

Dihydropyrimidinase Deficiency — Comprehensive Disease Characterization

Disease: Dihydropyrimidinase Deficiency (DHP deficiency / DPYS deficiency) **MONDO ID:** MONDO:0009111 · **OMIM:** 222748 · **Orphanet:** 35707 **Causal gene:** *DPYS* (dihydropyrimidinase), 8q22 · **Category:** Mendelian (autosomal recessive inborn error of metabolism)

Summary

Dihydropyrimidinase (DHP) deficiency is a rare autosomal recessive inborn error of the **pyrimidine degradation pathway**, caused by biallelic loss-of-function variants in *DPYS* on chromosome 8q22. The gene encodes dihydropyrimidinase (EC 3.5.2.2), a zinc-dependent amidohydrolase and the **second enzyme** of the three-step catabolism of uracil and thymine. Loss of enzyme activity blocks the ring-opening of 5,6-dihydrouracil and 5,6-dihydrothymine, producing the biochemical hallmark of the disease — **dihydropyrimidinuria**, with markedly elevated dihydrouracil and dihydrothymine in urine, plasma, and cerebrospinal fluid.

The clinical phenotype is **strikingly variable and incompletely penetrant**, ranging from completely asymptomatic individuals to severe neurodevelopmental disease. Reported features include global/developmental delay, seizures and infantile spasms, intellectual disability, autistic features, microcephaly, white matter abnormalities, and brain atrophy, together with gastrointestinal problems (feeding difficulties, vomiting) and, more rarely, congenital heart disease. A 2024 report expanded the phenotype to include **levodopa-responsive juvenile parkinsonism**. Critically, residual enzyme activity does **not** reliably predict whether an individual will be symptomatic, and identical mutations can produce both symptomatic and asymptomatic carriers — pointing to strong genetic/environmental modification.

The single most clinically actionable aspect of the disorder is a **pharmacogenetic predisposition to severe or lethal 5-fluorouracil (5-FU)/capecitabine toxicity**, which can occur even in otherwise asymptomatic individuals and even in partial deficiency. An intriguing biological puzzle — resolved during this investigation using GTEx expression data — is that

DPYS is expressed almost exclusively in **liver and kidney and essentially not in brain**, which reframes the prominent neurological phenotype as a **circulating-metabolite (non-cell-autonomous) effect** rather than a primary brain enzyme defect. A naturally occurring feline case provides the principal animal model, and population genetics (gnomAD) confirm that *DPYS* tolerates loss-of-function (as expected for a recessive disorder), with pathogenic missense alleles enriched in East Asian populations.

Key Findings

Finding 1 — Molecular identity: an autosomal recessive defect of pyrimidine degradation (F001)

Dihydropyrimidinase deficiency is caused by biallelic mutations in *DPYS*, which encodes the **second enzyme of the pyrimidine degradation pathway**. DHP (EC 3.5.2.2) catalyzes the ring-opening (hydrolysis) of 5,6-dihydrouracil to N-carbamyl- β -alanine and of 5,6-dihydrothymine to N-carbamyl- β -aminoisobutyric acid. The pathway runs: **uracil/thymine $\rightarrow$ (DPD) $\rightarrow$ dihydrouracil/dihydrothymine $\rightarrow$ (DHP/DPYS) $\rightarrow$ N-carbamyl- β -alanine/N-carbamyl- β -aminoisobutyrate $\rightarrow$ (β -ureidopropionase/UPB1) $\rightarrow$ β -alanine/ β -aminoisobutyrate**.

The human *DHP* gene spans **>80 kb**, consists of **10 exons**, and maps to **8q22** by FISH ([PMID: 9718352](#): "The human *DHP* gene spans >80 kb and consists of 10 exons. It has been assigned to 8q22, by FISH."). The enzyme reaction is defined precisely in the clinical literature ([PMID: 17383919](#): "Dihydropyrimidinase (*DHP*) is the second enzyme of the pyrimidine degradation pathway and it catalyses the ring opening of 5,6-dihydrouracil and 5,6-dihydrothymine to N-carbamyl-beta-alanine and N-carbamyl-beta-aminoisobutyric acid, respectively."). Inheritance is autosomal recessive — patients are homozygous or compound heterozygous, with heterozygous (carrier) parents.

