Available online at www.sciencedirect.com

ScienceDirect

Biochimica et Biophysica Acta 1763 (2006) 1733 – 1748

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.elsevier.com/locate/bbamcr

Review

Peroxisome biogenesis disorders

Steven J. Steinberg [a,b,*], Gabriele Dodt [c], Gerald V. Raymond [a,b], Nancy E. Braverman [d], Ann B. Moser [a,b], Hugo W. Moser [a,b]

[a] *Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[b] *Kennedy Krieger Institute, Baltimore, MD, USA*
[c] *Interfakultäres Institut für Biochemie, Eberhard Karls Universität, Tübingen, Germany*
[d] *Department of Pediatrics and Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Received 25 May 2006; received in revised form 5 September 2006; accepted 6 September 2006
Available online 14 September 2006

Abstract

Defects in PEX genes impair peroxisome assembly and multiple metabolic pathways confined to this organelle, thus providing the biochemical and molecular bases of the peroxisome biogenesis disorders (PBD). PBD are divided into two types—Zellweger syndrome spectrum (ZSS) and rhizomelic chondrodysplasia punctata (RCDP). Biochemical studies performed in blood and urine are used to screen for the PBD. DNA testing is possible for all of the disorders, but is more challenging for the ZSS since 12 PEX genes are known to be associated with this spectrum of PBD. In contrast, PBD-RCDP is associated with defects in the *PEX7* gene alone. Studies of the cellular and molecular defects in PBD patients have contributed significantly to our understanding of the role of each PEX gene in peroxisome assembly.

Keywords: Zellweger syndrome; Neonatal adrenoleukodystrophy; Infantile refsum disease; Rhizomelic chondrodysplasia punctata; PEX

1. Introduction

Our current understanding of peroxisome biology recognizes two groups of peroxisomal disorders–peroxisome biogenesis disorders (PBD) and isolated enzyme deficiencies. This view of peroxisomal disease evolved over the past three decades. Although peroxisomes were first recognized over 60 years ago, they were not associated with human disease until 1973 when Dr. Sidney Goldfischer recognized that kidney and liver tissue from Zellweger syndrome patients were devoid of peroxisomes [1]. It was not until 1984 that a specific biomarker was identified that could be used for screening patients and the first gene defect associated with a PBD was not identified until 1992 [2,3]. Thus, the four clinical syndromes comprising PBD – Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), infantile Refsum disease (IRD) and rhizomelic chondrodysplasia punctata (RCDP) – were described before the biochemical and molecular bases of the disorders were understood. The PBD are of historic significance, as they constituted the first description of a malformation syndrome resulting from a metabolic error, and thus highlight the importance of biochemical pathways in development.

The elucidation of the peroxisomal biochemical pathways permitted more precise characterization of the PBD and highlighted their relatedness. Many of the defects in peroxisome metabolism were measured in cultured fibroblasts. Comprehensive biochemical studies showed that some patients with clinical features similar to the PBD actually had defects limited to a single enzyme and thus had a different pathogenetic basis. The assays used in cultured fibroblasts became valuable markers for complementation studies that were used to determine whether different gene defects were associated with each clinical syndrome. Three main research groups performed these studies and identified the complementation groups shown in Table 1. It was of great interest that early complementation studies suggested that patients with ZS, NALD and IRD could have the same gene defect. Likewise, the early studies showed that some gene defects were more common than others. RCDP patients had a unique clinical and biochemical phenotype, so it

* Corresponding author. Room 500H Kennedy Krieger Institute 707 N. Broadway, Baltimore, MD 21205, USA. Tel.: +1 443 923 2759; fax: +1 443 923 2755.
E-mail address: Steven.Steinberg@kennedykrieger.org (S.J. Steinberg).

0167-4889/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2006.09.010

Table 1
Complementation groups and PEX gene defects in Peroxisome Biogenesis Disorders

Gene	CG-Dutch	CG-Japan	CG-KKI	Clinical Phenotypes	Proportion of ZSS*	Locus	Gene Size (kb)	Number of Exons	mRNA (kb)	Number of Amino Acids	Remarks	References
PEX1	2	E	1	ZS NALD IRD	70%	7q21–q22	41.5	24	4.343	1283	1	[68,131]
PEX2	5	F	10	ZS IRD	3%	8q13–21	17.5	4	1.796	626	2,3	[2]
PEX3		G	12	ZS	<1%	6q23–q24	39.0	12	1.963	373	4	[109,110]
PEX5	4		2	ZS NALD	<2%	12p13.31	21.1	14 (no e8)	3.082	602	5	[99]
PEX5						12p13.31	21.1	15	3.193	639	5,6	
PEX6	3	C	4 and 6	ZS NALD IRD	10%	6p21.1	15.4	17	3.478	980	1	[81]
PEX7	1	R	11	RCDP	–	6q22–q24	91.3	10	1.451	323	7	[132–134]
PEX10		B	7	ZS NALD	3%	1p36.22	7.8	6	2.043	326 and 346	2,8	[91]
PEX12			3	ZS NALD IRD	5%	17q12	3.8	3	2.608	359	2	[95]
PEX13		H	13	ZS NALD	<1%	2p15	31.2	4	1.723	403	9	[107]
PEX14		K		ZS	<1%	1p36.2	155.8	9	1.915	377	10	[108]
PEX16		D	9	ZS	<1%	11p12–p11.2	8.4	11	1704	346	4	[111]
PEX19		J	14	ZS	<1%	1q22	8.3	8	3.651	299	11	[114]
PEX26		A	8	ZS NALD IRD	5%	22q11–21	13.6	7	3.081	305	4	[83]

CG=complementation group; Dutch=group at University of Amsterdam; KKI=Kennedy Krieger Institute; Japan=group at Gifu University School of Medicine; *Estimates of CG frequency is derived from the KKI data; ZSS=Zellweger syndrome spectrum; ZS=Zellweger: syndrome; NALD=neonatal adrenoleukodystrophy; IRD=infantile Refsum disease; RCDP=rhizomelic chondrodysplasia punctata; kb=kilobases; e=exon.
1—AAA-ATPase; 2—Zn-binding Ring finger domain and two transmembrane domains; 3—1.5 kb and 2.4 kb isoforms detected on Northern [135]; 4—Membrane protein; 5—TPR domain (tetratricopeptide repeat); 6—long form binds PEX7; 7—WD repeats; 8—the short form is predominant; the long form may have 345 or 346 amino acids; 9—SH3 domain; 10—docking protein for PEX5; 11—farnesylated, binds all peroxisomal membrane proteins.

was not surprising that one major group existed that was separate from the other PBD. Now there are 13 known complementation groups that are associated with defects in unique PEX genes and additional PEX genes remain candidates for human disease.

2. Clinical Phenotypes

2.1. Zellweger syndrome spectrum (ZSS) disorders

2.1.1. Zellweger syndrome

Zellweger syndrome or cerebrohepatorenal syndrome is a multiple congenital anomaly syndrome characterized by craniofacial abnormalities, eye abnormalities, neuronal migration defects, hepatomegaly, and chondrodysplasia punctata. The craniofacial features include a high forehead, hypoplastic supraorbital ridges, epicanthal folds, midface hypoplasia, and a large anterior fontanel [4,5]. Affected children present in the newborn period with profound hypotonia, seizures, and inability to feed. There is absence of neonatal and deep tendon reflexes and little spontaneous movement. Given this severe hypotonia some children with this condition have been initially thought to have Down syndrome, Prader–Willi syndrome, or spinal muscular atrophy.

Reflecting the ubiquitous nature of the peroxisome nearly every organ system is affected. The eyes may demonstrate corneal clouding, cataracts, glaucoma, optic atrophy, and retinal anomalies. Infants with this condition have absent responses on electroretinograms. The liver is enlarged and there are renal cysts. Bony stippling is seen at the patella and in other bones in approximately 50% of patients. The brain demonstrates a unique and striking abnormality of neuronal migration. There are areas of polymicrogyria, Purkinje cell heterotopia, and abnormalities of the olivary nucleus [6,7]. The adrenal glands show cytoplasmic lamellar inclusions of cholesterol esterified very long chain fatty acids [8]. Children with this condition do not show any significant development and usually die in the first year of life [5].

2.1.2. Neonatal adrenoleukodystrophy and infantile Refsum disease

Historically milder clinical presentations were named based on the primary biochemical abnormalities identified and include the disorders NALD, IRD, and hyperpipecolic acidemia [9–11]. It was later determined that these were not isolated biochemical abnormalities, but rather less severe presentations of PBD. The milder presentations and longer life spans of these disorders result in a more varied initial presentation and natural history. There has been survival of mildly affected patients into adulthood. The boundaries between children labeled with NALD or IRD is often blurred and it seems more appropriate now to consider these disorders a continuum of peroxisome deficiency.

While many children will present in the newborn period, others may not come to attention until later. Most children have hypotonia, but unlike ZS there is a degree of psychomotor development—achieving head control, sitting unsupported, and even walking independently. Many communicate and although language is rare, there have been children who have near normal language for age. In terms of other features, the craniofacial features are similar but less pronounced. Seizures may be present in the neonatal period or manifest later. Renal cysts and bony stippling are not seen routinely. Biochemical liver disease may be present. In some individuals, a leukodystrophy develops with degeneration of myelin in the central nervous system, the loss of previously acquired skills, and development of spasticity. This may stabilize, or progress and be fatal.

The most common manifestation in this group of patients that is less apparent in ZS is the development of sensorineural hearing loss and retinitis pigmentosa. This may occur in the first years of life. Affected individuals may initially be diagnosed as having a variant of Usher syndrome, but the other manifestations provide clues to the appropriate diagnosis. Other diagnostic entities considered in patients subsequently found to have a PBD include Leber congenital amaurosis [12,13], a number of congenital myopathies in which severe hypotonia is a presenting feature [14] and late onset leukodystrophy [15].

2.2. Rhizomelic chondrodysplasia punctata

The main clinical features of this distinct PBD include shortening of the proximal long bones (rhizomelia) with metaphyseal cupping, coronal clefts of the vertebral bodies, generalized epiphyseal stippling (chondrodysplasia punctata) and other evidence of disturbed ossification. There are resultant severe contractures. Affected infants also have bilateral cataracts, abnormal facies with frontal bossing, depressed nasal bridge, and small nose. Ichthyosis can develop. Abnormalities of the central nervous system include cerebral and cerebellar atrophy, abnormalities of myelination and neuronal migration defects involving the midbrain [16]. Cervical spine stenosis secondary to the chondrodysplasia may be under appreciated [17]. Most RCDP children have manifested profound growth and psychomotor retardation. Lifespan is broad with some children dying in the first year and others surviving into young adulthood. Seizure disorders are common. Respiration may be significantly compromised and recurrent respiratory complications are a frequent source of morbidity.

A retrospective review of RCDP patients by White et al. provides guidelines for health maintenance and has helped define the clinical progression of this disorder [18]. Few patients with RCDP have been identified with milder phenotypes. These patients have congenital cataracts and chondrodysplasia, but variable rhizomelia and milder mental and growth deficiency. Atypical patients with *PEX7* defects have also been recognized and include a patient with congenital cataracts and a mild learning disability, as well as some patients with a phenotype similar to adult Refsum disease (ARD; see Section 3.3.2, α-oxidation) [19,20].

