

Defective *PEX* gene products correlate with the protein import, biochemical abnormalities, and phenotypic heterogeneity in peroxisome biogenesis disorders

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Revised version received 26 April 1999
 Accepted for publication 26 May 1999

Abstract

Peroxisome biogenesis disorders (PBD) comprise three phenotypes including Zellweger syndrome (ZS) (the most severe), neonatal adrenoleucodystrophy, and infantile Refsum disease (IRD) (the most mild), and can be classified into at least 12 genetic complementation groups, which are not predictive of the phenotypes. Several pathogenic genes for PBD groups have been identified, but the relationship between the defective gene products and phenotypic heterogeneity has remained unclear. We identified a mutation in the *PEX2* gene in an IRD patient with compound heterozygosity for a missense mutation and the known nonsense mutation detected in ZS patients. In transfection experiments using the peroxisome deficient CHO mutant, Z65 with a nonsense mutation in the *PEX2* gene, we noted the E55K mutation had mosaic activities of peroxisomal protein import machinery and residual activities of peroxisomal functions, including dihydroxyacetone phosphate acyltransferase and β oxidation of very long chain fatty acids. The nonsense mutation severely affects these peroxisomal functions as well as the protein import. These data suggest that allelic heterogeneity of the *PEX* gene affects the peroxisomal protein import and functions and regulates the clinical severity in PBD. (*J Med Genet* 1999;36:779-781)

Keywords: Zellweger syndrome; infantile Refsum disease; *PEX* gene; mosaic

Zellweger syndrome (ZS, McKusick 214100) is the most severe phenotype of peroxisome biogenesis disorders (PBD) and is characterised by the absence of peroxisomes and by multiple metabolic deficiencies, including very long chain fatty acids. Other metabolites accumulate and plasmalogens are deficient. Patients with ZS have a typical face, severe weakness and hypotonia, feeding difficulty, eye abnormalities, and liver dysfunction at birth and death usually occurs within the first year of life. Infantile Refsum disease (IRD, McKusick 266510), the mildest phenotype of PBD, is also characterised by absent peroxisomes and biochemical abnormalities. Patients with IRD

show mental retardation, minor facial dysmorphism, and retinitis pigmentosa. Many survive over 10 years old and can walk, as compared with death within a few months in ZS patients, even within the same complementation group. Why IRD patients can survive for over 10 years without peroxisomes has not been elucidated. The milder phenotypes may be because the defective gene products retain partial function.¹ Nine *PEX* genes have been identified as pathogenic genes for PBD and almost all of these products are involved in the uptake of peroxisomal matrix proteins.² We report here expression studies of mutated *PEX2* genes from patients with ZS and IRD from PBD group F (group 10 at the Kennedy Krieger Institute), using the *PEX2* deficient CHO mutant. The relationship between the phenotypes, biochemical features, protein import, and gene products at the molecular level was clarified.

Patients F-01 and F-04 had the phenotype of classical ZS and died of heart failure at 8 and 3 months, respectively. Mutation analysis of the *PEX2* gene in these patients showed a homozygous mutation of R119X in F-01³ and a heterozygous mutation of R119X and R125X in F-04.⁴ Patient F-05 was a Welsh female and presented at 1 year with delayed milestones and extreme joint laxity. Later, her retinitis pigmentosa was manifested and biochemical investigations at this stage showed accumulation of very long chain fatty acids and phytanic acid and an abnormal bile acid profile. The activities of dihydroxyacetone phosphate acyltransferase (DHAP-AT) were reduced in platelets and fibroblasts and a diagnosis of infantile Refsum disease was made. She died at 3 years of age following an episode of bronchiolitis. Transfectants with human *PEX2* cDNA³ into the F-05 fibroblasts showed peroxisomes (fig 1D), which meant that F-05 was affected by the *PEX2* gene deficiency. Mutation analysis showed a heterozygous mutation of R119X and E55K in the *PEX2* gene.⁵

Immunofluorescence studies showed no catalase containing particle peroxisome in any of three patients' fibroblasts (fig 1B, C), in contrast with numerous particles in control fibroblasts (fig 1A). In the study of peroxisomal ghost membrane protein, using anti-human 70 kDa peroxisomal integral membrane protein (PMP70) antibody, slightly fewer particles were seen in F-05 fibroblasts (fig 1H) and