Ontology anchors: MONDO:0009111; gene HGNC:3012 (*DPYS*); GO:0006208 (pyrimidine nucleobase catabolic process); GO:0004157 (dihydropyrimidinase activity); CHEBI 5,6-dihydrouracil, CHEBI 5,6-dihydrothymine.

Finding 2 — Biochemical hallmark: dihydropyrimidinuria across body fluids (F002)

The defining laboratory abnormality is marked accumulation of the two DHP substrates. Patients show **markedly elevated 5,6-dihydrouracil and 5,6-dihydrothymine in plasma, cerebrospinal fluid, and urine** (PMID: 20362666: *"Patients presented mainly with neurological and gastrointestinal abnormalities and markedly elevated levels of 5,6-dihydrouracil and 5,6-dihydrothymine in plasma, cerebrospinal fluid and urine."*). The defect is detectable by urine metabolomics: gas chromatography–mass spectrometry (GC-MS) screening reveals elevated dihydrouracil and dihydrothymine, confirmed by quantitative LC-MS/MS (PMID: 30384990: *"Gas chromatography-mass spectrometry-based urine metabolomics demonstrated significant elevations of dihydrouracil and dihydrothymine, which were subsequently confirmed by a quantitative analysis using liquid chromatography-tandem mass spectrometry."*). Comprehensive HPLC–electrospray ionization tandem MS panels reliably detect the entire spectrum of purine/pyrimidine disorders, including DHP deficiency, in a single run (PMID: 16613999).

Finding 3 — Highly variable, incompletely penetrant phenotype (F003)

Fewer than ~40 patients have been published. The phenotypic range spans **asymptomatic → severe neurological disease**. Reported features include global/developmental delay, seizures and infantile spasms, intellectual disability, dysmorphic facies, autistic features, microcephaly, white matter abnormalities, and brain atrophy, plus gastrointestinal abnormalities (feeding problems, vomiting). Congenital heart disease (atrioventricular septal defect, ventricular septal defect) has been reported as a rare feature.

Key quotes: (PMID: 38958169: *"Clinical findings are variable and some patients may remain asymptomatic. Global developmental delay and increased susceptibility to 5-fluorouracil are commonly reported."*) and (PMID: 29054612: *"a highly variable clinical presentation, ranging from asymptomatic to infantile spasm and reduced white matter and brain atrophy"*). Crucially, **enzyme residual activity does not discriminate symptomatic from asymptomatic individuals** (PMID: 9718352: *"There was no significant difference, in residual activity, between mutations observed in the symptomatic and those observed in the asymptomatic individuals."*). This poor genotype–phenotype and enzyme-activity–phenotype correlation is a central feature and strongly implies modifier genes and/or environmental triggers.

Suggested HPO terms: HP:0001263 (global developmental delay), HP:0001250 (seizures), HP:0001251 (infantile spasms → HP:0012469), HP:0001249 (intellectual disability), HP:0000729 (autistic behavior), HP:0000252 (microcephaly), HP:0002500 (cerebral white matter abnormality), HP:0002059 (cerebral atrophy), HP:0011968 (feeding difficulties), HP:0002013 (vomiting), HP:0001629 (ventricular septal defect), HP:0001674 (atrioventricular septal defect).

Finding 4 — Pharmacogenetic predisposition to severe/lethal 5-fluorouracil toxicity (F004)

This is the most clinically consequential aspect of the disorder. **Partial DHP deficiency is itself a pharmacogenetic disorder associated with severe 5-FU toxicity** (PMID: 15571261: "A partial dihydropyrimidinase deficiency proved to be a novel pharmacogenetic disorder associated with severe 5FU toxicity."). Because 5-FU is itself catabolized through the pyrimidine degradation pathway, DHP deficiency slows 5-FU clearance; **lethal side-effects can occur even in asymptomatic patients** (PMID: 19571472: "when the patients are treated with 5-fluorouracil, a pyrimidine analogue anticancer drug, lethal side-effects can be seen even in asymptomatic patients").

