Letters to the Editor

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Genetic Basis of Peroxisome-Assembly Mutants of Humans, Chinese Hamster Ovary Cells, and Yeast: Identification of a New Complementation Group of Peroxisome-Biogenesis Disorders Apparently Lacking Peroxisomal-Membrane Ghosts

To the Editor:

Complementation analysis has been used to study the genetic basis of peroxisome-biogenesis disorders (PBDs; MIM 601539) at the Academic Medical Centre (AMC) in the Netherlands (Brul et al. 1988), Kennedy Krieger Institute (KKI) in the United States (Roscher et al. 1989), and Gifu University in Japan (Yajima et al. 1992). These initial studies led to identification of 15 complementation groups. When we standardized these complementation groupings to establish the true number of different complementation groups, we found a total of 9 independent groups (Shimozawa et al. 1993). In only 5 years, the molecular study of PBDs has advanced rapidly: (1) Several peroxisome-deficient mutants of Chinese hamster ovary (CHO) cells and yeast were isolated, and these mutants were used to clone *PEX* genes, by functional complementation, that are required for peroxisome assembly. (2) Five *PEX* genes involved in peroxisome biogenesis—*PEX1, -2, -5, -6,* and *-12*—have been identified as apparently responsible for PBD groups E (group 1 at KKI), F (group 10 at KKI), 2, C (group 4 at KKI), and 3, respectively (Shimozawa et al. 1992*b;* Dodt et al. 1995; Fukuda et al. 1996; Yahraus et al. 1996; Chang et al. 1997; Okumoto and Fujiki 1997; Portsteffen et al. 1997; Reuber et al. 1997); and *PEX7* was found to be responsible for rhizomelic chondrodysplasia punctata (RCDP) (Braverman et al. 1997; Motley et al. 1997; Purdue et al. 1997). (3) The role of these six *PEX* genes may be importing peroxisomal-matrix protein, since empty peroxisomal-membrane structures (peroxisomal ghosts) were seen in fibroblasts from PBD groups C (4 at KKI), E (1 at KKI), 2, and 3 (Santos et al. 1988; Wendland and Subramani 1993).

We have now identified a new complementation group of PBDs, group J (we are leaving out "I" to avoid confusion with group 1 at KKI), which is genetically different from the 11 currently known groups, including complementation groups G (Poulos et al. 1995) and H (Shimozawa et al. 1998). Complementation tests on human fibroblasts from various PBD patients were performed by restoration of peroxisomes by means of immunocytochemical staining of catalase in fused cells (Yajima et al. 1992). Formation of peroxisomes in the majority of multinucleated cells was detected after fusion between fibroblasts from the patient and fibroblasts from the 11 complementation groups (A–H, 2, 3, and 6) of PBD (data not shown). These observations mean that this patient can be regarded as representing a new complementation group, J (table 1). Interestingly, careful immunofluorescence-microscopy studies of fibroblasts from a patient belonging to the newly identified group J, performed with an antibody directed against human 70-kD peroxisomal-membrane protein (PMP [PMP70]) (Imanaka et al. 1996), revealed the absence of empty peroxisomal-membrane structures (ghosts) (fig. 1*a* and *b*), as well as, when performed with anti–human catalase antibody, catalase-containing particles—that is, peroxisomes (fig. 2*a* and *b*). Furthermore, among the 11 complementation groups so far tested, fibroblasts from all patients belonging to group D very rarely have peroxisomal ghosts (fig. 1*c*) and those from group G have none (fig. 1*d*), whereas peroxisomal ghosts were detected in the fibroblasts from PBD groups A–C, E, F, H, 2, 3, and 6 (fig. 1*e–m*). In fibroblasts from a patient with RCDP, both catalase-containing (fig. 2*c*) and PMP70-containing particles were seen (fig. 1*n*). In addition, we performed immunofluorescent staining with an anti–adrenoleukodystrophy protein (ALDP; 75-kD PMP) antibody. As in the case of PMP70, ALDP-positive particles were not detected in fibroblasts from PBD complementation groups G and J, and ALDP-positive particles were rarely found in those from group D. In contrast, ALDP-positive particles that were larger and fewer than those in control fibroblasts were detected in fibroblasts from the other nine complementation groups (data not shown). These results suggest that the primary defect in PBD groups D, G, and J may not be matrix-protein import but, rather, synthesis or maintenance of PMP (Santos et al. 1988; Wendland and Subramani 1993; Baerends et al. 1996; Dodt and Gould 1996; Wiemer et al. 1996).

