δ-thal occurred on the non-β-thal chromosome because it was present in the father but not in his son; a similar observation was made by Galanello et al. (1990) in the family which they studied, which originated in the Ferrara region. This unfortunate situation again emphasizes the importance of adequate testing of these unusual families; data from in vitro chain-synthesis analyses would already have ruled out a possible α-thal-1 heterozygosity or an α-thal-2 homozygosity in the father and should have stimulated the more sophisticated laboratory studies reported here.

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Standardization of Complementation Grouping of Peroxisome-deficient Disorders and the Second Zellweger Patient with Peroxisomal Assembly Factor-1 (PAF-1) Defect

To the Editor:

Eight genetic groups of peroxisome-deficient disorders —including Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD)—were identified at the Kennedy-Krieger Institute and in our laboratory, by using somatic cell fusion (Roscher et al. 1989; Yajima et al. 1992). There was no obvious relation between genotypes and phenotypes. Although the primary etiology of these eight groups has not been elucidated, we recently clarified the primary defect in the ninth new complementation group (group F), including an infant with typical ZS; there was a point mutation that resulted in premature termination of peroxisomal assembly factor-1 (PAF-1) (Shimozawa et al. 1992). The presence of nine complementation groups in these disorders indicated that at least nine different genes are involved in the assembly of peroxisomes. Several laboratories independently reported original classification of complementation groups; however, the relation of complementation groups among these laboratories and the number of genes which affect the assembly of peroxisomes have not been clarified.

We compared three cell lines obtained from Zellweger patients (Z1–3; all belonged to different groups of Amsterdam University [Brul et al. 1988]) with our cell lines of nine complementation groups, according to our methods (Yajima et al. 1992). We found that our groups C, E, and F corresponded to Brul's groups 3 (Z2), 2 (Z1), and 5 (Z3), respectively. In addition, further collaborative study with the Kennedy-Krieger Institute (in conjunction with Ann Moser and Hugo Moser) revealed that a new U.S. complementation group 8 was the same as our group A (table 1).

As group 5 of Brul et al. was the same as our group F, whose primary etiology was PAF-1, we investigated whether PAF-1 restored the assembly of peroxisomes

Table 1

Comparison of Complementation Groups of Peroxisome-deficient Disorders

Phenotype(s)	Complementation Group: Gifu University	Kennedy-Krieger Institute	Amsterdam University
ZS, NALD, and IRD	A	8	
ZS	B		
ZS	C	4	3 (Z2)
ZS	D		
ZS, NALD, and IRD	E	1	2 (Z1)
ZS	F		5 (Z3)
ZS and NALD		2	4 (NALD)[a]
ZS		3	
NALD		6	

[a] Data are from Roscher et al. (1989).

in the Z3 fibroblasts, by using a mammalian-expression vector pcD2. Numerous peroxisomes were evident in most of the transfected cells (data not shown). Next, to research the mutation in this patient, we determined the nucleotide sequence of human PAF-1 cDNA from the patient's fibroblasts, by following PCR amplification. As compared with the control, nucleotide C at position 355 mutated to T in cDNA of the patient, in all the cDNA subcloned (data not shown). This mutation produced termination codon TAG instead of coding for arginine, and this is the same mutation found in the Japanese patient with ZS who belongs to group F (Shimozawa et al. 1992). To determine whether this point mutation in Z3 was homozygous, the nucleotide sequence in this region of genomic DNA, which was obtained by PCR from the Z3 fibroblasts, was examined. The same mutation was present in the entire cloned genome (data not shown).

A clear classification of genotypes of these disorders and a standard complementation group must be prepared, especially for genetic analyses of peroxisome-deficient disorders. Further efforts are in progress to isolate other genes responsible for peroxisome assembly of more than eight complementation groups.

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