Hypomyelinating Leukodystrophy 10 (HLD10 / PYCR2 Deficiency): Comprehensive Disease Characterization

Disease: Hypomyelinating Leukodystrophy 10 (HLD10) **Gene:** *PYCR2* (Pyrroline-5-Carboxylate Reductase 2) **Category:** Mendelian, autosomal recessive **Key identifiers:** OMIM #616420 · MONDO:0014635 · locus 1q42.12

Summary

Hypomyelinating leukodystrophy 10 (HLD10) is a rare autosomal recessive neurometabolic leukodystrophy caused by biallelic loss-of-function and missense variants in **PYCR2**, the gene encoding the mitochondrial enzyme pyrroline-5-carboxylate reductase 2, which catalyzes the final step of L-proline biosynthesis. The disorder was first defined molecularly in 2015 by Nakayama et al., who mapped it to chromosome 1q42.12 and demonstrated that biallelic *PYCR2* mutations cause postnatal microcephaly with hypomyelination (PMID: 25865492). Affected children characteristically present with postnatally acquired (progressive) microcephaly, moderate-to-profound global developmental delay, failure to thrive, craniofacial dysmorphism, hyperkinetic movements, axial hypotonia with variable appendicular spasticity, and seizures. Brain MRI shows hypomyelination/delayed myelination, thin corpus callosum, and generalized white-matter volume loss. Severely affected patients do not survive beyond the first decade of life.

Mechanistically, the disease is now understood as a mitochondrial and amino-acid metabolic disorder with a specific neurotoxic endpoint. Loss of PYCR2 destabilizes the enzyme, depletes PYCR1 in neural lineages, decreases mitochondrial membrane potential, and increases susceptibility to apoptosis under oxidative stress. The pivotal mechanistic insight, provided by Escande-Beillard et al. (2020), is that loss of PYCR2 upregulates **SHMT2**, driving excessive cerebral glycine that produces axonal beading and neurodegeneration; knockdown of SHMT2 partially rescues these deficits, nominating the glycine metabolic pathway as a therapeutic

target (PMID: 32330411). Notably, routine peripheral/serum metabolic profiles are normal, so the pathology is a compartmentalized cerebral metabolic derangement rather than a systemically measurable one.

The phenotypic spectrum is broader than originally appreciated. While the canonical presentation is a severe, often lethal infantile leukodystrophy, a milder allele (p.Val128Ala) has been reported to cause hereditary spastic paraplegia (HSP) in late childhood without overt hypomyelinating leukodystrophy. Population genetics are shaped by consanguinity and founder effects, exemplified by the Thai c.400G>A (p.Val134Met) founder allele on a shared 2.3 Mb haplotype estimated at ~1450 years old. There is no disease-specific or curative therapy; management is supportive, and SHMT2/glycine-lowering represents the leading mechanism-based experimental direction.

Key Findings

Finding 1 — HLD10 is an autosomal recessive disorder caused by biallelic *PYCR2* variants

HLD10 (OMIM #616420) maps to chromosome **1q42.12** and is caused by biallelic (homozygous or compound heterozygous) loss-of-function and missense variants in *PYCR2*, encoding pyrroline-5-carboxylate reductase 2, a **mitochondrial enzyme catalyzing the final step of proline biosynthesis**. Zaki et al. identified 11 consanguineous families, establishing autosomal recessive inheritance. The disorder's molecular architecture is consistent across cohorts: the majority of reported cases are homozygous with consanguineous family histories, with only rare compound-heterozygous exceptions.

> *"This is an autosomal recessive disorder mapped to chromosome 1q42.12 due to mutations in the PYCR2 gene, encoding an enzyme involved in proline synthesis in mitochondria."* — PMID: 27130255
>
> *"PYCR2 pathogenic variants lead to an autosomal recessive hypomyelinating leukodystrophy 10 (HLD10), characterized by global developmental delay, microcephaly, facial dysmorphism, movement disorder, and hypomyelination."* — PMID: 34037307

Finding 2 — Core clinical phenotype: postnatal microcephaly, developmental delay, hypomyelination, poor survival

The characteristic presentation includes postnatally acquired (**progressive**) microcephaly, moderate-to-profound global developmental delay, failure to thrive, craniofacial dysmorphism, hyperkinetic movements, axial hypotonia with variable appendicular spasticity, and seizures. Brain MRI shows hypomyelination/delayed myelination, thin corpus callosum, global brain/white-matter atrophy, and T2 white-matter hyperintensities. Severely affected patients do not survive beyond the first decade. A crucial diagnostic clue is that **routine serum metabolic profiles are unremarkable/normal**, distinguishing HLD10 from classical inborn errors of metabolism with peripheral biochemical signatures.

