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The 22-kD Peroxisomal Integral Membrane Protein in Zellweger Syndrome—Presence, Abundance, and Association with a Peroxisomal Thiolase Precursor Protein

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ABSTRACT. The primary genetic defect of Zellweger syndrome may be related to defective synthesis or impaired import of peroxisomal proteins. We analyzed the presence and measured the abundance of the 22-kD peroxisomal integral membrane protein (PMP) in patients with Zellweger syndrome. We determined the intracellular localization of the 22-kD PMP and compared it with the localization of a peroxisomal 44-kD thiolase precursor protein. The 22-kD PMP was quantified by immunoblot analyses in liver tissue (n = 7 patients). Immunoblot signals were evaluated using transmission photometry. The intracellular localization of the 22-kD PMP and the peroxisomal 44-kD thiolase precursor protein were determined by immunoblot analyses on fibroblast subcellular fractions prepared by Nycodenz (n = 5 patients) or sucrose density gradient centrifugation (n = 2 patients). The 22-kD PMP was present and associated with membrane fractions in all patients. Its abundance varied in patients as compared with normal human liver controls. The 22-kD PMP was located in subcellular membrane fractions having a lower density than normal peroxisomes or mitochondria. Using two different gradient techniques, the 22-kD PMP and the peroxisomal 44-kD thiolase precursor protein were found in the same low-density gradient fractions. These results suggest that in Zellweger syndrome peroxisome-like elements containing both the 22-kD PMP and a 44-kD thiolase precursor protein are formed. Globally defective synthesis or import of peroxisomal proteins is therefore unlikely to be the primary genetic defect in the patients we studied. (*Pediatr Res* 29: 141–146, 1991)

Abbreviations

PMP, peroxisomal integral membrane protein
SISP, signal intensity surface product

The cerebrohepatorenal syndrome of Zellweger (McKusick 21410) is an autosomal recessive disorder of multiple congenital anomalies (1, 2). It is characterized by a reduced number or absence of peroxisomes (3) and regarded as the prototype of peroxisomal deficiency disorders. Multiple peroxisomal biochemical processes are defective, including β-oxidation of very long chain fatty acids (4), phytanic acid oxidation (5, 6), pipecolic acid oxidation (7), plasmalogen biosynthesis (8), and bile acid metabolism (9).

Normally, peroxisomal proteins are synthesized in the cytosol on free polyribosomes (10, 11). Peroxisomes are formed by the import of newly synthesized proteins into preexisting peroxisomes, followed by growth and division (10–14). The integral membrane proteins, especially the 22-kD PMP, are speculated to have important roles in maintaining the integrity of the peroxisomal membrane and in transporting peroxisomal matrix components (10, 11, 15). The primary defect in Zellweger syndrome is unknown but may be related to defective synthesis or impaired import of peroxisomal proteins (12, 16–19). Previous studies have shown that in patients with Zellweger syndrome the 22-kD PMP is absent (20), present in normal amounts (18, 21), or varies from absent to markedly reduced (22, 23). Santos *et al.* (18) found the 22-kD PMP to be associated with "largely empty" aberrant membrane vesicles. To test current hypotheses for the pathogenesis of Zellweger syndrome, we analyzed the presence and abundance of the 22-kD PMP and determined its intracellular localization in comparison with the localization of a 44-kD precursor protein of the peroxisomal matrix protein, 3-ketoacyl-CoA thiolase.

MATERIALS AND METHODS

Patients and controls. Immunoblot analyses of autopsy liver specimens were performed in seven patients with Zellweger syndrome (AH, AW, CS, DM, FS, NM, and PW), three normal human controls, and two animal controls (rat and monkey). Immunoblot analyses of skin fibroblasts were carried out in five patients with Zellweger syndrome (BB, BH, ES, NS, and EW) and two normal human controls. Data on peroxisomal metabolite levels and clinical characteristics were obtained from the patients' medical records. All patients showed the features of "classical" Zellweger syndrome (1, 2). Fibroblast or other living cultures for complementation group analysis (24, 25) were not available from patients on whom liver analyses had been performed. Patients CS and FS have been included in previous reports (26).

Preparation of liver samples. Liver specimens were obtained at autopsy and frozen at −70°C until use. Pieces (0.5–1.5 g) of thawed human liver and fresh monkey and rat liver were minced in three volumes of buffer [100 mM KCl, 50 mM Tris (Cl^-), pH 8.33, 1 mM EGTA], homogenized by four to five passes using a Potter-Elvehjem homogenizer, and centrifuged for 30 s at 3900 × g. The supernatants were used to prepare total membranes.