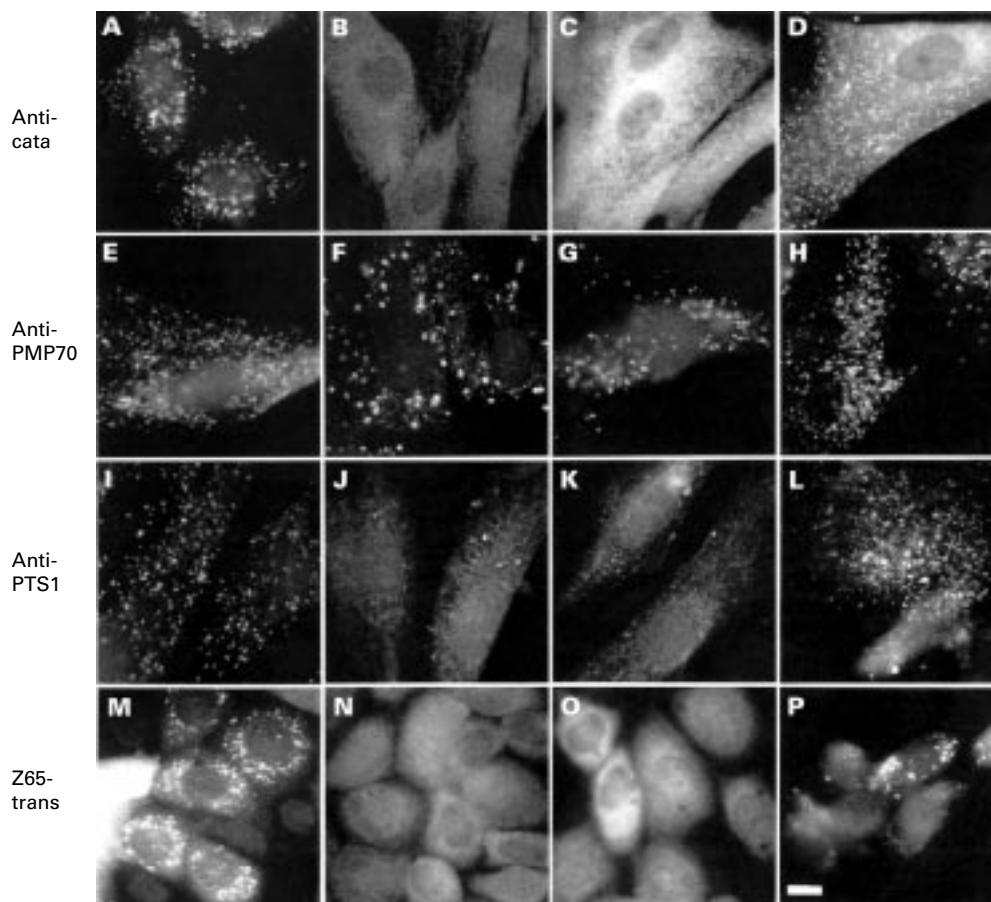


Figure 1 Immunofluorescence staining by antihuman catalase antibody (A-D), anti-human PMP70 antibody (E-H), anti-PTS1 antibody (I-L, P), and anti-rat catalase antibody (M-O). (A, E, I) Fibroblasts from control. (B, F, J) Fibroblasts from F-01. (G, K) Fibroblasts from F-04. (C, H, L) Fibroblasts from F-05. (D) F-05 fibroblasts transfected with PEX2-wild cDNA. (M) Z65 transfected with PEX2-wild cDNA. (N) Z65 transfected with PEX2-R125X cDNA. (O, P) Z65 transfected with PEX2-E55K cDNA. Bar=15 μ m.

larger and still fewer particles were seen in F-01 and F-04 fibroblasts (fig 1F, G), as compared with findings in control fibroblasts (fig 1E). Further immunofluorescence studies, using anti-peroxisome targeting signal (PTS) 1 antibody, showed hardly any particles in F-01 and F-04 fibroblasts (fig 1J, K), in contrast with numerous particles in control fibroblasts (fig 1I). A somewhat different picture was found in F-05 fibroblasts, in that some cells showed hardly any PTS1 containing particles and others showed numerous particles with PTS1 (fig 1L) (table 1), which means the PTS1 import machinery in F-05 fibroblasts displayed mosaicism.

