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Human Peroxisome Assembly Factor-2 (PAF-2): A Gene Responsible for Group C Peroxisome Biogenesis Disorder in Humans

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Summary

Peroxisome-biogenesis disorders (PBD) are genetically heterogeneous and can be classified into at least ten complementation groups. We recently isolated the cDNA for rat peroxisome assembly factor-2 (PAF-2) by functional complementation using the peroxisome-deficient Chinese-hamster-ovary cell mutant, ZP92. To clarify the novel pathogenic gene of PBD, we cloned the full-length human PAF-2 cDNA that morphologically and biochemically restores peroxisomes of group C Zellweger fibroblasts (the same as group 4 in the Kennedy-Krieger Institute) and identified two pathogenic mutations in the PAF-2 gene in two patients with group C Zellweger syndrome. The 2,940-bp open reading frame of the human PAF-2 cDNA encodes a 980-amino-acid protein that shows 87.1% identity with rat PAF-2 and also restored the peroxisome assembly after gene transfer to fibroblasts of group C patients. Direct sequencing of the PAF-2 gene revealed a homozygous 1-bp insertion at nucleotide 511 (511 insT) in one patient with group C Zellweger syndrome (ZS), which introduces a premature termination codon in the PAF-2 gene, and, in the second patient, revealed a splice-site mutation in intron 3 (IVS3+1G→A), which skipped exon 3, an event that leads to peroxisome deficiency. Chromosome mapping utilizing FISH indicates that PAF-2 is located on chromosome 6p21.1. These results confirm that human PAF-2 cDNA restores peroxisome of group C cells and that defects in the PAF-2 produce peroxisome deficiency of group C PBD.

Introduction

The peroxisome is an ubiquitous organelle bounded by a single membrane containing >50 matrix enzymes that catalyze many important metabolic reactions, including those involved in the β-oxidation of fatty acids, H_2O_2 metabolism, and biosynthesis of plasmalogens, cholesterol, bile acid, and glyoxylate (Lazarow and Moser 1995). Peroxisome-biogenesis disorder (PBD) is caused by an ineffective assembly of peroxisomes. Zellweger syndrome (ZS) is the most severe phenotype of PBD and is characterized by the absence of peroxisomes and by multiple metabolic deficiencies, including abnormal accumulation of very-long-chain fatty acids (VLCFA), phytanic acid and bile acid intermediates, and reduced tissue levels of plasmalogens. Patients with ZS have severe neurological abnormalities, dysmorphic features, hepatomegaly, and multiple renal cysts, and most die within 6 mo of birth. Neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD) are milder phenotypes of PBD (Lazarow and Moser 1995). Besides the phenotypic heterogeneity, there is considerable genetic heterogeneity, with at least 10 complementation groups for patients with PBD (complementation groups A–F of our institutes; 2, 3, and 6 of the Kennedy-Krieger Institute; and the newly identified group G of Adelaide) (Shimozawa et al. 1993; Poulos et al. 1995). We isolated three different types of peroxisome-deficient mutant Chinese-hamster-ovary cells—Z24, Z65, and ZP92—as models for human groups E, F, and C, respectively, corresponding to 1, 10, and 4 of the Kennedy-Krieger Institute, respectively, and clarified that mutation in PAF-1 is the primary defect in the group F patient (Tsukamoto et al. 1991; Shimozawa et al. 1992*a*, 1992*b*). Dodt et al. (1995) reported that the peroxisome-targeting signal 1 (PTS1) receptor gene, PXR1, is the gene defective in group 2 PBD patients; it was isolated from expressed sequence-tagged databases, as a human homologue of PTS1 of the yeast PAS8 gene of *Pichia pastoris* (Dodt et al. 1995).

We cloned and characterized the rat cDNA for peroxisome-assembly factor-2 (PAF-2), which restores peroxisomes of ZP92, the model Chinese-hamster-ovary mutant for the group C cell, by functional complementation (Tsukamoto et al. 1995). Rat PAF-2 is a peroxisome peripheral membrane protein of 978 amino acids containing ATP-binding sites and an AAA-protein-family

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Table 1

Primer Sequence for RT-PCR of Normal DNA and Patients' DNA

Name	Sequence (5′→3′)	Direction	Position
Primer A	CACCCTCCTCGTTGGTGTCCTGTC	Forward	−27→−3
Primer 32	CTGAATTCTGAGCTCTCCCA	Reverse	882→863
Primer 24	TCCAGGGCGAATGGGTGT	Forward	653→672
Primer 6	CTGTGGGTCAGAAGGGCAT	Reverse	1925→1907
Primer 7	GCTTTGTGGATGGGGATCTCT	Forward	1886→1906
Primer 23	GATCTCTCTGTGGGCTATCAAGGTA	Reverse	3009→2985
Primer B	CTAGCAGGCAGCAAACTTCGGCTGGAT	Reverse	2943→2917

NOTE.—The normal cDNA was reverse transcribed with primer B, and the resultant product was amplified by primers A/32, primers 24/6, and primers 7/B. Patients' cDNA was synthesized by random hexamer. PCR was done by use of the same primers, except for 7/23.

signature. A homology search revealed PAF-2 to be a member of a putative ATPase family that includes two yeast genes essential for peroxisome assembly. Transformants of group C cells, as well as of ZP92 cells with the rat PAF-2 cDNA, restored peroxisomes morphologically, thereby suggesting that PAF-2 is the causal gene of peroxisome deficiency in both ZP92 and human group C cells (Tsukamoto et al. 1995). To elucidate the mechanism of peroxisome assembly in human cells and to better understand the molecular basis of group C PBD, we cloned and defined the primary structure of human PAF-2. We then demonstrated and characterized the mutations in PAF-2 cDNA that are responsible for the peroxisome deficiency of group C PBD. We also mapped the chromosomal localization of PAF-2, utilizing FISH. The present study clearly defines the primary defect in group C patients, the third genetic defect in peroxisome assembly among PBD to be defined at the molecular level.

