Peroxisome Biogenesis Disorder 4A (Zellweger) — Comprehensive Disease Characteristics Report

Disease: Peroxisome Biogenesis Disorder 4A (Zellweger) — PBD4A **Gene:** *PEX6* (HGNC:8859; OMIM *601498) Category: Mendelian, autosomal recessive Suggested disease ontology mapping:** MONDO:0009279 (peroxisome biogenesis disorder), within the Zellweger spectrum; OMIM phenotype #614862 (Peroxisome biogenesis disorder 4A, Zellweger); Orphanet ORPHA:912 (Zellweger syndrome); ICD-10 E71.510; ICD-11 5C57.0; MeSH D015211 (Zellweger Syndrome)

Summary

Peroxisome Biogenesis Disorder 4A (Zellweger), abbreviated **PBD4A**, is the **severe, neonatal-lethal extreme of the *PEX6-related Zellweger Spectrum Disorder (ZSD). It is an autosomal recessive multisystem disease caused by biallelic loss-of-function variants in PEX6***, a gene encoding an **AAA+ ATPase** that — together with its partner ATPase PEX1 and the tail-anchored membrane anchor PEX26 — extracts and recycles the ubiquitinated peroxisomal targeting signal-1 (PTS1) receptor PEX5 back to the cytosol after cargo delivery. When this receptor-recycling machinery fails, peroxisomal matrix proteins can no longer be imported, peroxisome assembly collapses, and the full complement of peroxisomal metabolic functions is lost (F001, F002).

The biochemical consequence is a signature profile of **accumulated very-long-chain fatty acids (VLCFA; C26:0, elevated C26:0/C22:0 and C24:0/C22:0 ratios), phytanic and pristanic acid, and toxic C27 bile-acid intermediates, together with deficiency of plasmalogens and docosahexaenoic acid (DHA)** (F003, F005). These metabolic derangements drive the hallmark pathology of Zellweger syndrome: **impaired neuronal migration** (cerebral hemispheres, cerebellum, inferior olivary complex), abnormal Purkinje-cell arborization, demyelination, and post-developmental neurodegeneration, alongside severe hypotonia, seizures, craniofacial dysmorphism, hepatic dysfunction, and sensory (retinal and auditory) loss (F005, F007). Classic Zellweger presents at birth and typically leads to death within the first year of life (F007).

PBD4A sits at one end of a **continuous phenotypic spectrum**: hypomorphic or missense *PEX6* alleles that preserve residual peroxisomal function produce progressively milder disease — neonatal adrenoleukodystrophy, infantile Refsum disease, and, at the mildest end, **Heimler syndrome (PBD4B)** (F006). There is **no curative therapy**; management is supportive. The best-evidenced pharmacologic intervention is **oral cholic acid**, which significantly improves urinary bile-acid metabolite scores and serum transaminases in ZSD (F004), while **DHA supplementation was refuted by a double-blind randomized controlled trial** (F009). Liver transplantation can normalize toxic metabolites in mild ZSD (F010), and **AAV-mediated gene augmentation is an emerging preclinical therapy** for the ZSD retinopathy (F013).

Key Findings

F001 — PBD4A is caused by biallelic *PEX6* variants with a genotype–severity correlation

PBD4A is inherited in an **autosomal recessive** manner and arises from **biallelic pathogenic variants in *PEX6***. Case series and reviews demonstrate a **genotype–phenotype gradient**: missense variants that retain partial protein function trend toward milder disease, whereas **truncating variants (nonsense, frameshift, canonical splice-site)** that trigger nonsense-mediated decay or produce non-functional protein cause the severe, classic Zellweger (PBD4A) phenotype. A documented severe genotype is compound heterozygosity for **c.315G>A (p.Trp105Ter)** plus the splice variant **c.2095-3T>G**, both predicted to abolish functional protein; a milder-end example is the missense **c.1992G>C (p.Glu664Asp)**.

> *"Genetic variations in PEX6, an important peroxisome biogenesis factor, contribute significantly to this phenotypic diversity, with missense variants often associated with less severe disease compared to truncating mutations."* — PMID: 41787707
>
> *"WES identified compound heterozygous PEX6 variants: c.315G>A (p. Trp105Ter) and c.2095-3 T>G."* — PMID: 39013483

Ontology suggestions: gene *PEX6* (HGNC:8859); inheritance HP:0000007 (autosomal recessive inheritance).

F002 — *PEX6* encodes an AAA+ ATPase that recycles the PTS1 receptor PEX5

PEX6 encodes a member of the **AAA+ (ATPases Associated with diverse cellular Activities) family**. It forms a **heterohexameric ATPase complex with PEX1**, anchored to the peroxisomal membrane by the tail-anchored protein **PEX26**. After the cytosolic PTS1 receptor PEX5 delivers matrix cargo into the peroxisome, the PEX1–PEX6–PEX26 complex **extracts (dislocates) ubiquitinated PEX5 from the membrane back to the cytosol** for another round of import. Loss of PEX6 function halts PEX5 export; the receptor is instead proteasomally degraded, and matrix-protein import fails — the direct molecular lesion of PBD4A.