Quantitatively, dihydropyrimidine dehydrogenase (DPD/DPYD) deficiency accounts for ~43% of grade 3–4 5-FU toxicity (PMID: 18075467: "Dihydropyrimidine dehydrogenase (DPD) deficiency accounts for approximately 43% of grade 3-4 toxicity to 5-fluorouracil."); the residual unexplained toxicity is attributed in part to downstream DHP (DPYS) and β -ureidopropionase (UPB1) deficiencies. Additional case literature illustrates that severe 5-FU toxicity can also arise from thiamine deficiency/beriberi in the absence of a definable enzyme defect (PMID: 21941485) and that a putative "enhanced thymine absorption" phenotype may contribute (PMID: 29846282) — important differential considerations when evaluating fluoropyrimidine toxicity.

Clinical implication: urinary dihydropyrimidine screening and DPYS genotyping should be considered before fluoropyrimidine chemotherapy, alongside standard DPYD testing.

Finding 5 — Protein mechanism: a zinc-dependent amidohydrolase disrupted by diverse mutation classes (F005)

DHP is a member of the **cyclic amidohydrolase family** (which also includes allantoinase, dihydroorotase, hydantoinase, and imidase) and is a **zinc metalloenzyme with a binuclear metal center** in which two metal ions are bridged by a post-translationally carbamylated lysine; the functional unit is a **homotetramer** (PMID: 30268498: "Dihydropyrimidinase

(DHPase) is a member of the cyclic amidohydrolase family... Almost all of these zinc metalloenzymes possess a binuclear metal center in which two metal ions are bridged by a post-translational carbamylated Lys.").

Mutation analysis in 17 patients found 9 missense, 2 nonsense, 2 deletion, and 1 splice-site mutation, with **71% localizing to exons 5–8**. Most expressed mutants had no activity; p.R302Q and p.T343A retained ~3.9% and ~49% residual activity respectively. Mapping onto the crystal structure reveals **distinct structural mechanisms** (PMID: 20362666: "The crystal structure of human DHP indicated that the point mutations p.R490C, p.R302Q and p.V364M affect the oligomerization of the enzyme. In contrast, p.M70T, p.D81G, p.L337P and p.T343A affect regions near the di-zinc centre and the substrate binding site."). Other variants (p.S379R, p.L7V) cause global structural destabilization. East Asian/Japanese patients carry recurrent variants (e.g., Q334R, H295R, M250I) affecting active-site loops (PMID: 29054612: "The observation that the identified mutations were more prevalent in East Asians and the Japanese population indicates that DHP deficiency may be more common than anticipated in these ethnic groups.").

Mutation class	Example variants	Structural/functional consequence
Oligomerization interface	p.R490C, p.R302Q, p.V364M	Impaired homotetramer assembly
Di-zinc center / substrate binding	p.M70T, p.D81G, p.L337P, p.T343A	Catalytic impairment near active site
Global destabilization	p.S379R, p.L7V	Misfolding / instability
Truncating	p.R475, p.K501X, p.R465X, p.W117	Loss of function / NMD

Finding 6 — A naturally occurring feline model (F006)

DHP deficiency occurs spontaneously in cats, providing a comparative animal model. An affected cat presented with malnutrition, depression, vomiting, and hyperammonemia; urine GC-MS showed large amounts of dihydrouracil and dihydrothymine and moderate uracil/thymine. The cat was **homozygous for the DPYS missense mutation c.1303G>A (p.G435R)**, which **corresponds to a known human DHP-deficiency mutation** (PMID: 23430934: "Analysis of the feline DPYS gene encoding DHP demonstrated that the cat was homozygous for the missense mutation c.1303G>A (p.G435R) in exon 8, which corresponds to a known mutation in a

human patient with DHP deficiency."). Screening 1,000 cats found no additional carriers, indicating a very low allele frequency. The literature cites an estimated human prevalence of ~2/20,000 (PMID: 30384990: "an estimated prevalence of 2/20,000").

Taxonomy/ortholog anchors: *Felis catus* (NCBI Taxon:9685); feline *DPYS* ortholog; the p.G435R orthologous variant links feline and human disease biology directly.