> *"The characteristic clinical presentation of patients with PYCR2 mutations included failure to thrive, microcephaly, craniofacial dysmorphism, progressive psychomotor disability, hyperkinetic movements, and axial hypotonia with variable appendicular spasticity. Patients did not survive beyond the first decade of life."* — PMID: 27130255
>
> *"All patients presented with postnatally acquired microcephaly, moderate to profound global developmental delay, and failure to thrive. Brain MRI in these patients showed thin corpus callosum, delayed myelination, and generalized white-matter volume loss."* — PMID: 27860360

Finding 3 — Mechanism: PYCR2 loss raises cerebral glycine via SHMT2 upregulation, driving neurodegeneration

Escande-Beillard et al. (2020) solved the PYCR2 apo-enzyme crystal structure, showed that a p.Gly249Val mutation at the dimer interface lowers enzymatic activity, and demonstrated that *Pycr2*-knockout mice phenocopy the human disorder and deplete PYCR1 in neural lineages. In situ neurotransmitter quantification in mutant mouse and patient brains revealed encephalopathy driven by **excessive cerebral glycine caused by SHMT2 upregulation**; SHMT2 knockdown partially reversed axonal beading and rescued neurite length in *Pycr2*-KO neurons. Zaki et al. independently showed that both missense and nonsense mutations impair PYCR2 protein multimerization, the biophysical basis of loss of function.

> *"loss of PYCR2 upregulates SHMT2, which is responsible for glycine synthesis. This hyperglycemia could be partially reversed by SHMT2 knockdown, which rescued the axonal beading and neurite lengths of cultured Pycr2 knockout neurons."* — PMID: 32330411
>
> *"knocking out Pycr2 in mice phenocopies the human disorder and depletes PYCR1 levels in neural lineages"* — PMID: 32330411
>
> *"Both nonsense and missense mutations were identified, which impaired protein multimerization."* — PMID: 27130255

Finding 4 — Gene identification (2015): variants destabilize the protein and sensitize cells to oxidative-stress apoptosis

Nakayama et al. (2015, *Am J Hum Genet*) identified biallelic *PYCR2* mutations as the cause of postnatal microcephaly with hypomyelination through linkage mapping plus whole-exome sequencing in two consanguineous families (homozygous **c.355C>T p.Arg119Cys** and **c.751C>T p.Arg251Cys**). Patient lymphoblastoid cells showed strongly reduced PYCR2; transfected variant proteins retained normal mitochondrial localization but were present at lower amounts, indicating **reduced protein stability** as the loss-of-function mechanism. A CRISPR-Cas9 PYCR2-knockout HEK293FT line showed decreased mitochondrial membrane potential and increased susceptibility to apoptosis under oxidative stress, linking PYCR2 loss to mitochondrial dysfunction.

> *"A PYCR2-deficient HEK293FT cell line generated by genome editing with the clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 system showed that PYCR2 loss of function led to decreased mitochondrial membrane potential and increased susceptibility to apoptosis under oxidative stress."* — PMID: 25865492
>
> *"both variant proteins retained normal mitochondrial localization but had lower amounts than the wild-type protein, suggesting that the variant proteins were less stable"* — PMID: 25865492

Finding 5 — Zebrafish *pycr1b* knockdown recapitulates microcephaly, rescued by wild-type human *PYCR2* mRNA

Nakayama et al. (2015) performed morpholino-based knockdown of the zebrafish *PYCR2* ortholog *pycr1b*, which recapitulated the human microcephaly phenotype. The phenotype was rescued by **wild-type** human *PYCR2* mRNA but **not** by mutant (p.Arg119Cys / p.Arg251Cys) mRNAs, confirming both the pathogenicity of the specific variants and the functional conservation of the gene across vertebrates.

> *"Morpholino-based knockdown of a zebrafish PYCR2 ortholog, pycr1b, recapitulated the human microcephaly phenotype, which was rescued by wild-type human PYCR2 mRNA, but not by mutant mRNAs"* — PMID: 25865492

Finding 6 — Phenotypic spectrum extends to a milder late-childhood hereditary spastic paraplegia

Sager et al. (2023) reported a novel homozygous missense *PYCR2* variant (**NM_013328 c.383T>C, p.Val128Ala**) in 5 male patients from 2 related families presenting as **hereditary spastic paraplegia (HSP) in late childhood WITHOUT hypomyelinating leukodystrophy**. Developmental milestones were normal without dysmorphic features; ~80% had mild intention tremor from ~6 years and ~80% had progressive lower-limb spasticity/gait difficulty from age 8–12 years; ages ranged 6–26 years. This is the first report of *PYCR2* variants causing HSP and considerably widens the recognized clinical spectrum, with implications for genetic diagnosis of milder cases.