> *"After cargo delivery, a complex of the PEX1 and PEX6 ATPases and the PEX26 tail-anchored membrane protein removes ubiquitinated PEX5 from the peroxisomal membrane." —* PMID: 28742939
>
> *"in AWP1 knock-down cells, Pex5 stability was decreased, similar to fibroblasts from patients defective in Pex1, Pex6 and Pex26, all of which are required for Pex5 export" —* PMID: 21980954

Ontology suggestions: GO:0016887 (ATP hydrolysis activity); GO:0016558 (protein import into peroxisome matrix); GO:0005778 (peroxisomal membrane); GO:0043335 (protein unfolding).

F003 — Elevated plasma VLCFA is the key biomarker but can be normal

Impaired peroxisomal β-oxidation elevates **very-long-chain fatty acids (C26:0) and the diagnostic ratios C26:0/C22:0 and C24:0/C22:0**, along with **phytanic acid, pristanic acid, and abnormal bile-acid intermediates**. This constitutes the primary biochemical screening approach. However, **rare *PEX6* cases present with normal plasma VLCFA** (e.g., homozygous c.1992G>C, p.Glu664Asp), so a normal VLCFA result does not exclude ZSD. Definitive diagnosis therefore requires **combined clinical, biochemical, and molecular (WES/WGS) evaluation**.

> *"While elevated levels of very-long-chain fatty acids (VLCFAs) remain a key diagnostic feature, the existence of unusual cases with normal plasma VLCFA levels highlight the limitations of relying solely on this biochemical marker for diagnosis." —* PMID: 41787707
>
> *"A homozygous variant of uncertain significance (VUS) in PEX6 NM_000287.4: c.1992G > C (p. Glu664Asp) was identified" —* PMID: 39604887

Ontology / chemical suggestions: CHEBI:74102 (hexacosanoic acid / C26:0); CHEBI:37723 (phytanic acid); HP:0410054 (abnormal circulating VLCFA).

F004 — Management is supportive; oral cholic acid improves liver disease in ZSD

There is **no curative therapy**. The best-evidenced pharmacologic intervention targets the hepatic bile-acid abnormality. In a **phase 3 open-label study** (n=70 modified intention-to-treat; 20 with ZSD), **oral cholic acid (10–15 mg/kg/day)** significantly improved urinary atypical bile-acid metabolite scores (**P<0.0001**) and serum AST/ALT (**P<0.0001**), reduced direct bilirubin (**P<0.001**), and stabilized or improved liver histology. Other supportive measures include DHA, Lorenzo's oil, batyl alcohol, **fat-soluble vitamin (A, D, E, K) supplementation**, and dietary restriction of VLCFA and branched-chain fatty acids.

> *"Cholic acid significantly improved urine bile acid metabolite scores (P < 0.0001) and serum aspartate aminotransferase and alanine aminotransferase (P < 0.0001) in patients with SED and ZSD."* — PMID: 28644367
>
> *"There is some support for the pharmacologic therapies of Lorenzo's oil, docosohexanoic acid, and batyl alcohol in altering symptoms; however, systematic long-term studies are lacking. Cholic acid (CA) therapy has demonstrated treatment efficacy in patients with PBD-ZSD"* — PMID: 34625341

Ontology suggestions: NCIT — cholic acid therapy; CHEBI:16359 (cholic acid).

F005 — Neuropathology arises from combined loss of plasmalogens/DHA and VLCFA accumulation impairing neuronal migration

The hallmark neuropathology comprises **abnormal neuronal migration** affecting the cerebral hemispheres, cerebellum, and inferior olivary complex; **abnormal Purkinje-cell arborization**; **demyelination**; and **post-developmental neuronal degeneration**. Mouse models establish causality: the **Pex5 knockout** (Zellweger model) shows that peroxisomal metabolism in both brain and extraneuronal tissues affects neocortical development, and tissue-selective Pex5 reconstitution corrects the migration defect. The **Pex2 knockout** reproduces delayed cortical migration, cerebellar/Purkinje defects, embryonic lethality on an

inbred background, VLCFA accumulation, plasmalogen deficiency, and reduced brain DHA. Additional downstream contributors include mitochondrial dysfunction, oxidative stress, and inflammation.

> *"Neuropathological changes include abnormal neuronal migration affecting the cerebral hemispheres, cerebellum and inferior olivary complex, abnormal Purkinje cell arborisation, demyelination and post-developmental neuronal degeneration."* — PMID: 24607700
>
> *"Functional peroxisome deficiency, as encountered in Zellweger syndrome, causes a specific impairment of neuronal migration."* — PMID: 14586000
>
> *"Biochemical analysis of PEX2 mutant mice shows the characteristic accumulation of very long chain fatty acids and deficient plasmalogens in a wide variety of tissues."* — PMID: 11478384

Ontology suggestions: HP:0002269 (abnormality of neuronal migration); HP:0002079 (hypoplasia of the corpus callosum); HP:0001272 (cerebellar atrophy); GO:0001764 (neuron migration); CL:0000121 (Purkinje cell); CL:0000127 (astrocyte).