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Subcellular fractionation of cultured fibroblasts. Skin fibroblasts were maintained in culture as previously described (27). Cells were grown in 850 cm^2 roller bottles (four to six bottles per experiment). Confluent cells were washed with PBS, harvested by trypsinization, and collected by centrifugation for 10 min at 1000 × *g*. The cell pellet was washed twice with PBS and once with ice-cold homogenization buffer [0.25 M sucrose, 1 mM Tris (Cl^-), pH 7.5, 0.1 mM EDTA]. All subsequent steps were performed at 4°C. Cells were resuspended in homogenization buffer and homogenized in a precision ball-bearing homogenizer (28) using 11 passes. Homogenates were loaded directly either onto a linear Nycodenz (Accurate Chemical & Scientific Corp., Westbury, NY) gradient of increasing Nycodenz concentration (15 to 40%) and decreasing sucrose concentration (8.6 to 0%) or onto a linear sucrose gradient of increasing sucrose concentration (12 to 60%). All gradient solutions contained 1 mM Tris (Cl^-), pH 7.5, and 0.1 mM EDTA. A cushion of Maxidens (Accurate Chemical & Scientific Corp.) was at the bottom of the tube. Gradients were centrifuged in a vertical ultracentrifuge rotor (Beckman VTi 65.1; Beckman Instruments, Inc., Palo Alto, CA) for 35 min at 74 300 × *g*. Fractions of 750 μL were collected from the bottom of the tube and aliquots of each fraction assayed for marker enzymes. Catalase activity was measured by the method of Peters *et al.* (29) and succinate dehydrogenase activity by the method of Pennington (30). Four hundred-eighty μL of each fraction were used to prepare membranes.

Isolation of membranes. Total membranes were purified from liver postnuclear supernatant fractions and fibroblast density gradient fractions by a carbonate procedure (31). In the case of fibroblast fractions, red blood cell ghosts (20 μg of protein/fraction) were added as carrier before sodium carbonate extraction. The use of carrier did not result in nonspecific binding of antisera in the subsequent immunoblots. For liver membrane fractions, the protein content was determined by the method of Lowry (32) with BSA as standard. Fifty μg of total liver membrane protein and the total fibroblast membrane pellet were used for immunoblot analyses.

Immunoblot analyses. Antibodies against purified rat liver peroxisomal 3-ketoacyl-CoA thiolase (β-ketothiolase), 22-kD PMP, and mitochondrial 3-hydroxyacyl-CoA dehydrogenase were the gift of Dr. Takashi Hashimoto (Shinshu University, Japan). Purified protein A (P-6650; Sigma Chemical Co., St. Louis, MO) was labeled with ^{125}I after the procedure of Greenwood *et al.* (33). SDS-PAGE and immunoblotting were carried out as previously described (34).

Fibroblast subcellular fractions (240 μL/fraction) for immunoblot analysis of peroxisomal β-ketothiolase were kept in ice-cold 10% trichloroacetic acid at 4°C overnight. Precipitates were collected by centrifugation for 10 min at 2000 × *g*, washed once with diethyl ether, solubilized in sample buffer [67.5 mM Tris (Cl^-), pH 6.8, 1% SDS, 100 mM DTT, 0.005% bromophenol blue, and 10% glycerol], sonicated, and boiled for 20 min.

Membrane fractions for immunoblot analysis of 22-kD PMP were solubilized in sample buffer, sonicated, and boiled for 20 min. Membrane proteins were separated by SDS-PAGE and transblotted to nitrocellulose paper under conditions optimized for hydrophobic proteins (35).

The blot signal was measured by transmission photometry using an image analysis system for quantitative autoradiography (36). The product of the signal area and signal intensity is referred to as SISP. The SISP is used in this context as a semiquantitative measurement of the 22-kD PMP, peroxisomal 44-kD thiolase precursor protein, and mitochondrial 3-hydroxyacyl-CoA dehydrogenase abundance, respectively. Its units are given in mm^2. Regression analyses revealed a linear correlation between the amount of rat liver membrane protein (10 to 100 μg) and the SISP of the 22-kD PMP immunoblot signal ($n = 3$, $r = 0.99$, $p = 0.018$).

Biochemical assays of peroxisomal function. Concentrations of very long chain fatty acids in plasma and cultured fibroblasts were measured by gas liquid chromatography-mass spectrometry (27, 37).

Statistics. The correlation between two parameters was analyzed with the rank correlation test (38). All p values of 0.05 or less were considered to be statistically significant.

RESULTS

Abundance of 22-kD PMP in Zellweger syndrome. Results of immunoblot analyses in total liver membranes using antibodies against 22-kD PMP are given in Figure 1. The 22-kD PMP was present in liver from all seven patients with Zellweger syndrome, as well as in three human and two animal controls. The apparent molecular weight of the 22-kD PMP in patients with Zellweger syndrome did not differ from controls. The SISP of the blot signal, namely the abundance of the 22-kD PMP, showed a remarkable variability in patients with Zellweger syndrome. The SISP ranged from 943 to 6580 mm^2 (4075 ± 2036 mm^2, mean ± SD). In contrast, the SISP of the three human liver controls was relatively equal, ranging from 5886 to 6196 (6029 ± 156 mm^2, mean ± SD). In patients with Zellweger syndrome and in controls, the 22-kD PMP was not extractable with sodium carbonate, indicating that the 22-kD PMP is embedded in membranes.