Patients, Material, and Methods

Patients

The cell line of patient 1 (GM04340), that is ZS with a typical clinical presentation and ghost peroxisomes, was purchased from Coriell Cell Repositories (Camden, NJ). The patient was the product of a father-daughter mating. This cell line was classified into PBD complementation group C (the same as group 4 in the Kennedy-Krieger Institute), and belonged to the same complementation group as CHO mutant ZP92 (Santos et al. 1988; Shimozawa et al. 1992*a*; Yajima et al. 1992). Patient 2 was a Japanese girl of nonconsanguineous parents. She had been delivered at term, weighed 2,468 g, had a severe asphyxia, profound hypotonia, and clonic convulsion. Craniofacial dysmorphism, including low nasal bridge, hypertelorism, epicanthus inversus, and upward-slanting of the palpebral fissures as well as hepatomegaly were present. X-rays showed an abnormal calcific stippling of both patella. Serum VLCFA was elevated. The immunohistochemical staining of cultured fibroblasts from this patient indicated the absence of peroxisomes and confirmed the diagnosis of ZS. The complementation group proved to be group C. She died of respiratory failure at age 7 mo. The cell line of patient 2 was transformed by transfecting SV40 ori$^-$ (i.e., lacking an origin of replication) DNA into the patients' fibroblasts with the use of an electroporator (Okamoto et al. 1992).

Material

A human liver λgt10 and a λgt11 cDNA library were purchased from Clontech. Restriction enzymes, *Taq* DNA polymerase, and other modifying enzymes were obtained from Takara Shuzo. Murine-leukemia-virus reverse transcriptase was from Gibco BRL, and α-[^{32}P]-dCTP was from Amersham. Anti–human catalase antibody was provided by Prof. T. Hashimoto (Shinshu University, Japan).

Screening of a Human Liver cDNA Library

Partial rat PAF-2 cDNA (1.7-kb *Eco*RI-*Bam*HI fragment) was used to screen a 1×10^6 plaque of the human liver λgt10 cDNA library by plaque hybridization (Sambrook et al. 1989, pp. 2.108–2.125). The positive human clone was isolated and sequenced. On the basis of the sequence of this clone, we prepared two human PAF-2–specific primers, one for the upstream and the other for the downstream direction. Repeating the gene-walking strategy by PCR, using the λgt11 arm primer (5′-AGCCCGTCAGTATCGGCGGAATTC-3′) and human PAF-2–specific primers, we extended the desired fragments and covered the entire length of human PAF-2 sequence from a human λgt11 liver cDNA library. All the coding sequence, as well as the 5′ and 3′ noncoding sequences of the fragment, were determined by the direct cycle-sequence method, by use of an automated DNA