F006 — *PEX6* causes a continuous spectrum from neonatal-lethal Zellweger (PBD4A) to mild Heimler syndrome (PBD4B)

Biallelic loss-of-function *PEX6* variants cause severe Zellweger syndrome (PBD4A). Genotypes carrying at least one **hypomorphic** / **missense** / **"leaky" allele** yield progressively milder disease: neonatal adrenoleukodystrophy, infantile Refsum disease, and — at the mildest end — **Heimler syndrome (PBD4B)**, defined by sensorineural hearing loss, amelogenesis imperfecta, retinal dystrophy, and nail changes. In a review of 46 molecularly confirmed Heimler cases, **retinal dystrophy (rod-cone type) was present in 89%** and **macular edema in 40%**. A recurrent hypomorphic allele (p.Arg601Gln) shares a common founder haplotype.

> *"We demonstrate that each HS-affected family has at least one hypomorphic allele that results in extremely mild peroxisomal dysfunction."* — PMID: 26387595
>
> *"The finding of HS-causing mutations in PEX1 and PEX6 shows that HS represents the mild end of the ZSSD spectrum"* — PMID: 27302843
>
> *"Retinal dystrophy, predominantly of the rod-cone type with pigment clumping, was present in 89% of reported cases, with macular edema noted in 40%."* — PMID: 41126390

Ontology suggestions: HP:0000510 (rod-cone dystrophy); HP:0000407 (sensorineural hearing impairment); HP:0000705 (amelogenesis imperfecta).

F007 — ZSD is clinically heterogeneous with shortened lifespan; the severe (PBD4A) form is neonatal-lethal

PBD-ZSD ranges from profound neurologic disease in newborns to progressive degeneration in adults, and typically results in **shortened life spans**. **Classic Zellweger syndrome (the severe end, PBD4A)** presents at birth with **severe hypotonia, seizures, feeding difficulty, craniofacial dysmorphism, hepatic dysfunction**, and usually **death within the first year of life**. Milder forms survive into childhood or adulthood.

> *"individuals with PBD-ZSD can manifest a complex spectrum of clinical phenotypes that typically result in shortened life spans"* — PMID: 26750748
>
> *"Common clinical presentations include hypotonia, seizure, hepatomegaly, craniofacial dysmorphism and early death."* — PMID: 38409970

Ontology suggestions: HP:0001252 (hypotonia); HP:0001250 (seizure); HP:0002240 (hepatomegaly); HP:0001999 (abnormal facial shape); HP:0003811 (neonatal death).

F008 — Newborn screening (C26:0-LPC in dried blood spots) can incidentally detect PBDs

LC-MS/MS quantification of **C26:0-lysophosphatidylcholine (C26:0-LPC)** in dried blood spots — implemented for X-linked adrenoleukodystrophy newborn screening — **also detects other peroxisomal disorders including PBDs**. In a screen of **43,653 newborns**, 2 of 32 screen-positives (**6.3%**) were diagnosed with peroxisomal disorders other than X-ALD. C26:0-LPC correlates strongly with plasma C26:0 (**r=0.952**) and the C26:0/C22:0 ratio (**r=0.801**).

> *"two (6.3%) were diagnosed with other peroxisomal disorders"* — PMID: 37977233

F009 — DHA supplementation did NOT improve vision or growth (negative RCT)

A **double-blind, randomized, placebo-controlled trial** (n=50 enrolled; DHA 100 mg/kg/day for ~1 year) in peroxisome assembly disorders found **no difference** between DHA-treated and placebo groups in biochemical function, electroretinogram, or growth (Class II evidence). Nine patients died during the trial of their underlying disorder, underscoring severity. This refutes an earlier hypothesis that DHA supplementation is disease-modifying in ZSD.

> *"DHA supplementation did not improve the visual function or growth of treated individuals with peroxisome assembly disorders"* — PMID: 20805528

F010 — Liver transplantation can normalize toxic metabolites in mild ZSD

In mild ZSD (infantile Refsum-like), **living-donor liver transplantation** normalized plasma **phytanic, pristanic, and pipecolic acid** levels, stabilized hearing and vision, and improved neurodevelopment, with sustained benefit up to **17 years** post-transplant (2/3 patients survived and improved; 1 died). Separately, an **oral cholic acid extension study** (n=17, 21 months) showed durable suppression of bile-acid synthesis and reduced toxic C27 intermediates.

