometry (LC–MS/MS) to detect elevated levels of VLCFAs in newborn blood spots was validated as a diagnostic approach for X-linked adrenoleukodystrophy (X-ALD) [270, 271], a single enzyme/protein defect of peroxisome function in which there is only accumulation of VLCFA [reviewed in [272]]. X-ALD screening was recently included in the US Recommended Uniform Screening Panel [273]. The implications of X-ALD newborn screening include the ability to perform clinical surveillance for early detection and treatment of leukodystrophy in affected males, and counseling for carrier females [274]. Newborn screening for X-ALD should also detect the majority of PBD-ZSD cases that feature elevated blood VLCFA levels, thereby permitting early diagnosis and thus more timely institution of supportive management. It will also enable family counseling, determination of
accurate incidence figures and continued expansion of the clinical phenotype as variant patients are identified.

6. Management

There are no curative therapies for PBD. Management is multidisciplinary, symptomatic, and based on surveillance of the multiple systems involved. This includes regular developmental, neurological, auditory, visual, nutritional, and orthopedic assessment, and monitoring of liver and adrenal functions [261, 275]. Vitamin K and other fat soluble vitamin supplements are usually prescribed. Care is often palliative for those with severe disease. Genetic counselling and family support organizations provide crucial assistance to parents in their decision making process. The geneticist, neurologist and nutritionist should be involved early in the management of patients with PBD.

Dietary interventions have included restriction of metabolites that accumulate and replacement of those that are deficient. Reports of these approaches in patients are mostly anecdotal and have not been systematically studied. Furthermore, the extensive inter- and intrafamilial variations in the intermediate and milder ZSD patients requires better understanding of the natural history of these disorders and the establishment of reliable clinical endpoints for future interventional trials.

In a small case series in ZSD, recovery of erythrocyte plasmalogens was achieved by administration of the oral plasmalogen precursor batyl alcohol [276], but this did not improve clinical outcome. Oral bile acid supplementation (chenodeoxycholic and ursodeoxycholic acids) in two infants with severe ZSD reduced C27 bile acid intermediates (D/THCA) and improved hepatobiliary function, but did not change the clinical course [278]. In a third infant with severe ZSD, cholic acid supplementation also improved histology with a decrease in bile duct proliferation and inflammation [277]. An infant with AMACR deficiency presenting with cholestasis has done well on long term cholic acid supplementation with complete suppression of C27 bile acids and improvement in liver histology [279]. However, the few other AMACR patients reported were adults with neurological symptoms attributed to pristanic acid accumulation and improved on a phytanic acid restricted diet [213, 280]. Thus the clinical significance of early intervention with bile acid therapy remains unknown.

Patients have also received DHA supplementation, with controversial clinical benefit. In a small case series, improvements in electroretinograms (ERG) and growth were shown [281]. In a double-blind randomized placebo controlled trial that included 48 patients over one year of study, there were no overall improvements in ERG and growth in the treated group compared to the placebo group [207]. Due to the benefit of dietary phytanic acid restriction in adult Refsum disease [282], and the fact that it is not a difficult diet regimen, this is often prescribed for surviving PBD patients but formal studies have not been done to determine if this improves the disease course.

In two siblings with an intermediate-milder ZSD phenotype, the older child received an hepatocyte transplantation [283] and the younger received a liver transplant [284]. Peroxisome biochemical functions improved, but clinical outcome of the younger sibling was relatively better. Another patient received a liver transplant, and biochemical functions improved but it is not yet clear that the clinical outcome has changed [285].