3. Biochemical phenotypes and diagnosis of PBD

Without considering clinical criteria, the two spectra of PBD can be distinguished by the measurement of plasma very long chain fatty acid (VLCFA) levels and erythrocyte membrane plasmalogens from the same blood sample. RCDP patients have deficient plasmalogens, but normal VLCFA. ZSS patients have elevated VLCFA and usually deficient plasmalogens. Since 15–20% of patients with clinical features in the ZSS and elevated plasma VLCFA have a single enzyme defect in peroxisomal fatty acid metabolism, it is necessary to perform further studies for accurate diagnosis. Likewise, a small proportion (<10%) of RCDP patients have an isolated enzyme deficiency in peroxisomal plasmalogen synthesis. Furthermore, more comprehensive studies are necessary to characterize PBD patients outside of the classic spectra. The screening tests in blood and

Table 2
Biochemical markers and assays used for the characterization of patients with suspected defects in peroxisome assembly

	PBD: Zellweger spectrum		β-oxidation single enzyme defects		Rhizomelic Chondrodysplasia Punctata		
	Severe	Mild	DBP	ACOX1	RCDP1	RCDP2	RCDP3
Screening Tests:							
Very long chain fatty acids (VLCFA)	↑↑	↑	↑↑	↑	Normal	Normal	Normal
Phytanic acid	Normal*	↑	Normal*—↑↑	Normal	Normal*—↑	Normal	Normal
Pristanic acid	Normal*	↑	Normal*—↑	Normal	Normal	Normal	Normal
Plasmalogens	↓↓	↓— Normal	Normal	Normal	↓↓—↓	↓↓—↓	↓↓—↓
Pipecolic acid, plasma	↑	↑↑	Normal	Normal	Normal	Normal	Normal
Pipecolic acid, urine	↑↑	↑	Normal	Normal	Normal	Normal	Normal
Bile acid metabolites	↑↑	↑	↑	Normal	Normal	Normal	Normal
Follow up Tests (in fibroblasts):							
VLCFA	↑↑	↑	↑↑	↑	Normal	Normal	Normal
Plasmalogen synthesis	↓↓	↓—Normal	Normal	Normal	↓↓↓	↓↓	↓↓
Phytanic acid oxidation	↓↓	↓	↓↓	Normal	↓	Normal	Normal
Pristanic acid oxidation	↓↓	↓	↓↓	Normal	Normal	Normal	Normal
Catalase solubility	↑↑	↑↑	Normal	Normal	Normal	Normal	Normal
Fibroblast peroxisome morphology	Enlarged, reduced number or Absent	Enlarged, reduced number	Enlarged, reduced number	Enlarged, reduced number	Normal	Normal	Normal
Fibroblast PTS1 import	↓↓	↓	Normal	Normal	Normal	Normal	Normal
Fibroblast PTS2 import	↓↓—N	↓—N	Normal	Normal	↓↓	Normal	Normal
Molecular Tests:	*PEX1, 2, 3, 5, 6, 10, 12, 13, 14, 16, 19,* or *26*		DBP gene	*ACOX1*	*PEX7*	DHAPAT gene	ADHAPS gene

↑=higher than normal; ?=lower than normal; * since a severe case would present in the new born period and branched chain fatty acids accumulate with dietary exposure, these fatty acids are usually normal at that time; DBP=D-bifunctional protein; *ACOX1*=straight chain acyl-CoA oxidase; DHAPAT=dihydroxyacetonephosphate acyltransferase; ADHAPS=alkyldihydroxyacetonephosphate synthase.

urine and follow-up tests in cultured fibroblasts are summarized in Table 2.

3.1. Biochemical diagnosis of PBD-ZSS

The primary screening test is measurement of plasma VLCFA (26:0, C26:1, C24:0/C22:0 and C26:0/C22:0). Although most ZSS patients have elevations in all four VLCFA parameters, some atypical patients have equivocal results. False positive results can be associated with a ketogenic diet [21], hemolysis or elevated total lipid due to collection of a non-fasting specimen or generalized hyperlipidemia. An algorithm for the diagnosis of PBD-ZSS based on plasma VLCFA results is shown in Fig. 1.

Plasma fatty acid analysis also usually includes measurement of the branched chain fatty acids phytanic acid and pristanic acid. These accumulate with dietary exposure to phytanic acid, so are normal at birth. In contrast, plasma pipecolic acid is elevated in most ZSS patients, although in the first year of life it is usually higher in urine due to immaturity of renal reabsorption. Of historical note pipecolic acidemia was the first biomarker identified in ZSS patients [22]. The bile acid intermediates 3α,7α-dihydroxy-5β-cholest-26-oic acid (DHCA) and 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) are elevated in plasma and urine due to impaired peroxisomal β-oxidation of the C27-intermediates. The degree of DHCA and THCA elevation may vary based on clinical severity and there is

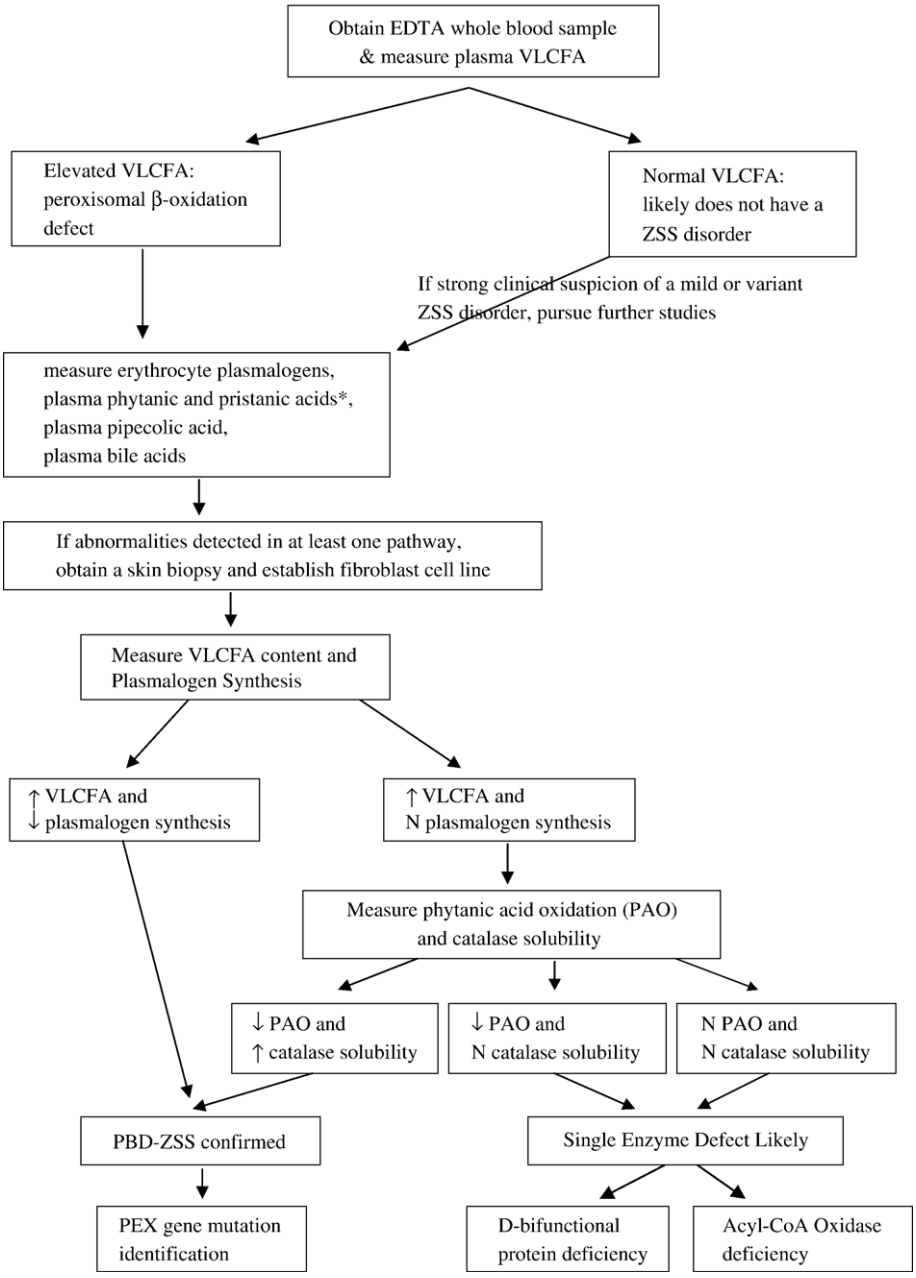

Fig. 1. Algorithm for suspected ZSS disorder. N=normal; *=not elevated in newborn period.

a good correlation between the VLCFA elevation and bile acid metabolites in plasma from PBD-ZSS patients [23].

Erythrocyte plasmalogen levels are generally severely deficient in ZS patients, but levels may be intermediate or normal in NALD and IRD patients [24]. If urine organic acid analysis is performed, epoxydicarboxylic acids may be detected and aside from their excretion in normal individuals following ricinoleic acid dietary exposure (castor oil) these compounds appear to be specific to peroxisomal fatty acid defects [25,26]. Hyperoxaluria was recently reported in >80% of a cohort of ZSS patients who survived >1 year; about 3/5 of these patients also had hyperglycolic aciduria [27]. Many ZSS patients have lower than normal plasma docosahexaenoic acid (DHA), a fatty acid believed to have an important role in brain development and function that requires peroxisomal β-oxidation for its synthesis [28,29].

It is generally recommended that a skin biopsy be collected to establish a cell line that can be used for biochemical, immunocytochemical and molecular studies. A variety of approaches can be used to document the perturbations in peroxisome metabolism indicated by the biomarkers studied in blood and urine. The deficiency in peroxisome fatty acid β-oxidation can be documented indirectly by measurement of VLCFA content or by determining the specific activity of C24:0 or C26:0 β-oxidation [3,30]. Plasmalogen synthesis can be assessed by an in situ assay that compares the initial peroxisomal steps of synthesis to the later microsomal steps of synthesis [31]. Alternatively, the individual enzymes dihydroxyacetonephosphate acyltransferase (DHAPAT) and alkyl-dihydroxyacetonephosphate synthase (ADHAPS) can be measured using cell homogenates [32,33]. The enzyme pipecolic acid oxidase is not measurable in dermal fibroblasts. The α-oxidation of phytanic acid or β-oxidation of pristanic acid can be measured by collecting water soluble products and CO_2 [34,35]. Catalase solubility is an indirect assessment of peroxisome assembly in cultured fibroblasts. PBD-ZSS patients have a high percentage of soluble catalase compared to controls [36].

To assess whether biochemical results had any prognostic value, Gootjes et al compared a number of biochemical markers to the age of death [37]. They found the best correlation occurred when DHAPAT activity and C26:0 β-oxidation in fibroblasts were used in combination to distinguish patients who died <1 year of age versus those who survived >5 years.

Immunocytochemistry can be used to visualize peroxisomes in cultured cells. A variety of antibodies to peroxisome matrix proteins (PMatP) and peroxisome membrane proteins (PMemP) are used. When immunostained with a PMemP antibody a small proportion of ZSS patients have a complete absence of peroxisome structures. So far this phenotype has only been observed in association with specific PEX gene defects (*PEX3, PEX16* and *PEX19*). Otherwise, in the majority of cases PMemP antibodies yield cells that show a reduced number of peroxisomal structures that appear larger than normal and have been referred to as peroxisome ghosts [38] (see Fig. 2c). Immunostaining the same cells with PMatP antibodies may yield very different results, depending on the antibody used. Table 3 lists some of the key PMatP by function and designates their peroxisome targeting signal (PTS). Catalase is a matrix protein with a weak PTS1, as it has the carboxy terminal sequence-KANL rather than the classic PTS1 consensus sequence-SKL [39]. This likely explains why catalase import is very sensitive to any perturbation in peroxisome assembly. In ZSS cultured fibroblasts catalase often has a cytosolic localization (see Fig. 2g).

Some ZSS patients have normal PTS2 import and, thus, thiolase, ADHAPS and phytanoyl-CoA hydroxylase are localized to peroxisome structures. The degree of impaired PTS1 and PTS2 import can be highly variable, depending on the PEX gene involved and the mutation(s) present. In sum, three types of import defects have been described [40]. Impaired PTS1 import alone is a type I defect, impaired PTS2 import alone is a type II defect and impaired PTS1 and PTS2 import is a type III defect. Patients in the ZSS may have types I or III defects, whereas a type II defect is consistent with RCDP. Type I defects were associated with a specific mutation in the *PEX5* gene, which encodes the PTS1 receptor. The majority of ZSS patients have type III defects. Only a small proportion of ZSS cells (15 %) are unable to import any PTS1 and PTS2 matrix proteins [40]. Thus, the majority of fibroblasts from these patients have only a reduced capacity for matrix protein import, rather than total abrogation.

3.2. Peroxisomal mosaicism and temperature sensitivity

Peroxisomal mosaicism is the descriptive term that has been applied to two types of observations regarding peroxisome function within and between tissues. Both types may be identified in the same patient. The first type of mosaicism (type 1) refers to a disparity between the biochemical profile in body fluids as compared to the studies performed in cultured fibroblasts [41]. When this arises it may not be possible to demonstrate a defect in the pathway in cultured cells even though the blood metabolites were abnormal. This potential is especially relevant to biochemical prenatal diagnosis and highlights why it is important to perform confirmatory studies in cultured fibroblasts when possible.

The second type of peroxisomal mosaicism (type 2) refers to variable phenotypes in cells with the same genotype and is observed when assessing peroxisome morphology and matrix protein import in cultured cells or other fixed tissues. In this case the results for adjacent cells may be incongruent. For example, several investigators have demonstrated that one population of liver cells may be able to import catalase but neighboring cells do not [41,42]. Shimozawa et al. demonstrated that when a CHO cell line lacking any *PEX2* expression was transfected with a construct harboring the *PEX2* mutation E55K, transformants were mosaic for PTS1 import, similar to the phenotype of the patient's cell line that expressed *PEX2*-E55K [43]. This case demonstrated that type 2 peroxisomal mosaicism is a biological phenomenon not caused by genomic mosaicism.