Relationship between 22-kD PMP abundance and age at death, histologic characteristics and biochemical parameters in patients with Zellweger syndrome. To determine whether or not the observed variable 22-kD abundance in patients with Zellweger syndrome was due to different degrees of liver degeneration before death, the 22-kD PMP SISP was correlated with the age at death, liver histology, and hepatic catalase activity. Statistical analysis revealed no correlation between the 22-kD PMP SISP and the age at death ($n = 7$, $r = -0.5$, $p > 0.5$). High 22-kD PMP SISP values were found in one severely affected patient who survived 94 d (patient PW) and in a patient who died as

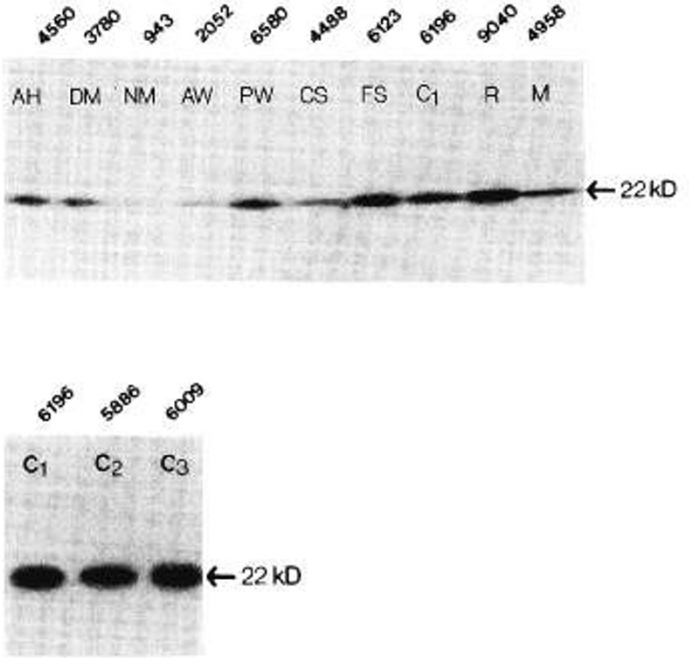

Fig. 1. Presence and abundance of the 22-kD PMP in liver from patients with Zellweger syndrome (*AH, DM, NM, AW, PW, CS, FS*), human controls (C_1, C_2, C_3), rat (*R*), and monkey (*M*). Fifty μg of total membrane protein prepared from postmortem liver specimens were analyzed by SDS-PAGE and immunoblot analysis using antibodies against purified rat liver 22-kD PMP. The numbers represent the SISP in mm^2. The SISP is the product of the autoradiographic signal intensity and the signal area. The SISP was variable in Zellweger syndrome and relatively equal in controls.

soon as 14 d after birth (patient FS). The results are also not indicative of a positive correlation between 22-kD PMP SISP and hepatic fibrosis. For example, patient PW with liver fibrosis had a relatively high 22-kD PMP SISP, whereas patient NM with no evidence for liver fibrosis had a relatively low 22-kD PMP SISP. In addition, the total soluble hepatic catalase activity did not correlate with the 22-kD PMP SISP ($n = 7$, $r = -0.4$, $p > 0.05$).

To exclude postmortem liver damage, the SISP of the 44-kD peroxisomal thiolase precursor protein and mitochondrial 3-hydroxyacyl-CoA dehydrogenase were analyzed in the same liver samples used for the PMP analyses. Although the SISP for 22-kD PMP in patients AH and AW differed by a factor of 2 (Fig. 1), there was no such difference between AH and AW for the 44-kD peroxisomal thiolase precursor protein (factor: 1; Fig. 2*A*) or mitochondrial hydroxyacyl-CoA dehydrogenase (factor: 1; Fig. 2*A*). The same was true for patients DM and NM. There was a 4-fold difference in the SISP of 22-kD PMP (Fig. 1), but no difference in the SISP of thiolase precursor (factor: 1; Fig. 2*B*) and hydroxyacyl-CoA dehydrogenase (factor: 1; Fig. 2*B*).

Relationship between 22-kD PMP abundance and impairment of peroxisomal functions in Zellweger syndrome. To determine if the 22-kD PMP abundance correlates with the degree of impairment of peroxisomal metabolic functions, plasma concentrations of very long chain fatty acids were measured. A statistically significant correlation could not be shown (C26:0: $n = 4$, $r = -0.4$, $p > 0.05$; C26:0/C22:0: $n = 4$, $r = -0.4$, $p > 0.05$; C24:0/C22:0: $n = 4$, $r = 0.4$, $p > 0.05$). For example, patients NM and PW had equally elevated plasma concentrations of very long chain fatty acids but a 7-fold difference in the SISP of the 22-kD PMP.

Subcellular localization of the 22-kD PMP in Zellweger syndrome. Results of immunoblot analyses of subcellular membrane fractions as prepared by Nycodenz gradient centrifugation and carbonate extraction are shown in Figure 3. In control fibroblasts, the 22-kD PMP was recovered in fractions 2 and 3 (Fig. 3*A*). These fractions were enriched in a portion of catalase activity (Fig. 4*A*), which is associated with intact peroxisomes, whereas fractions 7 to 9 (Fig. 3*A*) were enriched in succinate dehydrogenase activity (Fig. 4*A*), a marker for mitochondria. In contrast, in Zellweger syndrome the 22-kD PMP was located in fractions 11 and 12 (Fig. 3*B*). These fractions had a lower density than normal