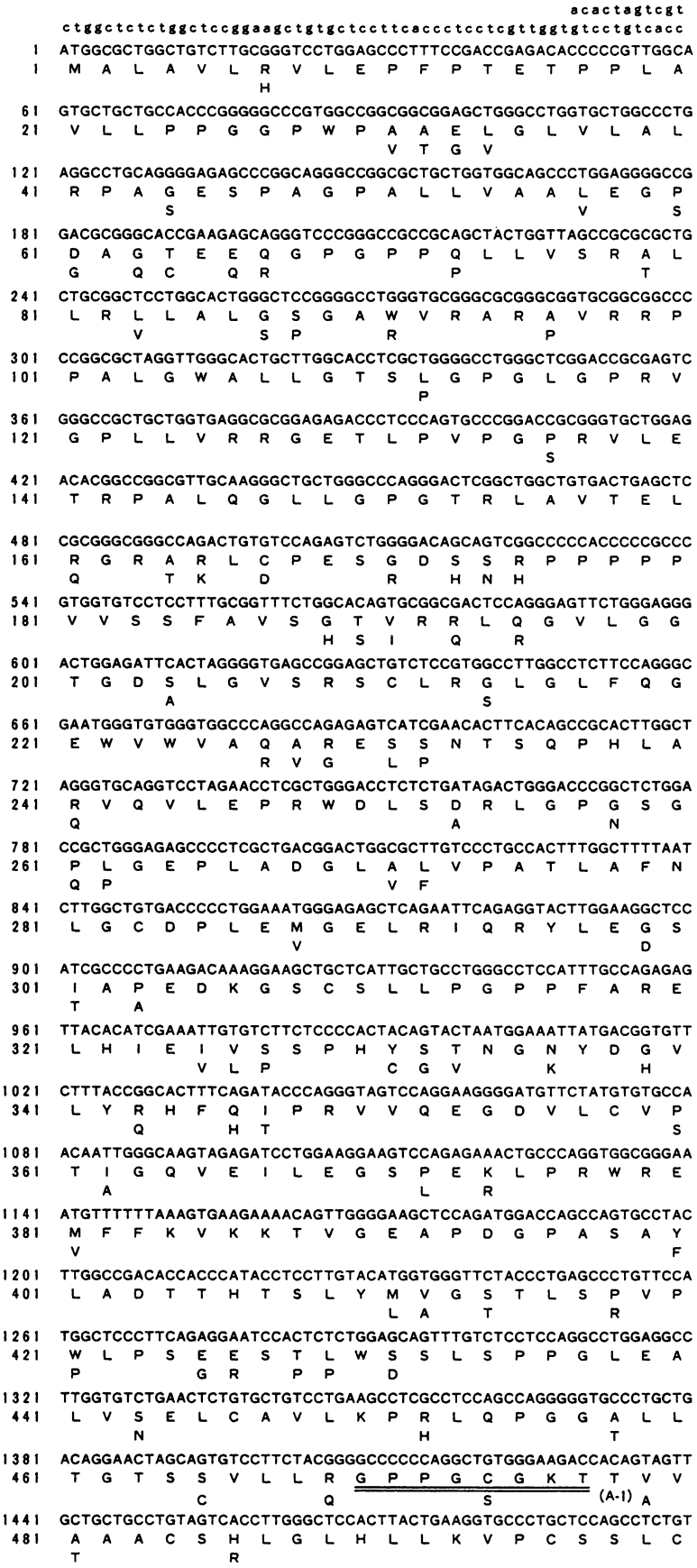
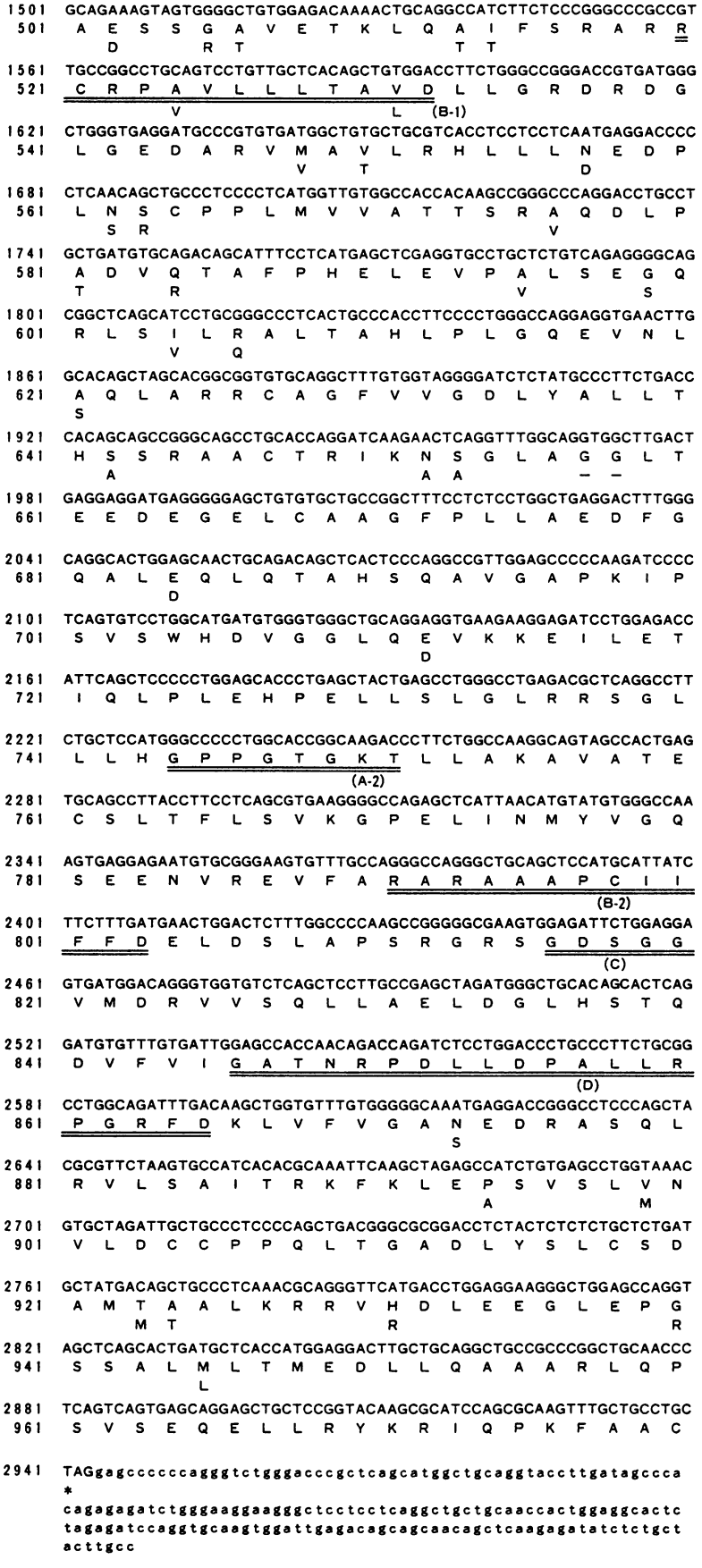


Figure 1 Nucleotide sequence and deduced amino acid sequence of human PAF-2. Nucleotides and amino acids are numbered from the first nucleotide of the initiation ATG and the initiation methionine as +1, respectively. The coding sequence (*uppercase letters*) and the noncoding sequence (*lowercase letters*) are shown. For comparison, the rat PAF-2 amino acids that differ from the human cDNA are indicated below the human sequence. The ATP/GTP-binding motifs A (A1 and A2) and B (B1 and B2), the serine protease active site (C), and the AAA-protein family signature (D) are underlined.


(B-1)
(A-2)
(B-2)
(C)
(D)

sequencer (model 373A; Perkin Elmer Japan Applied Biosystems).

Construction of Full-Length cDNA for Human PAF-2

Control normal human liver mRNA was obtained, with informed consent, from the subjects. This mRNA was reverse transcribed by use of a 3′-noncoding primer specific for human PAF-2, followed by PCR amplification in three fragments with high-fidelity LA-*Taq* (Takara Shuzo). Primer sequences for PCR are listed in table 1. Thirty cycles of amplification consisted of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and elongation at 72°C for 2 min, followed by final extension at 72°C for 7 min. The first −27-to-952 fragment was digested with *Eco*RI (Nucleotides are numbered from the first nucleotide, adenine, of initiation ATG.) The second 722-to-1995 DNA and the third 1771-to-3013 (the last nucleotide of termination codon) were treated by *Eco*RI and *Xho*I and by *Xho*I, respectively. The digested fragments were ligated, and the construct was cloned into T-vector (Amersham). The sequence of the clone was reconfirmed.

