ONLINE MUTATION REPORT

Genetic and clinical aspects of Zellweger spectrum patients with *PEX1* mutations

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Objective: To analyse the *PEX1* gene, the most common cause for peroxisome biogenesis disorders (PBD), in a consecutive series of patients with Zellweger spectrum.
Methods: Mutations were detected by different methods including SSCP analyses as a screening technique on the basis of genomic or cDNA, followed by direct sequencing of PCR fragments with an abnormal electrophoresis pattern.
Results: 33 patients were studied. Two common mutations, c.2528G→A, G843D and c.2098_2098insT, I700YfsX42, accounted for over 80% of all abnormal PEX1 alleles, emphasising their diagnostic relevance. Most *PEX1* mutations were distributed over the two AAA cassettes with the two functional protein domains, D1 and D2, and the highly conserved Walker motifs. Phenotypic severity of Zellweger spectrum in CG1 depended on the effect of the mutation on the PEX1 protein, peroxin 1. PEX1 mutations could be divided into two classes of genotype–phenotype correlation: class I mutations led to residual PEX1 protein levels and function and a milder phenotype; class II mutations almost abolished PEX1 protein levels and function, resulting in a severe phenotype. Compound heterozygote patients for a class I and class II mutation had an intermediate phenotype.
Conclusions: Molecular confirmation of the clinical and biochemical diagnosis will allow the prediction of the clinical course of disease in individual PBD cases.

Peroxisomes are ubiquitous components of eukaryotic cells. Metabolic functions of these organelles are widespread and include hydrogen peroxide based respiration, β oxidation of very long chain fatty acids (VLCFA), and biosynthesis of ether phospholipids, bile acids, and isoprene compounds.[1] Several human diseases are caused by mutations in genes encoding peroxisomal metabolic enzymes,[2] but the most dramatic loss of peroxisome function is observed in the peroxisome biogenesis disorders (PBD; MIM 601539).[3]

For PBD two broad clinical spectra can be distinguished, the Zellweger spectrum, accounting for about 80% of all PBD patients, and the rhizomelia chondrodysplasia punctata (RCDP) spectrum.[3 4] The Zellweger spectrum consists of three overlapping clinical phenotypes that represent a continuum of disease severity, including Zellweger syndrome (MIM 214100) as the prototype and most severe example of this group, neonatal adrenoleucodystrophy (NALD, MIM 202370) as an intermediate form, and infantile Refsum disease (IRD, MIM 266510) as the mildest variant. Zellweger syndrome patients have characteristic dysmorphic features, severe neurological dysfunction including hypotonia, seizures and poor feeding; they have eye abnormalities like cataracts, liver dysfunction and skeletal defects. They rarely survive the first year of life. Patients with NALD, IRD and atypical Zellweger syndrome have similar but less severe clinical signs and can survive up to several decades. RCDP is clinically and genetically distinctive from the Zellweger syndrome spectrum and includes classical RCDP as the prototype and also milder variants. Patients with classical RCDP have unique clinical symptoms including proximal shortening of the limbs (rhizomelia), cataracts, and profound psychomotor retardation.

PBDs can be caused by defects in any of several processes in organelle formation, including the synthesis of peroxisome membranes, the recognition of newly synthesised peroxisomal matrix proteins, or any of the downstream steps in peroxisomal protein import. Progress over the last two decades has led to the identification of 13 different human *PEX* genes involved in peroxisome biogenesis, explaining the primary genetic defect of all 13 known complementation groups (CG) for PBD patients. The defective genes are *PEX1* for CG1,[5 6] *PEX5* for CG2,[7] *PEX12* for CG3,[8 9] *PEX6* for CG4,[10] *PEX10* for CG7,[11 12] *PEX26* for CG8,[13] *PEX16* for CG9,[14] *PEX2* for CG10,[15] *PEX7* for CG11,[16 17] *PEX3* for CG12,[18] *PEX13* for CG13,[19 20] *PEX19* for CG14,[21] and *PEX14* for CGK.[22] *PEX1* mutations are the most common cause and account for two thirds of all PBD patients.