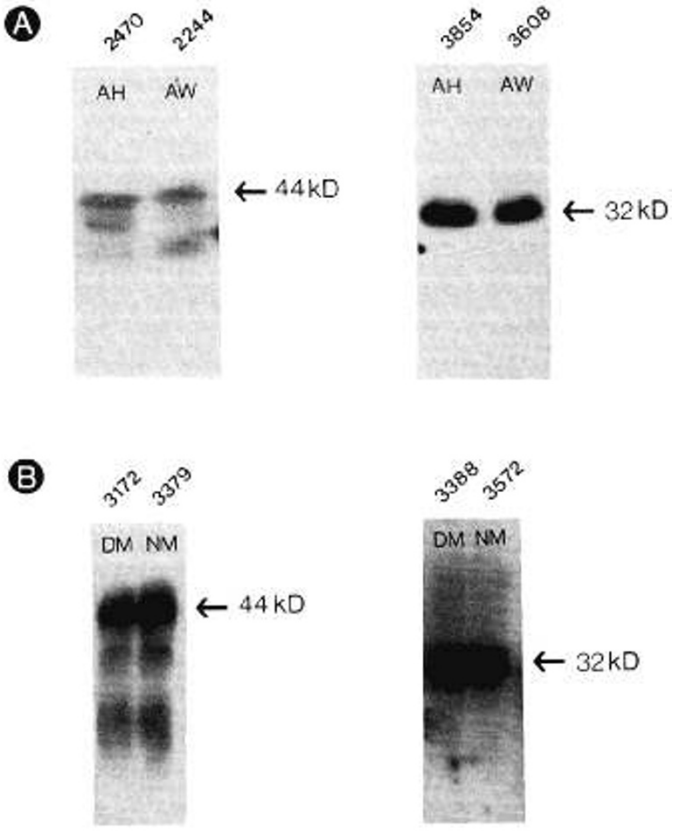

Fig. 2. Abundance of the 44-kD peroxisomal thiolase precursor protein and mitochondrial 3-hydroxyacyl-CoA dehydrogenase (32 kD) in patients with Zellweger syndrome (*A*: AH, AW; *B*: DM, NM). The same postmortem liver specimens as for the 22-kD PMP analyses (Fig. 1) were investigated. Analyses were performed by SDS-PAGE and immunoblot analysis using 50 μg of liver homogenate and antibodies against the purified rat liver enzymes. The immunoblot signals were quantified by transmission photometry. The numbers represent the SISP in mm^2. The SISP is the product of the autoradiographic signal intensity and the signal area. The SISP of the enzymes in patients AH and AW (*A*) as well as in patients DM and NM (*B*) were of a similar order of magnitude.

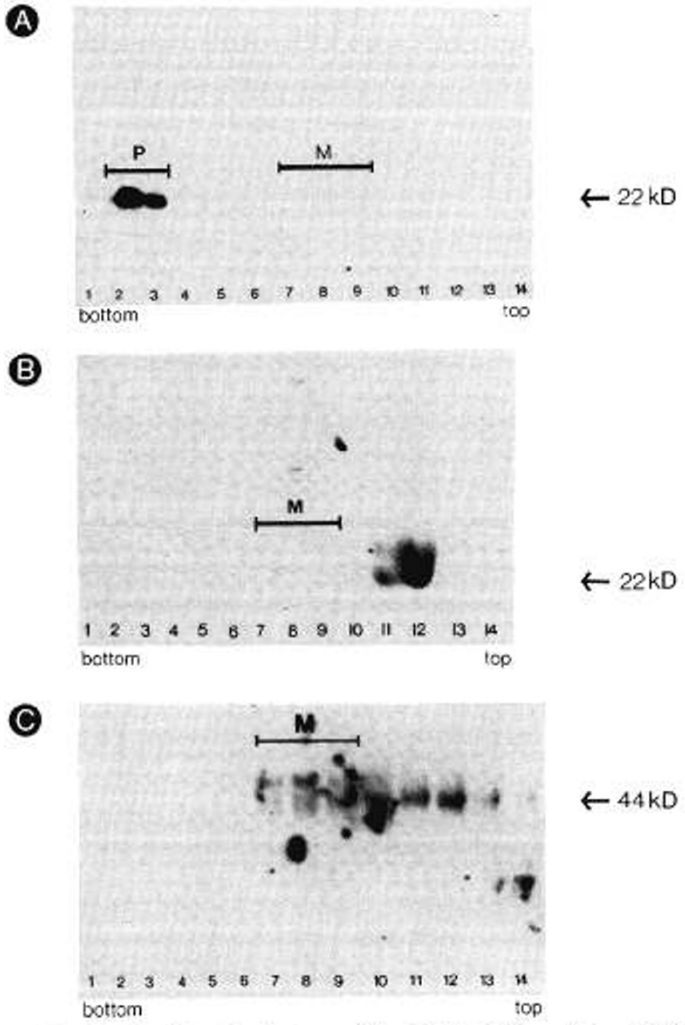

Fig. 3. Subcellular localization of the 22-kD PMP and the 44-kD peroxisomal thiolase precursor protein in Zellweger syndrome—immunoblot analyses of Nycodenz gradients. Fibroblast homogenates from a human control (*A*) and patient BH with Zellweger syndrome (*B* and *C*) were fractionated by Nycodenz gradient technique. Fractions 1 to 14 in *B* and *C* are from the same gradient. Fibroblast membrane fractions were taken for the 22-kD PMP analysis and total fibroblast fractions for the 44-kD thiolase analysis. The samples were subjected to SDS-PAGE and immunoblot analysis using antibodies against the purified rat liver proteins. *P* and *M* indicate positions of the peroxisomal and mitochondrial markers, respectively. In control fibroblasts, the 22-kD PMP was located in the peroxisomal fractions (2 and 3). In patient BH, the 22-kD PMP and the 44-kD peroxisomal thiolase precursor protein were found in the same nonperoxisomal fractions (11 and 12)