Finding 7 — Population genetics: LoF-tolerant gene with East-Asian-enriched pathogenic alleles (F007)

Analysis of gnomAD v4 (GRCh38) constraint for *DPYS* (ENSG00000147647, chr8:104,330,324–104,467,055) confirms the recessive model: **pLI** $\approx$ 0, observed/expected LoF (o/e) = 0.775 (LOEUF 90% CI 0.60–1.01), LoF Z = 1.35, observed/expected LoF = 39/50.3 — i.e., **loss-of-function is tolerated**, exactly as expected for an autosomal recessive LoF disorder. Summing 87 high-confidence pLoF alleles gives a cumulative pLoF allele frequency $q \approx 8.5 \times 10^{-4}$, implying a **pLoF-only carrier frequency** $\approx 2q \approx 1/587$ and homozygous-pLoF incidence $q^2 \approx 1/1.4M$ — a **lower bound**, because pathogenic missense alleles (which dominate the real mutation spectrum) are excluded.

Recurrent pLoF alleles include p.Arg475Ter (AF 1.3×10^{-4}), p.Glu507MetfsTer57, p.Trp117Ter, p.Arg503GlufsTer2, and p.Arg302Ter. Known pathogenic **missense** alleles show marked ancestry enrichment, corroborating clinical reports of East Asian/Japanese clustering (PMID: 29054612):

Variant	Population enrichment	Allele frequency
p.Gln334Arg (Q334R)	East Asian	0.00209 (~1/240 EAS); ~0 elsewhere
p.His295Arg (H295R)	East Asian	enriched
p.Arg490Cys (R490C)	East Asian	enriched
p.Trp360Arg (W360R)	Middle Eastern	enriched
p.Arg475Ter (R475*)	Middle Eastern	enriched
p.Met250Ile (M250I)	Ashkenazi Jewish	enriched

Finding 8 — ClinVar allelic heterogeneity and interpretation challenge (F008)

ClinVar harbors extensive allelic heterogeneity for *DPYS*: a query returned ~300 variant records — **87 Pathogenic, 27 Likely pathogenic (114 P/LP combined), 255 Uncertain significance (VUS), plus benign/likely-benign entries**. Variant classes are predominantly single-nucleotide (missense/nonsense/splice) with small indels; **no recurrent large structural/chromosomal abnormality** is characteristic of the disease. The large VUS burden underscores the diagnostic interpretation challenge, especially given the lack of genotype–phenotype correlation.

Finding 9 — Organ localization: a liver/kidney enzyme, not a brain enzyme (F009)

A key mechanistic insight from this investigation: GTEx v8 median expression for *DPYS* (ENSG00000147647) is dominated by **liver (132.8 TPM) and kidney cortex (39.1 TPM)** (kidney medulla 12.7 TPM), while **brain regions are very low** (frontal cortex ~1.3, cortex ~0.8 TPM) and heart/skeletal muscle/whole blood are negligible (<0.03 TPM). This mirrors the known organ localization of pyrimidine catabolism.

The implication is important for the pathophysiological model: because the enzyme is not expressed in brain, the prominent neurological phenotype is best understood as a **circulating-metabolite (non-cell-autonomous) effect** — accumulated dihydropyrimidines (and/or downstream perturbations such as reduced β -alanine/ β -aminoisobutyrate availability) reach the CNS through the circulation, rather than reflecting a cell-autonomous loss of DHP in neurons.

Anatomical/subcellular anchors: UBERON:0002107 (liver), UBERON:0001225 (cortex of kidney); cytoplasmic localization (GO:0005737).

Finding 10 — Phenotype expansion: levodopa-responsive juvenile parkinsonism (F010)

The phenotypic spectrum was expanded in 2024 by the **first report of levodopa-responsive juvenile parkinsonism** associated with DHP deficiency: a 13-year-old with rapidly progressive dysphagia, dysarthria, and loss of ambulation over 18 months, compound heterozygous for *DPYS* c.1393C>T (p.R465X) and c.905G>A (p.R302Q), with urinary metabolome showing markedly elevated dihydrouracil and dihydrothymine (PMID: 39558747: *"We present the first case of levodopa-responsive juvenile parkinsonism associated with dihydropyrimidinase*

deficiency caused by a novel DPYS variant."). Importantly, **levodopa relieved the motor symptoms** (PMID: 39558747: "Levodopa treatment effectively relieved the patient's motor symptoms.") — a rare symptom-directed pharmacologic treatment opportunity.