PEX1 maps to 7q21–q22 and encodes peroxin 1 (PEX1), a 147 kDa member of the AAA protein family of ATPases (ATPases associated with diverse cellular activities). PEX1 is classified as a type II AAA ATPase as it comprises two ATPase domains (AAA cassettes) containing two functional domains D1 and D2, each spanning over about 200 amino acids (D1: 198 amino acids (p.436–633); D2: 185 amino acids (p.717–901)) with the second one being highly conserved.[23] Both AAA cassettes contain a Walker A and B motif (Walker A1 and B1; Walker A2 and B2).[24] Given that PEX1 has not been crystallised completely, knowledge of the structure is limited.[23] So far, only the structure of the N terminal domain is known. It shows striking similarity to that of two other type II AAA ATPases, namely valosin containing protein (VCP) and N-ethylmaleimide sensitive factor (NSF). VCP and NSF also seem to be involved in maintaining organelle function.

We analysed the *PEX1* gene in a consecutive series of 33 patients with Zellweger spectrum. We report the full spectrum of molecular defects in these patients as well as of those who have already been reported. We also describe the clinical features related to *PEX1* gene mutations and establish a genotype–phenotype correlation.

METHODS

The study included 168 Zellweger spectrum patients with *PEX1* mutations. Thirty three patients were analysed in our laboratory. The study was approved by the local university

Abbreviations: HGVS, Human Genome Variation Society; IRD, infantile Refsum disease; NSF, N-ethylmaleimide sensitive factor; NTD, N terminal domain; PBD, peroxisome biogenesis disorder; RCDP, rhizomelia chondrodysplasia punctata; VCP, valosin containing protein

Table 1 Allele frequency regarding 288 mutant *PEX1*genes from 168 patients

cDNA level, protein level	Exon	Number of alleles	Allele frequency
Splice site mutations		**9**	**0.0313**
c.1670+5G→T	9	2	0.0069
c.2071+1G→T	Intron 12, splice donor	2	0.0069
c.2926+1G→A	Intron 18, splice donor	3	0.0104
c.2926+2T→C	Intron 18, splice donor	1	0.0035
c.3207+1G→C	Intron 20, splice donor	1	0.0035
Insertions and deletions		**134**	**0.4653**
c.434_448delTTGGGTTGATCAACAinsGCAA, V145GfsX24	4	1	0.0035
c.788_789delCA, T263IfsX6	5	1	0.0035
c.904delG, A302QfsX23	5	1	0.0035
c.911_912delCT, S304CfsX4	5	1	0.0035
c.1865_1866insGAGTGTGGA, H623insSVC	11	2	0.0069
c.1900_2070del171bp, G634_H690del57	12	1	0.0035
c.2083_2085delATG, M695del	13	2	0.0069
c.2085_2089delGATAA, M695IfsX45	13	1	0.0035
c.2097_2098insT, I700YfsX42	**13**	**102**	**0.3542**
c.2227_2416del190bp, ?	14	3	0.0104
c.2537_2545delATGAAGTTAinsTCATGGT, H846LfsX52	15	1	0.0035
c.2730delA, L910FfsX51	17	4	0.0139
c.2814_2818delCTTTG, F938LfsX2	18	1	0.0035
c.2916delA, G973AfsX16	18	10	0.0347
c.3180_3181insT, G1061WfsX16	20	1	0.0035
c.3691_3694delCAGT, Q1231HfsX3	23	2	0.0069
Nonsense mutations		**10**	**0.0347**
c.781C→T, Q261X	5	1	0.0035
c.1897C→T, R633X	11	1	0.0035
c.2368C→T, R790X	14	1	0.0035
c.2383C→T, R795X	14	1	0.0035
c.2614C→T, R872X	16	3	0.0104
c.2992C→T, R998X	19	1	0.0035
c.3287C→G, S1096X	21	1	0.0035
c.3378C→G, Y1126X	21	1	0.0035
Missense mutations		**134**	**0.4653**
c.274G→C, V92L	3	2	0.0069
c.1777G→A, G593R	10	1	0.0035
c.1991T→C, L664P	12	1	0.0035
c.2008C→A, L670M	12	1	0.0035
c.2387T→C, L796P	14	1	0.0035
c.2392C→G, R798G	14	1	0.0035
c.2528G→A, G843D	15	**124**	**0.4306**
c.2846G→A, R949Q	18	1	0.0035
c.3038G→A, R1013H	20	1	0.0035
c.3850T→C, X1284Q	24	1	0.0035
Duplications		**1**	**0.0035**
c.1951_1959dupCAGTGTGGA, W653_M654insTVW	12	1	0.0035