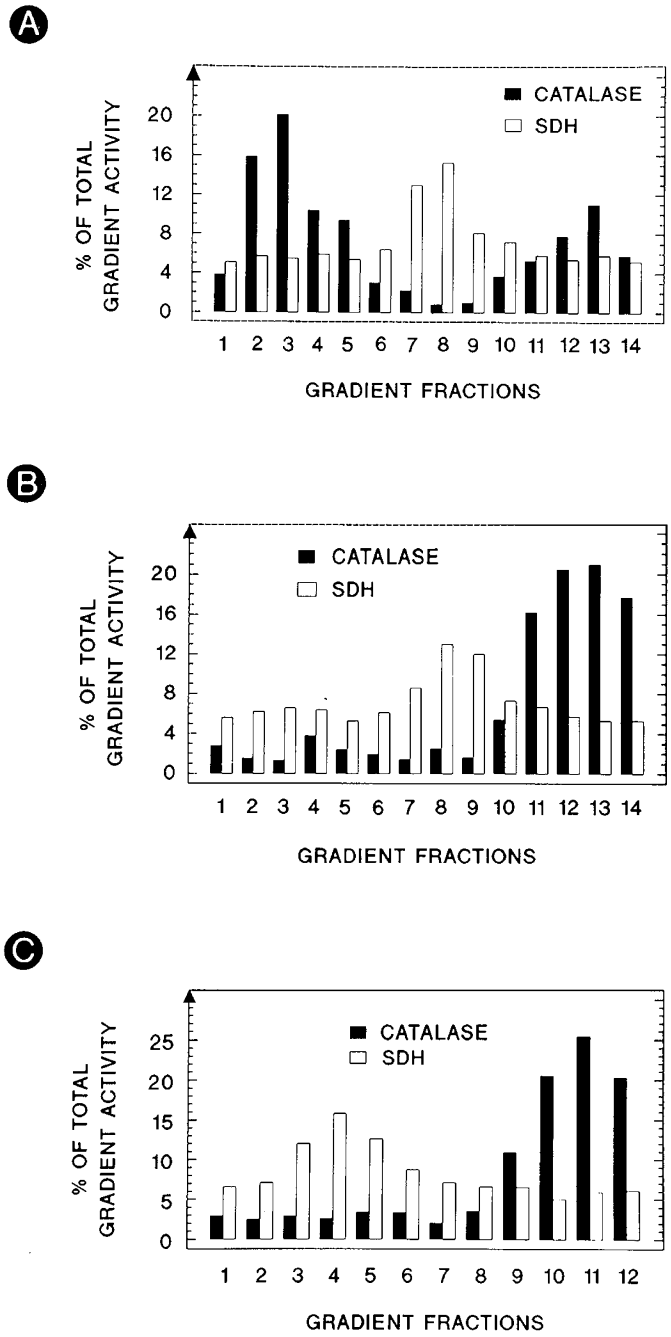

Fig. 4. Subcellular distribution of total enzyme activity on Nycodenz gradients (*A*, human control; *B*, patient BH) and sucrose gradient (*C*, patient BH). Fractions were analyzed for activity of the peroxisomal marker enzyme catalase and the mitochondrial marker enzyme succinate dehydrogenase. Units of enzyme activity equal percent of total gradient activity recovered in each faction. Fractions enriched in catalase activity were well separated from those enriched in succinate dehydrogenase activity.

peroxisomes and mitochondria. In Zellweger syndrome, the majority of the activity of the peroxisomal marker catalase was found at the top of the gradient (fractions 11 to 14; Fig. 4*B*), suggesting that normal peroxisomes are missing. As in normal fibroblasts, fractions 7 to 9 for Zellweger syndrome fibroblasts (Fig. 3*B*) were enriched in activity of the mitochondrial marker, succinate dehydrogenase (Fig. 4*B*).

Presence of 22-kD PMP and thiolase precursor protein in same density fractions in Zellweger syndrome. To determine whether in Zellweger fibroblasts the 22-kD PMP associated membranous elements include intraperoxisomal proteins, the localization of the 22-kD PMP and the peroxisomal 44-kD thiolase precursor protein was analyzed in Nycodenz gradients as well as in sucrose gradients. In Nycodenz gradients, the 44-kD thiolase precursor was located in the same subcellular fractions (11 and 12) as the 22-kD PMP (Fig. 3*B* and *C*). The likely association of the 22-kD PMP with the peroxisomal 44-kD thiolase precursor protein within the same membranous elements was also supported by their comigration by sedimentation transport in sucrose density gradients (Fig. 5, fractions 10 to 12).

DISCUSSION

Although the primary genetic defect in Zellweger syndrome is unknown, defective synthesis and impaired import of peroxisomal proteins into peroxisomes are often proposed as possible primary defects (16–18). However, the results of our study and others (18, 21–23, 26, 39) show that in Zellweger syndrome the 22-kD PMP is not absent, but present in variable amounts and associated with membranous elements. We have found that the 22-kD PMP is localized in exactly the same subcellular fraction as a 44-kD precursor protein of the peroxisomal matrix protein 3-ketoacyl-CoA thiolase.

