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PEX1 Mutations in Complementation Group 1 of Zellweger Spectrum Patients Correlate with Severity of Disease

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ABSTRACT

The peroxisome biogenesis disorders (PBD) are a group of autosomal-recessive diseases with complex developmental and metabolic phenotypes, including the Zellweger spectrum and rhizomelic chondrodysplasia punctata. The diseases are caused by defects in peroxisomal matrix protein import and are characterized by the loss of multiple peroxisomal metabolic functions. In humans, 12 complementation groups have been identified, with complementation group 1 accounting for more than two thirds of all PBD patients. Mutations in the *PEX1* gene encoding a member of the AAA protein family of ATPases are responsible for the defects in this group, and a variety of *PEX1* mutant alleles have been described. We characterized the *PEX1* gene mutations and associated haplotypes in a group of thoroughly documented Zellweger spectrum patients in complementation group 1 who represent the broad range of phenotypic variation. We compared the type of mutation with the age of survival, clinical manifestations, and biochemical alterations and found a close relationship between genotype and age of survival. Missense mutations cause a milder form of disease, whereas insertions, deletions, and nonsense mutations are associated with severe clinical phenotypes. Thus, knowing the *PEX1* gene mutation is helpful in predicting the course of disease in individual cases. **(*Pediatr Res* 51: 706–714, 2002)**

Abbreviations

AAA, ATPases associated with diverse cellular activities
PBD, peroxisome biogenesis disorders
PTS, peroxisomal targeting signal
SSCP, single-strand conformation polymorphism
VLCFA, very-long-chain fatty acids

Peroxisomes are ubiquitous components of eukaryotic cells and are involved in multiple metabolic processes (1). Examples are hydrogen peroxide–based respiration, β-oxidation of VLCFA, and biosynthesis of ether phospholipids, bile acids, and isoprene compounds. Biogenesis of peroxisomes appears to proceed mainly by growth and division of preexisting peroxisomes (1, 2). Newly synthesized peroxisomal proteins contain specific PTS—PTS1 or PTS2 for matrix enzymes and mPTS for membrane proteins—that direct them to and into peroxisomes. The proteins involved in and necessary for peroxisome biogenesis are peroxins, encoded by *PEX* genes. To date, 15 human *PEX* genes have been identified.

In humans, defective peroxisome biogenesis results in PBD, a group of autosomal-recessive diseases, including the Zellweger spectrum with four overlapping clinical phenotypes, classical Zellweger syndrome, atypical Zellweger syndrome ("pseudo"-Zellweger syndrome), neonatal adrenoleukodystrophy, and infantile Refsum disease, as well as a distinct phenotype, rhizomelic chondrodysplasia punctata (1). Patients with the classical Zellweger syndrome display characteristic dysmorphic features, severe neurologic dysfunction, eye abnormalities, hepatorenal defects, and patellar calcific stippling. They rarely survive the first year of life. Patients with atypical Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease exhibit similar clinical characteristics to those of classical Zellweger syndrome patients, but can survive up to several decades (1, 3). Zellweger spectrum patients are characterized biochemically by defective import of virtually all peroxisomal matrix enzymes, as well as by the inability of many peroxisomal enzymes to function properly when mislocalized to the cytosol. Patients with rhizomelic chondrodysplasia punctata have unique clinical symptoms, including proximal shortening of the limbs (rhizomelia) and cataracts. At the biochemical level, these patients have a limited set of impaired peroxisomal pathways, namely plasmalogen biosynthesis and branched-chain fatty acid oxidation (1, 3).

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Somatic cell fusion studies indicate that the Zellweger spectrum is genetically heterogeneous. Thus far, 11 complementation groups have been established, with complementation group 1 accounting for more than two thirds of Zellweger spectrum patients (1, 4). Mutations in the *PEX1* gene are responsible for the defects in this group, and a variety of mutant alleles have been described (5–13). The *PEX1* gene encodes a member of the AAA protein family of ATPases, peroxin 1, and interacts with another ATPase of this AAA protein family, peroxin 6 (13–15). Although the exact function of peroxin 1 is unknown, several recent studies favor a role in the process of peroxisomal matrix enzyme import.

The purpose of this study is to examine the relationship between the genotype and the clinical as well as the biochemical phenotype of Zellweger spectrum patients contained within complementation group 1. We have performed mutation and haplotype analyses of the *PEX1* gene in 16 thoroughly documented patients who represent the broad range of phenotypic variation within this complementation group. The study has been approved by the local ethical committee.