Northern-Blot Analysis of PAF-2

Multiple human-tissue northern-blot filters were purchased from Clontech. The blots were hybridized with full-length cDNA of PAF-2 labeled with α-[^{32}P]-dCTP (Amersham), as recommended by the manufacturer.

Identification of Mutations

The mRNA was obtained from cultured fibroblasts, by use of QuickPrep mRNA purification kits (Pharmacia) that was followed by reverse transcription–PCR (RT-PCR) with a random hexamer and three pairs of PAF-2–specific PCR primers, as described above (table 1). Nucleotide sequence was determined by direct sequencing, as described above.

Site-Directed Mutagenesis of the Mutation and Transfection of Human PAF-2 cDNA into Group C Peroxisome-Deficient Cells

The 896-bp *Eco*RI-*Xho*I fragment of the normal cDNA clone was replaced as a cassette with the corresponding fragment harboring the deletion identified in patient 2. Both the normal and the mutant cDNA was religated into expression vector pcD2SRα and transfected into group C peroxisome-deficient fibroblasts by the calcium phosphate transfection method, as described elsewhere (Shimozawa et al. 1992*b*). Peroxisomes of the transfectant were identified by immunofluorescence staining, using an anti–human catalase antibody, as described elsewhere (Shimozawa et al. 1992*a;* Yajima et al. 1992). [1-^{14}C] palmitate and [1-^{14}C] lignocerate oxidation in the transfectant were determined as described by Suzuki et al. (1991).

FISH

The direct-mapping method combined with FISH and replicated prometaphase R-bands, as based on cell synchronization with excess thymidine followed by bromodeoxyuridine release, has been described elsewhere (Masuno et al. 1992, 1994). A plasmid clone harboring a full-length human PAF-2 cDNA was the probe used. Intensity of the fluorescent signals was amplified according to the reported procedure but with slight modifications (Viegas-Pequignot et al. 1989; Hori et al. 1990).

Results

Cloning and Characterization of Full-Length cDNA of Human PAF-2

Using rat PAF-2 cDNA as a probe, we isolated from a human liver λgt10 cDNA library one positive clone with a frequency of 1/1,000,000 recombinant phage. This clone covers the region corresponding to bp 1880–2310 of the rat PAF-2 cDNA (where A of the initiation codon methionine is designated as "residue 1"). After repeating the screenings utilizing PCR, we obtained full-length human PAF-2 cDNA, which covered all of the open reading frame. It included 2,940 nucleotides that encode 980 amino acids. The nucleotide homology between human and rat cDNA was 83.8% (fig. 1). The average amino acid identity was 87.1%. The C-terminal half was more conserved (92.4%) than the N-terminal half (81.8%), and 97% identity was found in the region from Pro700 to Ser878 related to the putative ATPase family. The putative ATP/GTP-binding motifs (Walker et al. 1982; Chin et al. 1988), serine protease active site, and AAA-protein family signature have a 99% homology. The human cDNA is 6 bp longer than rat cDNA and contains codons for two glycine residues inserted at amino acid positions 657 and 658. The calculated molecular mass was 104,055 daltons, almost the same as that of rat cDNA. PTS-1 and PTS-2 motifs were absent. The 5′ noncoding and 3′ noncoding regions were not conserved. The homology was <35%, and neither a poly-A sequence nor a poly-A cleavage/addition signal was found in the cDNA.

Northern Blot Analysis of PAF-2 in Various Tissues

The mRNA was hybridized as a single band at ~3.5–4 kb for liver, kidney, skeletal muscle, lung, brain, pancreas, and placenta, with the most prominent expression being in the kidney and skeletal muscle (fig. 2).

Characterization of PAF-2 Mutant Alleles of Group C PBD Patients

Amplification of PAF-2 cDNA confirmed the expression of messenger RNA in the patients (fig. 3). Direct sequencing of the cDNA fragments of patient 1 revealed a one-base insertion; one thymine residue was inserted

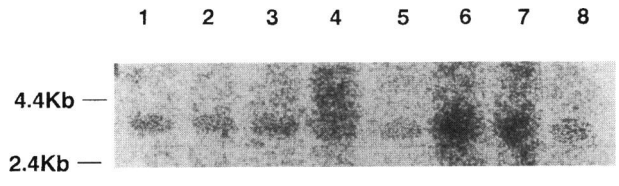


Figure 2 Northern-blot analysis of multiple human tissues. 3.5–4-kb single bands were detected in all lanes. Poly A (+) RNA was blotted in lanes 1–8, which show tissue from heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas.

at nucleotide 511 (the first residue of amino acid 171; nucleotides are numbered from the A of the initiator methionine codon). This insertion disrupts the reading frame of the downstream sequence, completely changing the amino acids starting from codon 171 and introducing a termination codon at amino acid 235. This results in a 234-amino-acid, completely altered, truncated polypeptide (fig. 4). For confirmation, we amplified the genomic DNA of the patient, using human PAF-2–specific primers, on the basis of our preliminary data on rat PAF-2 genomic structure. The insertion also was present in the genomic sequence, confirming the authenticity of the insertion mutation (designated "511insT"). Only the 511insT allele was present in the direct sequence, and neither the normal allele nor a second mutation was detected. This means that the 511insT mutant allele, which led to the premature termination of PAF-2 polypeptide, was apparently the only allele. Since patient 1 was a product of father-daughter mating, the mutation is likely to be homozygous.