> *"We documented a sustained improvement of biochemical functions, with a complete normalization of plasma phytanic, pristanic, and pipecolic acid levels. This was associated with stabilization of hearing and visual functions, and improved neurodevelopmental status"* — PMID: 29453832
>
> *"Bile acid synthesis was still suppressed after 21 months of CA treatment"* — PMID: 30793331

F011 — Mitochondria-mediated oxidative stress is a downstream effector of peroxisomal failure

Brain-restricted **PEX13-deficient** mice (Zellweger model) show cerebellar maldevelopment, impaired granule-cell migration, astro-/microgliosis, and — in cultured E19 PEX13-null cerebellar neurons — **elevated reactive oxygen species, increased mitochondrial MnSOD (SOD2), enhanced apoptosis, and mitochondrial dysfunction**; plasmalogens were reduced

while VLCFA were normal in this brain model. PBD models (Pex2$^{-/-}$, Pex5$^{-/-}$, Pex13$^{-/-}$) also show increased **α-synuclein oligomerization/phosphorylation and cytoplasmic deposition**, linking peroxisomal lipid changes to neurodegenerative protein aggregation.

> *"cultured cerebellar neurons from E19 PEX13-null mice exhibit elevated levels of reactive oxygen species and mitochondrial superoxide dismutase-2 (MnSOD), and show enhanced apoptosis together with mitochondrial dysfunction"* — PMID: 20959636
>
> *"We found increased alphaS oligomerization and phosphorylation and its increased deposition in cytoplasmic inclusions in these PBD mouse models."* — PMID: 19830841

Ontology suggestions: GO:0006915 (apoptotic process); GO:0006979 (response to oxidative stress); GO:0005739 (mitochondrion).

F012 — ~80% of PBD patients fall in the Zellweger spectrum; ~90% carry mutations in PEX1/PEX6/PEX10/PEX12/PEX26

Within the peroxisome biogenesis disorders, **approximately 80% of all PBD patients** are classified as PBD-ZSS, and mutations in **PEX1, PEX6, PEX10, PEX12, or PEX26 are found in ~90% of PBD-ZSS patients** (cohort of 58 PBD-ZSS cases; 71 unique sequence variants, 18 novel). Rare digenic cases with deleterious mutations across two PEX genes were observed. This places *PEX6* among the five major ZSS genes.

> *"Approximately 80% of PBD patients are classified in the Zellweger syndrome spectrum (PBD-ZSS). Mutations in the PEX1, PEX6, PEX10, PEX12, or PEX26 genes are found in approximately 90% of PBD-ZSS patients."* — PMID: 19105186

F013 — Preclinical AAV gene-augmentation therapy improves vision in a mild ZSD mouse model

AAV-mediated **PEX gene augmentation** was tested in the humanized **PEX1-Gly844Asp** mouse model of mild ZSD, which develops retinal dysfunction and vision loss. Ocular AAV delivery improved visual/retinal function — a **proof-of-concept preclinical gene therapy** for the ZSD retinopathy (not yet clinical).

> *"Patients with Zellweger spectrum disorder (ZSD) commonly present with vision loss due to mutations in"* — PMID: 34703844

Section-by-Section Report

1. Disease Information

PBD4A (Zellweger) is the **most severe form of the Zellweger Spectrum Disorder**, a group of autosomal recessive peroxisome biogenesis disorders. Peroxisomes are membrane-bound organelles essential for VLCFA β-oxidation, plasmalogen (ether-phospholipid) biosynthesis, bile-acid synthesis, and reactive-oxygen detoxification. In PBD4A, functional peroxisomes are essentially absent from patient fibroblasts, and multiple organ systems are affected (F001, F007).

Key identifiers: Gene *PEX6* (OMIM *601498; HGNC:8859). Phenotype OMIM #614862 (Peroxisome biogenesis disorder 4A, Zellweger). Orphanet ORPHA:912 (Zellweger syndrome, the broader clinical entity). ICD-10 E71.510; ICD-11 5C57.0; MeSH D015211. Suggested MONDO mapping within the peroxisome biogenesis disorder branch (MONDO:0009279 and related ZSD terms).

Synonyms / alternative names: Zellweger syndrome (severe end); cerebrohepatorenal syndrome; PBD4A; *PEX6*-related Zellweger spectrum disorder. The broader continuum encompasses neonatal adrenoleukodystrophy, infantile Refsum disease, and Heimler syndrome (PBD4B) (F006).

Information source type: Predominantly **aggregated disease-level resources** (OMIM, Orphanet, GeneReviews, case series and cohort reviews), supplemented by individual case reports; not derived from large EHR cohorts.

2. Etiology

Causal factor: Purely **genetic** — biallelic loss-of-function variants in *PEX6* (F001). There is no environmental or infectious cause.

Genetic risk factors: The causal variants are the *PEX6* alleles themselves. **Consanguinity** increases risk (many reported cases are from consanguineous unions, e.g., Egyptian, Iranian, Saudi, and Mixteco founder populations described in the literature). **Founder effects** exist (recurrent hypomorphic p.Arg601Gln allele; a Mixteco *PEX6* founder mutation reported in neonates). No common susceptibility loci or modifier genes are established beyond the allele-specific severity gradient (F001, F006).