ethics committee. Genomic DNA from cultured skin fibroblasts or leucocytes was used as template. Genomic DNA was obtained from leucocytes in 50 white controls. Mutations were detected by different methods including single strand conformation polymorphism (SSCP) analyses as a screening technique on the basis of genomic or cDNA, followed by direct sequencing of polymerase chain reaction (PCR) fragments with an abnormal electrophoresis pattern.[5 25 26] It is well known that SSCP analyses fail to detect 10–20% of mutations depending on fragment size and electrophoresis conditions.[27] Thus we and others have also used reverse transcriptase PCR (RT-PCR) to amplify the coding sequence of *PEX1*, followed by direct sequencing,[6 24 28 29] or direct sequencing of *PEX1* exons that were amplified by PCR with intronic primers.[30–33] For so far undescribed mutant alleles, polymorphism was excluded by analysing 100 white control alleles.

The mutation nomenclature used follows the recommendations of the Human Genome Variation Society (HGVS, http://www.genomic.unimelb.edu.au/mdi/rec.html).[34] All mutations were adjusted to mRNA reference sequence NM_000466 (version: NM_000466.1; source sequence: AF026086; version: AF026086.1; protein product: NP_000457; version: NP_000457.1).

RESULTS AND DISCUSSION

Mutation data analyses from 168 patients with 288 described mutant *PEX1* genes revealed 46% missense mutations, 46% insertions and deletions, 3% splice site mutations, 3% nonsense mutations, and 0.3% duplications (table 1).

Table 2 summarises all currently known *PEX1* mutations as well as the available clinical phenotypes.

The most common *PEX1* mutation, with an allele frequency of 0.43, is c.2528G→A, G843D in exon 15. The second most common mutation is the insertion c.2097_2098insT, I700YfsX42, with an allele frequency of 0.35. Thus these two common *PEX1* mutations comprise about 80% of all abnormal alleles in CG1. The vast majority of *PEX1* mutations are distributed over the two AAA cassettes with the functional protein domains, D1 and D2, and the highly conserved Walker motifs (fig 1).

c.2528G→A, G843D is localised in the second functional domain (D2). c.2097_2098insT, I700YfsX42 is localised before the second functional domain, resulting in a truncated