Temperature sensitivity refers to fluctuations in the phenotype of cell lines cultured at temperatures lower (30 °C) or

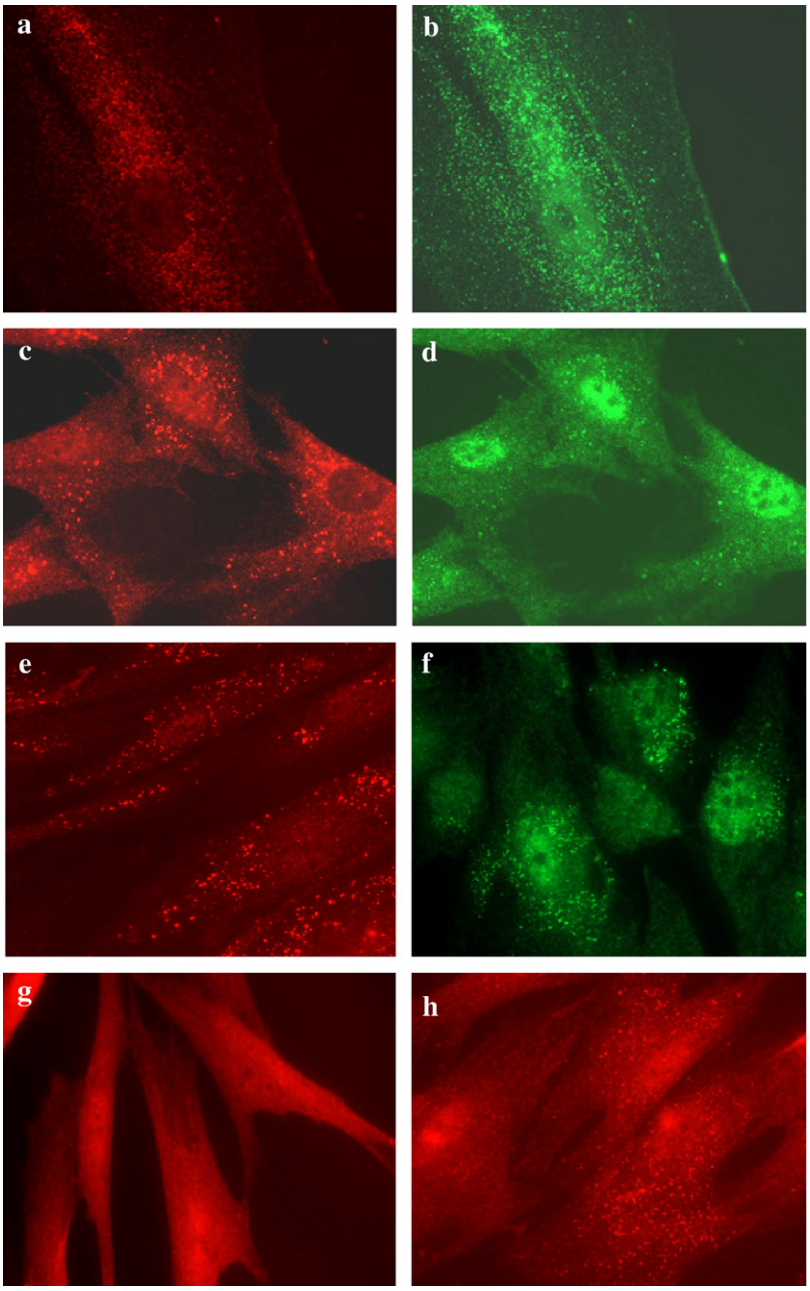

Fig. 2. Immunocytochemical analysis of cultured dermal fibroblasts. All cells were fixed, permeabilized with triton and then immunostained with anti-PMP70 to identify peroxisome structures, anti-thiolase or anti-catalase to identify peroxisome matrix proteins. a, normal control, anti-PMP70; b, normal control, anti-thiolase; c, ZS, anti-PMP70; d, ZS, anti-thiolase; e, IRD, anti-PMP70; f, IRD, anti-thiolase; g, IRD cultured at 37 °C, anti-catalase; h, IRD cultured at 30 °C, anti-catalase. Cells from a and b and c and d were dual labeled. Frame c shows cells with a reduced number of enlarged peroxisomes, and frame d shows these peroxisomes had a limited capacity to import a matrix protein. Frame f demonstrates peroxisome mosaicism. Cells used in frames g and h are from the same patient and thus demonstrate temperature sensitivity.

higher (40 °C) than body temperature. Culturing fibroblasts from some PBD patients at 30 °C improves peroxisome assembly, and thus the mutation(s) would be considered temperature sensitive. Conversely, culturing PBD cells with a modest or mosaic defect in peroxisome assembly at 40 °C may exacerbate the defect in peroxisome assembly [44]. The effect of culturing cells at 30 °C suggests that some peroxins harboring missense alleles may be amenable to improved function using small molecules as chaperones that would mimic the effect of lowering the temperature. In contrast, growing cells at 40 °C has been used to identify the gene defect in cell lines that do not have a marker that can be used for complementation analysis using standard culture conditions [44].

3.3. Metabolic pathways and their role in pathogenesis

3.3.1. β-oxidation

Fatty acids with a chain length >C22, including saturated and unsaturated, are degraded exclusively in the peroxisome. In addition to VLCFA, this pathway is vital to the synthesis of bile

Table 3
Peroxisome matrix proteins and their peroxisome targeting signal (PTS)

Peroxisome matrix protein	Type of PTS	PTS* amino acid sequence	Role in peroxisome metabolism
Acyl-CoA oxidase I	1	–SKL	Fatty acid metabolism
Alanine glyoxylate aminotransferase	1	–KKL	Glyoxylate metabolism
Alkyldihydroxyacetonephosphate synthase	2	–RLVLSGHL–	Plasmalogen synthesis
Catalase	1	–KANL	H_2O_2 metabolism
D-bifunctional protein	1	–AKL	Fatty acid metabolism
Dihydroxyacetonephosphate acyltransferase	1	–AKL	Plasmalogen synthesis
β-ketothiolase	2	–RLQVVLGHL–	Fatty acid metabolism
Phytanoyl-CoA hydroxylase	2	–RLQIVLGHL–	Fatty acid metabolism
L-pipecolic acid oxidase	1	–AHL	Amino acid metabolism
Sterol carrier protein 2	1	–AKL	Fatty acid metabolism

PTS= peroxisome targeting signal; *PTS amino acid sequences are listed as stated in the article by Wanders and Waterham [136].

acids and the inactivation of compounds such as prostaglandins. PBD patients have lamellar inclusions in the white and grey matter composed of VLCFA cholesterol esters [45]. In four ZS fetuses regions of the brain with abnormal development and cell ultrastructure had corresponding elevated cholesterol ester VLCFA and diminished plasmalogens [46]. Likewise, striated adrenocortical cells containing lamellae were detected in 7 of 8 ZS patients and these contained elevated VLCFA cholesterol esters [8]. ZS brain has an excess of >C32 polyenoic fatty acids predominantly in phosphatidylcholine species, with the sn-1 position harboring the polyunsaturated fatty acids and the sn-2 position containing fatty acids <C24 [47]. VLCFA in red blood cell sphingomyelin is increased in ZS patients [48]. In neonatal rat brain polyenoic VLCFA from C24–C38 enrich the sn-1 position of phosphatidylcholine [49]. Recently the amount of C26:0 in lysophosphatidylcholine isolated from whole blood spots and analyzed by tandem mass spectrometry has been shown to be significantly elevated in ZSS patients as compared to control [50]. The accumulation of VLCFA in specific lipid species may play an important role in pathogenesis.

3.3.2. α-oxidation

About 50–100 mg of phytanic acid is consumed daily in the average Western diet and approximately half is metabolized [51]. Chlorophyll and phytol may make a small dietary contribution [52]. Phytanic acid cannot be catabolized by β-oxidation due to the methylene group in the α-position. Patients with a defect in the gene encoding phytanoyl-CoA hydroxylase have adult Refsum disease (ARD) and accumulate vast amounts of phytanic acid in tissues. The constellation of clinical features associated with this defect includes retinitis pigmentosa, anosmia, deafness, cerebellar ataxia, and peripheral neuropathy. Cardiomyopathy and cardiac arrhythmias can occur. Mental developmental is normal and bone abnormalities are mild, including short metacarpals or metatarsals in 30% of patients [53]. Possible mechanisms for phytanic acid-associated pathology include direct toxicity and perturbations in cell signaling. Phytanic acid is not as significantly elevated in PBD as compared to ARD. Furthermore, ARD patients generally do not develop symptoms until the second decade. So its contribution to pathogenesis of the early onset PBD is not likely to be a major determinant of phenotype.

3.3.3. Plasmalogen biosynthesis

The first two steps of alkenyl ether lipid biosynthesis occur exclusively in the peroxisome. DHAPAT is a PTS1 protein and ADHAPS is a PTS2 protein. The precursor form of ADHAPS is not stable outside of the peroxisome [54]. Based on patients with isolated deficiencies of ADHAPS, the stability of DHAPAT appears to be dependent upon the presence of ADHAPS. Cell lines devoid of ADHAPS protein may also have reduced DHAPAT activity [55]. Plasmalogens comprise a significant proportion of mammalian cell membrane phospholipids. For example, ethanolamine phospholipids in myelin are 80–90% plasmalogens. Plasmalogen levels in ZS brain, liver, kidney, muscle and heart are <5% of control levels [56]. It has been suggested that plasmalogens protect cells from oxidative damage [57]. Other proposed cellular roles for plasmalogens include membrane fusion, ion transport, and cholesterol efflux [58]. The defect in plasmalogen biosynthesis is more severe in RCDP than ZS, and may therefore be the primary culprit in the skeletal dysplasia unique to RCDP. Bams-Mengerink et al. recently reported a direct correlation between plasmalogen levels and the RCDP clinical phenotype [16].

3.3.4. Pipecolic acid oxidation

Although pipecolic acid can be a useful biomarker for the diagnosis of PBD-ZSS, its role in pathogenesis is uncertain. Pipecolic acid elevation has been reported in association with liver disease and Vallat et al. reported one individual with grossly elevated pipecolic acid without any clinical features [59,60]. Pipecolic acid is also a marker for pyridoxine-dependent seizures, associated with a defect in Δ1-piperideine-6-carboxylate-α-aminoadipic semialdehyde dehydrogenase [61]. Wierzbicki et al has suggested that as many as 20% of ARD patients have mildly elevated plasma pipecolic acid [51]. Pipecolic acid metabolism in the CNS requires further study to determine whether it is a determinant of clinical phenotype in the PBD.

3.4. Biochemical Diagnosis of RCDP

RCDP is associated with three characteristic abnormalities: deficient ether lipid synthesis; deficient phytanic acid oxidation; and, failure to process peroxisomal β-ketothiolase to its mature form [62]. An infant suspected to have RCDP should have erythrocyte plasmalogens measured as a screening test (Fig. 3).

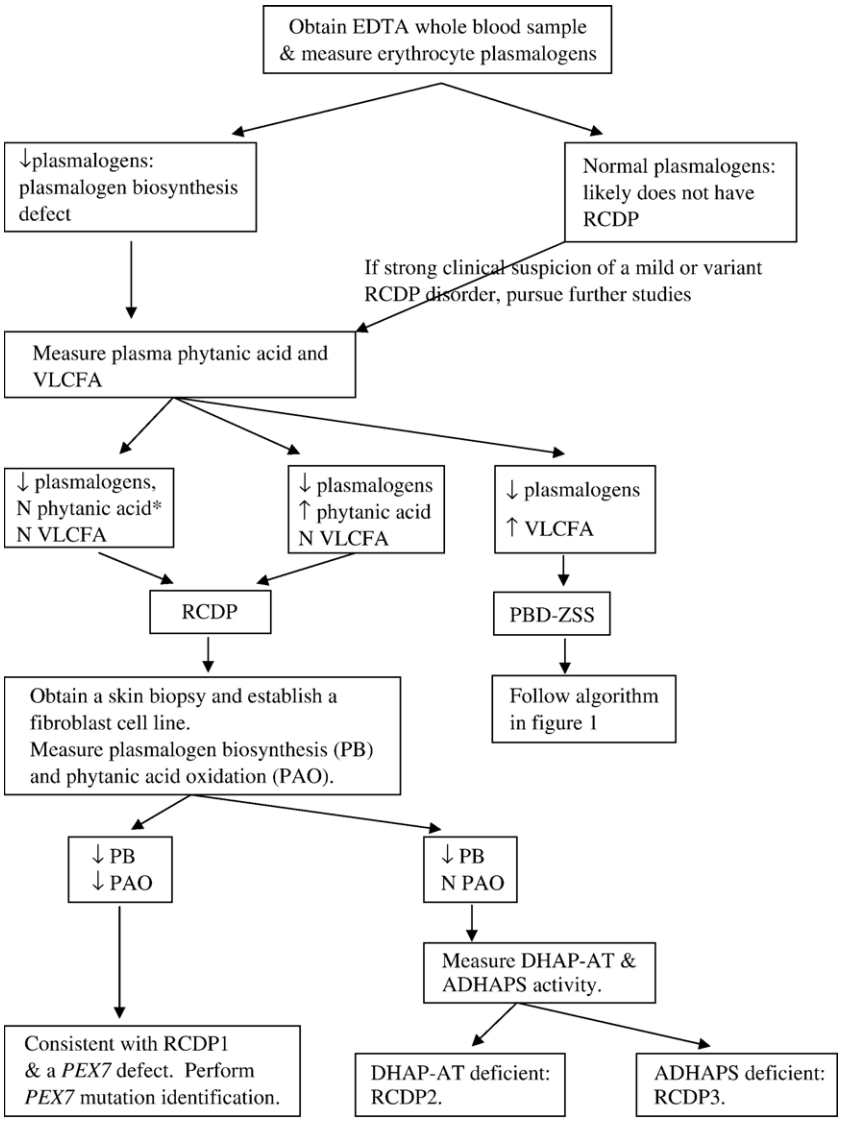

Fig. 3. Algorithm for suspected RCDP disorder.

