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Genetic and molecular bases of peroxisome biogenesis disorders

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Peroxisomes are ubiquitous organelles present in almost all eukaryotic cells and have a number of important metabolic pathways. They were discovered in 1954 and named peroxisomes in 1965.[1] Peroxisomes have their own system of fatty acid β-oxidation.[2,3] This system catalyzes a wide variety of substrates, including very long chain fatty acids (VLCFAs) such as lignoceric acid (C24:0) and cerotic acid (C26:0), bile acid intermediates such as trihydroxycholestanoic acid (THCA) and dihydroxycholestanoic acid (DHCA), and branched chain fatty acids like pristanic acid and phytanic acid. Plasmalogens are a special group of phospholipids, which are an important constituent of the brain and require peroxisomes for their synthesis.[4] Glyoxylate is a precursor of oxalate and is metabolized to glycine by alanine-glyoxylate aminotransferase.[5] Peroxisomes are also considered to play roles in the synthesis of docosahexaenoic acid (DHA)[6] and cholesterol.[7] Catalase, a marker enzyme of peroxisomal matrix, plays an important role in the degradation of hydrogen peroxide.[1]

Inborn errors of peroxisomes are classified into two categories: peroxisome biogenesis disorders (PBDs) and single enzyme deficiencies (Table 1). Generalized PBDs include Zellweger's cerebro-hepato-renal syndrome (ZS),[8] the most severe phenotype; neonatal adrenoleukodystrophy (NALD),[9] the intermediate; and infantile Refsum disease (IRD),[10] the least severe phenotype. Rhizomelic chondrodysplasia punctata (RCDP)[11] is a partial PBD. Deficiency of peroxisomes in ZS was first identified in 1973,[12] then the accumulation of VLCFAs was first reported in 1982.[13] These PBDs are caused by a defect in PEX genes, which encode peroxins, proteins necessary for peroxisome biogenesis and import of peroxisomal proteins.

CLINICAL, PATHOLOGICAL, AND BIOCHEMICAL FINDINGS OF PBDs

Zellweger syndrome was first reported in 1964 as an autosomal recessive disease with multiple congenital malformations.[8] Patients with ZS manifest severe hypotonia from the neonatal period, psychomotor retardation, facial dysmorphism such as high forehead and large fontanelle, hepatomegaly with prolonged jaundice and liver dysfunctions, calcific stippling of the patella, renal cortical microcysts, retinal degeneration, and congenital heart disease, and usually die during the early infantile period. Neonatal adrenoleukodystrophy is a phenotype with less severe clinical findings. Patients with NALD gradually develop, but then regression occurs, and patients usually die during the late infantile period. Infantile Refsum disease is characterized by hearing impairment, retinal degeneration, and mild psychomotor retardation, and patients usually survive longer.[14]

Defective neuronal migration is the main pathological finding in ZS.[15] Polymicrogyria, pachygyria, heterotopia of cerebral neurons and Purkinje cells in the cerebellum, dysplasia of olivary complex, and hypomyelination are also characteristic findings. Architecture of cerebral cortex is disorganized and abnormally thick. The cerebellar Purkinje layer is irregular, and heterotopic Purkinje cells are seen in the cerebellar white matter. The olivary nucleus is thick and poorly convoluted. The NALD brain shows demyelination. Lamellar inclusion bodies, which consist of cholesterol esters of VLCFAs, are observed in macrophages and adrenocortical cells.

Biochemical abnormalities of PBDs are as follows: accumulation of VLCFAs (C24:0, C25:0, C26:0), intermediate substances of bile acid synthesis (THCA, DHCA) and branched chain fatty acid (pristanic and phytanic acid); deficiency of plasmalogens and DHA; and absent or severely decreased peroxisomes. All phenotypes of PBDs show these findings; however, patients with milder phenotypes tend to have less severe abnormalities.[14] Peroxisomes are morphologically absent or severely reduced in hepatocytes (Fig. 1C) or fibroblasts (Fig. 1D). Peroxisomal ghosts, which are remnant membranous structures, can be detected in many PBD cell lines when the cells are stained with anti-PMP70,[16] a major peroxisomal membrane protein. However, some PBD cell lines lack ghosts,[17] which suggests that biogenesis of peroxisomal membrane itself is disordered. Peroxisomes also show another interesting phenomenon: temperature sensitivity (ts) of peroxisome biogenesis.[18] That is the biogenesis of peroxisomes and biochemical defects in the cells from patients with milder phenotypes such as NALD and IRD are restored at the lower temperature, 30°C, whereas the biogenesis is not restored in the severe Zellweger cells at 30°C. This phenomenon is useful for predicting the clinical severity and for investigating peroxisomal biogenesis.