Environmental / protective factors: None identified. There are no known environmental risk or protective factors, and no established gene–environment interactions — consistent with a monogenic, fully penetrant Mendelian disorder.

3. Phenotypes

Phenotype	Type	HPO term	Onset	Severity/Frequency
Severe hypotonia	Clinical sign	HP:0001252	Neonatal	Severe; near-universal (F007)
Seizures	Clinical sign	HP:0001250	Neonatal	Severe; common (F007)
Craniofacial dysmorphism	Physical	HP:0001999	Congenital	Characteristic (high forehead, large fontanelles, epicanthal folds) (F007)
Hepatic dysfunction / hepatomegaly	Lab/clinical	HP:0002240	Neonatal	Common; progressive (F004, F007)
Neuronal migration defect (polymicrogyria)	Imaging/ structural	HP:0002269	Congenital	Hallmark (F005)
Retinal dystrophy (rod-cone)	Clinical sign	HP:0000510	Infantile	89% in mild end (F006)
Sensorineural hearing loss	Clinical sign	HP:0000407	Infantile	Common (F006)
Elevated plasma VLCFA	Lab abnormality	HP:0410054	Congenital	Key biomarker (may be normal in rare cases) (F003)
Feeding difficulty / failure to thrive	Symptom	HP:0011968	Neonatal	Common (F007)
Amelogenesis imperfecta (mild end)	Physical	HP:0000705	Childhood	Heimler feature (F006)

Quality-of-life impact: In classic PBD4A, profound neurological impairment precludes normal development; infants are typically non-ambulatory, feeding-dependent, and die in infancy (F007). Milder spectrum survivors experience progressive vision and hearing loss, developmental delay, and hepatic complications.

4. Genetic/Molecular Information

Causal gene: *PEX6* (chromosome 6p21.1; OMIM *601498). Encodes a peroxisomal AAA+ ATPase** (peroxin-6) (F002).

Pathogenic variants: Documented ClinVar-type variants include **c.315G>A (p.Trp105Ter)** (nonsense), **c.2095-3T>G** (canonical splice, NMD-triggering) — severe; and **c.1992G>C (p.Glu664Asp)** (missense) — milder/normal-VLCFA (F001, F003). Variant classes span **missense, nonsense, frameshift, and splice-site**; classification ranges pathogenic/likely-pathogenic to VUS per ACMG/AMP. **Functional consequence: loss of function** (impaired PEX5 receptor recycling → failed matrix import) (F002). Origin is **germline**; no somatic role. Allele frequencies of pathogenic variants are very rare in gnomAD.

Modifier / genotype–severity relationship: Severity is chiefly determined by residual PEX6 function — the presence of a hypomorphic/leaky allele shifts phenotype toward milder disease (F001, F006). No independent trans-acting modifier genes are firmly established, though rare digenic PEX interactions are reported (F012).

Epigenetics / chromosomal abnormalities: No specific epigenetic mechanism or large-scale chromosomal abnormality is characteristic; PBD4A is a single-gene disorder.

5. Environmental Information

Not applicable. PBD4A is a monogenic disorder with no established environmental, lifestyle, or infectious contributors. Dietary VLCFA/branched-chain fatty acid intake is relevant only to *management* (restriction), not causation (F004).

6. Mechanism / Pathophysiology

Causal chain:

```
Biallelic PEX6 loss-of-function (F001)
        │
        ▼
AAA+ ATPase PEX1–PEX6–PEX26 complex cannot extract ubiquitinated PEX5 (F002)
        │
        ▼
PEX5 (PTS1 receptor) degraded → peroxisomal matrix-protein import fails
        │
        ▼
Loss of peroxisome function:
  • VLCFA β-oxidation ↓  → C26:0, phytanic/pristanic acid ↑ (F003)
  • Plasmalogen synthesis ↓ → ether-lipid deficiency (F005)
  • DHA ↓; bile-acid synthesis abnormal → toxic C27 intermediates (F004,F005)
        │
        ▼
Downstream cellular injury:
  • Mitochondrial dysfunction, ↑ROS, ↑MnSOD, apoptosis (F011)
  • α-synuclein aggregation (F011)
        │
        ▼
Tissue-level pathology:
  • Impaired neuronal migration, Purkinje defects, demyelination (F005)
  • Hepatic dysfunction; retinal/auditory degeneration
        │
        ▼
Clinical: neonatal hypotonia, seizures, dysmorphism, hepatic failure,
          sensory loss → death <1 year (severe PBD4A) (F007)
```

Molecular pathways: Peroxisomal matrix protein import (GO:0016558); peroxisomal β-oxidation of VLCFA; ether-lipid/plasmalogen biosynthesis; bile-acid synthesis. **Cellular processes:** apoptosis (GO:0006915), oxidative-stress response (GO:0006979), neuronal migration (GO:0001764). **Protein dysfunction:** loss of AAA+ ATPase activity → failed receptor recycling (upstream); secondary mitochondrial dysfunction and protein aggregation (downstream) (F002, F011). **Subcellular compartments:** peroxisome (GO:0005777), peroxisomal membrane (GO:0005778), mitochondrion (GO:0005739).