If plasmalogens are deficient, then plasma fatty acids should be analyzed to rule out a ZSS disorder and to determine phytanic acid levels. Confirmatory studies should be performed using cultured fibroblasts. Total plasmalogen synthesis and phytanic acid oxidation should be measured. If phytanic acid oxidation is normal, then the patient more likely has a single enzyme defect of plasmalogen synthesis. The specific enzymes DHAPAT and ADHAPS can be measured in cell lysates to assess this possibility; alternatively complementation studies or mutation detection could be used.

Although RCDP is caused by a defect in peroxisome assembly, cultured fibroblasts have peroxisomes that appear normal in size and number when stained with antibodies to PMemPs or PMatPs with a PTS1. However, some RCDP patients have been reported to have enlarged hepatic peroxisomes [63,64].

RCDP cells are unable to incorporate fatty alcohols into the sn-1 position of the phosphoglycerol backbone for plasmalogen synthesis. On this basis, Rizzo et al. measured plasma fatty alcohol levels and showed a 2.5- to 7-fold elevation of hexadecanol and octadecanol in six RCDP plasma samples as compared to normal control [65]. In contrast, two ZSS patients showed only a slight elevation. These authors speculated that fatty alcohols may play a role in the pathogenesis of ichthyosis and neurological features in RCDP patients, based on the overlap with Sjögren–Larsson syndrome [65].

4. Molecular Phenotype of PBD

To date thirteen PEX genes, encoding the peroxins required for peroxisome assembly, have been shown to be associated with human disease (Table 1). These genes encode proteins

essential to the assembly of functionally competent peroxisomes (see Fig. 4). The genes *PEX5* and *PEX7* encode the PTS1 and PTS2 receptors, respectively. The *PEX1, PEX6* and *PEX26* genes are most commonly associated with PBD-ZSS (~85%) and appear to play a role in the recycling of PTS1 and PTS2 matrix protein receptors. Three genes that encode peroxisome membrane proteins that are the site of matrix protein translocation, *PEX2, PEX10* and *PEX12*, are defective in at least 10% of ZSS patients. In contrast, genes associated with the synthesis of peroxisome membranes (*PEX3, PEX16* and *PEX19*) and that comprise the docking site for PTS1 and PTS2 receptor-matrix protein complexes (*PEX13* and *PEX14*) are rarely associated with human disease. In most cases, though, these less common defects were associated with ZS.

Characterization of the gene defect in PBD contributes to our understanding of the functional domains of each peroxin, but also has clinical utility. Although mutation identification is rarely needed to secure the diagnosis of a PBD, it has proven useful when the clinical and biochemical phenotypes do not fit the classic criteria. For instance a *PEX12* patient was initially thought to have an isolated deficiency of peroxisomal fatty acid metabolism [66]. In most cases mutation identification is sought because of its utility in prenatal diagnosis and for carrier detection, which is not possible by existing biochemical methods. There is increasing evidence of genotype–phenotype correlations, especially in regard to the common *PEX1* alleles described below.

Mutation identification is straightforward in RCDP. If patients have deficient plasmalogen synthesis and phytanic acid oxidation, then *PEX7* sequence analysis has a high degree of sensitivity. The situation is more complicated for ZSS patients. The biochemical profile does not help determine a candidate PEX gene. Complementation analysis by transfection of fibroblasts with plasmids encoding the different PEX genes has been used successfully to test for the restoration of peroxisome assembly. The limitation of this approach is that a fibroblast cell line is needed and the cells must express a consistent defect in assembly. We have used an alternate approach whereby genomic DNA from a patient is tested for mutations in select exons from the six PEX genes most commonly associated with PBD-ZSS. Our PEX gene screen algorithm identified at least one pathogenic mutation in ~80% of patients after testing 12 exons (14 amplicons) [67]. The PEX genes are listed below in five divisions based on their role in peroxisome assembly and we describe their contribution to molecular pathogenesis.

4.1. PEX genes involved in recycling of matrix protein receptors

4.1.1. PEX1

PEX1 deficiency is the most common cause of PBD-ZSS involving 70% of all PBD patients. This is mainly due to the existence of two common alleles, I700fs (Nt2098insT) and G843D (Nt2528G>A) [68, 69]. About 80% of patients suspected to have a *PEX1* deficiency based on complementation studies were found to have at least one of the common alleles [69–74]. A recent survey [74] estimates an allele frequency of 0.35 for I700fs and a frequency of 0.43 for G843D in *PEX1*-deficient patients. A third allele Nt2916delA (G973AfsX16) has a frequency of 0.034 and was mainly recognized in an

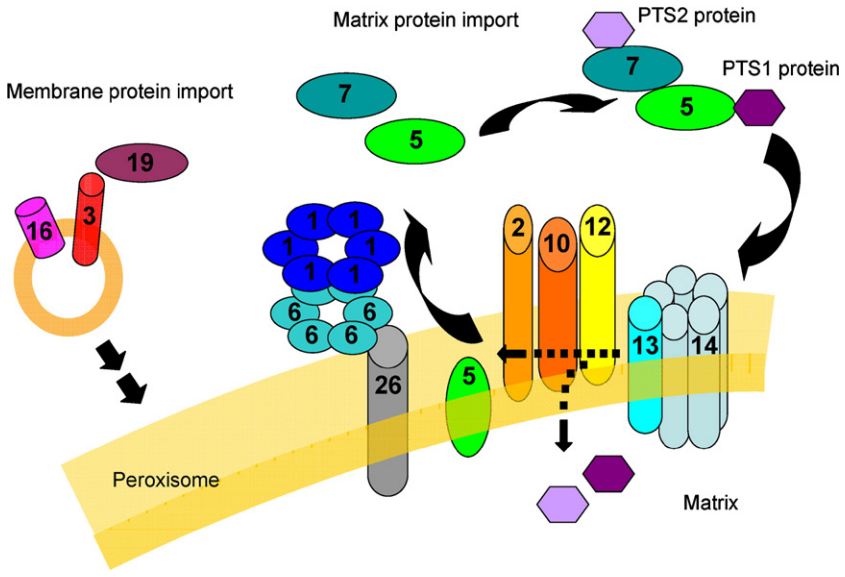

Fig. 4. Current working model for membrane protein and matrix protein import into mammalian peroxisomes. The biogenesis of peroxisomes in cells lacking peroxisomal structures starts with the early peroxins PEX3, PEX16 and PEX19 and proceeds via several steps. The import of membrane proteins into extant peroxisomes probably only needs PEX19 for recognition, targeting and insertion via docking at PEX3. Matrix proteins in the cytosol are recognized by their targeting signals – PTS1 via PEX5 and PTS2 via PEX7 – and transported to the docking complex PEX14 and PEX13 at the peroxisomal membrane. PEX5 seems to be integrated into the peroxisomal membrane and – in a yet unknown process – the cargo is imported by a mechanism involving the ring finger complex PEX2, PEX10 and PEX12. PEX5 is believed to exit the membrane with the help of a complex of the two mostly cytosolic AAA proteins PEX1 and PEX6 and the integral membrane protein PEX26, and then cycles back to the cytosol, ready for another round of import.

Australasian cohort. In general, 46% of all *PEX1* mutations are missense mutations, the same amount account for insertions and deletions, followed by 3% splice site mutations and 3% nonsense mutations [74]. Changes resulting in premature truncation codons are spread throughout *PEX1*; the missense mutations however seem to segregate within exons encoding the two AAA domains (exons 9 to 20) of the PEX1 protein [75]. The frequency data have been used for systematic screening of exons 13, 15 and 18 of *PEX1* in the recently developed *PEX* gene screen algorithm [67].

I700fs is associated with a reduction in the amount of mRNA and undetectable protein levels. As there is no evidence of aberrant splicing in association with this single nucleotide insertion and transfection of *PEX1* null cells with a plasmid harboring this sequence variation does not rescue peroxisome assembly, there is convincing evidence that this mutation results in a complete loss of function [69]. The same mechanism accounts for other alleles like Nt2916delA that introduce a premature termination codon [70,71].

Cells from patients harboring missense mutations on both alleles, especially the G843D allele, have RNA levels ranging from reduced to near normal and protein levels from 5 to 50% of normal. The G843D mutation results in a temperature-sensitive phenotype, leading to normal import of peroxisomal matrix proteins at the permissive temperature of 30 °C and to a low or absent import at 37 °C [70,71,76,77]. This is a strong indication that the mutant protein is misfolded at higher temperature and degraded, while at the permissive temperature the folding is improved and the protein level rises. This is accompanied by restoration of peroxisomal import. The mutant protein, if over expressed in *PEX1* defective cells, shows 15% restoration of import compared to the wild type protein [68]. Another temperature sensitive allele of *PEX1* is L66P [76].

In terms of genotype – phenotype correlation, the discrimination between null alleles and alleles having residual protein expression and function seems valuable. Throughout all reports patients with two *PEX1* null alleles all had a ZS phenotype and typically did not survive the first year. Poll-The [78] investigated a cohort of 17 milder affected children and detected no patient homozygous for a Nt2098insT mutation older than 4 years of age.

The genotype–phenotype relationship is less predictable for alleles resulting in residual PEX1 activity. One might expect that patients with two missense alleles fall into a group with a milder clinical spectrum compared to patients that are heterozygotes with one null and one missense allele (compound heterozygotes). However, a considerable overlap of phenotypes is apparent between these groups. Poll-The et al [78] found two G843D heterozygotes with an unexpected more severe phenotype and even one patient with an early lethal disease. This might indicate that other yet unknown factors in addition to the *PEX1* genotype determine the phenotype [78]. It is interesting to mention two recently studied polymorphisms in the promoter region of *PEX1* that can alter transcription level, one mutation reducing (Nt-137T>C) and one increasing (Nt-53C>G) the transcription level in human skin fibroblasts [73]. Both polymorphisms cosegregated in most but not all of the investigated cases. In vitro these variations alter expression levels, thus hypothetically they may contribute to phenotypic heterogeneity among ZSS patients.

4.1.2. PEX6

PEX6 is the second AAA-ATPase acting on matrix protein import. The interaction with PEX1 seems to be crucial for biogenesis of peroxisomes and mutations in *PEX1* affecting PEX6 binding lead to more severe phenotypes [76]. So far defects in *PEX6* have been described in 21 patients (summarized in Steinberg et al. [67]), with no evidence of common mutations [79–82]. Most mutations occur in exon 1, which encompasses one third of the coding region. The fact that about half of the patients have at least one defect in exon1 has been successfully used to identify new patients of this complementation group with the PEX gene screen algorithm [67]. Many of the identified alleles give rise to premature stop codons or deletions that manifest as ZS [79]. As with *PEX1* some of the missense alleles (L57P and T572I) result in a temperature sensitive phenotype, giving rise to a somewhat milder clinical phenotype [82].