We then analyzed the mutation sites of patient 2, using the same procedure. When amplified by use of primers 6 and 24, only the fragment of the expected size was obtained from the normal subjects, whereas both a major, unexpected band of a smaller size and a minor one with normal mobility were identified in the cDNA of patient 2. Direct sequencing revealed that the 84 nucleotides from 1047 to 1130 were absent in the smaller fragment and that 28 amino acids were eliminated, but without altering the reading frame (fig. 5*A*). Samples from the parents were unattainable.

To characterize the genomic defect resulting in the deletion, we screened the human genomic PAF-2 DNA, according to information on rat PAF-2 genomic DNA organization (authors' unpublished data). Interestingly, the rat PAF-2 genomic structure revealed that the deletion corresponds exactly to exon 3 of the rat PAF-2 gene, thereby suggesting that the deletion might result from a novel mutation that affects splice-site selection, rather than reflecting an intragenic 84-bp deletion. In agreement with this hypothesis, only one fragment of the same size as that of the control spanning exons 2–4 was amplified from patient genomic DNA. The fragment was sequenced directly, and a novel G→A substitution in the 5′ splice donor site of intron 3 (fig. 5*B*) was identified (IVS3+1G→A). As shown in figure 5*B*, the patient shares both the normal G and a mutated A, indicating that she was heterozygous for the mutation. Restriction analysis using *Eco*RII on the PCR fragment supports this result (data not shown). Analysis of exon 3 and nearly 100 bp immediately 5′ to exons 3 and 4 and 3′ to exon 2 revealed no additional changes.

Transfection and Expression of Human PAF-2 cDNA in Group C PBD Cells

To determine if human cDNA would complement the group C cell, the normal clone containing the full-length human PAF-2 cDNA was transfected into cells from both patients. The peroxisomes of the cells were morphologically restored by our human PAF-2 cDNA (fig. 6*B* and *D*), as had been observed for rat cDNA (Tsukamoto et al. 1995), and the ratio of [1-^{14}C] lignocerate and palmitate oxidation was elevated, as seen in the normal control cells (table 2). Thus, our human PAF-2 clone morphologically and biochemically restored the peroxisomes of group C PBD cells. We then checked to see if in-frame exon skipping led to the peroxisome deficiency in patient 2. As expected, none of the fibroblasts was complemented by the mutant clone (fig. 6*E* and table 2).

Chromosomal Localization

Of the 116 R-banded (pro)metaphases analyzed, 29 (25%) showed hybridization signals in region 6p21.1 (fig. 7). Distribution of the signals on 6p21.1 was as follows: twin spots on both homologues of chromosome 6 (3.4%); twin spots on one homologue and a single spot on the other (6.9%); twin spots on only one homologue (34.5%); single spots on both homologues (6.9%); and a single spot on only one homologue (48.3%). No such twin signals were detected on any other chromosomes.

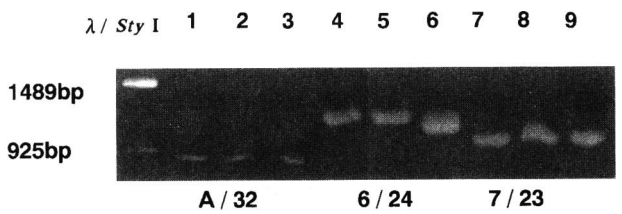


Figure 3 RT-PCR analysis of PAF-2 transcripts of the two patients and the normal control. Lanes 1, 4, and 7, Normal control. Lanes 2, 5, and 8, Patient 1. Lanes 3, 6, and 9, Patient 2. Lanes 1–3 show 910-bp N-terminal products amplified with primers A/32. Products shown in lanes 4–6 were amplified with primers 6/24, with an expected size of 1,273 bp. The 1,124-bp C-terminal transcripts were amplified by use of primers 7/23 and were electrophoresed (lanes 7–9). All transcripts except that amplified with primers 6/24 of patient 2 were of a size similar to that of control samples. The aberrant shorter transcript of patient 2 resulted from exon 3 skipping. λDNA/*Sty*I was used as a size marker.