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Table 1
Peroxisomal disorders

A. Peroxisome biogenesis disorders (PBDs)
1. Zellweger syndrome (ZS)
2. Neonatal adrenoleukodystrophy (NALD)
3. Infantile Refsum disease (IRD)
4. Rhizomelic type chondrodysplasia punctata (RCDP)
5. Zellweger-like syndrome
B. Isolated enzyme deficiencies
1. Adrenoleukodystrophy (ALD)
2. Acyl-CoA oxidase deficiency
3. D-Bifunctional protein deficiency
4. 3-Ketoacyl-CoA thiolase deficiency
5. Dihydroxyacetone phosphate (DHAP) acyltransferase deficiency
6. Alkyl-DHAP synthase deficiency
7. Refsum disease (phytanoyl-CoA hydroxylase deficiency)
8. Alpha-methylacyl-CoA racemase deficiency
9. Acatalasemia
10. Hyperoxaluria type I (alanine glyoxylate aminotransferase deficiency)
11. Mavalonate kinase deficiency
12. Glutaric aciduria type 3 (glutaryl-CoA oxidase deficiency)

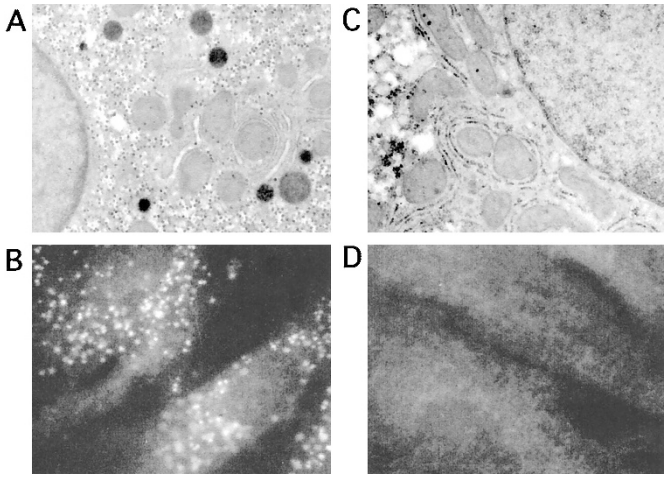

Fig. 1 Electron micrograph and immunofluorescence staining of peroxisomes. A: Control hepatocytes (electron micrograph with diaminobenzidine staining). B: Control fibroblasts (immunofluorescence staining with anticatalase). C: Zellweger hepatocytes (electron micrograph with diaminobenzidine staining). D: Zellweger fibroblasts (immunofluorescence staining with anticatalase).

GENETIC HETEROGENEITY AND PATHOGENIC GENES FOR PBDs

Genetic heterogeneity of PBDs was first identified in 1988.[19] Biochemical defects were restored when some pairs of PBD cell lines were fused.[20] In 1992, we identified five complementation groups using immunofluorescence staining of catalase as a marker.[21] If pathogenic genes are different, peroxisomes will be formed in multinuclear cells a few days after cell fusion. An international collaborative study has led to the identification of 12 groups at present (Table 2).[17] ZS, NALD, and IRD belong to 11 groups. RCDP is genetically heterogeneous and belongs to one PBD group and the single enzyme deficiency group. Patients with dihydroxyacetone phosphate (DHAP) acyltransferase deficiency and alkyl-DHAP synthase deficiency also manifest RCDP phenotype. Group 1(E) is the largest group, and more than half the number of patients belong to this group. Groups D, G, and J lack peroxisomal ghosts, and these groups contain only ZS, the most severe phenotype. At present, 11 of the genes associated with the different complementation groups have been identified. These genes are called *PEX* genes, and the gene products are called Pexp (peroxins).