> *"manifest Hereditary Spastic Paraplegia (HSP) is the only symptom without hypomyelinating leukodystrophy. This is the first study that report the PYCR2 gene variants as a cause of HSP in late childhood."* — PMID: 37141741
>
> *"A novel homozygous missense (NM_013328: c.383T > C, p.V128A) variant in the PYCR2 gene is detected in 5 patient from 2 related families."* — PMID: 37141741

Finding 7 — Founder effects and consanguinity-driven population genetics (Thai c.400G>A founder allele)

Manaspon et al. (2021) reviewed all 35 previously reported *PYCR2* patients: the majority were homozygous with consanguineous family history (except two compound-heterozygous cases); all had microcephaly and developmental delay; hypotonia and peripheral spasticity were common; hypomyelination/delayed myelination was the typical radiographic feature. In two unrelated Thai families, the **c.400G>A (p.Val134Met)** variant was found on a shared 2.3 Mb haplotype (estimated allele age ~1450 years), indicating a common ancestor/founder effect; it accounted for 3 of 4 mutant alleles in Thai patients.

> *"Haplotype analysis revealed that the two families' members shared a 2.3 Mb region covering the c.400G>A variant, indicating a common ancestry. The variant was estimated to age 1450 years ago."* — PMID: 34037307
>
> *"majorities of cases were homozygous with a consanguineous family history, except patient 1 and another reported case who were compound heterozygous. All patients had microcephaly and developmental delay. Hypotonia and peripheral spasticity were common."* — PMID: 34037307

Finding 8 — No disease-specific therapy; SHMT2/glycine-lowering is the leading mechanism-based target

No curative or disease-modifying therapy is approved for HLD10; care is supportive (anticonvulsants; spasticity, nutritional, and rehabilitation management). Because routine serum metabolic profiles are normal, peripheral proline supplementation is not clearly rational. The strongest mechanism-based lead is **lowering cerebral glycine**: Escande-Beillard et al. showed SHMT2 knockdown partially reversed axonal beading and rescued neurite lengths in *Pycr2*-knockout neurons, identifying the glycine metabolic pathway as a possible intervention point. No HLD10-specific clinical trials are currently registered.

> *"Our findings identify the glycine metabolic pathway as a possible intervention point to alleviate the neurological symptoms of PYCR2-mutant patients."* — PMID: 32330411

Section-by-Section Report

1. Disease Information

HLD10 is a rare autosomal recessive hypomyelinating leukodystrophy — a genetic white-matter disorder characterized by MRI evidence of absent or near-absent myelin development combined with postnatal (progressive) microcephaly, severe neurodevelopmental impairment, and failure to thrive. It is one of a numbered series of hypomyelinating leukodystrophies (HLD1–HLD~24+), each defined by a distinct causal gene; HLD10 is the *PYCR2*-associated entity.

Key identifiers:

Resource	Identifier
OMIM	#616420
MONDO	MONDO:0014635
Gene (HGNC)	*PYCR2*
Locus	1q42.12
MeSH	Hereditary Central Nervous System Demyelinating Diseases (closest); Leukodystrophy
Orphanet	Within genetic hypomyelinating leukodystrophy group

Synonyms / alternative names: Hypomyelinating leukodystrophy 10 with microcephaly; PYCR2-related microcephaly with hypomyelination; postnatal microcephaly with hypomyelination and failure to thrive; PYCR2 deficiency.

Source of information: Aggregated disease-level resources (OMIM, published case series/cohorts) and individual patient case reports; no large EHR-derived registry exists given the disorder's rarity.

2. Etiology

Primary cause: Biallelic (homozygous or compound heterozygous) pathogenic variants in *PYCR2* (genetic, Mendelian). The etiology is monogenic; there is no evidence of infectious, environmental, or acquired causation.

Genetic risk factors: The only established genetic risk factor is inheriting two pathogenic *PYCR2* alleles. **Consanguinity** is the dominant epidemiologic driver — most reported families are consanguineous, and homozygosity for founder or private variants predominates (Findings 1, 7).

Environmental risk factors: None identified. HLD10 is fully genetically determined. No toxin, exposure, dietary, or lifestyle factor has been implicated.

Protective factors: None specifically identified. In principle, heterozygous carriers are unaffected (recessive), and outbreeding in consanguineous populations reduces incidence.

Gene–environment interactions: No documented GxE interaction. Because peripheral metabolism is normal and the disorder is highly penetrant when biallelic, environmental modulation appears minimal; phenotypic variability is primarily allele-driven (e.g., the milder p.Val128Ala HSP allele — Finding 6).

3. Phenotypes

Phenotype	Type	Onset	Severity/ Progression	Frequency	Suggested HPO
Progressive (postnatal) microcephaly	Physical/ sign	Postnatal (acquired)	Severe, progressive	Nearly universal	HP:0005484 (Postnatal microcephaly)
Global developmental delay	Sign	Infantile	Moderate–profound	Universal	HP:0001263
Failure to thrive	Sign	Infantile	Severe	Very common	HP:0001508
Hypomyelination / delayed myelination (MRI)	Imaging/ lab	Infantile	Progressive	Universal	HP:0006808 (Hypomyelination)
Thin corpus callosum	Imaging	Congenital/ infantile	Static/ progressive	Common	HP:0033725
Cerebral/white-matter atrophy	Imaging	Infantile	Progressive	Common	HP:0002283 / HP:0012762
Axial hypotonia	Sign	Infantile	Variable	Common	HP:0008936
Appendicular / lower-limb spasticity	Sign	Infantile–childhood	Variable	Common	HP:0001257 / HP:0002061
Hyperkinetic movements	Sign	Infantile	Variable	Common	HP:0002487
Seizures	Sign	Infantile	Variable	Frequent	HP:0001250
Craniofacial dysmorphism	Physical	Congenital	Variable	Common	HP:0001999
Intention tremor (mild allele)	Sign	~6 yr	Mild	~80% of HSP-phenotype patients	HP:0002080
	Sign				HP:0001258