The variable abundances of the 22-kD PMP in Zellweger syndrome did not appear to be caused by different degrees of premortem liver degeneration, inasmuch as we found no correlation of the 22-kD PMP abundance to the age at death, liver

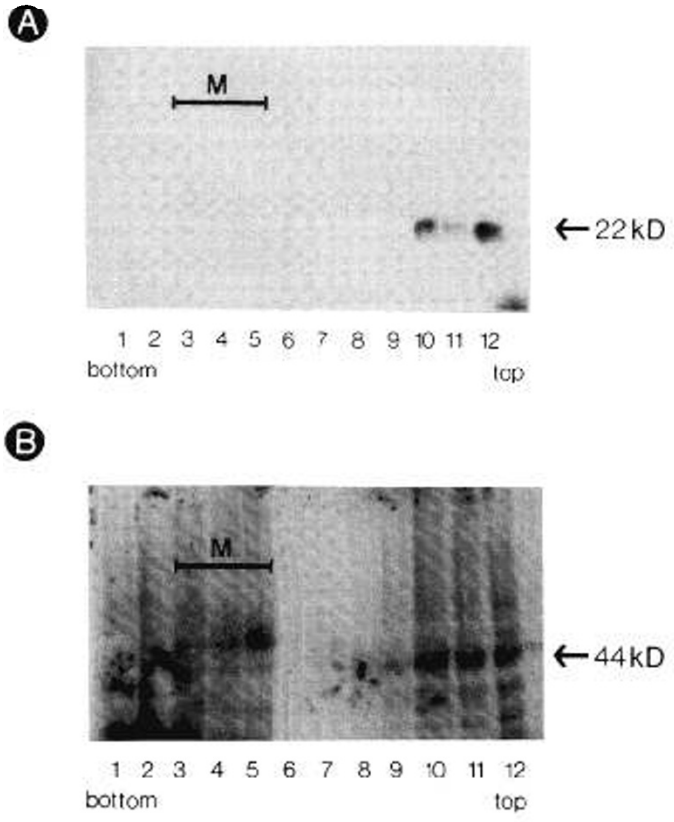

Fig. 5. Subcellular localization of the 22-kD PMP and the 44-kD peroxisomal thiolase precursor protein in Zellweger syndrome—immunoblot analyses of a sucrose gradient. Fibroblast homogenates from patient BH with Zellweger syndrome were fractionated by sucrose gradient technique. Fractions 1 to 12 in *A* and *B* are from the same sucrose gradient. Fibroblast membrane fractions were taken for the 22-kD PMP analysis and total fibroblast fractions for the 44-kD thiolase analysis. The samples were subjected to SDS-PAGE and immunoblot analysis using antibodies against the purified rat liver proteins. *M* indicates the position of the mitochondrial marker. The 22-kD PMP and the 44-kD thiolase were found in the same gradient fractions (10 to 12) and support the findings obtained by Nycodenz gradient technique (Fig. 3*B* and *C*).

histology, or liver catalase activity. Furthermore, using the same postmortem liver samples, we found the abundance of peroxisomal thiolase precursor and mitochondrial hydroxyacyl-CoA dehydrogenase to be relatively equal. The variable 22-kD PMP abundance in Zellweger syndrome may be due to different genetic defects. Somatic cell fusion studies have shown that patients with Zellweger syndrome are distributed among several different complementation groups (24, 25). Apparently, mutations in several different genes lead to a similar phenotype. However, the different 22-kD PMP abundances in patients AW and PW, who are siblings, suggest that nongenetic factors may affect the 22-kD PMP abundance in the membranous fractions.

Because the 22-kD PMP appears to be present in membranous structures, the synthesis and import mechanisms of the 22-kD PMP may be intact in Zellweger syndrome. The possibility that the 22-kD PMP is imported into some other intracellular oreganelle membranes due to the absence of peroxisomes has already been excluded by Santos *et al.* (18, 39). They demonstrated by immunoelectron microscopy and immunofluorescence that the peroxisomal integral membrane proteins were very specifically localized in rare, unusual membrane vesicles.

The 22-kD PMP is unique to peroxisomes. It is speculated to have important roles in maintaining the integrity of the peroxisomal membrane and in transporting peroxisomal matrix components (10, 11, 13–15). The 22-kD PMP was present in all our patients. There was no correlation between the 22-kD PMP abundance in the patients' liver and the degree of metabolic impairment of peroxisomal functions or the clinical severity of disease. However, in this study, the abundance of the 22-kD PMP was measured by immunoreactivity. It remains to be determined whether the structure and function of the 22-kD PMP are deficient in Zellweger syndrome.

Our results reveal that in Zellweger syndrome, the 22-kD PMP associated membranous bodies may not be "largely empty" membrane "ghosts" (18, 39) but also contain peroxisomal matrix elements. The 22-kD PMP associated membrane fraction and a peroxisomal thiolase precursor protein were consistently localized in the same low-density subcellular fractions, as already suspected by Balfe *et al.* (40). These results argue against a general import failure of peroxisomal matrix proteins as the primary defect in Zellweger syndrome. At least one matrix protein, the peroxisomal thiolase precursor, may be able to reach the peroxisome-like organelle.