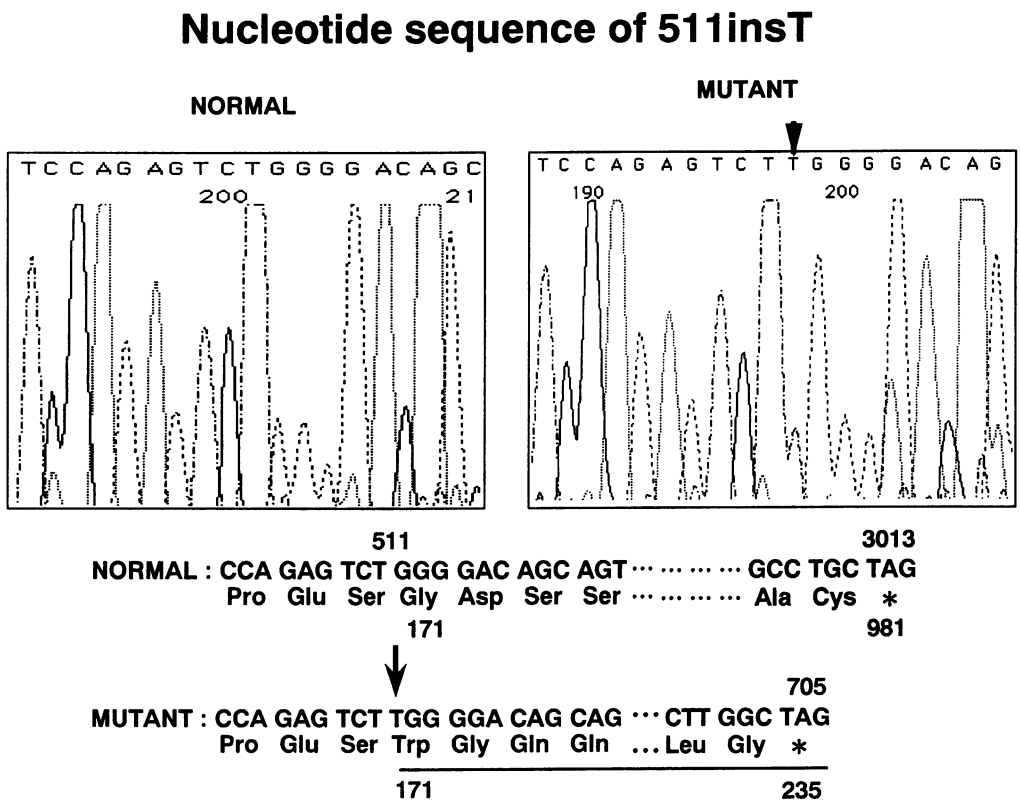


Figure 4 Partial nucleotide sequence and amino acid sequence of the mutation sites of the group C patient 1, determined by direct sequencing, by use of the forward primer. The normal sequence (*left*) and the 511insT sequence (*right*) are shown. The inserted T residue in patient 1 is indicated (*arrow* and *arrowhead*), as is the deduced amino acid sequence (*underlined*) that results from a frameshift. The antisense strand confirmed the same insertion (only data for the sense-strand sequence are shown).

Thus, the human PAF-2 cDNA was mapped to chromosome 6p21.1.

Discussion

To elucidate the mechanism of peroxisome assembly in human cells and to understand better the molecular basis of PBD, we cloned full-length human PAF-2 cDNA and identified two mutations responsible for the peroxisome deficiency of group C ZS. Our human cDNA clone morphologically and biochemically restored peroxisomes of group C patients' cell, thereby providing evidence that our clone encodes the full-length cDNA and that the product expressed from the PAF-2 cDNA, which complements group C PBD cells is essential and indispensable for peroxisome assembly. Human PAF-2 cDNA has a high homology with rat PAF-2 cDNA, especially in the C-terminal domain. They both share the AAA modules with yeast peroxisome biogenesis genes such as PAS5 (Spong and Subramani 1993), PAS8 (Voorn-Brouwer et al. 1993), and PAY4 (Nuttley et al. 1994). Regions with putative ATP/GTP-binding motifs and with the AAA-protein family signature are highly conserved between rat and human PAF-2 cDNA, suggesting that these domains are functionally important for PAF-2. The hypothesis that the human PAF-2 gene and other homologues arose from a common ancestral gene thus is given support.

The clinical phenotype of both patients was that of the most severe ZS. Our findings strongly suggest that patient 1 is most likely homozygous for the 511insT, because only one mutation was identified by direct sequencing of the PCR products, a procedure that allows for simultaneous analysis of both alleles, and because parents of patient 1 were consanguineous. The frameshift resulting from this mutation completely alters the amino acids of the downstream sequence and removes the C-terminal three-fourths of the PAF-2 protein, including the ATP/GTP-binding motifs and an AAA-protein family signature that appears to play an important role in PAF-2 function. These results explain why the homozygous 511insT leads to the complete absence of PAF-2 function—and, hence, to the severe form of the ZS.

The single base substitution (G→A), altering the highly conserved sequence at the 5′ splice site of intron 3 that skips the preceding exon, is most likely the cause of the deletion seen in patient 2's mRNA, since the sequence of exon 3, as well as both the 5′ and 3′ splice consensus sequences of the upstream intron 2 and