Phenotype	Type	Onset	Severity/ Progression	Frequency	Suggested HPO
Hereditary spastic paraplegia (mild allele)		Late childhood (8–12 yr)	Progressive, milder	Mild-allele families	

Quality of life impact: In the severe (classic) form, profound global disability, non-ambulatory/non-verbal status, feeding difficulty, and death within the first decade impose maximal burden on affected children and caregivers. In the milder HSP form, quality of life is affected principally by progressive gait impairment with preserved cognition (Finding 6). Formal QoL instrument data (EQ-5D, SF-36) are not available for this ultra-rare disease.

4. Genetic / Molecular Information

Causal gene: *PYCR2* (HGNC), 1q42.12; encodes pyrroline-5-carboxylate reductase 2, a mitochondrial enzyme catalyzing the final (NAD(P)H-dependent) step of L-proline biosynthesis (reduction of Δ^1-pyrroline-5-carboxylate to proline).

Pathogenic variants (representative):

Variant (cDNA)	Protein	Type	Phenotype	Population/Note	Source
c.355C>T	p.Arg119Cys	Missense (destabilizing)	Classic HLD10	Consanguineous; original 2015 report	PMID: 25865492
c.751C>T	p.Arg251Cys	Missense (destabilizing)	Classic HLD10	Consanguineous; original 2015 report	PMID: 25865492
c.400G>A	p.Val134Met	Missense	Classic HLD10	Thai founder allele (~1450 yr)	PMID: 34037307
(Gly249Val)	p.Gly249Val	Missense at dimer interface (↓ activity)	HLD10	Functional/ structural study	PMID: 32330411
c.383T>C	p.Val128Ala	Missense (mild)	HSP without leukodystrophy	2 related families, 5 males	PMID: 37141741

Variant classification: Reported disease alleles are classified pathogenic/likely pathogenic under ACMG/AMP, supported by functional evidence (reduced protein stability, impaired multimerization, decreased enzymatic activity, zebrafish rescue failure).

Variant types: Both **missense** (reduced stability / impaired multimerization / reduced activity) and **nonsense** variants have been reported; all converge on loss of function (Findings 3, 4).

Allele frequency: Pathogenic alleles are rare in population databases (gnomAD), consistent with a recessive, largely founder/consanguinity-driven disorder.

Origin: Germline (constitutional, biallelic). No somatic contribution.

Functional consequence: Loss of function — missense variants act principally by destabilizing the protein and impairing multimerization; nonsense variants truncate the protein. Downstream, PYCR1 is depleted in neural lineages (Finding 3).

Modifier genes: None formally established. *SHMT2* is a mechanistic effector (its upregulation drives glycine toxicity) rather than a classic modifier; *PYCR1* depletion is a downstream consequence. Phenotype severity tracks primarily with the specific *PYCR2* allele.

Epigenetic information: No disease-specific DNA-methylation or histone-modification signature has been reported for HLD10 (not available).

Chromosomal abnormalities: None; HLD10 is a single-gene disorder without recurrent structural/copy-number changes (not applicable).