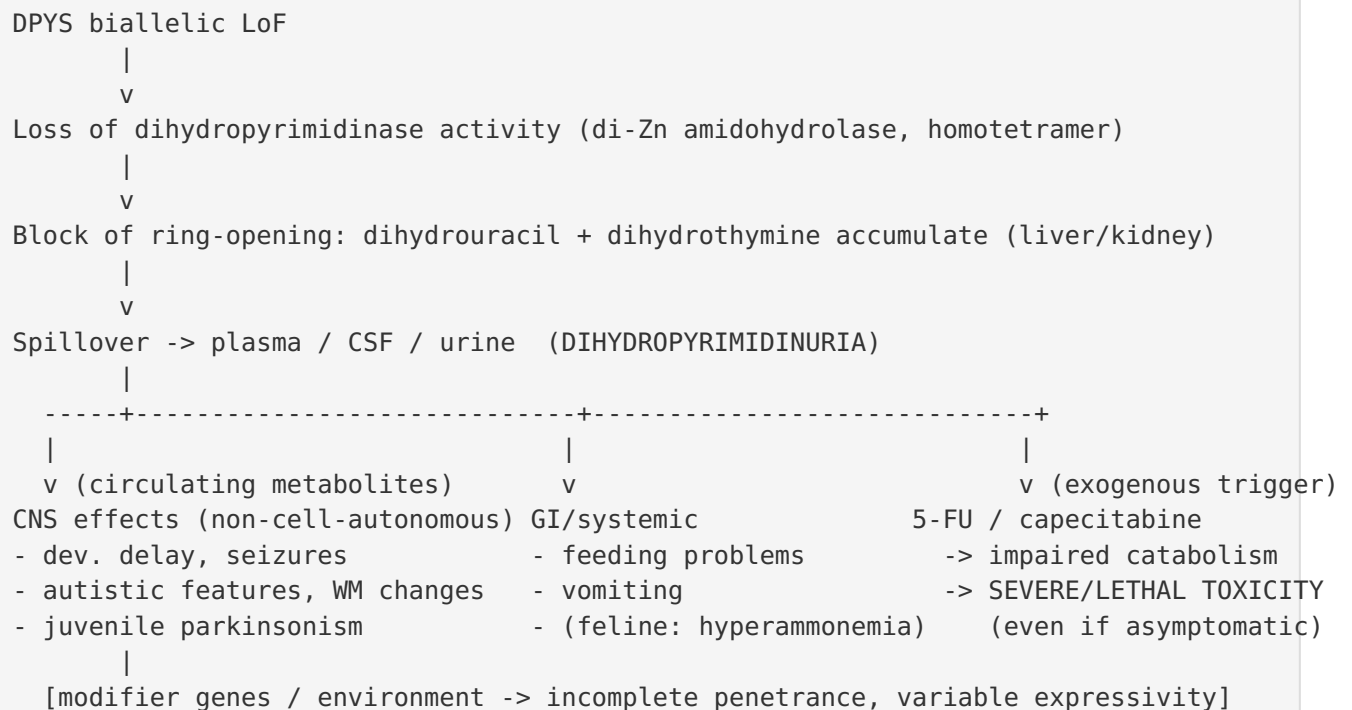
The variant p.R302Q (c.905G>A) is recurrent — also reported homozygously in a Chinese child (PMID: 33179229). Molecular-dynamics studies of 10 DHP variants show mutation-specific effects: oligomerization-interface disruption (T68R, D81G, G278D, L337P), stereochemistry-gate loops/substrate-binding pocket perturbation (W117R, T343A, R412M), and global collapse (W360R, G435R), while a near-normal-activity variant (R181W) showed only slight changes (PMID: 35961217: "In the T68R, D81G, G278D, and L337P variants, the flexibilities of structures related to the interaction for oligomer formation increased in comparison with those of the wild type.").

Mechanistic Model / Interpretation

Ordered causal chain (initiating lesion → clinical manifestation)