Table 2 *PEX1* sequence variants in patients with Zellweger spectrum

Mutation	cDNA level, protein level	Exon	Phenotype	Origin	References
Splice site mutations					
c.1670+5G→T	c.1670+5G→T, ?	Intron 9, splice donor	Classical ZS	Dutch	25
c.2071+1G→T	c.2071+1G→T, ?	Intron 12, splice donor	IRD	Dutch	25
c.2926+1G→A	c.2926+1G→A, ?	Intron 18, splice donor	ZSS	?	5, 6, 32, 33, 35
c.2926+2T→C	c.2926+2T→C, ?	Intron 18, splice donor	Classical ZS	Dutch	6, 25
c.3207+1G→C	c.3207+1G→C, ?	Intron 20, splice donor	NALD	?	6, 32
Insertions and deletions					
c.434_448delTTTGGGTTGATCAACinsGCAA	c.434_448delTTTGGGTTGATCAACinsGCAA, V145GfsX24	4	Classical ZS	Dutch	25
c.788_789delCA	c.788_789delCA, T263IfsX6	5	Classical ZS	Dutch	25
c.904delG	c.904delG, A302QfsX23	5	?	Australasian patient	31
c.911_912delCT	c.911_912delCT, S304CfsX4	5	Classical ZS	Italian	This study
c.1865_1866insGAGTGTGGA	c.1865_1866insGAGTGTGGA, A622_H623insSVC	11	NALD	?	This study; 26
c.1900_2070del171	c.1900_2070del171, G634_H690del57	12	ZS	?	24, 29
c.2083_2085delATG	c.2083_2085delATG, M695del	13	Classical ZS	Turkish	This study
c.2085_2089delGATAA	c.2085_2089delGATAA, M695IfsX45	13	ZSS	?	33
c.2097_2098insT	c.2097_2098insT, I700YfsX42	13	ZS, classical ZS, IRD, NALD, non-classical ZS, ZSS	Australasian, Dutch, German, ?	This study; 5, 6, 25, 26, 30–33, 36
c.2227_2416del190bp	c.2227_2416del190bp, ?	14	ZS, NALD	?	This study; 26
c.2537_2545delATGAAGTTAinsTCATGGT	c.2537_2545delATGAAGTTAinsTCATGGT, H846LfsX52	15	ZSS	?	33
c.2730delA	c.2730delA, L910FfsX51	17	Classical ZS	Dutch	25
c.2814_2818delCTTTG	c.2814_2818delCTTTG, F938LfsX2	18	ZS	?	This study; 26
c.2916delA	c.2916delA, G973AfsX16	18	?	Australasian, ?	30, 31, 33
c.3180_3181insT	c.3180_3181insT, G1061WfsX16	20	ZS	?	6, 32
c.3691_3694delCAGT	c.3691_3694delCAGT, Q1231HfsX3	23	Classical ZS	Turkish	This study
Nonsense mutations					
c.781C→T	c.781C→T, Q261X	5	ZS	?	29
c.1897C→T	c.1897C→T, R633X	11	NALD	?	29
c.2368C→T	c.2368C→T, R790X	14	?	Australasian	30, 31
c.2383C→T	c.2383C→T, R795X	14	ZS	?	6, 32
c.2614C→T	c.2614C→T, R872X	16	ZS, NALD	German, ?	This study; 26
c.2992C→T	c.2992C→T, R998X	19	?	Australasian	31
c.3287C→G	c.3287C→G, S1096X	21	NALD	German	This study
c.3378C→G	c.3378C→G, Y1126X	21	ZS	?	This study; 26
Missense mutations					
c.274G→C	c.274G→C, V92L	3	Non-classical ZS	Turkish	This study
c.1777G→A	c.1777G→A, G593R	10	IRD	Dutch	25
c.1991T→C	c.1991T→C, L664P	12	ZS	?	24, 29
c.2008C→A	c.2008C→A, L670M	12	Classical ZS	Dutch	25
c.2387T→C	c.2387T→C, L796P	14	Classical ZS	Italian	This study
c.2392C→G	c.2392C→G, R798G	14		Australasian	30, 31
c.2528G→A	c.2528G→A, G843D	15	ZSS, ZS, Classical ZS, NALD	Australasian, Dutch, German, ?	This study; 5, 6, 25, 26, 28–33, 37
c.2846G→A	c. 2846G→A, R949Q	18	Classical ZS	Dutch	25
c.3038G→A	c.3038G→A, R1013H	20	NALD	German	This study
c.3850T→C	c.3850T→C, X1284Q	24	NALD	Dutch	25
Duplications					
c.1952_1960dupCAGTGTGGA	c.1952_1960dupCAGTGTGGA, W653_M654insTVW	12			5, 6, 32

mRNA sequence: NM_000466 (version: NM_000466.1); source sequence: AF026086 (version: AF026086.1); product: NP_000457 (version: NP_000457.1).
IRD, infantile Refsum disease; NALD, neonatal adrenoleucodystrophy; ZS, Zellweger syndrome; ZSS, Zellweger syndrome spectrum.