On biochemical analysis, DHAP-AT activity, a membrane bound peroxisomal enzyme involved in the first step of biogenesis of plasmalogen, was severely diminished in fibroblasts from patients F-01 and F-04 with ZS (6.5% and 10.6% of control fibroblasts), determined as described previously.⁶ On the other hand, fibroblasts from F-05 with IRD had residual DHAP-AT activity (26.1% of the control) (table 1). Concerning β oxidation activity of lignoceric acid relative to that of palmitic acid (C24:0/C16:0), IRD fibroblasts also had residual activity (19.2% of the control), as compared to findings in fibroblasts from patients F-01 and F-04 with ZS (4.4% and

Table 1 Biochemical and morphological abnormalities which occur with PEX2 gene mutation

	DHAP-AT*		C24:0/C16:0		Cata \dagger	PMP70 \dagger	PTS1 \dagger
	No	%	No	%			
(1) Control	2.09	100	0.53	100	+++	+++	+++
(2) F-01 (ZS; R119X/R119X)	0.22	10.6	0.02	4.4	-	+	Rare
(3) F-04 (ZS; R119X/R125X)	0.14	6.5	0.03	5.9	-	+	Rare
(4) F-05 (IRD; R119X/E55K)	0.55	26.1	0.10	19.2	-	++	Rare -+++ \ddagger
(5) CHO-K1	2.02		14.3		+++		
(6) Z65	0.07		0.92		-		
(7) Z65/PEX2-wild	0.45	100	8.87	100	++		
(8) Z65/PEX2-R125X	0.10	21.9	0.56	6.3	-		
(9) Z65/PEX2-E55K	0.20	44.2	5.13	57.8	-		

*nmol/mg/120 min.

\dagger Immunofluorescent punctates with use of anti-human catalase (Cata), anti-PMP70, and anti-PTS1 antibodies (fig 1).

\ddagger Some fibroblasts showed hardly any PTS1 containing particles, others numerous particles with PTS1.

DHAP-AT activities and C24:0/C16:0 are the mean of duplicate experiments.

5.9% of the control), determined as described previously⁷ (table 1).

Next, we investigated the relationship between gene mutation, protein import machinery, and the residual activities using gene transfection. The *PEX2*-wild, *PEX2*-R125X, and *PEX2*-E55K gene sequences subcloned into the expression vector pUcD2SRαMCS⁸ were transfected to *PEX2* deficient CHO cell mutants, Z65,⁹ and stable transformants were produced. The transformants of *PEX2*-wild showed a punctate distribution of catalase, whereas no catalase positive granules were observed in transformants of either *PEX2*-R125X or *PEX2*-E55K (fig 1M-O). Immunofluorescence studies, using anti-PTS1 antibody, showed a mosaic distribution of PTS1 positive particles in the single colony of Z65 transformants with *PEX2*-E55K (fig 1P), in contrast to no particles in the transformants with *PEX2*-R125X (data not shown). Biochemical analysis showed that the transformant of *PEX2*-E55K had residual activities in DHAP-AT (44.2% of the *PEX2*-wild transformant) and C24:0/C16:0 (57.8% of the *PEX2*-wild transformant), as compared to the transformant of *PEX2*-R125X with severely diminished activities, in 21.9% and 6.3%, respectively, in duplicate experiments (table 1). Thus, the E55K mutation of the *PEX2* gene retains mosaic activities of peroxisomal import machinery and partial peroxisomal function and regulates the mild phenotype of IRD. On the other hand, the R125X mutation has no peroxisomes functionally and morphologically and regulates the severe phenotype of ZS.

Although there are 12 complementation groups in PBD,¹⁰ correlation of clinical heterogeneity, biochemical abnormalities, and genetic mutation in groups of PBD was not clear. Our present observations show that the difference between ZS and IRD is the result of different allelic heterogeneity, at least in the *PEX2* gene, and this heterogeneity plays an important role in peroxisomal import, determining the phenotypes of PBD, and the biochemical profiles. Even in group E (group 1 at the Kennedy Krieger Institute) resulting from *PEX1* deficiency, there were residual activities of both DHAP-AT and lignoceric acid oxidation in several patients with IRD.

The G843D mutation in *PEX1* which was thought to correspond to the IRD phenotype¹¹ also had residual activity of lignoceric acid oxidation (data not shown). We have noted that the clinical severity in PBD corresponds to temperature sensitive (TS) mutations, including the E55K mutation in *PEX2*⁵ and the TS phenomenon in F-05 may affect her death following infection. Thus, there are several factors which affect the severity and prognosis of PBD, and we are investigating the process by which the defective gene products retaining unstable peroxisomal import machinery result in the clinical heterogeneity and the TS phenomenon.

We thank K Hori for technical assistance, M Ohara for assistance with language, and T Imanaka for kindly providing the human PMP70 antibody. This study was supported in part by a Grant-in-Aid for Scientific Research (10670721) from the Ministry of Education, Science, Sports and Culture of Japan.

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