METHODS

Subjects. The study includes sixteen patients of German, Swiss, or Turkish origin with clinical and biochemical findings characteristic for the Zellweger spectrum of PBD. Biochemical assays of peroxisomal function were carried out at the time of diagnosis and included at least VLCFA and phytanic acid concentrations, catalase latency, and plasmalogen biosynthesis. All patients were assigned to complementation group 1 by cell fusion analyses.

Mutation analyses. Genomic DNA and total cellular RNA were isolated from peripheral blood samples or cultured skin fibroblasts according to standard protocols. cDNA was synthesized using the Superscript TM II reagents and protocol (Invitrogen, Carlsbad, CA, U.S.A.). For mutation and polymorphism screening, we amplified *PEX1* cDNA fragments covering the entire coding region or the 24 exons of the *PEX1* gene. The oligonucleotide primers are listed in Table 1, *A* and *B*. Twenty-five microliters of total reaction mixture contained 200 ng of genomic DNA or 50–100 ng of cDNA, specific primers (50 pmol each), reaction buffer (400 μM of each deoxyribonucleoside triphosphate, 10 mM Tris HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 2% formamide) and 1 U Taq DNA polymerase. Thirty cycles were performed (94°C for 60 s, 50–54°C for 60 s, 72°C for 60 s) followed by a final extension of 2 min at 72°C. The annealing temperatures of the oligonucleotide primers and the length of the amplification products are included in Table 1, *A* and *B*. The amplified genomic or cDNA fragments were subjected to SSCP analysis (16). Four to 8 μL of PCR product and 4 μL of SSCP buffer (0.25 mg bromphenol blue/xylene cyanol, 74% formamide) were denatured at 94°C for 3–5 min. Electrophoresis for SSCP was performed on 10% polyacrylamide-bisacrylamide gels (29:1) with 3 M urea in 1× TBE buffer (89 mM Tris HCl, 89 mM borate, 2.5 mM Na EDTA) at 4°C. Some of the gels also contained 5% glycerol to enhance migration differences between wild-type and mutant strands (17). DNA bands were detected by silver staining. PCR products showing an aberrant SSCP pattern were sequenced directly by the Sanger dideoxy chain termination method using reagents and the protocol of U.S. Biochemical Corporation (Cleveland, OH, U.S.A.) (18) or by semiautomated sequencing using reagents and the protocol of Applied Biosystems (Foster City, CA, U.S.A.).

Allele-specific oligonucleotide hybridization. 14mer or 19mer oligonucleotides were synthesized to the human mutant sites in exons 13, 14, and 15 and the corresponding human wild-type sites. The allele-specific oligonucleotides (ASO) used are 5'-AAGATTGGTGGGTT-3' (wild-type ASO) and 5'-AAGATTGATGGGTT-3' (mutant ASO) for G843D, 5'-TAAAAGAGTTTATCTCCAT-3' (wild-type ASO) and 5'-TAAAAGAGTTTTATCTCCA-3' (mutant ASO) for c.2097–2098insT, and 5'-AACTGGAGGGTTTG-3' (wild-type ASO) and 5'-AACTGGCGGGTTTG-3' (mutant ASO) for G777G. The oligonucleotides were end-labeled as described (19). Samples of PCR-amplified cDNA or genomic fragments for the regions of interest were arrayed on a nitrocellulose filter with

Table 1*A.* *Oligonucleotides for PEX1 cDNA amplification*

Product Length (bp)	Primer Sense (5′–3′)	Position	Primer Antisense (5′–3′)	Position	Annealing Temperature (°C)
267	gaacccagagggacgctc	c.(−25)–(−8)	ccaacttgtctgttaatttc	c.223–242	50
283	gatcaaggtgaaaatgtg	c.202–219	gtattagtgcaacaatttg	c.466–484	52
294	catcttctagatcaaattc	c.384–403	caactggaatctctgactc	c.694–712	50
312	ctgtgggaatcactgaatc	c.665–683	gctctacatcaaaatattc	c.958–976	50
310	cataaacactgtgccattc	c.919–937	caagatggagaacttctac	c.1210–1228	54
285	cttgaagaattgaacaatg	c.1171–1189	ctctgatattaccaaagg	c.1435–1452	50
297	ctacagcagtctactacc	c.1408–1425	ctccaaggaggatacgcc	c.1687–1704	54
291	gctgagctctttgggagg	c.1656–1673	gtgaaagccacctctagg	c.1929–1946	52
295	gactgtaaagctttacgag	c.1882–1900	gagcagaaacaagtaaga	c.2159–2176	52
305	gtcagtctcagcaatctc	c.2135–2152	gtccaatgttgttaaaac	c.2422–2439	50
294	gtcagagtatatccaccag	c.1393–2411	gtgcaattaccccagctag	c.2668–2686	50
293	ggaatactgttgtatggtc	c.2626–2644	ccttctactccatccaac	c.2901–2918	52
291	gtagttaaccagttgctg	c.2878–2895	caattgggcattgtaaag	c.3151–3168	50
286	ctttactggagctgatctg	c.3123–3141	ctgattcataagagcttcc	c.3391–3409	52
285	caatatgtaccggctctac	c.3369–3387	gttcatggattcgtcctc	c.3637–3654	52
301	caaaggcagataccggag	c.3609–3626	caacatatggaaaagccatc	c.3891–3910	52