Table 3 Genotype–phenotype correlation for so far unpublished *PEX1* alleles

Patient	G, D	V, N	K, K	G, A	K, M
1st allele	**c.911_912delCT, S304CfsX4**	**c.3287C→G, S1096X**	**c.3691_3694delCAGT, Q1231HfsX3**	**c.274G→C, V92L**	**c.2083_2085delATG, M695del**
2nd allele	**c.2387T→C, L796P**	**c.3038G→A, R1013H**	**c.3691_3694delCAGT, Q1231HfsX3**	**c.274G→C, V92L**	**c.2083_2085delATG, M695del**
Present age, age at death (years, months) (d)	0, 5	0, 4 (d)	0, 2 (d)	1, 11 (d)	0, 3 (d)
Clinical signs					
Dysmorphic features					
Large fontanelle, wide sutures	+	+	+	ND	+
High forehead	+	+	+	+	+
Broad nasal bridge	+	+	+	+	+
Hypertelorism	+	+	+	+	+
Epicanthus	+	−	+	−	+
External ear deformity	−	+	+	+	+
Sickle foot	−	+	+	+	+
Cerebral					
Poor sucking	+	+	+	+	+
Gavage feeding	−	+	+	−	+
Hypotonia	+	+	+	+	+
Severe psychomotor retardation	+	+	+	+	+
Seizures	−	+	+	−	+
Ocular					
Cataract	−	−	ND	−	ND
Retinitis pigmentosa	−	−	ND	−	ND
Optic atrophy	+	+	ND	−	ND
Nystagmus	−	−	ND	−	ND
Hepato-renal					
Hepatomegaly	−	−	+	−	+
Liver fibrosis	−	−	+	−	−
Raised liver enzymes	+	+	+	−	−
Renal cysts	+	−	+	ND	ND
Skeletal system					
Calcific stipling	ND	ND	ND	ND	+

ND, not determined; −, not present; +, present.

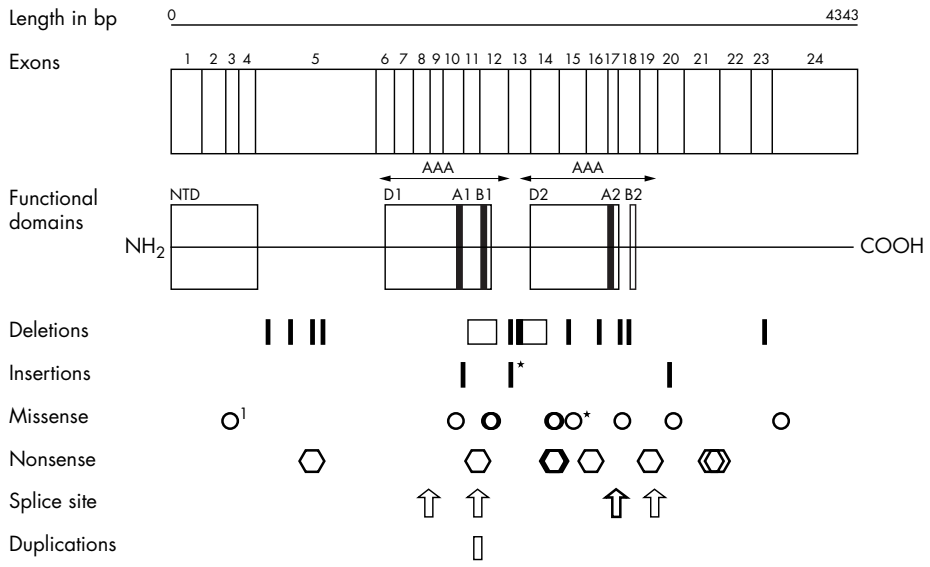

Figure 1 Distribution of *PEX1* mutations over the functional protein domains. The protein has two AAA domains (AAA) with two functional domains, D1 and D2, and the highly conserved Walker A and B motifs (A1, B1, A2, B2). Asterixes mark the two most common *PEX1* mutations, c.2528G→A, G843D and c.2097_2098insT, I700YfsX42; [1] = the first mutation identified in the crystallised NTD. NTD, N-terminal domain.

protein containing only one of the two nucleotide binding folds. In this study we identified seven mutations that have not been described so far—namely, c.911_912delCT, S304CfsX4; c.2083_2085delATG, M695del; c.3691_3694delCAGT, Q1231HfsX3; c.3287C→G, S1096X; c.274G→C, V92L; c.2387T→C, L796P; and c.3083G→A, R1013H. c.274G→C. V92L is the first mutation that has been detected in the crystallised NTD.