4.1.3. PEX26

Amongst the common complementation groups identified in the 1990's, *PEX26* was the last remaining gene to be identified [83]. Based on their studies Matsumoto et al. suggested that PEX26 interacts with a PEX1/PEX6 heterooligomeric complex. More recent analysis suggests that PEX26 has a critical N-terminal binding domain that interacts with PEX6 and that this complex then activates PEX1 [84]. So far 18 patients, covering the full spectrum of clinical phenotypes, have been tested. More than half the mutations have been missense changes, and all occur in the N-terminal half of the protein thought to be critical for interacting with PEX6 [67,84–86]. One allele, R98W, accounts for 39% of the mutant *PEX26* genes from 18 genotyped patients, possibly representing a founder effect or to a recurrent mutation at a CpG dinucleotide in codon 98 [84]. Several alternative transcripts have been identified, including one that omits exon 5 (*PEX26*Δe5). *PEX26* exon 5 encodes this protein's single transmembrane domain and its omission does not change the reading frame. *PEX26*Δe5 is able to rescue *PEX26* deficiency in cultured cells. This finding in conjunction with other functional in vitro studies suggest that PEX26 and PEX6 do not need to be localized to the peroxisome membrane to contribute to peroxisome biogenesis and that binding to PEX1 is dynamic [84]. Furuki et al. showed R98W in compound heterozygosity with L44P had a temperature sensitive cellular phenotype [86]. Several probands that were either homozygous or heterozygous for R98W had milder clinical phenotypes ranging from NALD to IRD [84,86].

4.1.4. PEX genes encoding membrane proteins involved in the translocation of matrix proteins

All three of these genes encode membrane proteins with two transmembrane domains and a zinc-finger binding domain toward the carboxy-terminus (Fig. 5).

```
hsPEX10 LCTLCLEERRHPTATP-CGHLFCWECITA--WCSSKAECPLCRE
hsPEX12 VCPLCRKTRVNDTVLATSGYVFCYRCVFH--YVRSHQACPITGY
hsPEX2  -CALCGEWPTMPHTIG-CEHIFCYFCAKSSFLFDVYFTCPKCGT
         * **                ** *           **
```

* = conserved in all three proteins

Fig. 5. Highlighted in yellow are the amino acid residues that comprise the PEX10 C3HC4 domain. Highlighted in green is PEX12-S320, the amino acid residue associated with peroxisome mosaicism in conjunction with a phenylalanine substitution (S320F) [44].

4.1.5. PEX2

Fourteen patients with *PEX2* defects have been reported, including five who were R119X homozygotes (Nt355C>T) [2,67,77,87,88,89]. One patient (F-05) was compound heterozygous for E55K/R119X [77] and one patient was homozygous for C247R [87]. All other patients had other frame shift or nonsense mutations predicted to be null alleles. The E55K heterozygote had IRD and the allele was shown to be temperature sensitive [77]. In contrast, the C247R patient had a severe phenotype (ZS). This missense change abrogates the second cysteine residue of the zinc-binding domain. In contrast, two patients who retain both transmembrane domains of *PEX2*, but are predicted to have no zinc-binding domain (Q214fs and W233X) are more mildly affected (IRD). Thus, it can be hypothesized that C247R acts in a dominant negative fashion that may perturb the interactions of other PEX proteins [87].

4.1.6. PEX10

There are two isoforms due to alternative splicing of the acceptor site of intron 3 and the shorter isoform predominates [90]. Mutations have been reported in the literature for 19 patients [66,67,90,91,92,93]. Thirteen patients were homozygous for Nt815–815delCT, which is the most common cause of PBD-ZSS in the Japanese population and has been associated with a founder haplotype [92]. Only two missense mutations have been reported and both were in conjunction with a null allele. Both of these patients had NALD. Otherwise, all of the *PEX10* patients had ZS.

4.1.7. PEX12

Mutation analysis on about 31 patients has been reported in the literature [44,66,67,94,95,96,97]. S320F (Nt959C>T) was the most common mutation reported, with 10 individuals purported to be homozygous. This mutation is associated with type 2 mosaicism (see Section 3.2). In general there is a reasonably good correlation between genotype and phenotype. All of the patients with at least one missense allele had NALD/IRD or milder. All patients with two null alleles had ZS or NALD, with the exception of one patient. PBD099 was an IRD patient with the mutations IVS+1G>T/Nt26–27delCA [95,98]. Further study indicated that an internal methionine (M94) had the Kozak consensus sequence needed for translation initiation and in vitro studies showed that the Nt26–27del CA allele yielded a protein consistent with a shorter protein starting at M94. Thus, the single anomaly between genotype and phenotype could be explained by this molecular mechanism.

4.2. PEX genes encoding the matrix protein receptors

4.2.1. PEX5

Two NALD patients (PBD018 and PBD093) and one ZS patient (PBD005) have been reported to have *PEX5* defects [40,99]. The two NALD patients are both homozygous for N489K (Nt1467T>G) and though unrelated, both are of Middle Eastern ancestry[1]. The ZS patient is homozygous for R390X (Nt1168C>T). The NALD patients are unable to import PTS1 proteins, but have nearly normal PTS2 import. In contrast, the ZS patient has no PTS1 or PTS2 import. *PEX5* is expressed in two forms (PEX5S) and (PEX5L), which are generated by alternative splicing [100]. Both isoforms recognize PTS1 proteins using their C-terminal TPR (tetratricopeptide) domain. N489K disturbs the binding to PTS1 [101]. Only the long isoform interacts with the PTS2 receptor encoded by *PEX7* and mediates the translocation of PTS2 cargo to the peroxisome membrane docking site [102]. The binding site is partly encoded by the alternatively spliced exon in the N-terminal half of the protein [103]. Thus, N489K must not disrupt the role PEX5L plays in PTS2 protein import while R390X results in complete absence of the PEX5 proteins and thus PTS1 and PTS2 import.

4.2.2. PEX7

Two groups have described *PEX7* mutations in a large number of RCDP patients. Motley et al. and Braverman et al. studied 73 and 60 unrelated patients, respectively [19,104]. L292X (Nt875T>A) is the most common allele, with a frequency of 50–52% in the two groups. Braverman et al. had previously reported evidence that L292X is a founder allele that arose in the Caucasian population [105]. Other *PEX7* alleles with a frequency>5% in at least one of the studies included A218V (Nt653C>T), IVS9+1G>C, G217R (Nt649G>A) and Nt370–396del27bp. A second allele was not found in 6% of patients, most of whom were heterozygous for L292X, thus yielding a test sensitivity of 94% for sequence analysis of the coding region. Most of the common alleles are located in exons 7 and 9. When PEX7 is superimposed on the crystal structure of another WD40 protein, B transducin, it is apparent that most of the mutations that cause classical RCDP are located in residues contributing to the rigid structure of this protein. In contrast, missense alleles located in more flexible regions are associated with the milder phenotypes, as well as 'leaky' mutations that can result in a small amount of normal transcript [19,20,104].

4.3. PEX genes encoding membrane proteins comprising the docking site

4.3.1. PEX13

Two patients with *PEX13* mutations have been reported in the literature. Cell lines H-01 (or PBD222) and H-02 are derived from patients with NALD and ZS phenotypes, respectively [106,107]. H-01 is homozygous for I326T and demonstrated

[1] Recently we identified one other patient (PBD 668) of similar ethnicity who is also apparently homozygous for N489K.

temperature sensitivity at 30 °C. H-02 is homozygous for W324X. Both mutations are located in the SH3 domain.

4.3.2. PEX14

A mutation in *PEX14* is the newest gene defect associated with human PBD [108]. The one patient identified (K-01) had ZS and was homozygous for Q185X (Nt553C>T). Peroxisome membrane structures were present, but PTS1 and PTS2 import were both absent.

4.4. PEX genes encoding proteins required for peroxisome membrane synthesis

4.4.1. PEX3

Two patients with ZS (PBD G-01 and PBD G-02) have been reported to have *PEX3* defects [109,110]. Cell lines from both patients lacked detectable peroxisome membrane remnants. Both patients were apparent homozygotes for null mutations, IVS10-8T>G which causes exon 11 skipping, and Nt543insT. Muntau et al. showed that cell fusion of either *PEX3* deficient cell line with the *PEX19* deficient cell line PBDJ-01, also lacking PMemP remnants, restored peroxisome biogenesis.

4.4.2. PEX16

A *PEX16* defect was described in one ZS patient, PBD061, who had the homozygous mutation R176X (Nt526C>T) [111,112]. Cells from this patient had no evidence of any peroxisome membrane structures, indicating that *PEX16*, like *PEX3* and *PEX19,* is indispensable for membrane biogenesis. Restoration of peroxisome biogenesis by *PEX16* cDNA complementation provides some evidence that preexisting peroxisomes are not required for organelle formation. Shimozawa et al. identified two further unrelated patients that were homozygous for exon 10 skipping caused by a splice site mutation (IVS10+2T>C) [113]. This mutation changes the amino acid sequence starting from codon 298 and introduces a termination codon at codon 336. Both patients displayed the classical ZS phenotype.

4.4.3. PEX19

One ZS patient, PBDJ-01, has been reported to be *PEX19* deficient and is homozygous for a frame shift mutation (Nt764insA) that introduces 24 novel amino acids toward the C-terminus of the protein [114]. The protein encoded by *PEX19* is partly farnesylated and the farnesylation consensus sequence CLIM is in the carboxy portion of PEX19. However, a natural splice variant that lacks this consensus sequence is able to restore peroxisome assembly and function in PBDJ-01, so farnesylation must not be essential to its role in peroxisome biogenesis [115].

5. Prenatal Testing

Due to the severe nature and inability to treat these disorders, many couples with an affected child seek prenatal counseling for future pregnancies. Prenatal diagnosis is possible by biochemical or molecular analysis. Most biochemical analyses that have been verified in cultured fibroblasts from the index case can be used for prenatal testing with cultured cells derived from chorionic villi or amniotic fluid cells. If the molecular defect has been identified, then DNA can be isolated from uncultured or cultured cells from chorionic villus samples (CVS) or amniotic fluid for targeted DNA analysis. Due to the risk of maternal cell contamination (MCC), especially when using CVS, it is essential to perform DNA testing to rule out this possibility [116]. When performing biochemical analysis, testing to rule out MCC is only important if the results are normal. Cells from obligate carriers for ZSS and RCDP disorders do not express partial defects, thus there is no biological basis for MCC causing a false positive result. Analysis of peroxisomal β-oxidation and plasmalogen synthesis are the two pathways most commonly assessed for prenatal testing [117–119]. Once the specific gene defect has been identified, it is also possible to offer a couple the option of preimplantation genetic diagnosis (PGD) [120]. Although CVS or amniocentesis is recommended to confirm that only unaffected embryos were implanted, PGD significantly improves the chance of a normal pregnancy. Recently fetal magnetic resonance imaging in the third trimester was shown to be able to confirm defects consistent with ZSS disorders, including abnormal cortical gyral patterns and renal cysts [121]. The skeletal abnormalities of RCDP have been noted on ultrasound as early as 18–19 weeks gestation [122].

6. Therapy

The multiple biochemical abnormalities that result from the failure of peroxisome assembly and their importance in embryogenesis lead to significant developmental abnormalities present at birth and further progression postnatally. Current treatment is supportive and focuses on treating seizures and liver dysfunction, providing hearing aids, ophthalmologic interventions, and meeting other developmental needs. However, the recognition of a larger number of PBD patients with milder phenotypes who are living longer, has prompted renewed interest in experimental therapies.

Thus far therapeutic interventions have targeted individual biochemical defects and the effects have not been studied in a systematic fashion. A diet low in phytanic acid has been successful in the treatment of ARD. Thus, its use in milder individuals with PBD has been proposed but has not been demonstrated to result in measurable clinical improvement. Similarly, oral DHA therapy can normalize blood DHA levels [123], but its affect on clinical outcome has not yet been proven [124]. Oral bile acid administration improved hepatobiliary function in several infants with ZS [125,126]. A few RCDP patients were supplemented with oral plasmalogen precursors, but this did not improve clinical outcome [127]. Liver transplantation has been reported in one patient with IRD [128], but it is too early to determine the benefit. Furthermore, potential therapies have been proposed that would improve peroxisome assembly based upon their effect in cell culture models. For example, Wei et al. have demonstrated that peroxisome proliferation in the presence of 4-phenylbutyrate

could improve β-oxidation in cultured fibroblasts from ZSS patients [129,130]. Mouse models have been developed for *PEX2, PEX5, PEX7* and *PEX13* deficiency and are discussed in more detail in another section of this journal. These models are useful for studying the underlying pathophysiology, for investigating existing therapies and for developing new approaches to treatment.

References

[1] S. Goldfischer, C.L. Moore, A.B. Johnson, A.J. Spiro, M.P. Valsamis, H.K. Wisniewski, et al., Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome, Science 182 (107) (1973) 62–64.