Table 1*B.* *Oligonucleotides for exon amplification of the PEX1 gene*

Exon	Product Length (bp)	Primer Sense (5′–3′)	Position	Primer Antisense (5′–3′)	Position	Annealing Temperature (°C)
1	257	cgatctcctccggctccg	IVS0(−32)–(−15)	ggctgaagatcaggtggc	IVS1(+36)–(+19)	54
2	266	catgtgaattgtaataatag	IVS1(−87)–(−68)	tagaagaaagttattgcc	IVS2(+30)–(+13)	50
3	262	catagactacatatacac	IVS2(−140)–(−123)	ccttaaatgagatagttc	IVS3(+35)–(+18)	47
4	217	ctagatatggagtggac	IVS3(−57)–(−41)	gctatagtgtagaatatg	IVS4(+44)–(+27)	50
5	315	gaatgtgcactaatgagc	IVS4(−33)–(−16)	gcttcctatcatagtcc	c.737–753	50
5	321	cagttgactcatcatag	c.707–724	gctttggagaaagtagc	c.1011–1027	50
5	301	gctttactgtgacatatgg	c.980–998	gccacataaaatttctcc	IVS5(+41)–(+24)	50
6	264	cacctaaattcatagaagc	IVS5(−102)–(−84)	cgtgtaaaagaattttg	IVS6(+42)–(+26)	47
7	252	gttaagttcagatatgagg	IVS6(−49)–(−31)	gtatgacaggttgcaag	IVS7(+78)–(+62)	60
8	244	gtttcagtactaactctgc	IVS7(−43)–(−25)	cacttcacaatgcaaggtg	IVS8(+96)–(+78)	50
9	180	gacccgagaaaaacatgc	IVS8(−68)–(−51)	ctaacatgctagtttggc	IVS9(+31)–(+14)	50
10	261	cagtcttttatcatgtaac	IVS9(−37)–(−19)	caccctatataatagatgg	IVS10(+90)–(+72)	52
11	239	ggttgccacaatgacag	IVS10(−71)–(−51)	gcattatgtataacattcc	IVS11(+68)–(+50)	50
12	249	cagcactgaaatgatactg	IVS11(−44)–(−26)	caagaccttaagccagtg	IVS12(+32)–(+15)	50
13	220	gcttttgcactatatgc	IVS12(−27)–(−10)	ggacataattcaataatcc	IVS13(+39)–(+21)	50
14	273	cagttgaatacaattgg	IVS13(−44)–(−28)	gaagattccaagttcag	IVS14(+37)–(+21)	50
15	297	ccagctaagatgatggc	IVS14(−80)–(−64)	cagtggttcttctgggag	IVS15(+80)–(+60)	56
16	286	gctgctacttagtcatttctg	IVS15(−64)–(−44)	gccagtgaatttacactttg	IVS16(+34)–(+15)	54
17	254	catttagacttagtcag	IVS16(−75)–(−59)	cataaaccacaggtttc	IVS17(+96)–(+112)	47
18	320	cactttgccaactatgaag	IVS17(−54)–(−36)	gggcgactagtagcagcc	c.2940–2957	54
19	262	caaaatgtcatcatgtc	IVS18(−54)–(−38)	gctggtcttctaaggac	IVS19(+99)–(+107)	51
20	284	gaaaatcataagatacac	IVS19(−75)–(−58)	gtcataacaacttcctc	IVS20(+30)–(+14)	50
21	295	ctttaaactggttgtcc	IVS20(−30)–(−14)	cagaactgtataatgatg	IVS21(+32)–(+15)	50
22	285	ccatctttcattactac	IVS21(−30)–(−14)	ggtgaaacaatttttaag	IVS22(+56)–(+39)	50
23	252	gtaacaactggactaccac	IVS22(−73)–(−55)	gtcacttgtaatagtagctg	IVS23(+48)–(+29)	54
24	286	gccaacaatccattatc	IVS23(−73)–(−57)	cagtattaatctcaatcc	c.4013–4030	50
24	345	gctataattatgtaatg	c.3992–4008	gaattatgtgtaaatttc	IVS24(+26)–(+8)	47