Peroxisomal thiolase is synthesized in the cytosol, imported into peroxisomes in a precursor form, and subsequently processed in the peroxisomal matrix (41 42). Our results, consistent with those of previous studies (40, 43), suggest that the processing of the enzyme precursor is defective in Zellweger syndrome. Our results further suggest that in Zellweger syndrome peroxisome-like elements containing the 22-kD PMP and a 44-kD precursor protein of the β-oxidation enzyme thiolase are formed. Globally defective synthesis or import of peroxisomal proteins are therefore unlikely in the patients we studied. The primary defect preventing full development of peroxisomes remains to be determined.

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REFERENCES

1. Bowen P, Lee CNS, Zellweger H, Lindenberg R 1964 A familial syndrome of multiple congenital defects. Bull Johns Hopkins Hosp 114:402–414
2. Kelley RI 1983 Review: the cerebrohepatorenal syndrome of Zellweger, morphologic and metabolic aspects. Am J Med Genet 16:503–517
3. Goldfischer S, Moore CL, Johnson AB, Spiro AJ, Valsamis MP, Wisniewski HK, Ritch RH, Norton WT, Rapin I, Gartner lM 1973 Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. Science 182:62–64
4. Moser AE, Singh I, Brown FR, Solish GI, Kelley RI, Benke PJ, Moser HW 1984 The cerebro-hepato-renal (Zellweger) syndrome: increased levels and impaired degradation of very long chain fatty acids and their use in prenatal diagnosis. N Engl J Med 310:1141–1146
5. Poulos A, Sharp P, Whiting M 1984 Infantile Refsum's disease (phytanic acid storage disease): a varient of Zellweger syndrome? Clin Genet 26:579–586
6. Stokke O, Skrede S, Ek J, Björkhem I 1984 Refsum's disease, adrenoleucodystrophy, and the Zellweger syndrome. Scand J Clin Lab Invest 44:463–464
7. Danks DM, Tippett P, Adams C, Campbell P 1975 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 86:382–387
8. Heymans HSA, Schutgens RBH, Tan R, van den Bosch H, Borst P 1983 Severe plasmalogen deficiency in tissues of infants without peroxisomes (Zellweger syndrome). Nature 306:69–70
9. Monnens L, Bakkeren J, Parmetier G, Jaussen G, van Haelst U, Trijbels F, Eyssen H 1980 Disturbances in bile acid metabolism of infants with the Zellweger (cerebro-hepato-renal) syndrome. Eur J Pediatr 133:31–35
10. Fujiki Y, Rachubinski RA, Lazarow PB 1984 Synthesis of a major integral membrane polypeptide of rat liver peroxisomes on free polysomes. Proc Natl Acad Sci USA 81:7127–7131
11. Lazarow PB, Fujiki Y 1985 Biogenesis of peroxisomes. Annu Rev Cell Biol 1:489–530
12. Borst P 1986 How proteins get into microbodies (peroxisomes, glyoxysomes, glycosomes). Biochim Biophys Acta 866:179–203
13. Köster A, Heisig M, Heinrich PC, Just WW 1986 *In vitro* synthesis of peroxisomal membrane polypeptide. Biochem Biophys Res Commun 137:626–632
14. Suzuki Y, Orii T, Takiguchi M, Mori M, Hijikata M, Hashimoto T 1987 Biosynthesis of membrane polypeptides of rat liver peroxisomes. J Biochem 101:491–496
15. Van Veldhoven PP, Just WW, Mannaerts GP 1987 Permeability of the peroxisomal membrane to cofactors of beta-oxidation. J Biol Chem 262:4310–4318
16. Goldlfischer S, Collins J, Rapin I, Coltoff-Schiller B, Chang CH, Nigro M, Black VH, Javitt NB, Moser HW, Lazarow PB 1985 Peroxisomal defects in neonatal onset and X-linked adrenoleukodystrophy. Science 227:67–70
17. Lazarow PB, Black V, Shio H, Fujiki Y, Hajra AK, Datta NB, Bangura BS, Dancis J 1985 Zellweger syndrome: biochemical and morphological studies on two patients treated with clofibrate. Pediatr Res 19:1356–1364
18. Santos MJ, Imanaka T, Shio H, Lazarow PT 1988 Peroxisomal integral membrane proteins in control and Zellweger fibroblasts. J Biol Chem 263:10502–10509
19. Borst P 1983 Animal peroxisomes (microbodies), lipid biosynthesis and the Zellweger syndrome. Trends Biochem Sci 8:269–272
20. Aikawa J, Ishizawa S, Narisawa K, Tada K, Yokota S, Hashimoto T 1987 The abnormality of peroxisomal membrane proteins in Zellweger syndrome. J Inherited Metab Dis 10(suppl 2):211–213
21. Lazarow PB, Fujiki Y, Small GM, Watkins P, Moser H 1986 Presence of the peroxisomal 22-kDa integral membrane protein in the liver of a person lacking recognizable peroxisomes (Zellweger syndrome). Proc Natl Acad Sci USA 83:9193–9196
22. Suzuki Y, Shimozawa O, Oril T, Hashimoto T 1989 Major peroxisomal membrane polypeptides are synthesized in cultured skin fibroblasts from patients with Zellweger syndrome. Pediatr Res 26:150–153
23. Wiemer EAC, Brul S, Just WW, van Driel R, Brouwer-Kelder E, van den Berg M, Weijers P, Schutgens RBH, van den Bosch H, Schram A, Wanders RJA, Tager JM 1989 Presence of peroxisomal membrane proteins in liver and fibroblasts from patients with the Zellweger syndrome and related disorders: evidence for the existence of peroxisomal ghosts. Eur J Cell Biol 50:407–417
24. Brul S, Westerfield A, Stijland A, Wanders RJA, Schram AW, Heymans HSA, Schutgens RBH, van der Bosch H, Tager JM 1988 Genetic heterogeneity in the cerebro-hepato-renal (Zellweger) syndrome and other inherited disorders with a generalized impairment of peroxisomal functions: a study using complementation analysis. J Clin Invest 81:1702–1710
25. Roscher AA, Hoefler S, Hoefler G, Paschke E, Paltauf F, Moser A, Moser H 1989 Genetic and phenotypic heterogeneity in disorders of peroxisome biogenesis. A complementation study involving cell lines from 19 patients. Pediatr Res 26:67–71
26. Small GM, Santos MJ, Imanak T, Poulos A, Danks DM, Moser HW, Lazarow PB 1988 Peroxisomal integral membrane proteins in livers of patients with Zellweger syndrome, infantile Refsum's disease and X-linked adrenoleukodystrophy. J Inherited Metab Dis 11:358–371
27. Moser HW, Moser AB, Kawamura N, Murphy J, Suzuki K, Schaumburg H, Kishimoto Y, Milunsky A 1980 Adrenoleukodystrophy: elevated C26-fatty acid in cultured skin fibroblasts. Ann Neurol 7:542–549
28. Balch WE, Rothman JE 1985 Characterization of protein transport between successive compartments of the Golgi apparatus: asymmetric properties of donor and acceptor activities in a cell free system. Arch Biochem Biophys 240:413–425
29. Peters TJ, Muller M, de Duve C 1972 Lysosomes of the arterial wall. Isolation and subcellular fractionation of cells from normal rabbit aorta. J Exp Med 136:1117–1139
30. Pennington RJ 1961 Biochemistry of dystrophic muscle: mitochondrial succinate tetrazolium reductase and adenosine triphosphatase. Biochem J 80:649–654
31. Fujiki Y, Hubbard AL, Fowler S, Lazarow PB 1982 Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplasmic reticulum. J Cell Biol 93:97–102