7. Research tools: mouse models

In order to gain further insight into the pathogenesis of ZSD, mouse models with complete deficiency of Pex2, Pex5, Pex13, and Pex11β have been engineered [286]. These mice recapitulate many aspects of severe ZSD, including the metabolic consequences of peroxisome elimination and abnormalities in brain formation, hypotonia, and early demise. Detailed investigations of these models revealed
neuronal migration defects commencing early in the cytogenetic epoch and demonstrated by birth dating experiments using 5-BrdU. Other abnormalities include delayed neuron differentiation and extensive neuronal apoptosis (Pex5, Pex11β). In the cerebellum, there was delayed neuronal layering, increased apoptosis in granular neurons and abnormal morphology of Purkinje cells (Pex2) [287]. In Pex11β null mice, there were reduced numbers of importing peroxisomes and only mild deficiencies of β-oxidation and plasmalogen biosynthesis, challenging the idea that PBD clinical features are directly related to the severity of the metabolic abnormalities [288, 289]. Overall, the null models survive gestation, but die soon thereafter, indicating that severe peroxisome deficiency is compatible with embryonic, but not postnatal life in both mice and humans. To gain further information, conditional Pex5 null mice were engineered to evaluate peroxisome dysfunction in key organs. Targeted elimination of peroxisomes in neural precursor cells results in moderate cortical migration defects that correct postnatally and might reflect migration delay [290]. Peroxisome elimination in hepatocytes results in postnatal arrest of neuron migration, particularly in the cerebellum, which still develops in the postnatal period [290]. Thus, peroxisome metabolism in the liver influences the development of the murine brain. It was suggested that elevated C27 bile acids, which are more hydrophobic than the terminal conjugated forms, may enter the brain and impair cerebellar development [290]. Survival of the mice with selective inactivation of Pex5 in neural cells, although neurodevelopmental defects were mild, resulted in a progressive motor and cognitive impairment with death before 6 months of age [291]. Myelinated axons and glial cells were severely affected in the brain and spinal cord, showing decreased compaction of myelin, loss of myelin and axonal damage. Mice with Pex5 inactivation in oligodendrocytes also developed similar axonal damage and demyelination [292]. Thus, peroxisomes may not be required for myelination, but for the preservation of axonal integrity and maintenance of myelin, and this role may be specific to oligodendrocyte peroxisomes [292].

Considering the interrelationship in mitochondrial and peroxisome functions, and the mitochondrial abnormalities noted in tissues from severe ZSD patients [4], mitochondrial abnormalities were investigated in the liver selective Pex5 null mouse model [293]. In this model, morphologically abnormal mitochondria with decreased respiratory chain complex activity were observed in liver. However, oxidative damage was not observed, possibly due to a compensatory increase in glycolysis and mitochondrial proliferation [293]. The decreased gluconeogenesis and glycogen synthesis resulted in the requirement for additional carbohydrates, increased food intake and lower body weights [294]. AMACR deficient mice show a metabolic adaptation that allows them to enhance excretion of C27 bile acids [295].

A PEX1-p.G843D equivalent knock-in mouse model (Pex1-p.G844D) was recently engineered to mimic the common human mutation, and to generate a model for the intermediate and milder phenotypes that feature progressive peroxisome dysfunction over time. Pex1-p.G844D homozygous mice exhibit many of the typical ZSD manifestations, including growth retardation, progressive retinopathy, bile acid defects resulting in fatty liver with cholestasis, elevated VLCFA, and decreased plasmalogens [296]. Further characterization of the course and mechanisms of disease pathophysiology are underway.

Mice engineered to study RCDP include Pex7 and Gnpat null mice, and Pex7 and Agps hypomorphic models [297–300]. The similarity of phenotypes amongst these models supports plasmalogen deficiency as the primary etiology for RCDP. Thus, they have been used to explore the normal functions of plasmalogens, which are largely unknown and likely to include tissue specific functions [299, 301]. Studies of these mice show requirements for plasmalogens in cerebellar, lens, and skeletal development, as well as spermatogenesis.

The PBD mouse models have also been used to test potential therapeutic interventions. In the Pex5 null models, supplementation with DHA, beginning in utero, normalizes DHA levels in brain but does not improve the neuronal migration disorder [302]. Bile acid supplementation in Pex2 null mouse
improves cerebellar abnormalities, implicating the accumulation of C27 bile acid intermediates in the cerebellar toxicity observed postnatally in these mice [303]. Supplementation with alkylglycerol, a plasmalogen precursor, can recover plasmalogen levels in PBD patient fibroblast lines [276]. Early alkylglycerol supplementation in the Pex7 null model improved growth and minimized cataract formation [45]. Although plasmalogen levels recovered and tissue pathology improved in somatic tissues, this was not the case in brain, where only around 1% of control plasmalogens were present after 2 months of treatment, and around 2% at 4 months of treatment.