a slot blotter. Filters were prehybridized for 2 h and hybridized for 1 h in 1% SDS, 1 M NaCl, and 10% dextran sulfate at 2°C below the calculated Tm of the wild-type or the mutant probe, respectively. The filters were rinsed twice in 2× SSC and 1% SDS at room temperature, and finally washed once for 10 min at the Tm in 2× SSC, 1% SDS. Samples showing the mutant pattern were sequenced for confirmation as described.

RESULTS

Clinical phenotype. To establish a possible genotype-phenotype correlation, we defined clinical criteria allowing us to score the severity of the clinical phenotype in every Zellweger spectrum patient in complementation group 1 (Table 2, *A* and *B*). We made the phenotypic assignment exclusively on the patients' present age or the age at death. A patient was scored as mildly affected if he survived beyond the age of 1 y. A patient was scored as severely affected if he died before that age. From a total of 16 thoroughly documented complementation group 1 patients, 9 could be classified as mildly affected and 7 as severely affected. The nine mildly affected patients had previously been assigned as atypical Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease, and the 7 severely affected patients as classical Zellweger syndrome.

Although clear differences appear between the ages of survival, the boundaries for the clinical manifestations between mildly and severely affected patients are imprecise. Their clinical signs, including dysmorphic features as well as cerebral, ocular, hepatorenal, and skeletal abnormalities, show considerable overlap. Typical dysmorphic features are present in both patient groups. Cerebral dysfunctions are less profound in mildly affected patients. In the mildly affected group, four of seven patients needed gavage feeding, five of seven patients were hypotonic, and only one of seven patients developed neonatal seizures. In contrast, severe psychomotor retardation is a consistent sign in severely and mildly affected patients. Regarding ocular signs, retinitis pigmentosa and optic atrophy are a common feature of mildly affected patients, whereas cataracts are exclusively present in severely affected patients. Renal cysts and calcific stippling are also typical features of severely affected patients.

Biochemical phenotype. We also defined biochemical criteria for scoring the severity of disease in Zellweger spectrum patients in complementation group 1. As for the characteristic abnormal clinical features of Zellweger spectrum patients, biochemical abnormalities could also not be taken into account for scoring. There was no clear difference in the extent of impairment of specific peroxisomal metabolic functions. As an example, the measurements of accumulated VLCFA in plasma revealed that four of seven patients with G843D have lower $C_{26:0}/C_{22:0}$ ratios than all other patients. Nevertheless, this is not a distinguishable marker. The $C_{26:0}/C_{22:0}$ ratio in plasma in the mildly affected patient group is 0.249 ± 0.163 (mean ± SD) and in the severely affected patient group 0.379 ± 0.078.

Mutations in the PEX1 gene. We found eight different mutations in the 16 Zellweger spectrum patients in complementation group 1. The mutational spectrum includes one missense mutation, two nonsense mutations, two insertions,

Table 2*A.* *Zellweger spectrum patients with mild clinical phenotype*

Clinical Signs	Range or Frequency of Clinical Signs	PBD-G008 G843D Homo	PBD-G001 G843D Homo	PBD-G012 G843D Homo	PBD-G014 G843D Homo	PBD-G010 G843D Homo	PBD-G015 G843D c.1865–1866ins CAGTGTGGA	PBD-G016 G843D c.1865–1866ins CAGTGTGGA	PBD-G009 c.2227–2416del ?
Present age (years, months)	3, 6–23, 9	9, 11	3, 6	13, 0	13, 8	2, 10	10, 5	23, 9	4, 0
Dysmorphic features									
Large fontanelle, wide sutures	5/5	+	+	+		+	+		
High forehead	5/5	+		+			+	+	+
Broad nasal bridge	5/5	+	+	+			+		+
Hypertelorism	7/7	+	+	+		+	+	+	+
Shallow orbital ridge	4/5	+	+	−		+		+	
Epicanthus	5/5	+		+			+	+	+
External ear deformity	2/3	−		+					+
Cerebral									
Poor sucking	6/7	+	+	+		+	+	+	−
Gavage feeding	4/7	+	+	+		−	+	−	−
Hypotonia	5/7	+	+	+		+	−	−	+
Severe psychomotor retardation	7/7	+	+	+		+	+	+	+
Neonatal seizures	1/7	−	−	−		−	−	−	+
Ocular									
Cataract	0/6	−	−	−			−	−	−
Retinitis pigmentosa	6/6	+	+	+			+	+	+
Optic atrophy	3/6	+	−	+			−	+	−
Nystagmus	5/6	+	+	+			+	+	−
Hepatorenal									
Hepatomegaly	5/7	+	+	+		−	+	+	−
Liver fibrosis	2/5	−		−		+	+	−	
Elevated liver enzymes	5/7	+	−	+		+	+	−	+
Renal cysts	1/7	−	−	−		+	−	−	−
Skeletal system									
Calcific stippling	0/3	−		−					−