The first isolated mammalian *PEX* gene is *PEX2,* which was first called peroxisome assembly factor-1 (PAF1).[22] This gene was cloned by means of functional complementation cloning using peroxisome-deficient Chinese hamster ovary (CHO) cells. Expression library was transfected into these CHO mutants, cells with restored peroxisome biogenesis were selected, and the cDNA was recovered from these cells. Pex2p is a 35-kDa peroxisomal membrane protein and has two membrane spanning regions and a RING finger motif. In 1992, we clarified that *PEX2* restored biogenesis of peroxisomes in fibroblasts from a ZS patient who belonged to complementation group F.[23] This patient carried a homozygous nonsense mutation R119Ter. A frameshift mutation del 550C, which destroys the second membrane spanning region, is associated with severe ZS, whereas another frameshift mutation del 642G, which destroys only the RING finger motif, is found in the milder IRD phenotype.[24] A missense mutation E55K, which was identified in a IRD patient, proved to be a ts mutation.[18] The second gene identified in PBD patients is *PEX5*.[25] This gene was cloned by expressed sequence tag (EST)-homology search using a human database and information on the yeast *PEX* gene. Pex5p is a cytosolic receptor for peroxisomal targeting signal (PTS)-1. A Zellweger patient who belonged to Group 2 had a nonsense mutation R390Ter, and an NALD patient had a missense mutation. The PTS-1 receptor binds tightly to the PTS-1 motif of acyl-CoA oxidase, Ser-Lys-Leu (SKL), whereas the binding potential to Ala-Lys-Leu (AKL) in D-bifunctional protein or Lys-Ala-Asn-Leu (KANL) in catalase is weaker.[26] *PEX6,* the gene for group C PBD, was also cloned using the functional complementation method.[27] Pex6p belongs to the group of AAA-proteins and is considered to interact with Pex1p.[28] A splice mutation and a one base insertion were identified in ZS patients.[29] *PEX12* encodes a peroxisomal integral membrane protein with RING finger motif. One ZS patient who belonged to Group 3 PBD had a frameshift mutation.[30] Pex12p is considered to play roles in protein import downstream to PTS1- or PTS2- receptor docking event.[31] *PEX1* is the pathogenic gene for Group 1(E), the largest group of PBDs.[32,33] A common one-base insertion 2097insT was found in severe ZS patients. Another common missense mutation G843D was found among patients with milder phenotypes, and it was a ts mutation.[34]

Table 2
Complementation groups, genes for PBDs, and *ts* mutations

Gifu	KKI	Phenotype	Ghosts	Gene	Mapping	*ts* Mutations
A	8	ZS, NALD, IRD	+			
B	7	ZS, NALD	+	*PEX10*		
C	4	ZS, NALD	+	*PEX6*	6p21.1	L57P
D	9	ZS	−	*PEX16*		
E	1	ZS, NALD, IRD	+	*PEX1*	7q21–22	G843D
F	10	ZS, IRD	+	*PEX2*	8q21.1	E55K
G		ZS	−	*PEX3*	6q23–24	
H	13	ZS, NALD	+	*PEX13*	2	I326T
J		ZS	−	*PEX19*	1	
	2	ZS, NALD	+	*PEX5*	12p13.3	
	3	ZS, NALD, IRD	+	*PEX12*		
R	11	RCDP		*PEX7*	6q22–24	

Gifu, Gifu University School of Medicine (Japan); KKI, Kennedy Krieger Institute (USA).

PEX7, the gene for RCDP, was cloned by EST homology search by several investigators.[35–37] Pex7p is a receptor for PTS-2 proteins such as 3-ketoacyl-CoA thiolase and alkyl-DHAP synthase. A common nonsense mutation L292Ter was shown to be caused by the founder effect.[38] *PEX10* is the gene for Group 7 PBD and encodes an integral membrane protein with a RING finger motif.[39,40] Pex10p is considered to play roles in protein import downstream to PTS1- or PTS2- receptor docking event.[31] *PEX16* encodes a peroxisomal membrane protein and restores the localization of both peroxisomal membrane proteins and matrix enzymes in group D PBD.[17] A ZS patient in group D had a nonsense mutation R176Ter.[41] *PEX19* is the gene for group J PBD, and it encodes a farnesylated protein with 299 amino acids. One base insertion 764insA was identified in a ZS patient.[42] Pex19p is considered to interact with various peroxisomal membrane proteins and ABC half transporters such as PMP70 and ALD protein[43,44] and is considered to be involved in the early stages of peroxisome biogenesis. *PEX13* encodes a membrane protein which had a SH3 docking site with PTS1 receptor, and it restores the biogenesis of peroxisomes in group H PBD.[45] A ZS patient had a nonsense mutation W234Ter which completely destroyed the SH3 binding domain, and an NALD patient had a missense mutation I326T in the SH3 domain which causes the ts phenomenon.[45] Pex13p is also required for the localization of Pex14p- and PTS-2-dependent protein import.[46] *PEX3* is the gene for group G PBD which lacks ghosts.[47,48] By transfecting *PEX3,* both matrix enzymes and membrane proteins are localized into peroxisomes. Pex3p is considered to initiate membrane assembly.[49]