8. Pharmacological therapies under development

8.1. Plasmalogen precursor supplementation

As discussed, oral alkylglycerol supplementation was shown to have some clinical efficacy in the Pex7 null murine model [45]. Additional plasmalogen precursor molecules are now being designed with properties that may improve clinical benefit, especially to recover plasmalogen levels in brain [304].

8.2. Induction of peroxisome numbers

Proliferation of peroxisomes in ZSD fibroblasts has been achieved using 4-phenylbutyrate and related compounds that act in a PPARα independent fashion through PEX11 stimulation [305, 306]. In cell lines from patients with intermediate and milder phenotypes, peroxisome proliferation results in improvement in peroxisome enzyme functions [305]. Presumably, an increased number of peroxisomes with partial function can improve overall cell functions. High throughput cell-based phenotype assays to identify additional compounds that can induce human peroxisome proliferation have been reported [67].

8.3. Nonsense suppression

The nonsense suppressor aminoglycoside drug, G418 (geneticin), was shown to promote peroxisome recovery in fibroblast lines from patients with PEX2 and PEX12 mutations that produce stable nonsense transcripts [307]. Thus, the development of safe and effective nonsense suppressor therapies might benefit a significant number of PBD patients.

8.4. Pharmacological enhancement of activity of a defective PEX protein

Studies on PEX1-p.G843D fibroblasts suggest that this is a misfolded protein amendable to improved folding and function when cells are cultured at 30°C or treated with chaperones drugs [268, 308]. The chemical chaperone arginine and betaine improved peroxisome biogenesis and functions in patient cell lines with missense mutations in PEX1, PEX6, and PEX12 [308, 309]. Chemical chaperones are small-molecules that non-selectively stabilize mutant proteins by altering the microenvironment around the protein, while pharmacologic chaperones are small molecules designed to selectively bind target proteins. The latter include enzyme substrates or inhibitors that bind to and facilitate folding of non-native protein intermediates to their native state. To determine if pharmacologic chaperones could be identified for PEX1-p.G843D, a high throughput cell-based phenotype assay was designed using a patient fibroblast cell line with this allele and expressing a GFP-tagged PTS1 reporter that was cytosolic at baseline [308]. After drug treatment the cells were evaluated for recovery of GFP-PTS1 peroxisome importation. Hit compounds, a flavonoid and a protein kinase C inhibitor, were previously shown to
bind the ATP binding site of ABC transporter proteins, suggesting they might act as pharmacological chaperones by binding to the ATP binding sites in the PEX1 protein [310, 311]. The advantages of using a cell-based assay include demonstrating that the molecules are able to cross at least one cell membrane and are functional in the cellular milieu. By measuring the downstream effect of peroxisome protein import recovery, these assays should enable the identification of any drug recovery mechanism. Hit compounds identified can be further developed into lead compounds if efficacious in animal models, and this task is currently being undertaken.

9. Conclusion

Enormous progress has been made in understanding mammalian peroxisome biology, biochemistry, and the molecular defects that underlie PBD. The PBD mouse models have already shown enormous utility in determining the disease pathophysiology, and are poised to contribute additional information in the future. Finally, pharmacological interventions for PBD that improve clinical outcomes may be possible. Additional therapeutic interventions can now be explored. The natural history of PBDs needs to be better defined and accurate clinical endpoints established in order to move forward in future systematic clinical trials.

Acknowledgments

We acknowledge the support of the Fonds de recherche du Québec – Santé (FRQS 32105) to CA, and the Canadian Institutes of Health Research (CIHR 126108) to NB. We also acknowledge the family organization: the Global Foundation for Peroxisomal Disorders, for their constant collaboration with us to focus research endeavours for best patient care.

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