Table 1

Complementation Groups of PBDs

Complementation Group								
Gifu	KKI[a]	AMC	Phenotype(s)[b]	Peroxisomal-Membrane Ghosts[c]	CHO Mutant(s)	Human Gene	Mapping	Yeast Gene
A	8		ZS, NALD, IRD	+				
B	7 (5)		ZS, NALD	+				
C	4	3	ZS, NALD	+	ZP92	*PEX6* (*PAF2*)	6p21.1	*Pex6*
D	9		ZS	−				
E	1	2	ZS, NALD, IRD	+	Z24, ZP101	*PEX1*	7q21-22	*Pex1*
F	10	5	ZS, IRD	+	Z65	*PEX2* (*PAF1*)	8q21.1	*Pex2*
G			ZS	−				
H			NALD	+				
J			ZS	−	ZP119[d]			
	2	4	ZS, NALD	+	ZP102	*PEX5*	12p13.3	*Pex5*
	3		ZS	+	ZP104, ZP109	*PEX12*		*Pex12*
	6		NALD	+				
					ZP110, ZP 111 ZP114			
R	11	1	RCDP			*PEX7*	6q22-24	*Pex7*

[a] The numbering listed under KKI is based on the study by Moser et al. (1995).
[b] NALD denotes neonatal adrenoleukodystrophy, and IRD denotes infantile Refsum disease.
[c] A plus sign (+) indicates presence, and a minus sign (−) indicates absence.
[d] Kinoshita et al. (1998).

The patient from the newly identified complementation group J had the phenotype of classic Zellweger syndrome (ZS; MIM 214100). Dihydroxyacetone phosphate acyltransferase activity was severely diminished in fibroblasts from the patient (0.11 nmol/120 min per mg protein), in comparison with findings in control fibroblasts (1.55 nmol/120 min per mg protein) (Shimozawa et al. 1988). β-Oxidation activity of lignoceric acid relative to that of palmitic acid in this patient's fibroblasts was also decreased (0.038), in comparison with findings in the control cells (0.58), determined as described by Suzuki et al. (1991). In addition, all of the patients from PBD groups D, G, and J had only the severe phenotype of ZS (Shimozawa et al. 1993; Poulos et al. 1995), whereas some patients from the other nine PBD groups had the severe phenotype but others had milder phenotypes, such as neonatal adrenoleukodystrophy and infantile Refsum disease.

We then performed cell fusion between fibroblasts from group J and CHO mutants ZP110 (Tateishi et al. 1997), ZP114 (Tateishi et al. 1997), and ZP119, the CHO mutant newly isolated by Kinoshita et al. (1998), which were found to belong to complementation groups other than the known PBD groups (A-H, 2, 3, 6, and RCDP) (Shimozawa et al. 1998). Numerous peroxisomes were detected after fusion by use of methods reported elsewhere (Shimozawa et al. 1992*a*), between fibroblasts and CHO mutants ZP110 (fig. 2*d*) and ZP114, whereas no peroxisome was detected after fusion between fibroblasts and ZP119 (fig. 2*e*). These observations imply that the newly identified CHO mutant ZP119 represents ZS fibroblasts from group J. Furthermore, this CHO mutant, like group J, had no peroxisomal ghosts (Kinoshita et al.; 1998), whereas large but fewer particles immunoreactive with anti-PMP70 antibody were detected in CHO mutants Z24, Z65, and ZP92, which belong to the same complementation groups as E, F, and C, respectively (Shimozawa et al. 1992*a*).

We then transfected human PMP70 cDNA (Kamijo et al. 1992) into fibroblasts lacking peroxisomal ghosts, from groups D, G, and J, according to methods reported elsewhere (Shimozawa et al. 1996). In all these transfectants, peroxisomes were not detected when we performed immunostaining with an anti–human catalase antibody (fig. 2*f–h*), and the same held true for transfectants of PMP70 into fibroblasts from groups A–C, E, F, H, 2, 3, and 6 (data not shown). Therefore, human PBD groups caused by defects in the PMP70 gene have heretofore not been identified. Furthermore, when we transfected, into the fibroblasts from the group J patient, human *PEX13* cDNA, which encodes an SH3 protein of the peroxisomal membrane (Gould et al. 1996). Peroxisomes were not evident in the transfectants (fig. 2*i*). In summary, (1) in mammalian cell lines there are 15 known peroxisomal-deficient complementation groups, including RCDP and CHO mutants; (2) abnormalities of PMP synthesis, not matrix-protein import, may be the primary defect, at least in PBD groups D, G, and J, and all patients from these groups manifested only the severe phenotype of ZS, whereas the other groups in-