Metabolic changes: ↑ VLCFA, phytanic/pristanic acid, pipecolic acid, C27 bile-acid intermediates; ↓ plasmalogens and DHA (F003, F005, F010). **Cell types involved:** migrating neurons and Purkinje cells (CL:0000121), astrocytes (CL:0000127), microglia, hepatocytes, photoreceptors.

7. Anatomical Structures Affected

- **Primary organs / systems:** Central nervous system (UBERON:0001017) — cerebral cortex, cerebellum (UBERON:0002037), inferior olivary complex; **liver** (UBERON:0002107); **kidney** (UBERON:0002113 — cortical renal cysts; hence "cerebro-hepato-renal syndrome"); **eye/retina** (UBERON:0000970 / UBERON:0000966); **ear** (cochlea). Skeletal: chondrodysplasia punctata (epiphyseal stippling) (F005, F006, F007).
- **Tissue/cell level:** Nervous tissue (migrating neurons, Purkinje cells CL:0000121); hepatocytes; retinal photoreceptors; cochlear hair cells.
- **Subcellular:** Peroxisome (GO:0005777) and peroxisomal membrane (GO:0005778) — the primary lesion; mitochondria (GO:0005739) — secondary.
- **Lateralization:** Bilateral / generalized (multisystem).

8. Temporal Development

- **Onset:** Congenital / neonatal for classic PBD4A; presentation at or shortly after birth (F007).
- **Onset pattern:** Insidious congenital multisystem involvement.
- **Progression:** Severe form is rapidly progressive and neonatal-lethal (death typically within the first year) (F007). The broader spectrum shows slower, progressive neurodegeneration and sensory decline in milder survivors (F006, F010).
- **Course:** Chronic, progressive; no remission. **Critical period:** prenatal/neonatal (neuronal migration occurs in utero, limiting the window for developmental rescue) (F005).

9. Inheritance and Population

- **Inheritance:** Autosomal recessive (HP:0000007) (F001).
- **Penetrance:** Complete for biallelic loss-of-function genotypes; **expressivity variable**, governed by residual PEX6 function (F001, F006).
- **Epidemiology:** ZSD overall estimated at roughly **1 in 50,000 births** (Orphanet range; regionally variable). *PEX6* accounts for a substantial fraction within the five major ZSS genes (~90% of PBD-ZSS cases carry PEX1/PEX6/PEX10/PEX12/PEX26 variants) (F012).
- **Founder effects / consanguinity:** Recurrent hypomorphic p.Arg601Gln (shared founder haplotype); Mixteco *PEX6* founder mutation reported; elevated incidence in consanguineous populations (F006).
- **Sex ratio:** Autosomal → approximately 1:1 male:female.
- **No genetic anticipation** (not a repeat-expansion disorder).

10. Diagnostics

- **Biochemical (first-line):** Plasma **VLCFA panel** (C26:0, C26:0/C22:0, C24:0/C22:0), phytanic/pristanic acid, plasmalogens (red-cell), pipecolic acid, and urinary bile-acid intermediates (F003, F010). Note: VLCFA can rarely be **normal**, so a normal panel does not exclude PBD4A (F003).
- **Molecular (confirmatory): WES/WGS** or targeted PEX gene panels are recommended, particularly when biochemistry is atypical or normal (F003). Single-gene *PEX6* testing where a familial variant is known.
- **Imaging:** Brain MRI showing neuronal migration abnormalities (polymicrogyria), white-matter changes; skeletal radiographs may show chondrodysplasia punctata (F005).
- **Newborn screening: C26:0-LPC** in dried blood spots (LC-MS/MS), primarily for X-ALD, incidentally detects PBDs (F008).
- **Differential diagnosis:** D-bifunctional protein deficiency (Zellweger-like), single-enzyme peroxisomal defects, Smith-Lemli-Opitz syndrome, other causes of neonatal hypotonia/seizures, chondrodysplasia punctata (F005).

11. Outcome / Prognosis

- **Life expectancy:** Classic PBD4A (severe Zellweger) is **neonatal-lethal**, with death typically **within the first year of life** (F007). Milder spectrum forms survive into childhood/adulthood with progressive morbidity (F006).
- **Morbidity:** Profound developmental disability, seizures, vision and hearing loss, hepatic dysfunction (F004, F006, F007).
- **Prognostic factors:** Genotype (truncating vs. hypomorphic/missense) is the dominant determinant of severity and survival (F001, F006). Residual peroxisomal function and VLCFA levels correlate with severity.
- **Interventions altering course:** Cholic acid stabilizes/improves liver disease (F004); liver transplantation normalizes toxic metabolites in mild ZSD (F010) but is not curative for CNS disease.

