Molecular Characterization of Peroxisome Biogenesis Disorders with Zellweger Syndrome Spectrum

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Abstract

Peroxisome biogenesis disorders, Zellweger syndrome spectrum (PBD, ZSS) are constituted of three different phenotypically disorders: Zellweger syndrome (ZS), the most severe; neonatal adrenoleukodystrophy (NALD); and infantile refsum disease (IRD), the least severe, that have been originally described based on their biochemical and molecular bases of these disorders which had been fully determined. Individuals with PBD, ZSS usually come to clinical attention in the newborn period or later in childhood. The diagnosis of PBD, ZSS can be definitively determined by biochemical assays. Measurement of plasma very-long-chain fatty acid (VLCFA) levels is the most commonly used and most informative initial screen. Mutations in thirteen different *PEX* genes - those that encode peroxins, the proteins required for normal peroxisome assembly - have been identified in PBD, ZSS. Mutations in *PEX1*, the most common cause of PBD, ZSS, are observed in about 68% of affected individuals. Sequence analysis is available clinically for the following seven genes: *PEX1*, *PXMP3 (PEX2)*, *PRXR1 (PEX5)*, *PEX6*, *PEX10*, *PEX12*, and *PEX26*.

Key Words: Zellweger syndrome; Neonatal adrenoleukodystrophy; Infantile refsum disease; *PEX*

Introduction

Peroxisome biogenesis disorders, Zellweger syndrome spectrum (PBD, ZSS) are defined by a continuum of three phenotypes described before the biochemical and molecular bases of these disorders had been fully determined: Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD) and infantile refsum disease (IRD)[1]. All of the peroxisome assembly disorders are serious disorders, which frequently cause death in the early stage of life. ZS is the most severe and IRD the least severe of these phenotypes. The generalizations that these labels represent are still useful when facing undiagnosed individuals and counseling their families, it should not emphasize too much on assigning an affected individual in placing to one of these categories. However the affected persons are categorized in broad range of phenotypic variations, as in

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individuals with PBD, ZSS Occasionally, the subtlety of symptoms caused delaying in diagnosis until adulthood. In the newborn period, affected children are hypotonic, feed poorly, and have distinctive facies, seizures, and liver cysts with hepatic dysfunction. Infants with ZS are significantly impaired and typically die during the first year of life, usually having made no developmental progress[1](Figure 1).

Older children have retinal dystrophy, sensori-neural hearing loss, developmental delay with hypotonia, and liver dysfunction[2]. The clinical phenotypes of NALD and IRD are more variable and may include developmental delays, hearing loss, vision impairment, liver dysfunction, episodes of hemorrhage, and intracranial bleeding. While some affected children can be very hypotonic, others learn to walk and talk. Mutations in the twelve genes listed in Table 1 are known to cause PBD, ZSS in humans. These genes encode proteins required for peroxisome biogenesis called "peroxins"; the nomenclature for naming these genes are "*PEX*" followed by a number. A few of the peroxins appear to be essential for peroxisome membrane formation [3]. However, the majority of known affected individuals have mutations in *PEX* genes encoding proteins essential for the import of peroxisomal matrix proteins[4]. Mutations in the two most commonly involved genes, *PEX1* and *PEX6*, are associated with the full continuous clinical phenotypes. This clinical variability, in general, is also found in individuals with mutations in *PEX10, PEX12*, and *PEX26*.

PEX3, PEX16, and *PEX19* mutations are associated exclusively with the most severe phenotype (ZS)[3]. Deficiencies in these three genes cause a cellular phenotype without peroxisome detected by immunocytochemical analysis. Peroxisomal membrane formation is completely absent in related patients fibroblast cell lines from these individuals. No direct association exists between the biochemical phenotype and the deficient *PEX* gene. Thus, it is not possible to identify the candidate gene based solely on the biochemical phenotype. However, a report suggests that two biochemical findings (DHAP-AT and C26:0 β-oxidation activity) are predictors of survival rate in individuals with PBD, ZSS[5].