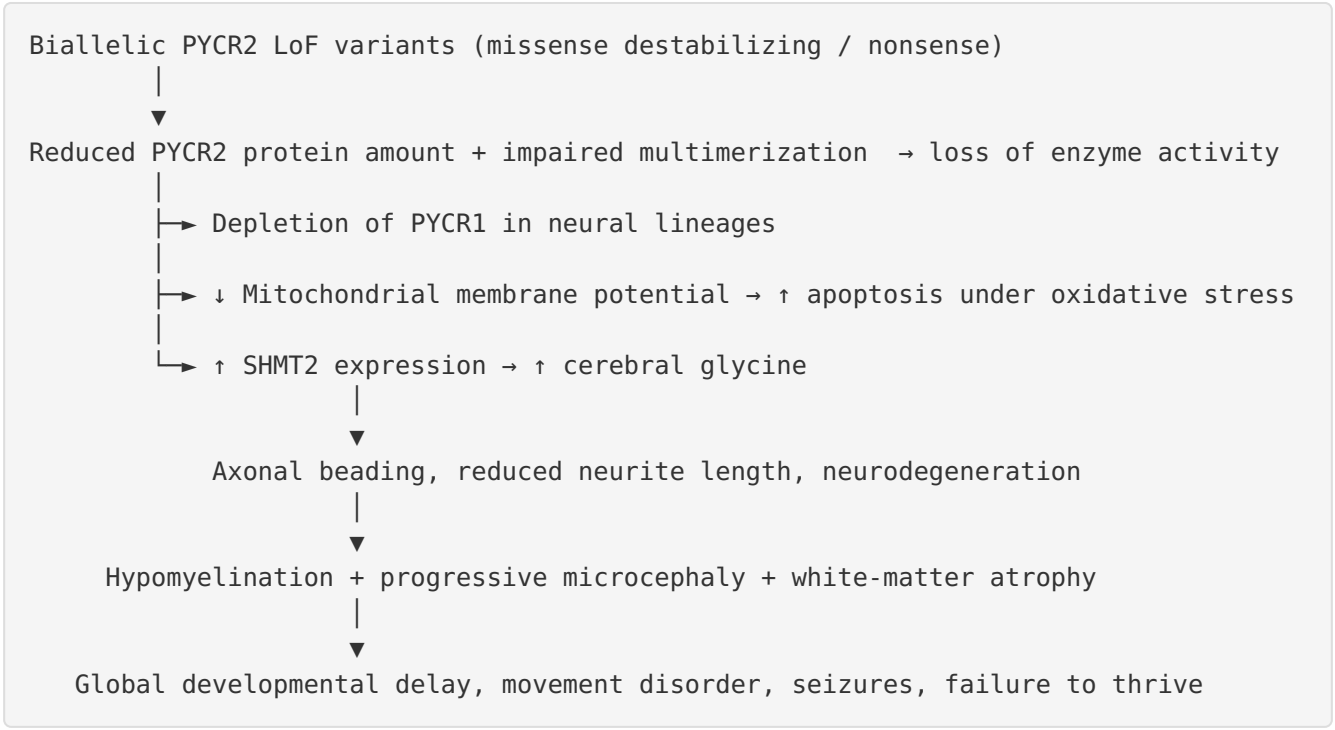
5. Environmental Information

No environmental, lifestyle, or infectious factors contribute to HLD10. It is a purely genetic Mendelian disorder. Consanguinity (a social/demographic rather than environmental exposure) increases the probability of homozygosity for pathogenic alleles but is not an environmental cause of the molecular defect. Infectious agents are not applicable.

6. Mechanism / Pathophysiology

Molecular pathway: Proline biosynthesis and one-carbon/serine–glycine metabolism. PYCR2 catalyzes the terminal reduction of pyrroline-5-carboxylate (P5C) to L-proline in mitochondria. Loss of PYCR2 activity perturbs this node and, critically, triggers compensatory **upregulation of SHMT2** (serine hydroxymethyltransferase 2), which synthesizes glycine — producing pathological cerebral glycine excess (Finding 3).

Causal chain (upstream → downstream):



Cellular processes: Apoptosis (increased under oxidative stress), mitochondrial dysfunction (decreased membrane potential), and neuronal/axonal degeneration (axonal beading, neurite shortening). The hypomyelination appears to be at least partly secondary to a primary neuronal/axonal defect (a "leuko-axonopathy"-type mechanism), consistent with the neurotransmitter/glycine-driven neurodegeneration.

Protein dysfunction: Missense variants retain correct mitochondrial localization but are present at lower amounts (reduced stability) and impair multimerization; the p.Gly249Val substitution at the dimer interface lowers catalytic activity — the apo-enzyme crystal structure was solved to demonstrate this (Findings 3, 4).

Metabolic changes: Elevated cerebral glycine (via SHMT2); perturbed proline biosynthesis. Importantly, **peripheral/serum metabolic profiles are normal**, indicating a CNS-compartmentalized metabolic derangement (Finding 2).

Immune involvement / tissue-damage mechanisms: No autoimmune or inflammatory driver; oxidative stress-sensitized apoptosis and glycine excitotoxic-type neurodegeneration are the operative injury mechanisms.

Suggested ontology terms: - GO biological process: proline biosynthetic process (GO:0006561); glycine biosynthetic process (GO:0006545); myelination (GO:0042552); apoptotic process (GO:0006915); neuron projection development (GO:0031175). - GO cellular component:

mitochondrion (GO:0005739); mitochondrial matrix (GO:0005759). - CL cell types: oligodendrocyte (CL:0000128); neuron (CL:0000540); central nervous system neuron. - CHEBI: L-proline (CHEBI:17203); glycine (CHEBI:15428); L-1-pyrroline-5-carboxylate (CHEBI:17388).

7. Anatomical Structures Affected

- **Primary organ / body system:** Central nervous system / brain (nervous system). UBERON: brain (UBERON:0000955); white matter (UBERON:0002316); corpus callosum (UBERON:0002336); cerebral hemisphere.
- **Tissue level:** Cerebral white matter (myelin) with generalized volume loss; thin corpus callosum; cerebral atrophy.
- **Cell level:** Oligodendrocytes (myelinating cells; CL:0000128) and neurons/axons (CL:0000540) are affected; PYCR1 depletion occurs in neural lineages.
- **Subcellular level:** Mitochondria (GO:0005739) — reduced membrane potential and increased oxidative-stress apoptosis.
- **Localization / lateralization:** Diffuse and **bilateral / symmetric** white-matter involvement, typical of hypomyelinating leukodystrophies.

