
Address for correspondence and reprints: Dr. J. Jaeken, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium. E-mail: Jo.Vencken@uz.kuleuven.ac.be
© 1998 by The American Society of Human Genetics. All rights reserved. 0002-9297/00/6206-0033$02.00


Temperature-Sensitive Phenotypes of Peroxisome-Assembly Processes Represent the Milder Forms of Human Peroxisome-Biogenesis Disorders

To the Editor:

Peroxisome-biogenesis disorders (PBDs) are lethal hereditary diseases caused by abnormalities in the assembly processes of peroxisomes (Moser et al. 1995). The peroxisome is a ubiquitous organelle involved in vital metabolic functions, such as oxidative processes involving H₂O₂, β-oxidation of fatty acids, and biosynthesis of plasmalogens (Van den Bosch et al. 1992). PBDs are characterized by multiple defects in these functions, as well as by the lack of morphologically normal peroxisomes. They are genetically classified into complementation groups (CGs), the number of which is \( \geq 11 \) (Shimozawa et al. 1993; Moser et al. 1995; Poulos et al. 1995). Each CG contains significantly different clinical phenotypes—for example, Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD). ZS patients have severe neurological abnormalities, dysmorphic features, hepatomegaly, and multiple renal cysts, and most die at age \(< 6\) mo. NALD patients have similar symptoms, but they survive considerably longer, dying during early childhood. In contrast, IRD patients do not exhibit significant abnormalities in the CNS, and they have the longest average life span among patients with PBDs (Lazarow and Moser 1995; Moser et al. 1995). Although the causal genes (PEXs) for several CGs have been cloned and the mutations have been identified at the molecular level (Shimozawa et al. 1992; Dodt et al. 1995; Wiemer et al. 1995; Fukuda et al. 1996; Yahraus et al. 1996; Chang et al. 1997; Okamoto and Fujiki 1997; Porte-sten et al. 1997; Reuber et al. 1997), it is unknown why such diverse clinical phenotypes occur in the same CGs although, in all CGs, the phenotypes are very similar. We report that milder forms of PBDs are characterized by temperature-sensitive (TS) phenotypes of peroxisome-assembly processes in the fibroblasts of patients.

In spite of the variations in the clinical features, the fibroblasts from patients of all three PBD phenotypes generally lack peroxisomes. Although the occurrence of a reduced number of peroxisomes occasionally has been noted in several PBD cell lines (Arias et al. 1985; Wiemer et al. 1991; Slawecki et al. 1995), no correlation with clinical features has been apparent. We assumed that limited types of leaky mutations in the PEX genes could be the causes of the milder forms of PBDs. As a possible parameter representing such leakiness, we examined temperature sensitivity. Fibroblasts from PBD patients with different CGs were incubated at 30°C and at 37°C and were subjected to immunofluorescence staining with anti-catalase antibody. After 72 h incubation at 30°C, punctate staining of catalase typical of peroxisomes was detected in the fibroblasts of all six patients with IRD and in three of five of those with NALD, belonging to four different CGs (fig. 1b and table 1), whereas no peroxisomes appeared in the same cells after incubation at 37°C (fig. 1a). Catalase and the 70-kD peroxisomal membrane protein (PMP70) were colocalized in these
Figure 1  Immunofluorescence staining of peroxisomes in patients’ fibroblasts and in Z65 mutant Chinese-hamster-ovary cells. Cells were cultured for 72 h at either 37°C (a, c, and e) or 30°C (b, d, and f) and were stained with either anti–human catalase antibody (a–d) or anti–rat catalase rabbit antibody (e and f). a and b, Fibroblasts of an IRD patient (F-05). c and d, Fibroblasts of a ZS patient (F-01). e and f, Z65 transformant with Pex2E55K. (Scale bar = 50 μm)

Table 1  Temperature Sensitivity of Peroxisome Biogenesis in Fibroblasts of PBD Patients