Metabolic Pathways

A variety of anabolic and catabolic pathways occur in the peroxisome. β-oxidation and plasmalogen synthesis are two fundamental pathways localized there. The peroxisomal β-oxidation enzymes are distinct from the mitochondrial β-oxidation system. Straight-chain VLCFA β-oxidation requires several enzymes with very-long-chain acyl CoA synthetase, acyl CoA oxidase, D-bifunctional protein (enoyl-CoA

Fig 1- Characteristic facial abnormalities in a neonatal affected by peroxisomal disorders**.** Peroxisomal disorders are associated with characteristic facial abnormalities (high forehead, frontal bossing, small face, low set ears, slanted eyes, etc.). Patients present as floppy children, due to their decreased muscle tone (hypotonia). Developmental delay and mental retardation is common to all patients, and vision and hearing are affected very soon. In general, these children are difficult to feed (personal communication with professor Fujiki, Kyushu University, Japan).

Table 1- Molecular Genetics of Peroxisome Biogenesis Disorders, Zellweger Syndrome Spectrum[5,6,7]

Gene Symbol	Chromosomal Locus	Protein Name
PEX1	7q21-q22	Peroxisome biogenesis factor 1
PEX10	1p36.32	Peroxisome assembly protein 10
PEX12	17q12	Peroxisome assembly protein 12
PEX13	2p15	Peroxisomal membrane protein PEX13
PEX14	1p36.2	Peroxisomal membrane protein PEX14
PEX16	11p12-p11.2	Peroxisomal membrane protein PEX16
PEX19	1q22	Peroxisomal biogenesis factor 19
PEX26	22q11-21	Peroxisome assembly protein 26
PEX3	6q23-q24	Peroxisomal biogenesis factor 3
PEX5	12p13.3	Peroxisomal targeting signal 1 receptor
PEX6	6p21.1	Peroxisome assembly factor 2
PXMP3	8q21.1	Peroxisome assembly factor 1

hydratase and 3-hydroxyacyl-CoA dehydro-genase), and peroxisomal β-ketothiolase. All of these proteins have PTS1 signals (Peroxisomal targeting signal type I) except for peroxisomal β-ketothiolase, which is imported to the peroxisome via a PTS2 signal[7]. These proteins also play an important role in the side-chain modification of bile acids. Thus, the defect in peroxisomal fatty acid β-oxidation accounts for the increase in very long chain fatty acids (VLCFA) and branched-chain fatty acids such as pristanic acid, and bile acid in the walls. A unique branched-chain acyl CoA oxidase is used for bile acids and pristanic acid, thus explaining why these metabolites are normal in acyl-CoA oxidase deficiency. The initial steps of plasma-logen synthesis occur in the peroxisome and the final stages of synthesis are completed in the endoplasmic reticulum. Dihydroxyacetone phosphate (DHAP)-acyl transferase and alkyl-DHAP-synthase are PTS1 and PTS2 containing proteins, respectively[8,9].

Peroxisome Biogenesis and Assembly

At least 29 peroxins are required for peroxisome membrane biogenesis, fission, and protein import to form competent organelles. Thus far, mutations in 13 genes that encode peroxins are associated with related human disorders. The biogenesis of membranes is not well understood, but mutations in three human *PEX* genes (*PEX3*, *PEX16*, and *PEX19*) are associated with the absence of peroxisome membrane structures [10, 11, 12]. The remaining proteins encoded by known *PEX* genes contribute to the import machinery required for matrix protein sorting, a complex process that is being studied recently. In general, peroxisomal matrix proteins are encoded by nuclear genes and are translated on free polyribosomes. *PXR1 (PEX5)* encodes a receptor that recognizes proteins containing peroxisomal targeting sequence 1 (PTS1), defined by the carboxy terminal consensus sequences: serine-lysine-leucine (SKL)[13]. *PEX7* encodes the PTS2 receptor which recognizes proteins containing an N-terminaly a peptide termed PTS2. Mutations in PEX7 are associated with the clinically distinct disorder RCDP[13]. Those two sub-complexes have been proposed to be anchored by product of in yeast which is associated with the more lumenal aspect of the peroxisomal membrane and its homologues has not been recognized in mammals so far[14]. The two sub complexes comprise the products of (a) *PEX14, PEX17*, and *PEX13*, and (b) *PEX10, PEX12*, and *PEX2* genes. The first sub-complex plays a role in the docking of the PTS1 and PTS2 receptors and their cargo proteins. The second sub-complex appears to be part of the translocation apparatus for matrix

proteins at peroxisomal membranes [15,16,17]. In contrast, *PEX1* and *PEX6* products form a complex that may play a role in the recycling of the PTS1 and PTS2 receptors. Epistatic studies in yeast indicate that *PEX1, PEX6, PEX4* and *PEX22* products act at late step in the import pathway, perhaps after the translocation process[17]. However, the recent identification of *PEX26* gene has shown that the encoded protein directly interacts with Pex1-Pex6 protein complexes. Thus, all three proteins may play a critical role in the presentation of PTS1 and PTS2 proteins to the peroxisomal membrane (Figure 2).