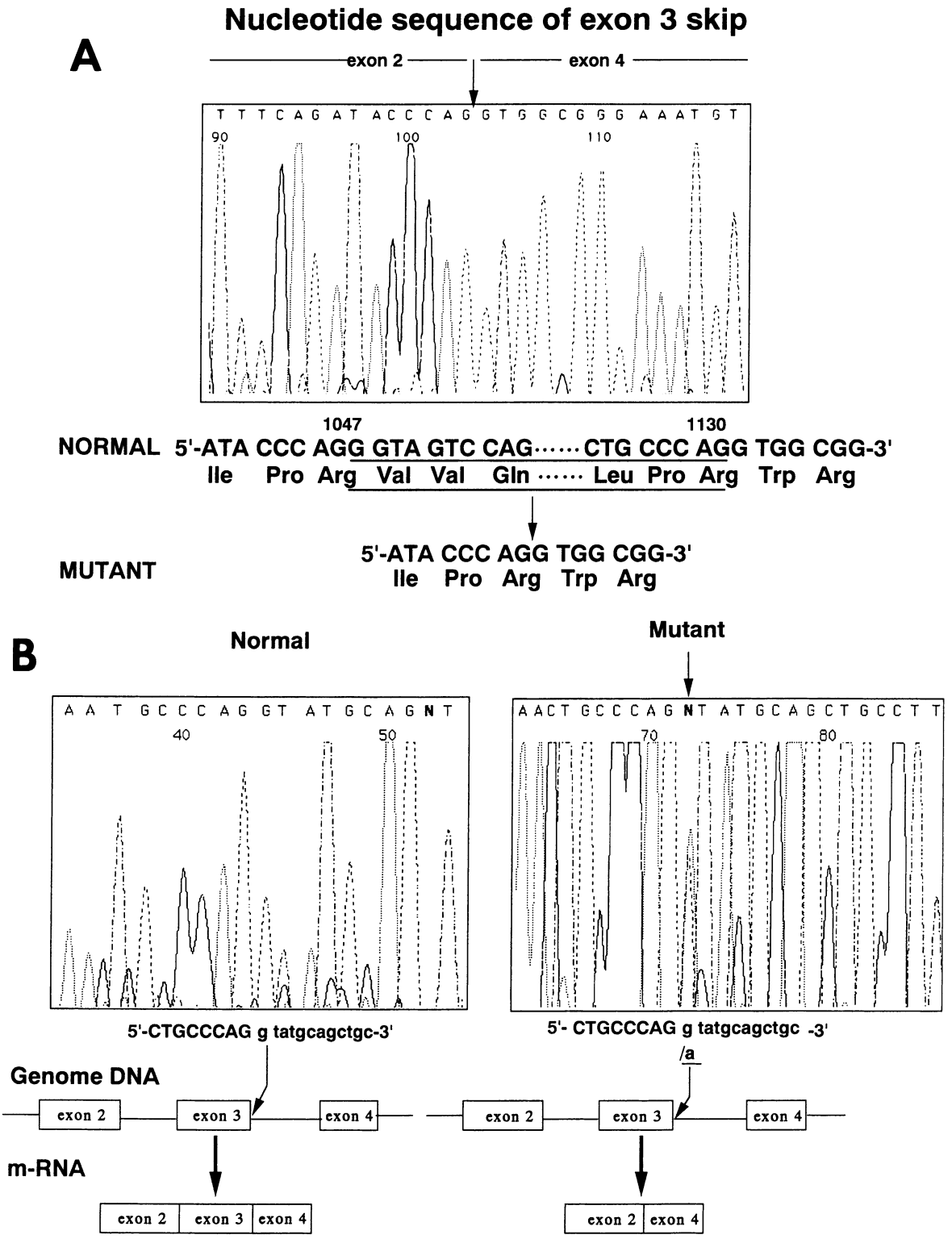


Figure 5 *A*, Nucleotide sequence and deduced amino acid sequence of exon 3 skipping in patient 2's cDNA (IVS3+1G→A). The skipped 84 nucleotides and 26 amino acids (*underlined*) are shown, as is a breakpoint (*arrow*) in the mutant allele. *B*, Nucleotide sequence of 5′ splice site of intron 3, detected by direct sequencing. The mutant sequence (*right-hand panel*), harboring both the normal G and a transversion from G→A at the splice-site consensus sequence, is shown, as are the exonic sequence (*uppercase letters*), the intron (*lowercase letters*), and the mutation site (*underlined*). The splicing patterns of the normal and the mutant are illustrated as a schema.