Homo, homozygous; +, present; −, absent.

Table 2*B.* *Zellweger spectrum patients with severe clinical phenotype*

Clinical Signs	Range or Frequency of Clinical Signs	PBD-G005 G843D Homo c.2097–2098insT	PBD-G003 c.2097–2098insT Homo	PBD-G011 c.2097–2098insT c.2916delA	PBD-G007 c.2097–2098insT c.2814–2818del	PBD-G013 c.2097–2098insT Y1126X	PBD-G002 c.2097–2098insT R872X	PBD-G004 c.2227–2416del Homo
Age of survival (months)	1–34	34	2	3	3	9	2	1
Dysmorphic features								
Large fontanelle, wide sutures	6/6	+	+	+	+	+		+
High forehead	6/6	+	+	+	+	+		+
Broad nasal bridge	4/4			+	+	+		+
Hypertelorism	6/6	+	+	+	+	+		+
Shallow orbital ridge	3/3		+			+	+	
Epicanthus	3/3		+			+		+
External ear deformity	5/5		+		+	+	+	+
Cerebral								
Poor sucking	7/7	+	+	+	+	+	+	+
Gavage feeding	7/7	+	+	+	+	+	+	+
Hypotonia	7/7	+	+	+	+	+	+	+
Severe psychomotor retardation	7/7	+	+	+	+	+	+	+
Neonatal seizures	6/7	+	+	−	+	+	+	+
Ocular								
Cataract	3/7	−	−	−	+	−	+	+
Retinitis pigmentosa	1/5	+	−	−		−		−
Optic atrophy	1/5	−	−	−		−		+
Nystagmus	4/5	+	+	−		+		+
Hepatorenal								
Hepatomegaly	4/6	+	+		−	+	−	+
Liver fibrosis	3/3	+	+			+		
Elevated liver enzymes	6/6	+		+	+	+	+	+
Renal cysts	5/7	−	+	+	+	−	+	+
Skeletal system								
Calcific stippling	1/4	−	−			+		−

Homo, homozygous; +, present; −, absent.

and three deletions. The most common mutation in 8 of 16 patients is a G to A transition at cDNA bp 2528 in exon 15 producing G843D. The mutation is located between the two nucleotide binding folds of peroxin 1 (Fig. 1). Six patients are homozygous for G843D. The second common mutation in our patient group is a 1 bp insertion in exon 13, c.2097–2098insT, which results in a frameshift and a premature termination codon 41 amino acids downstream at codon 740. The mutation results in a truncated protein containing only one of the two nucleotide binding folds (Fig. 1). Five patients are heterozygous for c.2097–2098insT, only one patient is homozygous. One of the patients studied (patient PBD-G005) includes both of these common mutations. He is homozygous for G843D and also heterozygous for c.2097–2098insT. His mother is heterozygous for the missense mutation and his father heterozygous for the insertion. The other *PEX1* gene mutations we identified include two nonsense mutations, R872X and Y1126X, a 190 bp deletion in exon 14, c.2227–2416del, a 5 bp deletion in exon 18, c.2814–1818delCTTTG, a 1 bp deletion in exon 19, c.2916delA, and a 9 bp insertion c.1960–1961insCAGTGTGGA in exon 12. Three of the eight mutations identified in our patient group, namely G843D, c.2097–2098insT, and c.1960–1961insCAGTGTGGA, have also been described in patients from other groups (5–9, 11–13). Twenty-two of the 30 PEX1 gene mutations described by others and us are located within the two nucleotide binding domains of peroxin 1 (Fig. 1).

Genotype-phenotype correlation. To study the genotype-phenotype correlation, we investigated how the clinical and biochemical phenotypes of the 16 Zellweger spectrum patients are distributed among the different *PEX1* mutations. We found that the missense mutation G843D gives rise to a milder phenotype than the truncation mutations, insertions, and deletions. When correlated with G843D or the other *PEX1* gene mutations, the present age or the age at death is the strongest indicator of mild or severe illness (Fig. 2). Figure 3 underlines that there is no discernible correlation between genotype and biochemical phenotype. Thus, the type of *PEX1* mutation can be helpful in predicting the assignment of an individual case to mild or severe disease.