Diagnosis of Peroxisomal Biogenesis Disorders

The diagnosis of PBD, ZSS can be determined by biochemical and Sequence analyses assays. Biochemical parameters could be detected in blood and/or urine which should be confirmed with data of cultured Patients fibroblasts.

Measurement of plasma very-long-chain fatty acid (VLCFA) levels is the most commonly used and most informative initial screen. Elevation of C26:0 and C26:1 and the ratios C24/C22 and C26/C22 are consistent with a defect in peroxisomal fatty acid metabolism[9,19].
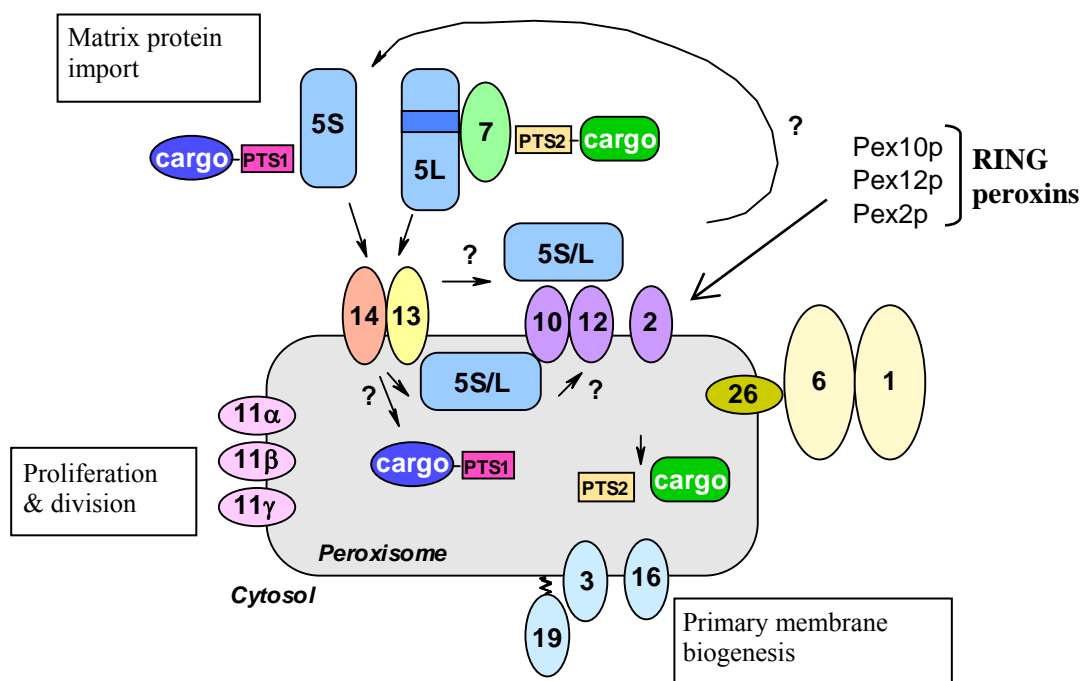
PTS1: Peroxisome targeting signal type-1, C-terminal tripeptides; -S-K-L-**COOH**

PTS2: Peroxisome targeting signal type-2, N-terminal cleavable presequence

Fig 2- Peroxisome biogenesis and matrix protein import.

The various proteins are directed to their correct positions in the peroxisome - either incorporated into the membrane or passing through it into the matrix by means of peroxisomal targeting signals (PTSs). A PTS receptor is a mobile protein which repeatedly shuttles between the cytosol - recognizing and binding the PTS protein - and the peroxisome, separating from it and leaving it for import. There are about fifteen other proteins known to be necessary to the correct assembly of a peroxisome. The biogenesis of peroxisomes starts with the peroxins Pex3p, Pex16p and Pex19p. The import of membrane proteins into extant peroxisomes needs Pex19p for recognition, targeting and insertion via docking at Pex3p. Matrix proteins in the cytosol are recognized by their targeting signals – PTS1 via Pex5p and PTS2 via Pex7p – and transported to the docking complex Pex14p and Pex13p at the peroxisomal membrane. Pex5p integrated into the peroxisomal membrane and the cargo is imported by Pex2p, Pex10p and Pex12p (personal communication with professor Fujiki, Kyushu University, Japan).