8. Temporal Development

- **Onset:** Congenital-to-infantile in the classic form; microcephaly is postnatally acquired and **progressive** (head circumference normal at birth then decelerating). The milder HSP allele has late-childhood onset (spasticity 8–12 yr; tremor from ~6 yr).
- **Onset pattern:** Insidious/chronic and progressive.
- **Progression:** Progressive neurodegeneration with failure to thrive; severe cases are fatal within the first decade. The mild HSP phenotype progresses more slowly, with survival into adulthood (ages reported up to 26 years).
- **Course:** Chronic, progressive, lifelong; no remission.
- **Critical periods:** Early postnatal myelination window is the period of maximal vulnerability and the theoretical window for any myelination-directed or glycine-lowering intervention.

9. Inheritance and Population

- **Inheritance:** Autosomal recessive.
- **Penetrance:** High/complete for the biallelic classic phenotype; **variable expressivity** exists across alleles (severe leukodystrophy vs. milder HSP).

- **Genetic anticipation:** Not applicable (not a repeat-expansion disorder).
- **Founder effects:** Documented — the Thai **c.400G>A (p.Val134Met)** founder allele on a shared 2.3 Mb haplotype, estimated age ~1450 years, accounting for 3 of 4 mutant alleles in Thai patients (Finding 7).
- **Consanguinity:** Central to the epidemiology; most families are consanguineous and patients homozygous.
- **Carrier frequency / prevalence / incidence:** Not precisely established; the disorder is ultra-rare with ~35+ patients reported in the literature as of the 2021 review. Prevalence in outbred populations is very low; locally elevated where founder alleles and consanguinity coincide.
- **Population demographics:** Reported across consanguineous populations (Middle Eastern, South/Southeast Asian including Thai and Indian patients). No strong sex bias in the classic form; the reported HSP-allele families comprised affected males (small sample). Age distribution skews pediatric owing to early mortality in the severe form.

10. Diagnostics

- **Recommended approach:** Molecular genetic diagnosis is definitive. Because **routine serum/urine metabolic tests are normal**, biochemical screening does not establish the diagnosis and can mislead.
- **Genetic testing:** Whole-exome sequencing (WES) is the highest-yield test and was the discovery method; whole-genome sequencing (WGS) or leukodystrophy/hypomyelination gene panels including *PYCR2* are appropriate. Single-gene testing is reasonable in populations with known founder alleles (e.g., Thai c.400G>A). Chromosomal microarray/karyotype/FISH are not indicated (single-gene disorder).
- **Imaging:** Brain MRI is the key phenotyping modality — hypomyelination/delayed myelination (delayed T2 hypointensity, often T1 hyperintensity), thin corpus callosum, cerebral and white-matter atrophy. Serial MRI helps distinguish primary hypomyelination from progressive atrophy.
- **Biomarkers:** No validated peripheral biomarker; cerebral glycine elevation is a mechanistic finding (MR spectroscopy could theoretically detect elevated glycine, but this is not an established clinical biomarker).
- **Differential diagnosis:** Other hypomyelinating leukodystrophies and microcephaly syndromes — e.g., PMD/PLP1 (HLD1), HIKESHI-related HLD (with febrile-illness crises, Ashkenazi founder), SLC25A12/AGC1-related leuko-axonopathy, and KIF1C-related spastic-ataxia/HLD. *PYCR2* disease is distinguished by progressive postnatal microcephaly, failure

to thrive, normal peripheral metabolics, and biallelic *PYCR2* variants. The milder allele overlaps clinically with hereditary spastic paraplegias.

- **Screening:** Cascade/carrier testing in affected consanguineous families; targeted founder-allele carrier screening is feasible where relevant (e.g., Thai c.400G>A).

11. Outcome / Prognosis

- **Survival/mortality:** Severe (classic) HLD10 is **fatal within the first decade of life**. The milder HSP-phenotype patients survive into adulthood.
- **Morbidity/function:** Profound, lifelong disability in the classic form (non-ambulatory, non-verbal, feeding-dependent, seizures). The mild form causes progressive gait impairment with relatively preserved cognition.
- **Complications:** Failure to thrive, feeding difficulty, seizures, aspiration/respiratory complications, and consequences of severe neurodisability.
- **Prognostic factors:** Genotype is the principal determinant — null/severely destabilizing biallelic variants predict the severe lethal phenotype, whereas partial-function alleles (e.g., p.Val128Ala) predict the milder HSP course.
- **Recovery potential:** None; the disorder is progressive and neurodegenerative.