MOLECULAR AND BIOCHEMICAL CORRELATION WITH THE PHENOTYPE

Patients with milder phenotypes tend to have less severe biochemical abnormalities.[50] The defects in plasmalogen synthesis and the degree of VLCFA accumulation in NALD are less severe than in Zellweger syndrome. A few residual peroxisomes could be detected in hepatocytes or in fibroblasts from patients with NALD or IRD. However, it is difficult to predict clinical severity or prognosis of a patient with PBDs solely on the basis of biochemical analysis. Molecular analysis of *PEX* genes revealed that many of the patients with severe Zellweger syndrome had severe mutations, including nonsense mutations, frameshift mutations, and deletions, whereas many of NALD or IRD patients had missense mutations.[23–25,29,30,32,33,35–42,45,48] There is also a relationship between severe phenotype and absence of ghosts. Defects of *PEX3, PEX16,* and *PEX19* genes (group G, D and J) lead to absence of ghosts and severe Zellweger phenotypes.[41,42,48] Severity of phenotypes also correlate with ts phenomenon. Many cell lines from patients with milder phenotypes showed ts phenotype. When the cells from mild phenotype are cultivated at 30°C, peroxisomes are formed in almost all cells and the biochemical abnormalities are normalized. Mutation analyses revealed the presence of specific ts mutations.[18,34,45,51] These are all missense mutations (Table 2).

PATHOPHYSIOLOGY OF PBDs

PEX gene products, peroxins, are encoded by nuclear genes. Several important functions of peroxins have been obtained from the investigations of PBDs (Fig. 2). Pex3p, Pex16p, and Pex19p are considered to play roles in the early stages of peroxisome biogenesis, membrane integrity and transport of various membrane proteins, including other peroxins and ABC half transporters.[41,43,44,49] Pex5p and Pex7p, the specific receptors for PTS-1 and PTS-2 proteins, are considered to bind PTS-1 and PTS-2 proteins, interact with Pex1p and Pex6p, and bind to the transport machinery in the membrane, followed by their recycling.[25,35,36,37] Pex2p, Pex10p, and Pex12p, the integral membrane protein with a RING finger, are considered to form part of the transport machinery.

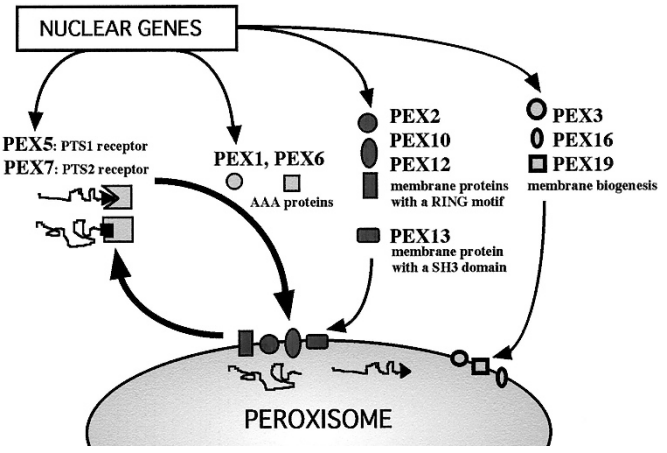

Fig. 2 Biogenesis of peroxisomes and functions of *PEX* gene products.

Defects of these *PEX* genes cause the dysfunction of transport machinery and lead to mislocalization of multiple enzymes and metabolic dysfunctions: accumulation of VLCFA, bile acid intermediates, and phytanic and pristanic acid; deficiency of plasmalogens and DHA. Defects of β-oxidation system per se would play a major role in migration disorders and demyelination process, since isolated deficiencies of peroxisomal β-oxidation enzymes manifest these neuropathology.[52] Deficiency of plasmalogen is considered to cause abnormal myelination and bone abnormalities, because DHAP-acyltransferase deficiency manifests these findings.[53] Phytanic acid may lead to retinal degeneration and hearing impairment. Deficiency of DHA is considered to relate to brain and visual dysfunction and abnormal myelination.[54,55]

Although 11 human *PEX* genes have been identified, there are over 20 yeast *PEX* genes. Novel complementation groups and responsible genes may be elucidated in the near future. While considerable information on the *PEX* genes has been obtained, precise structural and functional bases of pathophysiology of PBDs remains to be elucidated. Two mouse models for Zellweger syndrome have been generated by inactivating the murine *PEX5* and *PEX2* genes, respectively.[56,57] *PEX5*-deficient mice exhibited the typical clinical, biochemical, and pathological abnormalities of ZS, including severe hypotonia and impaired neuronal migration. These model mice will provide important knowledge on mechanisms of pathological changes and peroxisome biogenesis.

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