<table>
<thead>
<tr>
<th>CG AND PATIENT</th>
<th>PHENOTYPE</th>
<th>PEROXISOME-POSITIVE CELLS INCUBATED ATb (%)</th>
<th>AGE AT DEATH OR LAST FOLLOW-UPc</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (8):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-06</td>
<td>ZS</td>
<td>0</td>
<td>4 mo</td>
</tr>
<tr>
<td>A-05</td>
<td>NALD</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>A-08</td>
<td>NALD</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>A-04d</td>
<td>IRD</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>C (4):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-03a</td>
<td>ZS</td>
<td>0</td>
<td>8 mo</td>
</tr>
<tr>
<td>C-08</td>
<td>ZS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E (1):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-14</td>
<td>ZS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E-01</td>
<td>NALD</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>E-13</td>
<td>NALD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E-05e</td>
<td>IRD</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>E-24</td>
<td>IRD</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>E-25</td>
<td>IRD</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>E-26</td>
<td>IRD</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>F (10):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-01a</td>
<td>ZS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F-04e</td>
<td>ZS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F-05</td>
<td>IRD</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>... (6):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-01</td>
<td>NALD</td>
<td>5</td>
<td>80</td>
</tr>
</tbody>
</table>

* The letter designation is that provided by Gifu University (Japan), and the number designation (in parentheses) is that provided by the Kennedy Krieger Institute.

b Data are averages of several view fields, at ×200.

Annual data are for traceable cases only.

f Purchased from Coriell Cell Repositories (Camden, NJ); the cell line designations are GM08771 (A-04) and GM08770 (E-05).

g Source: Fukuda et al. (1996).

h Source: Maeda et al. (1990).

i Source: Shimozawa et al. (1992).

j No designation of CG was available from Gifu University.
55°C, and extension for 3 min at 72°C. Nucleotide-sequence comparison with the normal PEX2 gene revealed that this patient was heterozygous for two point mutations. One was a G→A substitution at nucleotide position 163, relative to the A residue of the initiation codon, causing an amino acid alteration (E55K). The other was a C→T substitution at nucleotide position 355, resulting in the change of the codon 119 to a stop codon, TGA (R119 Stop). These two mutations were also observed in the PEX2 cDNA obtained by reverse transcriptase-PCR of the mRNA from F-05. The latter nonsense mutation is identical to that reported by Shimozawa et al. in both ZS patient F-01 (Shimozawa et al. 1992) and another CG-F patient (Shimozawa et al. 1993) (both cases were homozygous for the mutation), and it previously had been established that this mutation is non-functional. Accordingly, we investigated the relationship between the E55K mutation and the TS phenotype, by gene transfection. The PEX2E55K gene sequence subcloned in the expression vector pUCD2SRaMCS (Tsukamoto et al. 1995) was transfected to a PEX2-deficient Chinese-hamster-ovary cell mutant (Z65) (Tsukamoto et al. 1991, 1994), and stable transformants were produced. The transformants revealed a punctate distribution of catalase after 72 h incubation at 30°C, whereas no catalase-positive granules were observed for incubation at 37°C (fig. 1e and f); Z65 transfected with wild-type PEX2 had catalase-positive granules at both 30°C and 37°C; and the cells transfected with the empty vector exhibited no peroxisomal staining at either 30°C or 37°C (data not shown). Thus, the TS phenotype of peroxisome biogenesis of the IRD fibroblasts (F-05) is caused by the E55K mutation of the PEX2 gene.

The present results indicate that the peroxisome-assembly process is TS in the fibroblasts of patients with the mildest form of PBD (i.e., IRD), irrespective of CGs, and that this phenotype is directly linked to the specific genotype of the responsible PEX gene, at least in F-05. Such a TS phenotype was not observed for the most severe form of PBD (i.e., ZS), whereas only a subset of the cell lines were TS for NALD, the intermediate form of PBD. In this regard, it is interesting to note that the NALD patients with the TS phenotype (patients A-08 and 6-01) had longer life spans than did those with the non-TS phenotype (patients E-01 and E-13), even though the latter two patients exhibited slight leakiness at both temperatures. Thus, the TS phenotypes of peroxisome assembly in the cultured fibroblasts represent the mildness of the clinical symptoms of PBD. Patients with the TS phenotypes may be mosaic for peroxisome occurrence from cell to cell in the body at normal body temperatures. A mosaicism of peroxisomes was indeed reported in the liver of a PBD patient who had a relatively long life span (Giros et al. 1996). It is also possible that TS patients have partially functional peroxisomes.