32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
33. Greenwood FC, Hunter WM, Glover JS 1963 The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem J 89:114–123
34. Chen WW, Watkins PA, Hashimoto T, Moser HW 1987 Peroxisomal beta-oxidation enzymes in adrenoleukodystrophy. Proc Natl Acad Sci USA 84:1425–1428
35. Small GM, Imanaka T, Lazarow PB 1988 Immunoblotting of hydrophobic integral membrane proteins. Anal Biochem 169:405–409
36. Yonekura Y, Brill AB, Som P, Bennett GW, Fand I 1983 Quantitative autoradiography with radiopharmaceuticals, part 1: digital film analysis system by videodensitometry: concise communication. J Nucl Med 24:231–237
37. Moser HW, Moser AB, Frayer KK, Chen WW, Schulman JD, O'Neil PB, Kishimoto Y 1981 Adrenoleukodystrophy: increased plasma content of saturated very long chain fatty acids. Neurology 31:1241–1249
38. Sachs L 1984 Angewandte Statistik. Springer Verlag, Berlin, pp 309–312
39. Santos MJ, Imanaka T, Shio H, Small GM, Lazarow PB 1988 Peroxisomal membrane ghosts in Zellweger syndrome aberrant organelle assembly. Science 239:1536–1538
40. Balfe A, Hoefler G, Chen WW, Watkins PA 1990 Aberrant subcellular localization of peroxisomal 3-oxoacyl-CoA thiolase in the Zellweger syndrome and rhizomelic chondrodysplasia punctata. Pediatr Res 27:304–310
41. Miura S, Mori M, Takiguchi M, Tatibana M 1984 Biosynthesis and intracellular transport of enzymes of peroxisomal beta-oxidation. J Biol Chem 259:6397–6402
42. Fujiki Y, Rachubinski RA, Mortensen RM, Lazarow PB 1985 Synthesis of 3-ketoacyl-CoA thiolase of rat liver peroxisomes on free polyribosomes as a larger precursor. Induction of thiolase mRNA activity by clofibrate. Biochem J 226:697–704
43. Suzuki Y, Orii T, Mori M, Tatibana T, Hashimoto T 1986 Deficient activities and proteins of peroxisomal beta-oxidation enzymes in infants with Zellweger syndrome. Clin Chim Acta 156:191–196

Announcement

1991 Annual Meetings

The American Pediatric Society, The Society for Pediatric Research, and The Ambulatory Pediatric Association will hold their annual meetings April 28–May 2, 1991 at the New Orleans Hilton and Rivergate Convention Center, New Orleans, LA. *For further information, contact:* APS/SPR Association Headquarters, 2650 Yale Blvd., S.E., Suite 104, Albuquerque, NM 87106, (505)764-9099, FAX (505)842-8227 *or* Ambulatory Pediatric Association, 6728 Old McLean Village, McLean, VA 22101, (703)556-9222.