PEX1 haplotypes. During the course of our mutation analyses work, we also identified two polymorphic synonymous mutations in the *PEX1* gene. These polymorphisms have already been reported in part (9, 20). The first polymorphism lies within exon 14. It is an A to C transition at cDNA bp 2331 and has a frequency in population of 0.08. Because GGA and GGC both code for glycine, this alteration produces the synonymous mutation G777G. The second polymorphism lies within intron 11. It is represented by the presence or absence of a 16 bp insertion upstream of the intron 11 donor splice site, IVS11+142insAGAAATTTTAAGTCTT (IVS11ins16bp). The frequency of this polymorphism in population is 0.86.

Among our 16 Zellweger spectrum patients in complementation group 1, the association of the G843D and c.2097–2098insT mutations with the G777G and IVS11ins16bp polymorphisms was striking (Table 3). Five patients who are homozygous for G843D are also homozygous for the GGA codon at G777 and the IVS11ins16bp. An identical haplotype association could be observed in patients PBD-G015 and PBD-G016, who are both compound heterozygous for G843D and c.1960–1961insCAGTGTGGA. Exceptions to this kind of haplotype association are patients PBD-G010 and PBD-G005. Patient PBD-G010 is homozygous for G843D, also homozygous for the GGA codon at G777, but compound heterozygous for the IVS11ins16bp. Patient PBD-G005, with three *PEX1* gene mutations, G843D homozygote, and c.2097–2098insT heterozygote, is heterozygous for the two polymorphisms. By

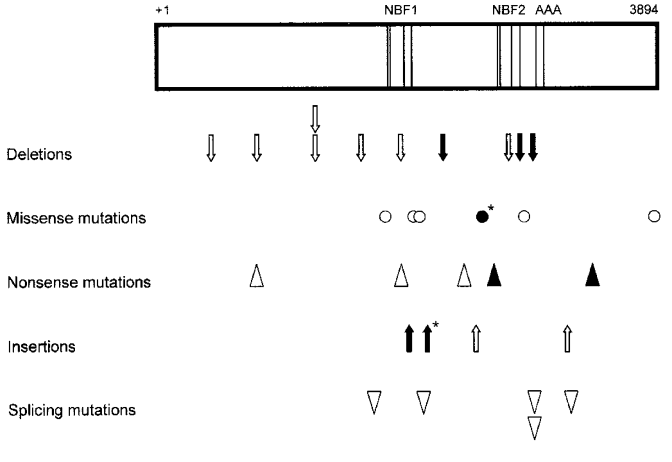

Figure 1. Distribution of mutations in the *PEX1* gene over the functional protein domains. The *boxes* represent the coding region of 1283 amino acids, including the two nucleotide binding folds (*NBF*) and the AAA protein family signature (*AAA*). *Asterisks* mark the two most common *PEX1* mutations, G843D and c.2097–2098insT. *Filled symbols* represent the mutations from this study and *open symbols* represent further mutations reported in the literature (5–13).

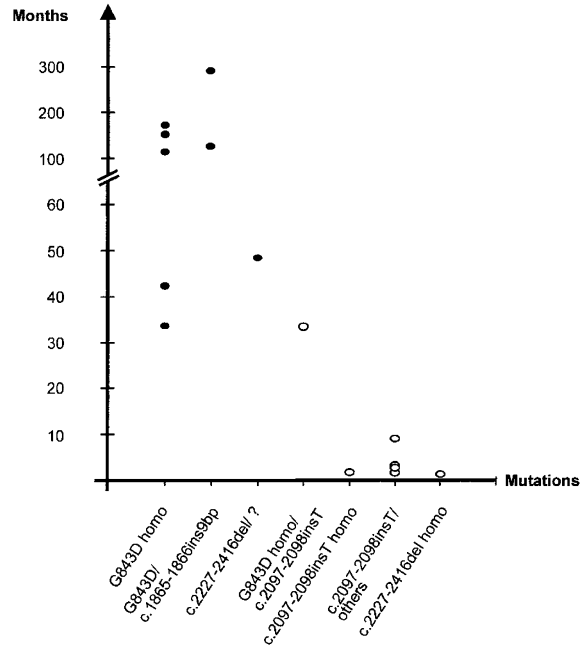

Figure 2. Age of death or age of last known survival of Zellweger spectrum patients in relation to the identified *PEX1* gene mutations. A correlation between the age of survival and the genotype could be shown. *Filled symbols* represent the age of patients who were alive at the time of this study and *open symbols* the age of death for already deceased patients.