Molecular genetic testing for carrier detection, prenatal diagnosis and prognostication through emerging genotype-phenotype correlations should be done. These are including sequence analysis of selected *PEX1* gene exons at first step and *PEX* gene screen algorithm at the second step. the pregnancy rate of mutation in *PEX1* exon 13 (where I700fs is localized) and exon 15 (where G843D is localized) in individual with a PBD, ZSS identified to be slightly more than 50% of individuals[19]. To circumvent the need for complementation studies, two slightly different algorithms for analysis a subset of other *PEX* genes and their exons have been developed: recently sequence analysis of *PEX1* exons 13, 15, and 18, *PEX2* exon 4, *PEX6* exon 1, *PEX10* exons 3-5, *PEX12* exons 2 and 3, and *PEX26* exons 2 and 3 has a sensitivity of 79%. Sequence analysis of *PEX1* exons 13 and 15, *PEX2* exon 4, *PEX10* exons 4 and 5, *PEX12* exons 2 and 3, and *PEX26* exons 2 and 3 has a sensitivity of approximately 72% for the identification of at least one mutation [20].

Differential diagnoses vary with the age of presentation and most prominent feature of presentation. PBD, ZSS in newborns is most often confused with other conditions that result in profound hypotonia including Down syndrome, other chromosomal abnormalities, Prader-Willi syndrome, spinal muscular atrophy, congenital myotonic dystrophy type 1, and congenital myopathies such as X-linked myotubular myopathy and multiminicore myopathy. Older children have been initially presumed to have Usher syndrome type I or Usher syndrome type II and other disorders of sensorineural hearing loss and retinitis pigmentosa, Leber congenital amaurosis, Cockayne syndrome, or congenital infections [6,21].

An increase in plasma VLCFA concentration consistent with a defect in peroxisomal fatty acid metabolism could be associated with four main disease types: a) PBD, ZSS; b) a single enzyme deficiency (SED) of the peroxisomal β-oxidation enzymes D-bifunctional protein (D-BP) or acyl-CoA oxidase (AOx)[22]; c) X-linked adrenoleuko-dystrophy (X-ALD) or adrenomyeloneuropathy (AMN), caused by mutations in ABCD1; and d) CADDS, a contiguous deletion syndrome with a critical region spanning the genes ABCD1 and BAP31[23] (table 2).

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 which can be used for prenatal testing with cultured cells derived from chorionic villi or amniotic fluid cells. To identify any molecular defect DNA can be isolated from cells from chorionic villus samples (CVS) or amniotic fluid for further DNA analysis. Due to the risk of maternal cell contamination (MCC), especially when using CVS, it is essential to perform mother DNA testing to rule out this possibility especially when

Table 3- Molecular Genetic Testing Used in Peroxisome Biogenesis Disorders, ZSS[20, 24, 25, 26]

Test Method	Mutations Detected	Mutation Detection Rate
Sequence analysis of select exons	*PEX1* mutations in: exon 13 (I700fs); exon 15 (G843D)	50%
	Sequence variations in *PEX1*, *PXMP3 (PEX2)*, *PEX10*, *PEX12*, *PEX26*	72%
Sequence analysis of all coding exons	Sequence variations in *PEX1*, *PXMP3 (PEX2)*, *PEX6*, *PEX10*, *PEX12*, *PEX5*	95%
Direct DNA	Mutations in *PEX3*, *PEX13*, *PEX14*, *PEX16*, *PEX19*	Unknown

cells from heterozygotic carriers for ZSS disorders do not express partial defects, thus there is no MCC causing for false positive result [27]. Analysis of peroxisomal β-oxidation and plasmalogen synthesis are the two pathways most commonly assessed for prenatal testing [28,29]. Once the specific gene defect has been identified, it is also possible to offer the couple an option of preimplantation genetic diagnosis (PGD) [30]. 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 [31].

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 have longer life 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[32], but its affect on clinical outcome has not yet been proven[33]. Oral bile acid administration improved hepatobiliary function in several infants with ZS[34,35]. Liver transplantation has been reported in one patient with IRD [36], 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[37,38]. Mouse models have been developed for *PEX2, PEX5, PEX7* and *PEX13* deficiency and are discussed in more details. These models are useful for studying the underlying pathophysiology, for investigating existing therapies and for developing new approaches to treatment.

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