[2] N. Shimozawa, T. Tsukamoto, Y. Suzuki, T. Orii, Y. Shirayoshi, T. Mori, et al., A human gene responsible for Zellweger syndrome that affects peroxisome assembly, Science 255 (5048) (1992) 1132–1134.

[3] A.E. Moser, I. Singh, F.R. Brown III, G.I. Solish, R.I. Kelley, P.J. Benke, et al., The cerebrohepatorenal (Zellweger) syndrome. Increased levels and impaired degradation of very-long-chain fatty acids and their use in prenatal diagnosis, N. Engl. J. Med. 310 (18) (1984) 1141–1146.

[4] H.S.A. Heymans, Cerebro-hepato-renal (Zellweger) syndrome, Clinical and Biochemical Consequences of Peroxiosmal Dysfunction, University of Amsterdam, 1984.

[5] G.N. Wilson, R.G. Holmes, J. Custer, J.L. Lipkowitz, J. Stover, N. Datta, et al., Zellweger syndrome: diagnostic assays, syndrome delineation, and potential therapy, Am. J. Med. Genet. 24 (1) (1986) 69–82.

[6] P. Evrard, V.S. Caviness Jr., J. Prats-Vinas, G. Lyon, The mechanism of arrest of neuronal migration in the Zellweger malformation: a hypothesis bases upon cytoarchitectonic analysis, Acta Neuropathol. (Berl.) 41 (2) (1978) 109–117.

[7] J.J. Volpe, R.D. Adams, Cerebro-hepato-renal syndrome of Zellweger: an inherited disorder of neuronal migration, Acta Neuropathol. (Berl.) 20 (3) (1972) 175–198.

[8] S. Goldfischer, J.M. Powers, A.B. Johnson, S. Axe, F.R. Brown, H.W. Moser, Striated adrenocortical cells in cerebro-hepato-renal (Zellweger) syndrome, Virchows Arch., A Pathol. Anat. Histopathol. 401 (3) (1983) 355–361.

[9] P.D. Gatfield, E. Taller, G.G. Hinton, A.C. Wallace, G.M. Abdelnour, M.D. Haust, Hyperpipecolatemia: a new metabolic disorder associated with neuropathy and hepatomegaly: a case study, Can. Med. Assoc. J. 99 (25) (1968) 1215–1233.

[10] J.M. Scotto, M. Hadchouel, M. Odievre, M.H. Laudat, J.M. Saudubray, O. Dulac, et al., Infantile phytanic acid storage disease, a possible variant of Refsum's disease: three cases, including ultrastructural studies of the liver, J. Inherit Metab. Dis. 5 (2) (1982) 83–90.

[11] J. Ulrich, N. Herschkowitz, P. Heitz, T. Sigrist, P. Baerlocher, Adrenoleukodystrophy. Preliminary report of a connatal case. Light- and electron microscopical, immunohistochemical and biochemical findings, Acta Neuropathol. (Berl.) 43 (1–2) (1978) 77–83.

[12] J. Ek, B.F. Kase, A. Reith, I. Bjorkhem, J.I. Pedersen, Peroxisomal dysfunction in a boy with neurologic symptoms and amaurosis (Leber disease): clinical and biochemical findings similar to those observed in Zellweger syndrome, J. Pediatr. 108 (1) (1986) 19–24.

[13] H.M. Michelakakis, D.I. Zafeiriou, M.S. Moraitou, J. Gootjes, R.J. Wanders, PEX1 deficiency presenting as Leber congenital amaurosis, Pediatr. Neurol. 31 (2) (2004) 146–149.

[14] M.R. Baumgartner, N.M. Verhoeven, C. Jakobs, F. Roels, M. Espeel, M. Martinez, et al., Defective peroxisome biogenesis with a neuromuscular disorder resembling Werdnig–Hoffmann disease, Neurology 51 (5) (1998) 1427–1432.

[15] A.B. Moser, M. Rasmussen, S. Naidu, P.A. Watkins, M. McGuinness, A.K. Hajra, et al., Phenotype of patients with peroxisomal disorders subdivided into sixteen complementation groups, J. Pediatr. 127 (1) (1995) 13–22.

[16] A.M. Bams-Mengerink, C.B. Majoie, M. Duran, R.J. Wanders, J. Van Hove, C.D. Scheurer, et al., MRI of the brain and cervical spinal cord in rhizomelic chondrodysplasia punctata, Neurology 66 (6) (2006) 798–803 discussion 789.

[17] A.J. Khanna, N.E. Braverman, D. Valle, P.D. Sponseller, Cervical stenosis secondary to rhizomelic chondrodysplasia punctata, Am. J. Med. Genet. 99 (1) (2001) 63–66.

[18] A.L. White, P. Modaff, F. Holland-Morris, R.M. Pauli, Natural history of rhizomelic chondrodysplasia punctata, Am. J. Med. Genet., A 118 (4) (2003) 332–342.

[19] N. Braverman, L. Chen, P. Lin, C. Obie, G. Steel, P. Douglas, et al., Mutation analysis of PEX7 in 60 probands with rhizomelic chondrodysplasia punctata and functional correlations of genotype with phenotype, Hum. Mutat. 20 (4) (2002) 284–297.

[20] D.M. van den Brink, P. Brites, J. Haasjes, A.S. Wierzbicki, J. Mitchell, M. Lambert-Hamill, et al., Identification of PEX7 as the second gene involved in Refsum disease, Am. J. Hum. Genet. 72 (2) (2003) 471–477.

[21] C. Theda, R.C. Woody, S. Naidu, A.B. Moser, H.W. Moser, Increased very long chain fatty acids in patients on a ketogenic diet: a cause of diagnostic confusion, J. Pediatr. 122 (5 Pt. 1) (1993) 724–726.

[22] D.M. Danks, P. Tippett, C. Adams, P. Campbell, Cerebro-hepato-renal syndrome of Zellweger. A report of eight cases with comments upon the incidence, the liver lesion, and a fault in pipecolic acid metabolism, J. Pediatr. 86 (3) (1975) 382–387.

[23] D.W. Johnson, H.J. ten Brink, R.C. Schuit, C. Jakobs, Rapid and quantitative analysis of unconjugated C(27) bile acids in plasma and blood samples by tandem mass spectrometry, J. Lipid Res. 42 (1) (2001) 9–16.

[24] I. Bjorkhem, L. Sisfontes, B. Bostrom, B.F. Kase, R. Blomstrand, Simple diagnosis of the Zellweger syndrome by gas–liquid chromatography of dimethylacetals, J. Lipid Res. 27 (7) (1986) 786–791.

[25] L. Hagenfeldt, L. Blomquist, T. Midtvedt, Epoxydicarboxylic aciduria resulting from the ingestion of castor oil, Clin. Chim. Acta 161 (2) (1986) 157–163.

[26] O. Stokke, E. Jellum, E.A. Kvittingen, O. Skjeldal, G. Hvistendahl, Epoxy acids in peroxisomal disorders, Scand. J. Clin. Lab. Invest. 46 (1) (1986) 95–96.

[27] C.S. van Woerden, J.W. Groothoff, F.A. Wijburg, M. Duran, R.J. Wanders, P.G. Barth, et al., High incidence of hyperoxaluria in generalized peroxisomal disorders, Mol. Genet. Metab. (2006).

[28] A.B. Moser, D.S. Jones, G.V. Raymond, H.W. Moser, Plasma and red blood cell fatty acids in peroxisomal disorders, Neurochem. Res. 24 (2) (1999) 187–197.

[29] S.A. Moore, E. Hurt, E. Yoder, H. Sprecher, A.A. Spector, Docosahexaenoic acid synthesis in human skin fibroblasts involves peroxisomal retroconversion of tetracosahexaenoic acid, J. Lipid Res. 36 (11) (1995) 2433–2443.

[30] R.J. Wanders, C.W. van Roermund, W.F. Visser, S. Ferdinandusse, G.A. Jansen, D.M. van den Brink, et al., Peroxisomal fatty acid alpha-and beta-oxidation in health and disease: new insights, Adv. Exp. Med. Biol. 544 (2003) 293–302.

[31] A. Roscher, B. Molzer, H. Bernheimer, S. Stockler, I. Mutz, F. Paltauf, The cerebrohepatorenal (Zellweger) syndrome: an improved method for the biochemical diagnosis and its potential value for prenatal detection, Pediatr. Res. 19 (9) (1985) 930–933.

[32] G. Schrakamp, C.F. Roosenboom, R.B. Schutgens, R.J. Wanders, H.S. Heymans, J.M. Tager, et al., Alkyl dihydroxyacetone phosphate synthase in human fibroblasts and its deficiency in Zellweger syndrome, J. Lipid Res. 26 (7) (1985) 867–873.

[33] R.B. Schutgens, G.J. Romeyn, R.J. Wanders, H. van den Bosch, G. Schrakamp, H.S. Heymans, Deficiency of acyl-CoA: dihydroxyacetone phosphate acyltransferase in patients with Zellweger (cerebro-hepato-renal) syndrome, Biochem. Biophys. Res. Commun. 120 (1) (1984) 179–184.

[34] J. Zenger-Hain, D.A. Craft, W.B. Rizzo, in: P.M. Coates, K. Tanaka (Eds.), New Developments in Fatty Acid Oxidation, Wiley-Liss, New York, 1992, pp. 399–407.

[35] B.C. Paton, P.C. Sharp, D.I. Crane, A. Poulos, Oxidation of pristanic acid in fibroblasts and its application to the diagnosis of peroxisomal beta-oxidation defects, J. Clin. Invest. 97 (3) (1996) 681–688.