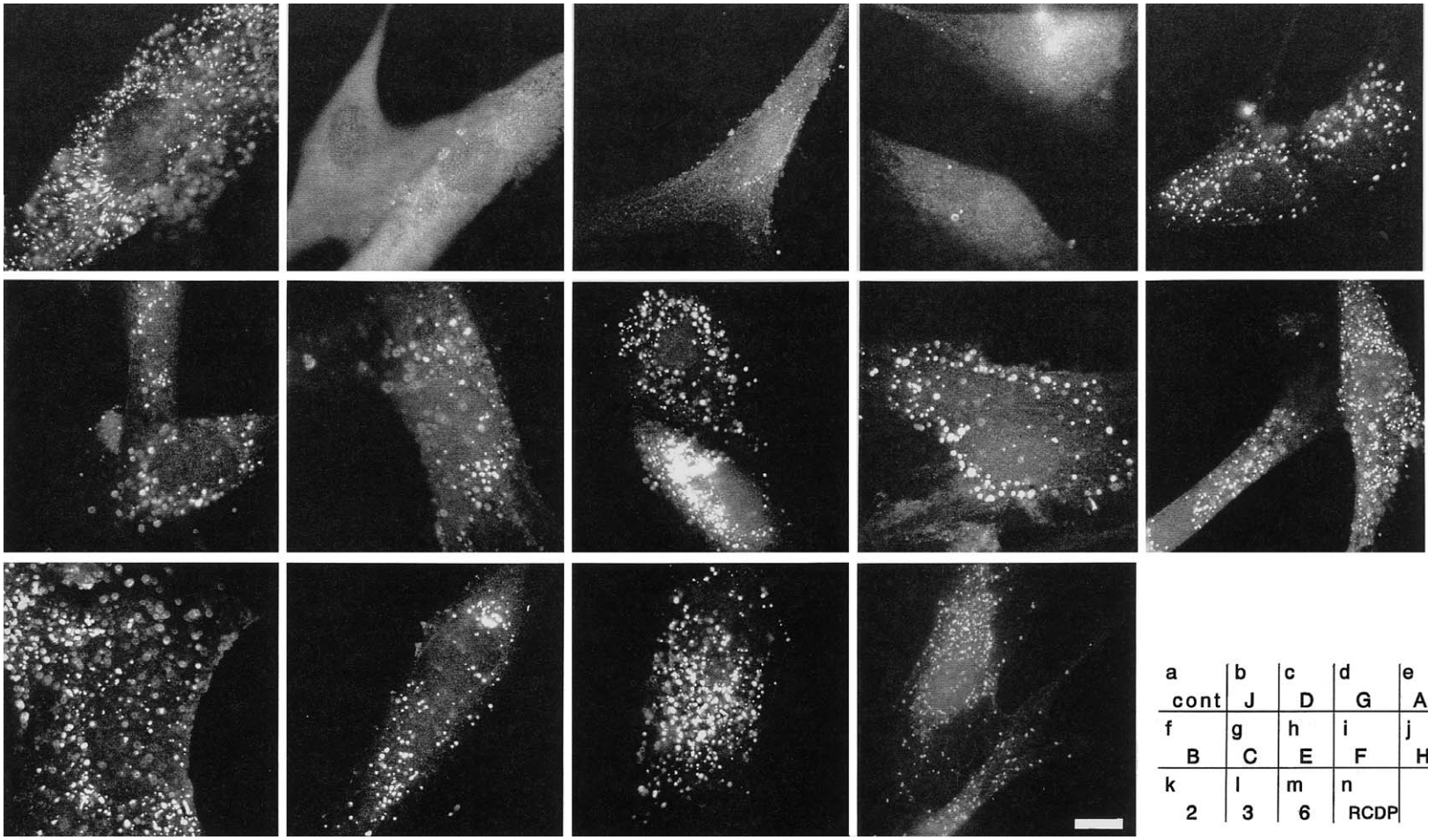

Figure 1 Immunofluorescent staining with anti–human PMP70 antibody. *a,* Control fibroblasts. *b,* Fibroblasts from group J patient. *c–m,* Fibroblasts from patients from groups D, G, A–C, E, F, H, 2, 3, and 6. *n,* Fibroblasts from RCDP patient. (Bar = 15 μm). In the fibroblasts from groups J and G, no PMP70-positive particles are visualized, except for the nonspecific puncta, and the cytosol is stained strongly and diffusely. Very few and various PMP70-positive particles are detected in the fibroblasts from group D and the other nine groups (A, B, C, E, F, H, 2, 3, and 6).