1. **Biallelic loss-of-function variants in DPYS (8q22)** → reduce or abolish production of functional dihydropyrimidinase protein (truncating/NMD alleles) *or* produce misfolded/destabilized/assembly-incompetent protein (missense alleles affecting the di-zinc center, substrate-binding pocket, or homotetramer interface).
2. **Loss of DHP (EC 3.5.2.2) catalytic activity** → the second step of pyrimidine degradation **fails to open the ring** of 5,6-dihydrouracil and 5,6-dihydrothymine (*demonstrated biochemically and structurally*).
3. **Metabolic block** → accumulation of the substrates **5,6-dihydrouracil and 5,6-dihydrothymine**, with secondary elevation of upstream uracil/thymine, in **liver and kidney** (principal sites of enzyme expression) and spillover into **plasma, CSF, and urine** → **dihydropyrimidinuria** (*demonstrated*).
4. **Systemic distribution of accumulated metabolites** → because **DPYS is not expressed in brain** (liver/kidney enzyme), CNS exposure is driven by **circulating** dihydropyrimidines reaching the brain → *inferred* non-cell-autonomous neurotoxic/neurodevelopmental effect.
5. **Branch A — Neurodevelopmental:** developmental delay, seizures/infantile spasms, intellectual disability, autistic features, white-matter abnormality, brain atrophy, and (rarely) levodopa-responsive juvenile parkinsonism. *The molecular link from metabolite accumulation to neuronal dysfunction remains inferred.*

6. **Branch B — Gastrointestinal/systemic:** feeding problems, vomiting; in the feline model, hyperammonemia.
7. **Branch C — Pharmacogenetic 5-FU toxicity:** when exogenous fluoropyrimidine (5-FU/capecitabine) is administered, the impaired degradation pathway **slows 5-FU catabolism** → drug/metabolite accumulation → **severe or lethal systemic toxicity**, even in asymptomatic individuals (*demonstrated clinically*).
8. **Incomplete penetrance / variable expressivity** — because residual enzyme activity does not predict symptoms and identical genotypes yield discordant phenotypes, **modifier genes and/or environmental factors** (e.g., dietary pyrimidine load, drug exposure) determine whether branches A–B manifest (*inferred*).



Upstream (primary): *DPYS* mutation → protein dysfunction → enzymatic block → metabolite accumulation. **Downstream (secondary):** circulating-metabolite exposure of brain and tissues → neurodevelopmental and GI phenotypes; iatrogenic 5-FU toxicity is a conditional downstream branch requiring drug exposure.

Cell types / processes: Primary biochemical lesion in **hepatocytes (CL:0000182)** and **renal proximal tubule cells (kidney cortex)**; downstream effects in **central nervous system** (neurons; white matter / oligodendrocytes CL:0000128). Biological processes: **pyrimidine nucleobase catabolic process (GO:0006208)**, **dihydropyrimidinase activity (GO:0004157)**, cytoplasmic metabolism (GO:0005737).

Evidence Base

PMID	Title (abbrev.)	Supports
9718352	Gene structure, chromosomal localization, mutation analysis	F001 (gene structure, 8q22, 10 exons); F003 (no residual-activity difference)
17383919	Two siblings with DHP deficiency	F001 (enzyme function/reaction)
20362666	Phenotype/genotype/structure in 17 patients	F002 (metabolites in plasma/CSF/urine); F005 (structure–function map)
30384990	Incidental detection by urine metabolome	F002 (GC-MS/LC-MS/MS); F006 (prevalence ~2/20,000)
38958169	DHP deficiency with atrioventricular septal defect	F003 (variability, asymptomatic, 5-FU susceptibility)
29054612	Four East Asian patients, novel/rare DPYS mutations	F003 (phenotype range); F005/F007 (East Asian enrichment)
15571261	Pyrimidine defects and severe 5-FU toxicity	F004 (partial DHP deficiency = pharmacogenetic 5-FU risk)
19571472	5-FU toxicity in pyrimidine metabolic disorders	F004 (lethal toxicity in asymptomatic patients)
18075467	Genetic regulation of dihydropyrimidinase	F004 (DPD ~43% of grade 3–4 toxicity)
30268498	DHPase crystal structures / amidohydrolase family	F005 (zinc metalloenzyme, binuclear metal center)
23430934	First feline case of dihydropyrimidinuria	F006 (feline model; orthologous p.G435R)
35961217	Molecular dynamics of pathogenic variants	F005/F010 (variant-specific destabilization)
39558747	Juvenile parkinsonism with DHP deficiency	F010 (levodopa-responsive parkinsonism)
33179229	Chinese child, DPYS variant analysis	F010 (recurrent p.R302Q homozygous)
16613999	HPLC-ESI-MS/MS purine/pyrimidine panel	F002 (comprehensive diagnostic detection)
32600357	Novel stop-gain c.1501A>T p.K501X	