12. Treatment

Treatment	Category	Evidence	NCIT/CHEBI
Oral cholic acid 10–15 mg/kg/day	Pharmacotherapy (bile-acid replacement)	Phase 3: ↑ bile-acid scores P<0.0001, ↓ AST/ALT P<0.0001 (F004); durable ≥21 mo (F010)	CHEBI:16359
Fat-soluble vitamins A, D, E, K	Supportive	Standard of care (F004)	—
VLCFA / branched-chain fatty acid dietary restriction	Supportive/dietary	Standard of care (F004)	—
DHA supplementation	Pharmacotherapy	**Refuted** by RCT — no vision/growth benefit (F009)	CHEBI:28125
Lorenzo's oil, batyl alcohol	Pharmacotherapy	Limited/weak evidence (F004)	—
Liver transplantation	Surgical	Normalizes phytanic/pristanic/pipecolic acid; mild ZSD only (F010)	—
AAV *PEX* gene augmentation	Gene therapy	Preclinical proof-of-concept (retinopathy) (F013)	—
Anti-seizure medication (e.g., levetiracetam), physiotherapy, nutritional support	Supportive/rehabilitative	Symptom management	—

There is **no curative therapy**. Management is supportive and multidisciplinary (F004, F007).

13. Prevention

- **Primary prevention:** Not possible for the individual (monogenic congenital disorder). **Genetic counseling** for at-risk families (25% recurrence risk per pregnancy for carrier couples) is central (F001).

- **Reproductive options:** Carrier testing, prenatal diagnosis (biochemical + molecular), and preimplantation genetic testing where a familial variant is known.
- **Secondary prevention:** Newborn screening via C26:0-LPC can enable earlier detection (F008); early cholic acid and supportive care can mitigate hepatic complications (F004).
- **Consanguinity counseling** in high-risk / founder populations (F006).

14. Other Species / Natural Disease

PEX6 orthologs and peroxisome-biogenesis function are **evolutionarily conserved** from yeast/fungi to mammals. In the basidiomycete *Cryptococcus neoformans*, PEX1 and PEX6 AAA-ATPases are required for peroxisome formation; pex1/pex6 mutants fail to localize peroxisomal proteins and cannot grow on fatty acids (PMID: 17041184). In *Arabidopsis*, pex6 and pex26 mutants show peroxisomal retrotranslocation and oil-body utilization defects (PMID: 28742939). No prominent naturally occurring companion-animal Zellweger disease is established; the disorder is chiefly modeled experimentally (see Section 15). No zoonotic potential (genetic disease).

Ontology: NCBI Taxon 9606 (human); orthologs conserved across *Mus musculus* (10090), *Danio rerio* (7955), *Saccharomyces cerevisiae* (4932).

15. Model Organisms

- **Mouse (mammalian):** *Pex5* knockout (Zellweger model) — establishes peroxisome-dependent neuronal migration; tissue-selective reconstitution rescues migration (F005). *Pex2* knockout — cortical migration delay, cerebellar/Purkinje defects, embryonic lethality (inbred background), VLCFA↑/plasmalogen↓/DHA↓ (F005). Brain-restricted *Pex13* knockout — cerebellar maldevelopment, gliosis, ROS↑, apoptosis, mitochondrial dysfunction (F011). Humanized *Pex1*-Gly844Asp mouse — mild ZSD retinopathy, used for AAV gene therapy (F013).
- **Phenotype recapitulation:** Mouse models faithfully reproduce the biochemical signature (VLCFA↑, plasmalogen↓, DHA↓) and neurodevelopmental pathology (migration defects, cerebellar abnormalities) (F005, F011).
- **Limitations:** Severe knockouts are neonatal/embryonic-lethal, limiting adult-phenotype study; brain-restricted and humanized hypomorphic models were developed to address this (F005, F011, F013).

- **Fungal/plant models:** *Cryptococcus* and *Arabidopsis* pex6 mutants illuminate the conserved receptor-recycling and retrotranslocation function (F002; PMIDs 17041184, 28742939).
- **Resources:** MGI (mouse), ZFIN (zebrafish), SGD (yeast), Alliance of Genome Resources.

Mechanistic Model / Interpretation

PBD4A is fundamentally a **disorder of a molecular machine**. *PEX6* is one subunit of the AAA+ ATPase engine (PEX1–PEX6, membrane-anchored by PEX26) that powers the **recycling step of peroxisomal matrix-protein import**. Because import is a receptor-shuttle cycle, disabling the recovery/extraction step (PEX5 export) is as catastrophic as disabling import itself: PEX5 is trapped and degraded, no further cargo enters, and peroxisomes become empty "ghosts" devoid of matrix enzymes (F002).

The clinical phenotype is then the sum of **multiple simultaneous metabolic failures**: loss of VLCFA β-oxidation (toxic lipid accumulation), loss of plasmalogen synthesis (membrane/myelin ether-lipid deficiency), loss of DHA and normal bile-acid synthesis, and impaired ROS detoxification. Uniquely, these converge on the **developing brain**, where peroxisome-dependent lipid metabolism is required for **neuronal migration** — an in-utero process, which is why the severe form is congenital and largely irreversible (F005). Downstream, **mitochondrial dysfunction, oxidative stress, apoptosis, and even α-synuclein aggregation** amplify tissue injury (F011).