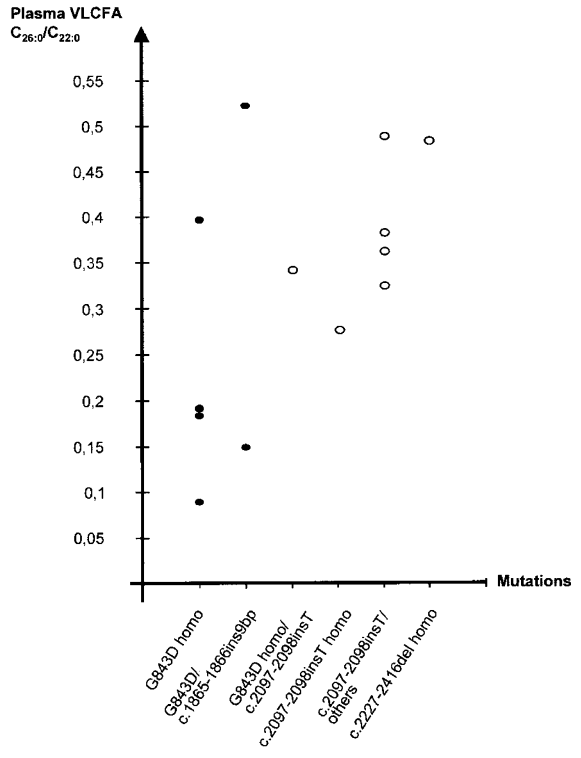

Figure 3. Concentrations of VLCFA in plasma of Zellweger spectrum patients at the time of diagnosis in relation to the *PEX1* gene mutations. There was no correlation between the biochemical phenotype and the patients' genotype. *Filled symbols* represent the VLCFA ratio of patients who were alive and *open symbols* the VLCFA ratio of those patients who were already deceased at the time of this study.

contrast to the haplotype association of G843D, patient PBD-G003, who is homozygous for c.2097–2098insT, and patients PBD-G007 and PBD-G002, who are compound heterozygous for c.2097–2098insT, are homozygous for the GGC codon at G777 and the absence of IVS11ins16bp. Patients PBD-G011 and PBD-G013, who are also compound heterozygous for c.2097–2098insT, are compound heterozygous for the two polymorphisms. These comprehensive analyses confirm a distinct haplotype association for the two most common *PEX1* mutations, G843D and c.2097–2098insT.

DISCUSSION

So far, 30 different *PEX1* mutations have been reported in Zellweger spectrum patients in complementation group 1. The spectrum includes missense and nonsense mutations, deletions, insertions, and splicing mutations. G843D is the most common mutation. The reported allele frequency ranges from 25% to 37% for Caucasian patients in the North American, Australasian, Dutch, and German population (6, 7, 9–12). The second common mutation in this group is c.2097–2098insT, with an allele frequency between 22% and 32% (9, 11, 12). All other *PEX1* mutations are private, occurring mostly in single patients.

The presence of common mutations in a population suggests either a high mutation rate at a single nucleotide position or a common evolutionary relationship between mutant alleles. Known mutation hot spots are at CpG dinucleotides or in regions that play an important role in the breakage and rejoining of DNA (21). *PEX1* sequence analyses on either side of these two most common patient mutations did not reveal motifs characteristic of hot spots. Two polymorphisms have been identified in the *PEX1* gene, G777G and a 16 bp insertion variation in intron 11 (9, 20). Comprehensive haplotype analyses revealed that there is a distinct relationship between the two most common *PEX1* mutations, G843D and c.2097–2098insT, and these polymorphisms. G843D alleles are associated with the GGA codon at G777 and the 16 bp insertion in intron 11, whereas c.2097–2098insT alleles are associated with the GGC codon at G777 and the absence of a 16 bp insertion in intron 11. The distinct haplotype associations for G843D and c.2097–2098insT suggest that a founder effect may have contributed to the high frequency of these mutations. The mutations may have arisen once during human evolution and since expanded among the Caucasian population, the origin of our Zellweger spectrum patients. An interesting example is patient PBD-G005, with three *PEX1* mutations. The two mutant alleles inherited from his parents are consistent with the founder-effect hypothesis. Furthermore, the paternal allele carries G843D as an additional *de novo* mutation. Although a founder effect for G843D is most likely, this *de novo* mutation points to a mutation hot spot in this region with so far unidentified DNA alteration elements.