12. Treatment

- **Disease-specific therapy:** None approved. Management is **supportive/symptomatic** — anticonvulsants for seizures, spasticity management (physiotherapy, antispasticity agents), nutritional support for failure to thrive, and multidisciplinary rehabilitation (physical, occupational, speech therapy).
- **Mechanism-based experimental direction: Glycine-lowering / SHMT2 inhibition** is the leading strategy, supported by the demonstration that SHMT2 knockdown partially rescues axonal beading and neurite length in *Pycr2*-KO neurons (Finding 8). Dietary/pharmacologic glycine reduction and SHMT2-targeted approaches are conceptually motivated but unproven clinically.
- **Rational cautions:** Because peripheral proline metabolism is normal, systemic proline supplementation lacks clear rationale.
- **Clinical trials:** No HLD10-specific registered trials.
- **Suggested NCIT terms:** Supportive Care; Anticonvulsant Agent; Physical Therapy; Nutritional Support (used generically; no disease-specific intervention exists).

13. Prevention

- **Primary prevention: Genetic counseling** for consanguineous couples and affected families; carrier testing and reproductive options (preimplantation genetic testing, prenatal diagnosis) are the principal preventive measures given the recessive, high-penetrance nature.
- **Screening:** Cascade carrier testing within families; founder-allele carrier screening where population-relevant (e.g., Thai c.400G>A).
- **Behavioral/public-health:** Awareness of consanguinity-associated recessive disease risk; no vaccine or environmental intervention is applicable.
- **Counseling:** 25% recurrence risk for carrier–carrier couples; genetic counseling is central.

14. Other Species / Natural Disease

- **Orthologs / model species:** Mouse *Pycr2*; zebrafish ortholog *pycr1b*. No naturally occurring animal disease has been documented (OMIA); model organisms are engineered/ experimental.
- **Comparative biology:** The gene and its function are evolutionarily conserved — zebrafish *pycr1b* knockdown reproduces microcephaly rescued by human *PYCR2* mRNA (Finding 5), and *Pycr2*-KO mice phenocopy the human disorder (Finding 3), demonstrating conserved requirement for PYCR2 in neurodevelopment.
- **Zoonotic/transmission:** Not applicable (genetic disorder).

15. Model Organisms

Model	Type	Key features	Recapitulation	Reference
Pycr2 knockout mouse	Mammalian, genetic KO	Phenocopies human disorder; depletes PYCR1 in neural lineages; elevated cerebral glycine via SHMT2; SHMT2 knockdown rescues axonal beading/ neurite length	High — reproduces neurodegeneration and the core mechanism	PMID: 32330411
Zebrafish *pycr1b* morphant	Vertebrate, morpholino knockdown	Recapitulates microcephaly; rescued by WT human *PYCR2* mRNA but not mutant mRNAs	Good for microcephaly; validates variant pathogenicity	PMID: 25865492
PYCR2-KO HEK293FT (CRISPR-Cas9)	Cellular, in vitro	↓ Mitochondrial membrane potential; ↑ apoptosis under oxidative stress	Models mitochondrial/ apoptotic mechanism	PMID: 25865492
Patient lymphoblastoid cells; transfected variant proteins	In vitro	Reduced PYCR2; variants normally localized but less stable	Models loss-of-function via reduced stability	PMID: 25865492
Recombinant PYCR2 (crystal structure)	Structural/ biochemical	Apo-enzyme structure; p.Gly249Val at dimer interface lowers activity	Structural basis of pathogenicity	PMID: 32330411

Model applications: Dissecting the SHMT2/glycine mechanism, testing glycine-lowering interventions, validating variant pathogenicity, and studying mitochondrial dysfunction. **Limitations:** Morpholino knockdown is transient and can carry off-target effects; the milder human HSP phenotype has not been separately modeled; therapeutic rescue to date is partial and in vitro/animal only.

Mechanistic Model / Interpretation

The evidence converges on a coherent model in which **PYCR2 is a mitochondrial enzyme whose loss produces a compartmentalized cerebral metabolic and mitochondrial crisis**. Two mechanistic arms operate downstream of the same biallelic loss-of-function lesion:

1. **A mitochondrial/apoptotic arm** (established 2015): destabilized or truncated PYCR2 → reduced enzyme → decreased mitochondrial membrane potential → heightened apoptosis under oxidative stress, with PYCR1 co-depletion in neural lineages.
2. **A glycine-excess arm** (established 2020): PYCR2 loss → SHMT2 upregulation → excess cerebral glycine → axonal beading, neurite shortening, and neurodegeneration.

The second arm is the more actionable one because it is **reversible in models** — SHMT2 knockdown partially rescues neuronal morphology. Both arms terminate in the same clinical endpoint: progressive postnatal microcephaly, hypomyelination/white-matter atrophy, and severe neurodevelopmental disability. The observation that peripheral metabolics are normal despite cerebral glycine elevation underscores that this is a brain-restricted metabolic disease, which also explains why classical biochemical newborn screening does not detect it and why genetic testing is essential.