The **genotype–severity gradient** (F001, F006) provides the unifying logic of the entire spectrum: the amount of *residual* PEX6 activity a genotype permits determines where a patient lands — from neonatal-lethal Zellweger (PBD4A, near-zero function) to Heimler syndrome (PBD4B, minimal residual dysfunction). This "dial" model directly rationalizes both the phenotypic continuum and the therapeutic rationale for gene augmentation (F013): restoring even partial PEX6 function should shift phenotype toward the milder end.

Evidence Base

PMID	Role	Supports
41787707	Review	Genotype–severity gradient; VLCFA limitations (F001, F003)
39013483	Case	Biallelic truncating/splice PEX6 → Zellweger (F001)
28742939	Mechanism	PEX1–PEX6–PEX26 removes ubiquitinated PEX5 (F002); plant model (§14)
21980954	Mechanism	PEX6 required for PEX5 export (F002)
39604887	Case	Normal-VLCFA PEX6 case (F003)
28644367	Phase 3	Cholic acid efficacy in ZSD (F004)
34625341	Review	Supportive therapy landscape (F004)
24607700	Review	Neuropathology (F005)
14586000	Mouse	Peroxisome deficiency → migration defect (F005)
11478384	Mouse	PEX2 KO biochemical signature (F005)
26387595	Genetics	Hypomorphic alleles → Heimler (F006)
27302843	Genetics	HS = mild end of spectrum (F006)
41126390	Review	Heimler phenotype frequencies (F006)
26750748	Guideline	Shortened lifespan (F007)
38409970	Case	Severe neonatal presentation (F007)
37977233	Screening	C26:0-LPC NBS detects PBDs (F008)
20805528	RCT	DHA refuted (F009)
29453832	Case series	Liver transplant normalizes metabolites (F010)
30793331	Extension	Durable cholic acid effect (F010)
20959636	Mouse	ROS/apoptosis/mitochondrial dysfunction (F011)
19830841	Mouse	α-synuclein pathology (F011)
19105186	Cohort	80% ZSS; ~90% five PEX genes (F012)
34703844	Preclinical	AAV gene therapy for retinopathy (F013)

Limitations and Knowledge Gaps

1. **PBD4A-specific epidemiology is imprecise.** Prevalence figures are for ZSD as a whole; the *PEX6*-specific, severe-end (PBD4A) incidence is not separately quantified in the reviewed literature.
2. **Mechanistic evidence relies heavily on non-PEX6 mouse models** (Pex2, Pex5, Pex13). While the peroxisomal defect is shared, PEX6-specific in-vivo models are less represented in this evidence set.
3. **Therapeutic evidence is largely from milder ZSD.** Cholic acid trials, liver transplantation, and AAV gene therapy data derive predominantly from milder-spectrum patients; benefit in neonatal-lethal PBD4A specifically is unproven, and CNS disease remains untreatable.
4. **No modifier genes** beyond the allele-intrinsic residual-function gradient are established.
5. **No natural animal disease** counterpart is documented; comparative pathology relies on experimental models and evolutionarily conserved fungal/plant orthologs.
6. **Variant interpretation gaps:** several *PEX6* variants remain VUS (e.g., p.Glu664Asp), and functional assays are not routinely available.

Proposed Follow-up Experiments / Actions

1. **PEX6-specific natural history and prevalence study** — stratify ZSD registries by causal gene and genotype class (truncating vs. hypomorphic) to quantify PBD4A-specific incidence, survival, and phenotype frequencies.
2. **Genotype–function assay** — develop a standardized cell-based peroxisomal-import assay to reclassify *PEX6* VUS (e.g., p.Glu664Asp) and predict severity, improving prenatal/prognostic counseling.
3. **PEX6 humanized mouse models** — generate patient-specific *Pex6* knock-in alleles spanning the severity spectrum to test whether AAV gene augmentation (extending F013) can rescue CNS as well as retinal phenotypes.
4. **Early cholic acid + metabolite trial in confirmed PBD4A neonates** — prospective evaluation of cholic acid initiated at newborn-screening diagnosis, with bile-acid intermediate and transaminase endpoints.

5. **Antioxidant / mitochondrial-protective adjuncts** — test targeted antioxidants against the ROS/apoptosis axis identified in PEX13 models (F011) as a neuroprotective strategy.
6. **Expand newborn screening validation** — assess sensitivity of C26:0-LPC screening specifically for *PEX6*-PBD, including the rare normal-VLCFA genotypes (F003, F008).

Report compiled from 13 confirmed findings and 52 reviewed papers across 5 investigation iterations. Evidence types span human clinical (case reports, cohorts, phase 3 and RCT trials), model organism (mouse, fungal, plant), and in-vitro studies.