[36] R.J. Wanders, M. Kos, B. Roest, A.J. Meijer, G. Schrakamp, H.S. Heymans, et al., Activity of peroxisomal enzymes and intracellular distribution of catalase in Zellweger syndrome, Biochem. Biophys. Res. Commun. 123 (3) (1984) 1054–1061.
[37] J. Gootjes, P.A. Mooijer, C. Dekker, P.G. Barth, B.T. Poll-The, H.R. Waterham, et al., Biochemical markers predicting survival in peroxisome biogenesis disorders, Neurology 59 (11) (2002) 1746–1749.
[38] M.J. Santos, S.C. Henderson, A.B. Moser, H.W. Moser, P.B. Lazarow, Peroxisomal ghosts are intracellular structures distinct from lysosomal compartments in Zellweger syndrome: a confocal laser scanning microscopy study, Biol. Cell 92 (2) (2000) 85–94.
[39] P.E. Purdue, P.B. Lazarow, Targeting of human catalase to peroxisomes is dependent upon a novel COOH-terminal peroxisomal targeting sequence, J. Cell Biol. 134 (4) (1996) 849–862.
[40] M.L. Slawecki, G. Dodt, S. Steinberg, A.B. Moser, H.W. Moser, S.J. Gould, Identification of three distinct peroxisomal protein import defects in patients with peroxisome biogenesis disorders, J. Cell. Sci. 108 (Pt. 5) (1995) 1817–1829.
[41] M. Pineda, M. Giros, F. Roels, M. Espeel, M. Ruiz, A. Moser, et al., Diagnosis and follow-up of a case of peroxisomal disorder with peroxisomal mosaicism, J. Child Neurol. 14 (7) (1999) 434–439.
[42] H. Mandel, M. Espeel, F. Roels, N. Sofer, A. Luder, T.C. Iancu, et al., A new type of peroxisomal disorder with variable expression in liver and fibroblasts, J. Pediatr. 125 (4) (1994) 549–555.
[43] N. Shimozawa, A. Imamura, Z. Zhang, Y. Suzuki, T. Orii, T. Tsukamoto, et al., Defective PEX gene products correlate with the protein import, biochemical abnormalities, and phenotypic heterogeneity in peroxisome biogenesis disorders, J. Med. Genet. 36 (10) (1999) 779–781.
[44] J. Gootjes, F. Schmohl, P.A. Mooijer, C. Dekker, H. Mandel, M. Topcu, et al., Identification of the molecular defect in patients with peroxisomal mosaicism using a novel method involving culturing of cells at 40 degrees C: implications for other inborn errors of metabolism, Hum. Mutat. 24 (2) (2004) 130–139.
[45] P.B. Lazarow, H.W. Moser, Disorders of peroxisome biogenesis, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw Hill, New York, 1995, pp. 2287–2324.
[46] J.M. Powers, R.C. Tummons, V.S. Caviness Jr., A.B. Moser, H.W. Moser, Structural and chemical alterations in the cerebral maldevelopment of fetal cerebro-hepato-renal (Zellweger) syndrome, J. Neuropathol. Exp. Neurol. 48 (3) (1989) 270–289.
[47] A. Poulos, P. Sharp, D. Johnson, C. Easton, The occurrence of polyenoic very long chain fatty acids with greater than 32 carbon atoms in molecular species of phosphatidylcholine in normal and peroxisome-deficient (Zellweger's syndrome) brain, Biochem. J. 253 (3) (1988) 645–650.
[48] K. Tanaka, K. Nishizawa, H. Yamamoto, T. Naruto, E. Izeki, T. Taga, et al., Analysis of very long-chain fatty acids and plasmalogen in the erythrocyte membrane: a simple method for the detection of peroxisomal disorders and discrimination between adrenoleukodystrophy and Zellweger syndrome, Neuropediatrics 21 (3) (1990) 119–123.
[49] B.S. Robinson, D.W. Johnson, A. Poulos, Unique molecular species of phosphatidylcholine containing very-long-chain (C24–C38) polyenoic fatty acids in rat brain, Biochem. J. 265 (3) (1990) 763–767.
[50] W. Hubbard, A. Moser, S. Tortorelli, A. Liu, D. Jones, H. Moser, Combined liquid chromatography-tandem mass spectrometry as an analytical method for high throughput screening for X-linked adrenoleukodystrophy and other peroxisomal disorders: preliminary findings, Molec. Genet. Metab. 89 (1–2) (2006) 185–187.
[51] A.S. Wierzbicki, M.D. Lloyd, C.J. Schofield, M.D. Feher, F.B. Gibberd, Refsum's disease: a peroxisomal disorder affecting phytanic acid alpha-oxidation, J. Neurochem. 80 (5) (2002) 727–735.
[52] A.S. Wierzbicki, Clinical significance of oxidation from phytol to phytanic acid in man, Mol. Genet. Metab. 83 (4) (2004) 347.
[53] G.R. Plant, D.M. Hansell, F.B. Gibberd, M.C. Sidey, Skeletal abnormalities in Refsum's disease (heredopathia atactica polyneuritiformis), Br. J. Radiol. 63 (751) (1990) 537–541.
[54] J. Biermann, J. Gootjes, R.J. Wanders, H. van den Bosch, Stability of alkyl-dihydroxyacetonephosphate synthase in human control and peroxisomal disorder fibroblasts, IUBMB Life 48 (6) (1999) 635–639.
[55] E.C. de Vet, L. Ijlst, W. Oostheim, C. Dekker, H.W. Moser, H. van Den Bosch, et al., Ether lipid biosynthesis: alkyl-dihydroxyacetonephosphate synthase protein deficiency leads to reduced dihydroxyacetonephosphate acyltransferase activities, J. Lipid Res. 40 (11) (1999) 1998–2003.
[56] H.S. Heymans, R.B. Schutgens, R. Tan, H. van den Bosch, P. Borst, Severe plasmalogen deficiency in tissues of infants without peroxisomes (Zellweger syndrome), Nature 306 (5938) (1983) 69–70.
[57] T. Brosche, D. Platt, The biological significance of plasmalogens in defense against oxidative damage, Exp. Gerontol. 33 (5) (1998) 363–369.
[58] A.A. Farooqui, L.A. Horrocks, Plasmalogens: workhorse lipids of membranes in normal and injured neurons and glia, Neuroscientist 7 (3) (2001) 232–245.
[59] J.C. Baas, R. van de Laar, L. Dorland, M. Duran, R. Berger, B.T. Poll-The, et al., Plasma pipecolic acid is frequently elevated in non-peroxisomal disease, J. Inherit Metab. Dis. 25 (8) (2002) 699–701.
[60] C. Vallat, S. Denis, H. Bellet, C. Jakobs, R.J. Wanders, H. Mion, Major hyperpipecolataemia in a normal adult, J. Inherit Metab. Dis. 19 (5) (1996) 624–626.
[61] P.B. Mills, E. Struys, C. Jakobs, B. Plecko, P. Baxter, M. Baumgartner, et al., Mutations in antiquitin in individuals with pyridoxine-dependent seizures, Nat. Med. 12 (3) (2006) 307–309.
[62] G. Hoefler, S. Hoefler, P.A. Watkins, W.W. Chen, A. Moser, V. Baldwin, et al., Biochemical abnormalities in rhizomelic chondrodysplasia punctata, J. Pediatr. 112 (5) (1988) 726–733.
[63] D. De Craemer, M.J. Zweens, S. Lyonnet, R.J. Wanders, B.T. Poll-The, R.B. Schutgens, et al., Very large peroxisomes in distinct peroxisomal disorders (rhizomelic chondrodysplasia punctata and acyl-CoA oxidase deficiency): novel data, Virchows Arch., A Pathol. Anat. Histopathol. 419 (6) (1991) 523–525.
[64] J.L. Hughes, A. Poulos, D.I. Crane, C.W. Chow, L.J. Sheffield, D. Sillence, Ultrastructure and immunocytochemistry of hepatic peroxisomes in rhizomelic chondrodysplasia punctata, Eur. J. Pediatr. 151 (11) (1992) 829–836.
[65] W.B. Rizzo, D.A. Craft, L.L. Judd, H.W. Moser, A.B. Moser, Fatty alcohol accumulation in the autosomal recessive form of rhizomelic chondrodysplasia punctata, Biochem. Med. Metabol. Biol. 50 (1) (1993) 93–102.
[66] J. Gootjes, F. Skovby, E. Christensen, R.J. Wanders, S. Ferdinandusse, Reinvestigation of trihydroxycholestanoic acidemia reveals a peroxisome biogenesis disorder, Neurology 62 (11) (2004) 2077–2081.
[67] S. Steinberg, L. Chen, L. Wei, A. Moser, H. Moser, G. Cutting, et al., The PEX Gene Screen: molecular diagnosis of peroxisome biogenesis disorders in the Zellweger syndrome spectrum, Mol. Genet. Metab. 83 (3) (2004) 252–263.
[68] B.E. Reuber, E. Germain-Lee, C.S. Collins, J.C. Morrell, R. Ameritunga, H.W. Moser, et al., Mutations in PEX1 are the most common cause of peroxisome biogenesis disorders, Nat. Genet. 17 (4) (1997) 445–448.
[69] C.S. Collins, S.J. Gould, Identification of a common PEX1 mutation in Zellweger syndrome, Hum. Mutat. 14 (1) (1999) 45–53.
[70] C. Walter, J. Gootjes, P.A. Mooijer, H. Portsteffen, C. Klein, H.R. Waterham, et al., Disorders of peroxisome biogenesis due to mutations in PEX1: phenotypes and PEX1 protein levels, Am. J. Hum. Genet. 69 (1) (2001) 35–48.
[71] M.A. Maxwell, T. Allen, P.B. Solly, T. Svingen, B.C. Paton, D.I. Crane, Novel PEX1 mutations and genotype–phenotype correlations in Australasian peroxisome biogenesis disorder patients, Hum. Mutat. 20 (5) (2002) 342–351.
[72] M.A. Maxwell, P.V. Nelson, S.J. Chin, B.C. Paton, W.F. Carey, D.I. Crane, A common PEX1 frameshift mutation in patients with disorders of peroxisome biogenesis correlates with the severe Zellweger syndrome phenotype, Hum. Genet. 105 (1–2) (1999) 38–44.
[73] M.A. Maxwell, P.B. Leane, B.C. Paton, D.I. Crane, Novel PEX1 coding mutations and 5' UTR regulatory polymorphisms, Hum. Mutat. 26 (3) (2005) 279.

[74] H. Rosewich, A. Ohlenbusch, J. Gärtner, Genetic and clinical aspects of Zellweger spectrum patients with PEX1 mutations, J. Med. Genet. 42 (9) (2005) e58.
[75] D.I. Crane, M.A. Maxwell, B.C. Paton, PEX1 mutations in the Zellweger spectrum of the peroxisome biogenesis disorders, Hum. Mutat. 26 (3) (2005) 167–175.
[76] S. Tamura, N. Matsumoto, A. Imamura, N. Shimozawa, Y. Suzuki, N. Kondo, et al., Phenotype–genotype relationships in peroxisome biogenesis disorders of PEX1-defective complementation group 1 are defined by Pex1p–Pex6p interaction, Biochem. J. 357 (Pt. 2) (2001) 417–426.
[77] A. Imamura, T. Tsukamoto, N. Shimozawa, Y. Suzuki, Z. Zhang, T. Imanaka, et al., Temperature-sensitive phenotypes of peroxisome-assembly processes represent the milder forms of human peroxisome-biogenesis disorders, Am. J. Hum. Genet. 62 (6) (1998) 1539–1543.
[78] B.T. Poll-The, J. Gootjes, M. Duran, J.B. De Klerk, L.J. Maillette De Buy Wenniger-Prick, R.J. Admiraal, et al., Peroxisome biogenesis disorders with prolonged survival: Phenotypic expression in a cohort of 31 patients, Am. J. Med. Genet. 126A (4) (2004) 333–338.
[79] Z. Zhang, Y. Suzuki, N. Shimozawa, S. Fukuda, A. Imamura, T. Tsukamoto, et al., Genomic structure and identification of 11 novel mutations of the PEX6 (peroxisome assembly factor-2) gene in patients with peroxisome biogenesis disorders, Hum. Mutat. 13 (6) (1999) 487–496.
[80] A. Raas-Rothschild, R.J. Wanders, P.A. Mooijer, J. Gootjes, H.R. Waterham, A. Gutman, et al., A PEX6-defective peroxisomal biogenesis disorder with severe phenotype in an infant, versus mild phenotype resembling Usher syndrome in the affected parents, Am. J. Hum. Genet. 70 (4) (2002) 1062–1068.
[81] S. Fukuda, N. Shimozawa, Y. Suzuki, Z. Zhang, S. Tomatsu, T. Tsukamoto, et al., Human peroxisome assembly factor-2 (PAF-2): a gene responsible for group C peroxisome biogenesis disorder in humans, Am. J. Hum. Genet. 59 (6) (1996) 1210–1220.
[82] A. Imamura, N. Shimozawa, Y. Suzuki, Z. Zhang, T. Tsukamoto, Y. Fujiki, et al., Temperature-sensitive mutation of PEX6 in peroxisome biogenesis disorders in complementation group C (CG-C): comparative study of PEX6 and PEX1, Pediatr. Res. 48 (4) (2000) 541–545.
[83] N. Matsumoto, S. Tamura, Y. Fujiki, The pathogenic peroxin Pex26p recruits the Pex1p–Pex6p AAA ATPase complexes to peroxisomes, Nat. Cell Biol. 5 (5) (2003) 454–460.
[84] S. Weller, I. Cajigas, J. Morrell, C. Obie, G. Steel, S.J. Gould, et al., Alternative splicing suggests extended function of PEX26 in peroxisome biogenesis, Am. J. Hum. Genet. 76 (6) (2005) 987–1007.
[85] N. Matsumoto, S. Tamura, S. Furuki, N. Miyata, A. Moser, N. Shimozawa, et al., Mutations in novel peroxin gene PEX26 that cause peroxisome-biogenesis disorders of complementation group 8 provide a genotype–phenotype correlation, Am. J. Hum. Genet. 73 (2) (2003) 233–246.
[86] S. Furuki, S. Tamura, N. Matsumoto, N. Miyata, A. Moser, H.W. Moser, et al., Mutations in the peroxin Pex26p responsible for peroxisome biogenesis disorders of complementation group 8 impair its stability, peroxisomal localization, and interaction with the Pex1p x Pex6p complex, J. Biol. Chem. 281 (3) (2006) 1317–1323.
[87] J. Gootjes, O. Elpeleg, F. Eyskens, H. Mandel, D. Mitanchez, N. Shimozawa, et al., Novel mutations in the PEX2 gene of four unrelated patients with a peroxisome biogenesis disorder, Pediatr. Res. 55 (3) (2004) 431–436.
[88] N. Shimozawa, Y. Suzuki, S. Tomatsu, H. Nakamura, T. Kono, H. Takada, et al., A novel mutation, R125X in peroxisome assembly factor-1 responsible for Zellweger syndrome, Hum. Mutat. (Suppl. 1) (1998) S134–S136.
[89] N. Shimozawa, Z. Zhang, A. Imamura, Y. Suzuki, Y. Fujiki, T. Tsukamoto, et al., Molecular mechanism of detectable catalase-containing particles, peroxisomes, in fibroblasts from a PEX2-defective patient, Biochem. Biophys. Res. Commun. 268 (1) (2000) 31–35.
[90] D.S. Warren, B.D. Wolfe, S.J. Gould, Phenotype–genotype relationships in PEX10-deficient peroxisome biogenesis disorder patients, Hum. Mutat. 15 (6) (2000) 509–521.
[91] D.S. Warren, J.C. Morrell, H.W. Moser, D. Valle, S.J. Gould, Identification of PEX10, the gene defective in complementation group 7 of the peroxisome-biogenesis disorders, Am. J. Hum. Genet. 63 (2) (1998) 347–359.
[92] N. Shimozawa, T. Nagase, Y. Takemoto, T. Ohura, Y. Suzuki, N. Kondo, Genetic heterogeneity of peroxisome biogenesis disorders among Japanese patients: evidence for a founder haplotype for the most common PEX10 gene mutation, Am. J. Med. Genet. 120A (1) (2003) 40–43.
[93] K. Okumoto, R. Itoh, N. Shimozawa, Y. Suzuki, S. Tamura, N. Kondo, et al., Mutations in PEX10 is the cause of Zellweger peroxisome deficiency syndrome of complementation group B, Hum. Mol. Genet. 7 (9) (1998) 1399–1405.
[94] J. Gootjes, F. Schmohl, H.R. Waterham, R.J. Wanders, Novel mutations in the PEX12 gene of patients with a peroxisome biogenesis disorder, Eur. J. Hum. Genet. 12 (2) (2004) 115–120.
[95] C.C. Chang, W.H. Lee, H. Moser, D. Valle, S.J. Gould, Isolation of the human PEX12 gene, mutated in group 3 of the peroxisome biogenesis disorders, Nat. Genet. 15 (4) (1997) 385–388.
[96] C.C. Chang, D.S. Warren, K.A. Sacksteder, S.J. Gould, PEX12 interacts with PEX5 and PEX10 and acts downstream of receptor docking in peroxisomal matrix protein import, J. Cell Biol. 147 (4) (1999) 761–774.
[97] K. Okumoto, N. Shimozawa, A. Kawai, S. Tamura, T. Tsukamoto, T. Osumi, et al., PEX12, the pathogenic gene of group III Zellweger syndrome: cDNA cloning by functional complementation on a CHO cell mutant, patient analysis, and characterization of PEX12p, Mol. Cell. Biol. 18 (7) (1998) 4324–4336.
[98] C.C. Chang, S.J. Gould, Phenotype–genotype relationships in complementation group 3 of the peroxisome-biogenesis disorders, Am. J. Hum. Genet. 63 (5) (1998) 1294–1306.
[99] G. Dodt, N. Braverman, C. Wong, A. Moser, H.W. Moser, P. Watkins, et al., Mutations in the PTS1 receptor gene, PXR1, define complementation group 2 of the peroxisome biogenesis disorders, Nat. Genet. 9 (2) (1995) 115–125.
[100] N. Braverman, G. Dodt, S.J. Gould, D. Valle, An isoform of pex5p, the human PTS1 receptor, is required for the import of PTS2 proteins into peroxisomes, Hum. Mol. Genet. 7 (8) (1998) 1195–1205.
[101] G.J. Gatto Jr., B.V. Geisbrecht, S.J. Gould, J.M. Berg, Peroxisomal targeting signal-1 recognition by the TPR domains of human PEX5, Nat. Struct. Biol. 7 (12) (2000) 1091–1095.
[102] H. Otera, T. Harano, M. Honsho, K. Ghaedi, S. Mukai, A. Tanaka, et al., The mammalian peroxin Pex5pL, the longer isoform of the mobile peroxisome targeting signal (PTS) type 1 transporter, translocates the Pex7p.PTS2 protein complex into peroxisomes via its initial docking site, Pex14p, J. Biol. Chem. 275 (28) (2000) 21703–21714.
[103] G. Dodt, D. Warren, E. Becker, P. Rehling, S.J. Gould, Domain mapping of human PEX5 reveals functional and structural similarities to Saccharomyces cerevisiae Pex18p and Pex21p, J. Biol. Chem. 276 (45) (2001) 41769–41781.
[104] A.M. Motley, P. Brites, L. Gerez, E. Hogenhout, J. Haasjes, R. Benne, et al., Mutational spectrum in the PEX7 gene and functional analysis of mutant alleles in 78 patients with rhizomelic chondrodysplasia punctata type 1, Am. J. Hum. Genet. 70 (3) (2002) 612–624.
[105] N. Braverman, G. Steel, P. Lin, A. Moser, H. Moser, D. Valle, PEX7 gene structure, alternative transcripts, and evidence for a founder haplotype for the frequent RCDP allele, L292ter, Genomics 63 (2) (2000) 181–192.
[106] N. Shimozawa, Y. Suzuki, Z. Zhang, A. Imamura, R. Toyama, S. Mukai, et al., Nonsense and temperature-sensitive mutations in PEX13 are the cause of complementation group H of peroxisome biogenesis disorders, Hum. Mol. Genet. 8 (6) (1999) 1077–1083.
[107] Y. Liu, J. Bjorkman, A. Urquhart, R.J. Wanders, D.I. Crane, S.J. Gould, PEX13 is mutated in complementation group 13 of the peroxisome-biogenesis disorders, Am. J. Hum. Genet. 65 (3) (1999) 621–634.
[108] N. Shimozawa, T. Tsukamoto, T. Nagase, Y. Takemoto, N. Koyama, Y. Suzuki, et al., Identification of a new complementation group of the peroxisome biogenesis disorders and PEX14 as the mutated gene, Hum. Mutat. 23 (6) (2004) 552–558.
[109] A.C. Muntau, P.U. Mayerhofer, B.C. Paton, S. Kammerer, A.A. Roscher, Defective peroxisome membrane synthesis due to mutations in human