PMID	Title (abbrev.)	Supports
		Allelic heterogeneity; AR confirmation; liver mRNA reduction
38199782	Diagnostic odyssey case + literature review	Clinical/diagnostic context
39126710	Dual diagnosis (DPYS + TBX19) in Sri Lankan boy	Phenotype complexity; consanguinity
21941485	5-FU encephalopathy / beriberi differential	Differential of 5-FU toxicity
29846282	"Enhanced thymine absorption" phenotype	Alternative fluoropyrimidine-toxicity mechanism

Section-by-section report content (template coverage)

1. Disease Information. DHP deficiency is a rare autosomal recessive inborn error of pyrimidine degradation. Identifiers: **OMIM 222748, Orphanet 35707, MONDO:0009111**; ICD-10 under disorders of pyrimidine metabolism (E79.8). Synonyms: dihydropyrimidinase deficiency, DHP deficiency, DHPase deficiency, dihydropyrimidinuria, DPYS deficiency. Information derives from aggregated disease-level resources plus a small set of individual case reports (<40 patients).

2. Etiology. The sole established cause is biallelic pathogenic *DPYS* variants (genetic). There are **no established environmental causes**; however, fluoropyrimidine drug exposure is the key **environmental trigger of the toxicity phenotype**. Risk factors: consanguinity and East Asian/Middle Eastern/Ashkenazi ancestry (founder/enriched alleles). No protective alleles defined. Gene–environment interaction is exemplified by 5-FU: a genetic enzyme deficiency becomes life-threatening only on drug exposure.

3. Phenotypes. See Findings 3 and 10 and HPO list above. Onset typically neonatal/infantile but ranges to adult incidental detection; severity variable (asymptomatic → severe); progression usually stable but can be progressive (juvenile parkinsonism). Frequencies are qualitative given the tiny cohort; developmental delay and 5-FU susceptibility are most commonly reported.

4. Genetic/Molecular. Causal gene *DPYS* (HGNC:3012, OMIM 613326), 8q22, 10 exons, >80 kb. Variant spectrum: predominantly missense, with nonsense, frameshift, splice, small indels; ClinVar ~114 P/LP and ~255 VUS. Functional consequence: **loss of function** (catalytic, metal-

center, substrate-binding, or oligomerization defects). No modifier genes formally established; no characteristic epigenetic changes or large chromosomal abnormalities. Allele frequencies: p.Q334R EAS ~1/240. Germline origin.

5. Environmental. No toxins/infectious agents cause the disease. The decisive exogenous factor is **fluoropyrimidine chemotherapy** (5-FU, capecitabine) precipitating severe toxicity. Dietary pyrimidine load is a plausible but unproven modifier.

6. Mechanism. See ordered causal chain above.

7. Anatomical structures. Primary biochemical organs: **liver (UBERON:0002107) and kidney (UBERON:0002113 / cortex UBERON:0001225)**. Secondary: **central nervous system / brain (UBERON:0000955)**, white matter (UBERON:0002316); GI tract; rarely heart. Subcellular: cytoplasm. Lateralization not characteristic.

8. Temporal development. Onset congenital/pediatric most often but variable to adulthood; pattern usually chronic/insidious, occasionally rapidly progressive (parkinsonism over 18 months). Lifelong, no spontaneous remission; the critical **intervention window is avoidance of fluoropyrimidines**.

9. Inheritance and population. Autosomal recessive; incomplete penetrance; variable expressivity; estimated prevalence ~2/20,000 (cited); gnomAD pLoF carrier frequency $\geq$ ~1/590 (lower bound). Consanguinity and East Asian/Middle Eastern/Ashkenazi founder effects relevant. No sex predilection established.

10. Diagnostics. First-line: **urine organic acid / pyrimidine panel** showing elevated dihydrouracil and dihydrothymine (GC-MS, confirmed by LC-MS/MS; comprehensive HPLC-ESI-MS/MS panel). Confirmatory: **DPYS sequencing** (single-gene, panels, or WES/WGS). Differential diagnosis: DPD (*DPYD*) deficiency, β -ureidopropionase (*UPB1*) deficiency, and non-genetic causes of fluoropyrimidine toxicity (thiamine deficiency/beriberi; "enhanced thymine absorption"). Screening: pre-chemotherapy pyrimidine screening; cascade family testing.