Mutations in the *PEX1* gene are the most common cause of peroxisome biogenesis disorders.[6] Alterations in this gene can lead to different clinical phenotypes comprising a continuum from severely affected patients with classical Zellweger syndrome through neonatal adrenoleucodystrophy, to infantile Refsum disease as the least severe. A summary of the data on genotype–phenotype correlations, including those already published and those derived from our own study (tables 2 and 3), confirms that there is a close link between the cellular impact of particular *PEX1* mutations and the severity of the clinical course.

Class I mutations result in a peroxin 1 with residual protein levels and function, while class II mutations abolish PEX1 protein levels and function. Class I mutations are missense mutations, with c.2528G→A, G843D being by far the most frequent allele. c.2528G→A, G843D is a temperature sensitive mutation leading to reduced protein levels and function.[25 31] c.2528G→A, G843D on both alleles results in PEX1 protein levels of 3 to 20% compared to wild type. Other missense mutations like c.2392C→G, R798G that affects the first conserved functional protein domain D1 shows comparable PEX 1 protein levels and activity.[31] Interaction of PEX1 with the second AAA-ATPase involved in peroxisome biogenesis, PEX6, in patients carrying c.2528G→A, G843D alleles is reduced by 70% when compared to wild type.[38] The most common class II mutation is c.2097_2098insT, I700YfsX42 resulting in a truncated PEX1 protein with a complete loss of the second functional domain, D2. In cells from CG1 patients bearing this mutation or other class II mutations including insertions, deletions, and splicing mutations, there is a total or nearly total loss of protein levels and function.[25 30] Patients with a class I mutation on both alleles present with a milder disease course, while those with class II mutation have a severe clinical phenotype. Patients who are compound heterozygotes for a class I and class II mutation (for example, c.2528G→A, G843D / c.2097_2098insT, I700YfsX42; c.2071+1G→T / c.1777G→A, G593R) show an intermediate clinical phenotype.[25 26]

The incomplete knowledge of peroxisome biogenesis in general and of the pathophysiology in Zellweger spectrum patients and other PBDs hampers the attempts at devising effective treatments. The combined approach of functional studies in vitro and in mouse models with conditional alleles in vivo, as well as studying human cellular and clinical phenotypes, should further improve our understanding of the role of peroxins including PEX1 protein in peroxisome assembly. As the milder PBD phenotypes correlate with an unstable mutant protein with some residual function, treatment directing towards the identification of peroxisome associated factors that regulate protein stability of mutant PEX1 proteins might be a useful approach to reduce the severity of the disease.

Conclusions

PEX1 mutations are the major primary cause of Zellweger spectrum. These conditions include, in decreasing order of clinical severity, Zellweger syndrome, neonatal adrenoleucodystrophy, and infantile Refsum disease. In a given patient the degree of phenotypic severity correlates closely with the functional consequences of the *PEX1* mutation on the encoded protein. In clinical practice, having a molecular confirmation of the clinical and biochemical diagnosis allows prediction of the clinical course of the disease in individual PBD cases. Furthermore, knowing the *PEX1* mutation in an affected family is the only way to identify carriers. It ensures accurate genetic counselling and prenatal diagnosis on DNA based mutation detection techniques to identify the genotype of a fetus at risk.

DATABASES

PEX1 - OMIM: No 214100, 602136; GenBank: AC000064 (*Homo sapiens*, BAC clone), AF026086, AF030356, AB008112 and BC035575 (*Homo sapiens*, mRNA), AB052090 (*Homo sapiens*, mRNA for Pex1p-634del690), AB052091 (*Homo sapiens*, mRNA for Pex1pL664P), AB052092 (*Homo sapiens*, mRNA for Pex1pG843D), AB052093 (*Homo sapiens*, mRNA for

Pex1pR633Ter), AB052094 (*Homo sapiens*, mRNA for Pex1pQ261Ter), CH236949 (*Homo sapiens*, genomic sequence).

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