Allelic severity maps onto phenotype: severe destabilizing/null biallelic genotypes produce the lethal infantile leukodystrophy, while partial-function alleles (p.Val128Ala) produce a milder, later-onset hereditary spastic paraplegia without overt leukodystrophy — a genotype–phenotype gradient rather than two separate diseases.

Evidence Base

PMID	Study	Contribution	Evidence type
25865492	Nakayama et al. 2015, *Am J Hum Genet*	Gene identification; reduced protein stability; CRISPR-KO mitochondrial/apoptosis phenotype; zebrafish *pycr1b* rescue	Human genetics + in vitro + model organism
27130255	Zaki et al. — *PYCR2 mutations cause a lethal syndrome*	11 consanguineous families; AR locus 1q42.12; impaired multimerization; core phenotype and lethal prognosis	Human clinical/ genetics
27860360	Homozygous *PYCR2* variants, progressive microcephaly & hypomyelination	Confirms postnatal microcephaly, MRI features, normal metabolics	Human clinical
32330411	Escande-Beillard et al. 2020 — *Loss of PYCR2 causes neurodegeneration via SHMT2*	Crystal structure; *Pycr2*-KO mouse; SHMT2/glycine mechanism and rescue; therapeutic target	Structural + model organism + mechanistic
34037307	Manaspon et al. 2021 — Thai cohort	Review of 35 patients; consanguinity dominance; Thai c.400G>A founder allele (~1450 yr)	Human genetics/ population
37141741	Sager et al. 2023 — *PYCR2 causes HSP in late childhood*	First HSP phenotype; p.Val128Ala; spectrum expansion	Human clinical/ genetics
34055512	Indian child case report	Compound-heterozygous HLD10; normal metabolics; MRI hypomyelination	Human clinical (case)
33771508	Disease variants of human Δ^1-pyrroline-5-carboxylate reductase	Biochemistry of PYCR enzymology	In vitro/ biochemical

Supporting/contextual literature on the hypomyelinating leukodystrophy landscape and differentials includes reviews of hypomyelinating disorders and MRI approaches (PMID: 26477299, PMID: 27235001), the expanded genetic white-matter disorder gene catalog (PMID: 32704519), and comparators such as HIKESHI-related HLD (PMID: 34111619), SLC25A12/AGC1 leuko-axonopathy (PMID: 31403263), and KIF1C-related classification ambiguity (PMID: 40794111).

Limitations and Knowledge Gaps

- **Small evidence base:** Only ~35+ patients reported; prevalence, incidence, carrier frequency, and sex ratio are not precisely quantified.
- **Genotype–phenotype correlations** are still coarse; the full determinants of the severe-vs-mild spectrum are not systematically mapped.
- **Biomarkers:** No validated peripheral or imaging biomarker (e.g., MRS glycine) is clinically established for diagnosis or monitoring.
- **Therapeutics:** Glycine-lowering/SHMT2 inhibition rescue is partial and demonstrated only in vitro/animal; no human therapeutic data or registered trials exist.
- **Two mechanistic arms** (mitochondrial-apoptotic vs glycine-excess) are not fully integrated — their relative contributions to hypomyelination versus neuronal loss remain to be resolved.
- **Epigenetics, immune involvement, and structural genomics** are not characterized (not applicable/unknown).
- **Model gaps:** The milder HSP phenotype lacks a dedicated model; morpholino data carry inherent caveats.

Proposed Follow-up Experiments / Actions

1. **Test glycine-lowering interventions in vivo:** Evaluate dietary glycine restriction and/or SHMT2 pharmacologic inhibition in *Pycr2*-KO mice for effects on myelination, brain growth, and survival — the most direct translation of the mechanistic finding.

2. **Genotype–phenotype registry:** Aggregate all reported and new *PYCR2* patients with standardized allele annotation, MRI phenotyping, and outcomes to define severity predictors and refine the severe-vs-HSP spectrum.
3. **MR spectroscopy for cerebral glycine:** Prospectively test whether MRS-detectable brain glycine elevation can serve as a diagnostic/monitoring biomarker.
4. **iPSC-derived oligodendrocyte/neuron models** from patients (severe and mild alleles) to dissect whether hypomyelination is primary (oligodendrocyte-autonomous) or secondary to axonal/neuronal glycine toxicity.
5. **Structure-guided variant functional classification:** Use the apo-enzyme structure to model additional missense VUS (stability, dimer-interface, activity) and improve ACMG classification.
6. **Population carrier screening** for founder alleles (e.g., Thai c.400G>A) in high-consanguinity communities, paired with genetic counseling programs.
7. **Integrate the two mechanistic arms:** Experiments manipulating oxidative-stress/mitochondrial function and glycine levels independently to determine their relative causal weight for the hypomyelination endpoint.

Report compiled from an autonomous multi-iteration literature investigation (8 confirmed findings, 17 papers reviewed). Evidence types span human clinical/genetics, model organism (mouse, zebrafish), in vitro/cellular, and structural/biochemical studies.