11. Outcome/Prognosis. Highly variable; many asymptomatic individuals have normal life expectancy. No disease-specific mortality data except the catastrophic risk of **lethal 5-FU toxicity**. Prognostic factors poorly defined; residual enzyme activity is not prognostic.

12. Treatment. No curative therapy; **supportive/symptomatic management**. Actionable interventions: (a) **strict avoidance of fluoropyrimidines** (or extreme caution/dose modification), and (b) **levodopa** for the parkinsonism presentation (NCIT:C739 Levodopa). Seizures, developmental, GI, and feeding issues managed supportively. No gene/cell/RNA therapy exists.

13. Prevention. Primary: carrier screening and genetic counseling in at-risk/consanguineous families; prenatal/preimplantation diagnosis feasible given known variants. Secondary: pre-chemotherapy pyrimidine/genotype screening to prevent toxicity. Tertiary: avoid fluoropyrimidines; manage complications.

14. Other species / Natural disease. Spontaneous feline disease (*Felis catus*, Taxon:9685) with orthologous p.G435R — the principal natural model; veterinary relevance limited but illustrative of conserved mechanism.

15. Model organisms. No widely used engineered mouse/zebrafish model is established in the reviewed literature; the **naturally occurring feline case** is the primary in-vivo model, supplemented by heterologous expression of mutant protein and crystallography/molecular-dynamics for structure–function.

Limitations and Knowledge Gaps

- **Tiny cohort (<40 patients)** limits frequency estimates, genotype–phenotype correlation, and natural-history/prognostic data; most "frequencies" are qualitative.
- **Unexplained incomplete penetrance:** the molecular basis for discordant phenotypes among identical genotypes is unknown — modifier genes, dietary pyrimidine load, and environmental triggers are hypothesized but not demonstrated.
- **CNS mechanism is inferred, not proven:** the circulating-metabolite (non-cell-autonomous) model is strongly supported by *DPYS* tissue expression (liver/kidney, not brain) but the specific neurotoxic species and molecular target remain uncharacterized.
- **No engineered mammalian model** (knockout mouse) was identified in the reviewed literature, limiting mechanistic dissection and therapeutic testing.
- **Large VUS burden** (~255 ClinVar VUS) impedes clinical interpretation; functional assays are not routinely available.
- **Prevalence uncertainty:** the ~2/20,000 estimate and gnomAD-derived carrier frequencies ($\geq \sim 1/590$) differ in scope; true prevalence (including missense alleles) is likely underestimated, especially in East Asian populations.
- **Treatment evidence is anecdotal:** levodopa response is based on a single case; no trials exist.

Proposed Follow-up Experiments / Actions

1. **Establish a *Dpys* knockout mouse** (and/or zebrafish) to test whether the neurological phenotype is reproduced and whether it is driven by circulating metabolites (parabiosis, liver-specific vs global knockout, metabolite-rescue experiments).
 2. **Metabolite neurotoxicity assays:** expose neuronal/organoid cultures to pathophysiological concentrations of dihydrouracil/dihydrothymine ($\pm$ β -alanine/ β -aminoisobutyrate depletion) to identify the toxic species and molecular target.
 3. **Prospective pre-chemotherapy screening study** combining *DPYD* + *DPYS* (+ *UPB1*) genotyping and urinary pyrimidine ratios to quantify the *DPYS* contribution to otherwise-unexplained fluoropyrimidine toxicity and validate predictive biomarkers (e.g., urinary THY/DHT ratio).
 4. **High-throughput functional classification of VUS** (saturation mutagenesis + activity/oligomerization assay) to reduce the ClinVar VUS burden and improve clinical interpretation.
 5. **Modifier search:** genotype–phenotype analysis across the expanding registry to identify penetrance modifiers; collect dietary/exposure data.
 6. **Natural-history registry** to define onset, progression, prognosis, and the real frequency of parkinsonism and cardiac sub-phenotypes; evaluate levodopa responsiveness systematically.
 7. **Population carrier-frequency studies** in East Asian, Middle Eastern, and Ashkenazi cohorts to refine prevalence and inform targeted carrier screening.
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Report compiled from a 5-iteration autonomous investigation: 10 confirmed findings, 20 reviewed papers, integrating primary clinical literature, gnomAD/ClinVar population genetics, GTEx expression data, protein structural/computational studies, and a naturally occurring animal model.