PEX3 causes Zellweger syndrome, complementation group G, Am. J. Hum. Genet. 67 (4) (2000) 967–975.
[110] K. Ghaedi, M. Honsho, N. Shimozawa, Y. Suzuki, N. Kondo, Y. Fujiki, PEX3 is the causal gene responsible for peroxisome membrane assembly-defective Zellweger syndrome of complementation group G, Am. J. Hum. Genet. 67 (4) (2000) 976–981.
[111] M. Honsho, S. Tamura, N. Shimozawa, Y. Suzuki, N. Kondo, Y. Fujiki, Mutation in PEX16 is causal in the peroxisome-deficient Zellweger syndrome of complementation group D, Am. J. Hum. Genet. 63 (6) (1998) 1622–1630.
[112] S.T. South, S.J. Gould, Peroxisome synthesis in the absence of preexisting peroxisomes, J. Cell Biol. 144 (2) (1999) 255–266.
[113] N. Shimozawa, T. Nagase, Y. Takemoto, Y. Suzuki, Y. Fujiki, R.J. Wanders, et al., A novel aberrant splicing mutation of the PEX16 gene in two patients with Zellweger syndrome, Biochem. Biophys. Res. Commun. 292 (1) (2002) 109–112.
[114] Y. Matsuzono, N. Kinoshita, S. Tamura, N. Shimozawa, M. Hamasaki, K. Ghaedi, et al., Human PEX19: cDNA cloning by functional complementation, mutation analysis in a patient with Zellweger syndrome, and potential role in peroxisomal membrane assembly, Proc. Natl. Acad. Sci. U. S. A. 96 (5) (1999) 2116–2121.
[115] P.U. Mayerhofer, T. Kattenfeld, A.A. Roscher, A.C. Muntau, Two splice variants of human PEX19 exhibit distinct functions in peroxisomal assembly, Biochem. Biophys. Res. Commun. 291 (5) (2002) 1180–1186.
[116] S. Steinberg, S. Katsanis, A. Moser, G. Cutting, Biochemical analysis of cultured chorionic villi for the prenatal diagnosis of peroxisomal disorders: biochemical thresholds and molecular sensitivity for maternal cell contamination detection, J. Med. Genet. 42 (1) (2005) 38–44.
[117] G.I. Solish, H.W. Moser, L.D. Ringer, A.E. Moser, C. Tiffany, E. Schutta, The prenatal diagnosis of the cerebro-hepato-renal syndrome of Zellweger, Prenat. Diagn. 5 (1) (1985) 27–34.
[118] L. Govaerts, J. Bakkeren, L. Monnens, J. Maas, F. Trijbels, W. Kleijer, Disturbed very long chain (C24–C26) fatty acid pattern in fibroblasts of patients with Zellweger's syndrome, J. Inherit. Metab. Dis. 8 (1) (1985) 5–8.
[119] S. Hoefler, G. Hoefler, A.B. Moser, P.A. Watkins, W.W. Chen, H.W. Moser, Prenatal diagnosis of rhizomelic chondrodysplasia punctata, Prenat. Diagn. 8 (8) (1988) 571–576.
[120] W.G. Kearns, R. Pen, J. Graham, T. Han, J. Carter, M. Moyer, et al., Preimplantation genetic diagnosis and screening, Semin. Reprod. Med. 23 (4) (2005) 336–347.
[121] F. Mochel, A.G. Grebille, A. Benachi, J. Martinovic, F. Razavi, D. Rabier, et al., Contribution of fetal MR imaging in the prenatal diagnosis of Zellweger syndrome, AJNR Am. J. Neuroradiol. 27 (2) (2006) 333–336.
[122] S.H. Sastrowijoto, K. Vandenberghe, P. Moerman, J.M. Lauweryns, J.P. Fryns, Prenatal ultrasound diagnosis of rhizomelic chondrodysplasia punctata in a primigravida, Prenat. Diagn. 14 (8) (1994) 770–776.
[123] M. Martinez, E. Vazquez, MRI evidence that docosahexaenoic acid ethyl ester improves myelination in generalized peroxisomal disorders, Neurology 51 (1) (1998) 26–32.
[124] M.J. Noetzel, Fish oil and myelin: cautious optimism for treatment of children with disorders of peroxisome biogenesis, Neurology 51 (1) (1998) 5–7.
[125] K.D. Setchell, P. Bragetti, L. Zimmer-Nechemias, C. Daugherty, M.A. Pelli, R. Vaccaro, et al., Oral bile acid treatment and the patient with Zellweger syndrome, Hepatology 15 (2) (1992) 198–207.
[126] K. Maeda, A. Kimura, Y. Yamato, H. Nittono, H. Takei, T. Sato, et al., Oral bile Acid treatment in two Japanese patients with Zellweger syndrome, J. Pediatr. Gastroenterol. Nutr. 35 (2) (2002) 227–230.
[127] A.K. Das, R.D. Holmes, G.N. Wilson, A.K. Hajra, Dietary ether lipid incorporation into tissue plasmalogens of humans and rodents, Lipids 27 (6) (1992) 401–405.
[128] L. Van Maldergem, A.B. Moser, M.F. Vincent, D. Roland, R. Reding, J.B. Otte, et al., Orthotopic liver transplantation from a living-related donor in an infant with a peroxisome biogenesis defect of the infantile Refsum disease type, J. Inherit. Metab. Dis. 28 (4) (2005) 593–600.
[129] H. Wei, S. Kemp, M.C. McGuinness, A.B. Moser, K.D. Smith, Pharmacological induction of peroxisomes in peroxisome biogenesis disorders, Ann. Neurol. 47 (3) (2000) 286–296.
[130] M.C. McGuinness, H. Wei, K.D. Smith, Therapeutic developments in peroxisome biogenesis disorders, Expert Opin. Investig. Drugs 9 (9) (2000) 1985–1992.
[131] H. Portsteffen, A. Beyer, E. Becker, C. Epplen, A. Pawlak, W.H. Kunau, et al., Human PEX1 is mutated in complementation group 1 of the peroxisome biogenesis disorders, Nat. Genet. 17 (4) (1997) 449–452.
[132] N. Braverman, G. Steel, C. Obie, A. Moser, H. Moser, S.J. Gould, et al., Human PEX7 encodes the peroxisomal PTS2 receptor and is responsible for rhizomelic chondrodysplasia punctata, Nat. Genet. 15 (4) (1997) 369–376.
[133] A.M. Motley, E.H. Hettema, E.M. Hogenhout, P. Brites, A.L. ten Asbroek, F.A. Wijburg, et al., Rhizomelic chondrodysplasia punctata is a peroxisomal protein targeting disease caused by a non-functional PTS2 receptor, Nat. Genet. 15 (4) (1997) 377–380.
[134] P.E. Purdue, J.W. Zhang, M. Skoneczny, P.B. Lazarow, Rhizomelic chondrodysplasia punctata is caused by deficiency of human PEX7, a homologue of the yeast PTS2 receptor, Nat. Genet. 15 (4) (1997) 381–384.
[135] M. Biermanns, J. Gärtner, Genomic organization and characterization of human PEX2 encoding a 35-kDa peroxisomal membrane protein, Biochem. Biophys. Res. Commun. 273 (3) (2000) 985–990.
[136] R.J. Wanders, H.R. Waterham, Peroxisomal disorders I: biochemistry and genetics of peroxisome biogenesis disorders, Clin. Genet. 67 (2) (2005) 107–133.