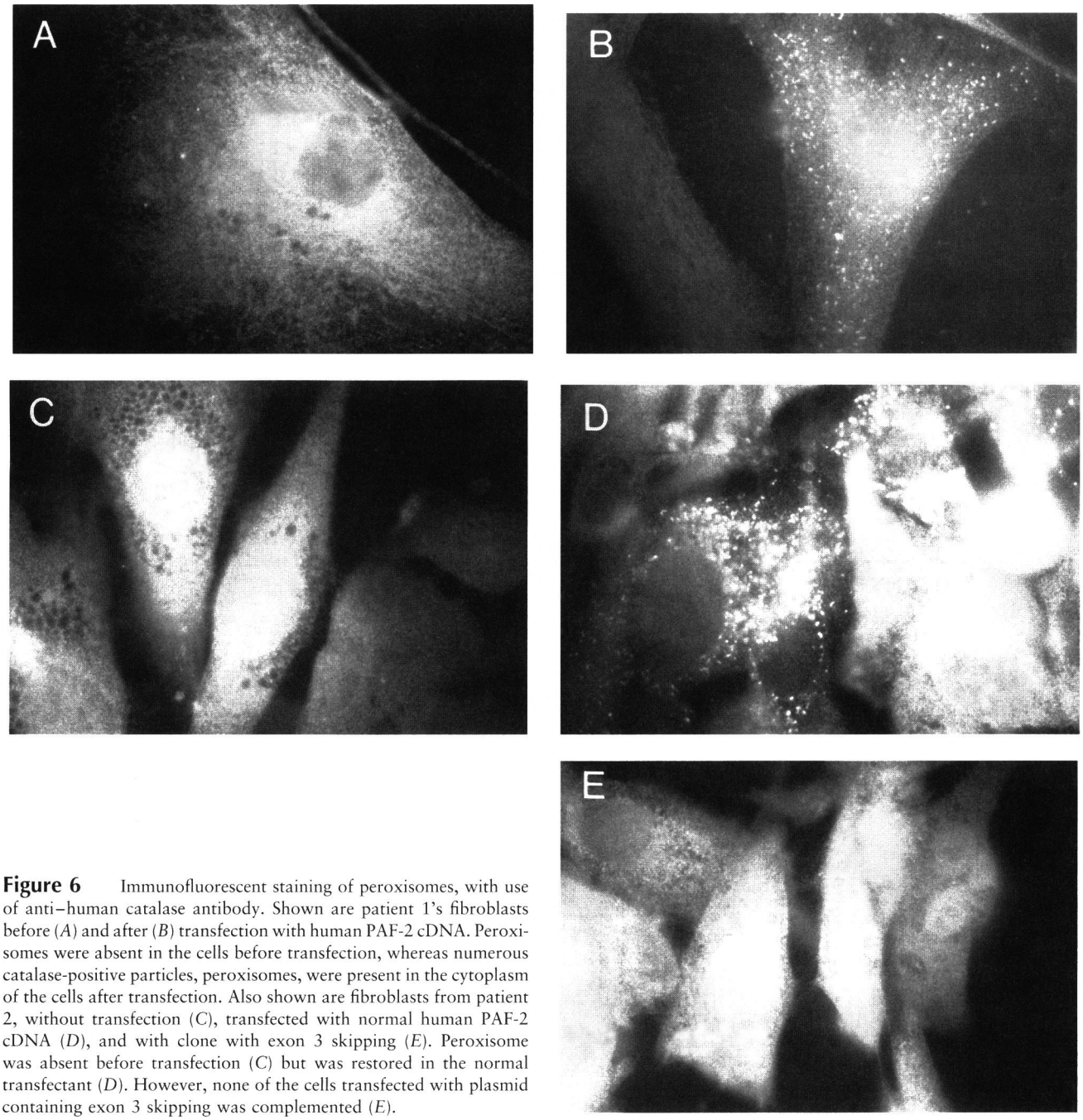


Figure 6 Immunofluorescent staining of peroxisomes, with use of anti–human catalase antibody. Shown are patient 1's fibroblasts before (*A*) and after (*B*) transfection with human PAF-2 cDNA. Peroxisomes were absent in the cells before transfection, whereas numerous catalase-positive particles, peroxisomes, were present in the cytoplasm of the cells after transfection. Also shown are fibroblasts from patient 2, without transfection (*C*), transfected with normal human PAF-2 cDNA (*D*), and with clone with exon 3 skipping (*E*). Peroxisome was absent before transfection (*C*) but was restored in the normal transfectant (*D*). However, none of the cells transfected with plasmid containing exon 3 skipping was complemented (*E*).

downstream intron 4, were intact. A preceding exon skipping has been noted in many cases of naturally occurring mutations that reside at the 5′ splice site of the intron (Hidaka et al. 1987; Weil et al. 1988; Kudo and Fukuda 1989; Carstens et al. 1991; Raben et al. 1993). The consensus sequence of exon 3 and of intron 3 boundary AG:GTATGC were well conserved in the normal control. The mutant AG:ATATGC destroys the consensus sequence and significantly reduces the splicing score for the exon-intron boundaries, from 82.8 to 64.6 (Shapiro and Senapathy 1987), thereby preventing normal splicing on the IVS3+1G→A allele. Although exon 3 skipping (IVS3+1G→A) does not disrupt the reading frame, this event greatly alters the secondary structure of the flanking region of PAF-2 polypeptides and leads to an internally deleted protein. These findings suggest that the IVS3+1G→A mutation can explain the PAF-2 deficiency in patient 2. Peroxisomes were never evident in cells transfected by the mutant clone without exon 3, thereby supporting the argument that this mutation is responsible for the PAF-2 deficiency that impairs peroxisome assembly in group C PBD patients. The minor transcript of normal length is the product of another allele not related to

Table 2

[1-^{14}C] Lignocerate Oxidation/[1-^{14}C] Palmitate Oxidation in Cells of Patient 2

Source	Ratio
Patient 2's cells	.08
Patient 2's cells transfected with human normal PAF-2 cDNA	.26
Patient 2's cells transfected with mutant (exon 3–skipping) cDNA	.09
Control cells	.35

NOTE.—In cells transfected with normal clone, [1-^{14}C] lignocerate oxidation was recovered, thereby indicating that the PAF-2 cDNA biochemically complements the group C cells. There was no change in the transfectant with the mutant clone.

IVS3+1G→A, since the splicing mutation was heterozygous. We have found a minor insertion that introduces a frameshift in this transcript, thereby suggesting that another allele in the patient also contributes to the PAF-2 deficiency (data not shown).

FISH with the PAF-2 cDNA clone revealed the chromosomal localization of PAF-2 to be 6p21.1. We elsewhere have reported data on a Japanese patient with group C ZS, in whom there was a microdeletion of chromosome 7 (Naritomi et al. 1988). Our present study indicates that the deletion of chromosome 7 in this patient was incidental and not related to the ZS phenotype.

Among the 10 complementation groups of PBD, only two pathogenic genes, PAF-1 for group F and PXR-1 for group 2, have been cloned and characterized. Nonsense mutations in the PAF-1 gene, as well as missense and nonsense mutations in PXR-1, were associated with peroxisome deficiency, in each group. The present study clearly defines the primary defect in group C patients.

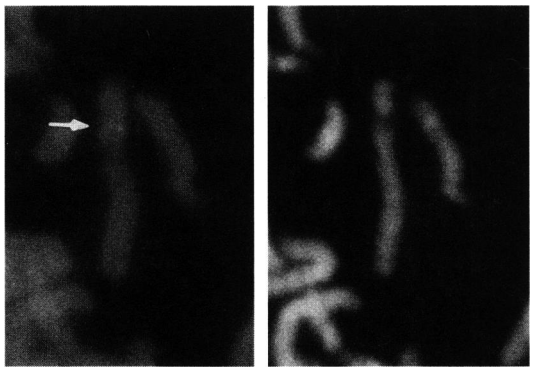


Figure 7 Assignment of human PAF-2 cDNA to chromosome 6p21.1, by FISH. The same prometaphase was sequentially photographed through different filter combinations (Nikon B-2A [*left*] and UV-2A [*right*]). *Left*, Partial R-banded prometaphase showing double spots (*arrow*) on both chromatids of chromosome 6. *Right*, G-banding pattern indicating fluorescent signals at 6p21.1.

These two PAF-2 mutations, the frameshift mutation 511insT and the splice-site mutation IVS3+1G→A, confirm the hypothesis that PAF-2 is the gene defective in peroxisome deficiency in group C PBD patients, the third genetic defect, in peroxisome assembly among PBD patients, to be defined at the molecular level. Studying the correlation between these mutational sites and the extent of peroxisome deficiency, especially with a different clinical phenotype, will shed light on functional domains of PAF-2.

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