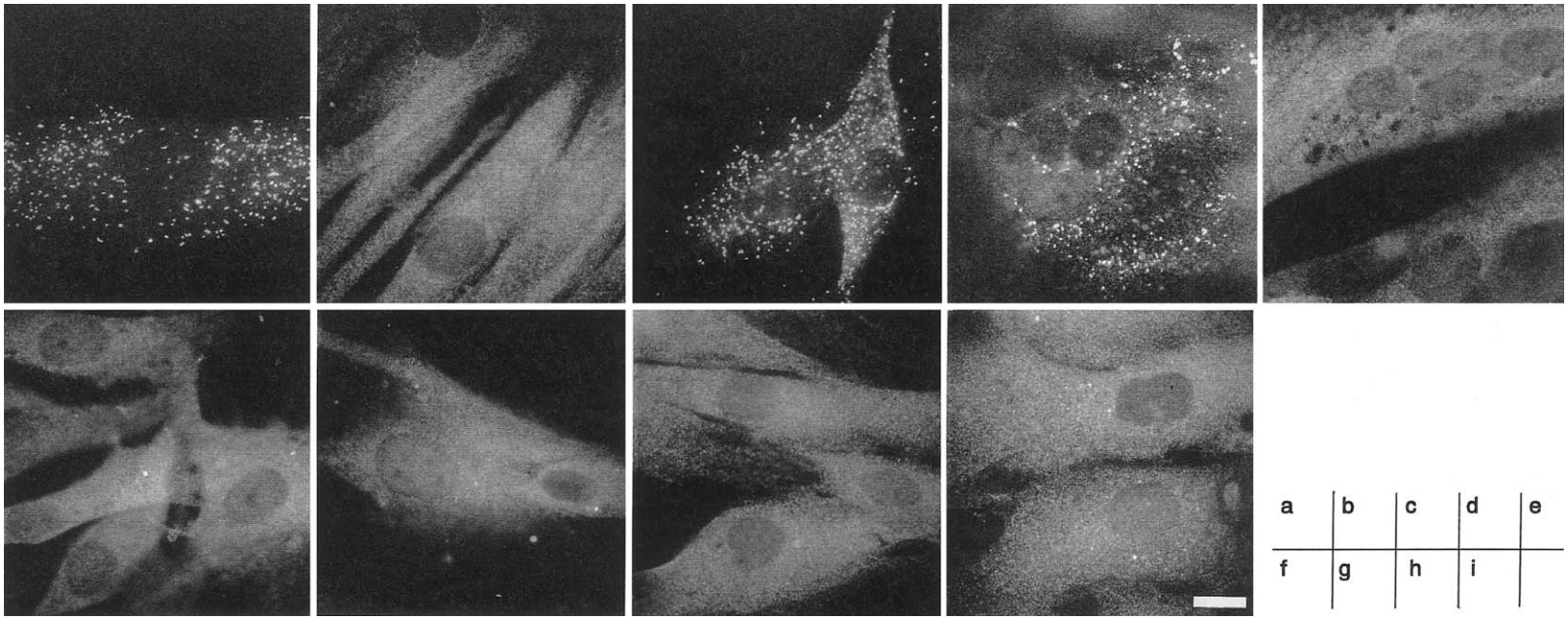

Figure 2 Immunofluorescent staining with anti–human catalase antibody. *a*, Control fibroblasts. *b*, Fibroblasts from group J patient. *c*, Fibroblasts from RCDP patient. *d* and *e*, Cell hybrids of fibroblasts from the group J patient with ZP110 and the group J patient with ZP119, respectively. *f–h*, Transfectants with human PMP70 cDNA into group D, G, and J fibroblasts, respectively. *i*, Transfectants with human *PEX13* cDNA into group J fibroblasts. (Bar = 15 μm)

cluded various phenotypes; and (3) there were no PBD groups complemented by human PMP70.

It was first reported that in ZS fibroblasts from complementation group 4 at KKI (group C at Gifu [*PEX6* defect]) the PMPs were located in unusual empty membrane structures (peroxisomal ghosts) of a larger size—a finding determined mainly by use of an anti-PMP70 antibody (Santos et al. 1988).Later, ghost size and abundance were noted in seven ZS fibroblasts belonging to five complementation groups (Santos et al. 1992), and detectable PMP70 in vesicles was noted in those from KKI groups 1 (E at Gifu), 2, 3, 6, and 8 (A at Gifu) (Wendland and Subramani 1993). ALDP-positive particles were also detected in two PBD cell lines from group 1 but were rare in ZS fibroblasts from group D (Mosser et al. 1994). All these data support our findings of heterogeneity of peroxisomal ghosts in PBD complementation groups.

At least 18 yeast *PEX* genes have been identified, and several human genes have been considered to be human orthologues of these *PEX* genes. It has been suggested that there are yeast mutants without peroxisomal ghosts—for example, *Hansenula polymorpha per9* or *Pichia pastoris pas2* (*PEX3* gene defect) (Baerends et al. 1996; Wiemer et al. 1996)—and that these *PEX* genes may play roles of synthesis or maintenance of peroxisomal membrane. Therefore, any of these *PEX* genes may be primary defects of PBD groups D, G, and J. We are using western blot and pulse-chase experiments with some PMP antibodies to perform detailed analyses of ghosts in these three groups, and we are examining genes responsible for these PBD groups by identifying human orthologues of these *PEX* genes and by performing functional cloning of peroxisome-deficient CHO mutants.

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