In any case, the TS patients probably have higher gross peroxisomal activities than do the patients with non-TS leaky phenotypes (patients E-01 and E-13).

Temperature- or cold-sensitive phenotypes have been noted in a few genetic diseases. In epidermolysis bullosa simplex, the disturbance of the skin becomes worse at higher temperatures, whereas, in paramyotonia congenita, exposure to lower temperatures causes myotonia. In these instances, the symptoms are understood to be direct effects of the temperature- and cold-sensitive phenotypes of the corresponding gene products, keratin (Morley et al. 1995) and Na+-channel protein (McClatchey et al. 1992), respectively. In maple syrup–urine disease, symptoms sometimes worsen when there is a high fever (Chuang and Shih 1995); however, the responsible mutation has not been identified. Thus, TS phenotypes directly linked to specific genotypes possibly occur in various genetic diseases. Among these, PBD cases are unique in that a complex cellular process, peroxisome assembly, becomes TS because of a single gene mutation, causing distinct clinical features.

Our present results would raise several clinical implications. First, the severity of prognosis could be diagnosed, by examination of the temperature sensitivity of peroxisome assembly in the fibroblasts of newborn PBD patients. Second, precaution against fever may be necessary in the treatment TS PBD patients. Third, hypothermal therapy might be applicable to TS PBD patients. Such therapy might increase the frequency of cells having functional peroxisomes, thereby improving clinical symptoms.

**Acknowledgments**

We thank T. Hashimoto and N. Usuda for anti-human and anti-rat catalase antibodies, respectively, and we thank H. W. Moser, A. B. Moser, R. J. A. Wanders, G. T. N. Besley, B. C. Paton, and A. Poulos for patients’ fibroblasts. This work was supported in part by Grants-in-Aid for Scientific Research and Exploratory Research, from the Ministry of Education, Science, Sports and Culture of Japan, and by grants from the Sumitomo Foundation and Uehara Memorial Foundation.

**ATSUSHI IMAMURA,**1 **TOSHIRO TSUKAMOTO,**1 **NORUYUKI SHIMOZAWA,**2 **YASUYUKI SUZUKI,**2 **ZHONGHI ZHANG,**3 **TSUENOSHIRO TSUKAMOTO,**1 **YUKIO FUJIKI,**1 **TADAO ORII,**3 **NAOMI KONDO,**2 AND **TAKASHI OSUMI**1

1. **Department of Life Science, Himeji Institute of Technology, Kamigori, Hyogo, Japan;** 2. **Department of Pediatrics, Gifu University School of Medicine, and** 3. **Faculty of Human Welfare, Chubu Gakuin University, Seki, Gifu, Japan;** 4. **Department of Microbiology and Molecular Pathology, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa, Japan; and** 5. **Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan**
Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

(for human peroxisome-assembly factor-1 [hsPEX2; accession number M86852])

References


A Breast Cancer Patient of Scottish Descent with Germ-Line Mutations in BRCA1 and BRCA2

To the Editor:
Ramus et al. (1997) previously described an Ashkenazi Jewish patient found to have germ-line mutations in both breast and ovarian cancer–susceptibility genes, BRCA1 and BRCA2. We report the first such example for the non-Jewish Caucasian population. The patient, who is indicated by an arrow in pedigree 232 (fig. 1), was of Scottish origin. She was diagnosed with breast cancer (grade 2 adenocarcinoma) at age 35 years. Simultaneous screening by protein truncation test of both BRCA1 (exon 11) and BRCA2 (exon 11) detected truncating mutations.