The *PEX1* gene is located on chromosome 7q21–q22 and encodes peroxin 1, a member of the AAA protein family of ATPases. Peroxin 1 is involved in peroxisome biogenesis and appears to act in peroxisomal matrix protein import (22, 23). Given the important function of peroxin 1 in organelle biogenesis, together with the various types of *PEX1* mutations and the broad clinical spectrum of abnormalities encountered in cases of mutations, we analyzed the genotype-phenotype correlation in *PEX1*-associated disease. We studied clinical, biochemical, and genetic characteristics in 16 Zellweger spectrum patients and compared the data with those described in the literature. *PEX1* mutations cause predictable clinical phenotypes and we propose to subdivide these mutations in two classes.

Class I mutations include only missense mutations. The presence of missense mutations on both alleles in a given patient causes mild disease. Eleven of 12 Zellweger spectrum patients with *PEX1* missense mutations on both alleles including G843D survived the first year of life, and their age of last known survival ranges from 2 to 45 y (the present study, 12). In addition, these patients had a lower frequency of clinical symptoms compared with patients with other forms of *PEX1* mutations (Table 2*A*). Class I mutations result in a peroxin 1 with residual protein levels and function. Most of these alleles carry G843D, and there is only a limited observation for other missense mutations. Fibroblasts of patients homozygous for class I mutations had detectable protein levels for peroxin 1 and the import of catalase, a peroxisomal matrix enzyme, was partially retained (6, 12, 13).

Class II mutations include the truncation mutations, insertions, deletions, and splicing mutations. The presence of a class II mutation on both patient alleles excludes mild disease. All 16 Zellweger spectrum patients homozygous for class II mu-

Table 3. *PEX1 haplotypes in Zellweger spectrum patients*

	Mutations			Polymorphisms	
Patients	G843D	2097–2098insT	Others	GGA at G777	IVS11ins16bp
PBD-G008	+/+			+/+	+/+
PBD-G001	+/+			+/+	+/+
PBD-G012	+/+			+/+	+/+
PBD-G006	+/+			+/+	+/+
PBD-G014	+/+			+/+	+/+
PBD-G010	+/+			+/+	+/−
PBD-G015	+/−		+/−	+/+	+/+
PBD-G016	+/−		+/−	+/+	+/+
PBD-G005	+/+	+/−		+/−	+/−
PBD-G003		+/+		−/−	−/−
PBD-G007		+/−	+/−	−/−	−/−
PBD-G002		+/−	+/−	−/−	−/−
PBD-G011		+/−	+/−	+/−	+/−
PBD-G013		+/−	+/−	+/−	+/−

+, present; −, absent; +/+ or −/−, homozygous; +/− or −/+, heterozygous.

tations died within the first 12 mo of age (the present study, 12). All these patients had profound cerebral dysfunctions. Cataracts, renal cysts, and calcific stippling are almost exclusive clinical signs in this patient group (Table 2*B*). Class II mutations lead to a total loss of peroxin function. In fibroblasts of nine patients homozygous for class II mutations, there were no detectable protein levels and peroxin 1 activities (9, 12).

The presence of both class I and class II mutations in a Zellweger spectrum patient gives rise to an intermediate and less well predictable clinical phenotype. The presence of the G843D allele or other class I mutations appears to have a moderating effect on the phenotype, whereas the presence of class II mutations aggravates disease. Patient PBD-G005 and 7 of 11 reported patients who are compound heterozygous for the G843D missense allele and the c.2097–2098insT allele have been diagnosed with an intermediate phenotype (9, 12). The difficulties in predicting the clinical phenotype for patients in this group with a broad variation in disease severity may be due to the fact that the amount of residual protein levels and function depend on the type of interactions of the two mutations of peroxin 1 or on secondary factors involved. To examine these possibilities, more information regarding the physiologic functions and intracellular interactions of peroxin 1 must be acquired.

In conclusion, phenotypic severity of Zellweger spectrum patients in complementation group 1 seems rather correlated with the consequences of the mutation on the function of peroxin 1 than with secondary factors. *PEX1* mutations that are expected to create the most significant loss in protein function are associated with the most severe clinical and cellular phenotypes. This information should help clinicians make informed decisions and recommendations to families that carry *PEX1* gene mutations. Furthermore, the presence of one or the other of the two most common *PEX1* mutations in more than two thirds of Zellweger spectrum patients improves our ability to offer genetic testing and prenatal diagnosis to all of these families.

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