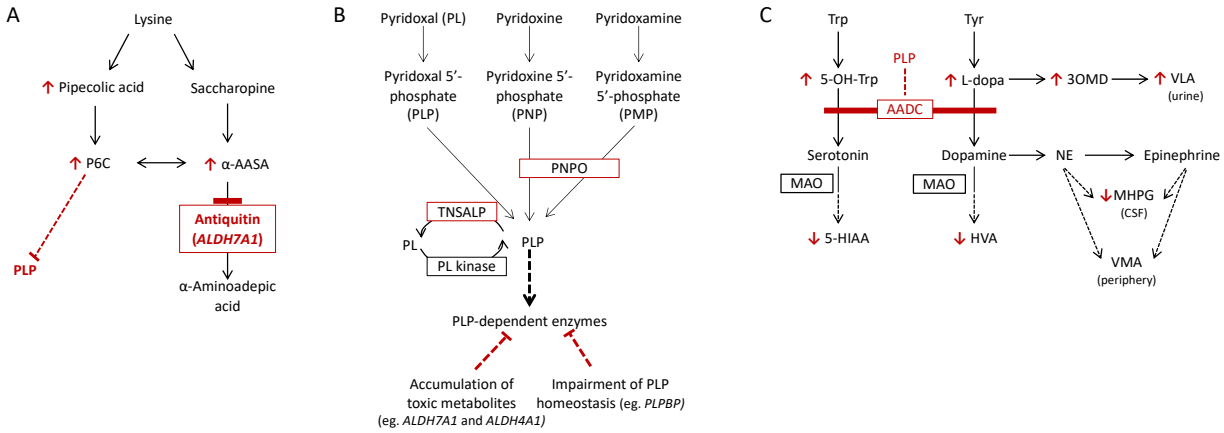
Supplementary Figure 1



Supplementary Figure 1. Pathways related to pyridoxine metabolism. ***(A) Lysine degradation pathway and its role in pyridoxine dependent epilepsy.*** Lysine is catabolised to pipecolic acid and Saccaropine. Antiquitin deficiency causes accumulation of α-aminoadipic semialdehyde (AASA) which is in equilibrium with L-Δ^1-piperideine 6-carboxylate (P6C). This P6C compound inactivates pyridoxal phosphate (PLP), leading to severe cerebral PLP deficiency. ALDH7A1: α-aminoadipic semialdehyde dehydrogenase***. (B) Simplified Pyridoxine 5'-phosphate synthesis pathway.*** Pyridoxal, pyridoxine and pyridoxamine (which are derived from diet or supplementation) are ultimately converted into pyridoxal-5'-phosphate (PLP) by PNPO. Responsive disorders result from disrupted B6 metabolism, either by leading to the accumulation of toxic metabolites that inactivate PLP (such as ALDH7A1 and ALDH4A1), interfering with the interconversion of B6 vitamers (such as PNPO and TNSALP), or impairing PLP homeostasis (such as PLPBP). PL: Pyridoxal, PLP: Pyridoxal 5'-phosphate, PMP: Pyridoxamine 5'-phosphate, PNP: Pyridoxine 5'-phosphate. (***C) Monoamine neurotransmitters synthesis***. Aromatic L-amino acid decarboxylase (AADC) is dependent on PLP as a co-factor. Therefore, mutations of PLPBP lead to dysfunction of AADC and accumulation of metabolites above the block and decrease in those below the block. AADC: L-aromatic amino acid decarboxylase, 5-HIAA: 5-Hydroxyindoleacetic acid, HVA: Homovanillic acid, MHPG: 3-methoxy-4-hydroxyphenylglycol, MOA: Monoamine oxidase, NE: Norepinephrine, 5-OH-Trp: 5-hydroxytryptophan, 3OMD: 3-ortho-methyldopa, PLPBP: Pyridoxal-5'-phosphate binding protein, Trp: Tryptophan, Tyr: Tyrosine, VLA: Vanillactic acid.

Supplementary Table 1. Summary of clinical, genetic, and biochemical features of vitamin B6-responsive disorders

Vitamin B6-responsive disorders	Antiquitin deficiency (PDE)	Hyperprolinemia type II	PNPO deficiency	ALP enzyme dysfunction Hypo/Hyperphosphatasia	Molybdenum cofactor deficiency (MoCD) / Sulfite oxidase deficiency	PLPHP deficiency
Gene	*ALDH7A1*	*ALDH4A1*	*PNPO*	Hypophosphatasia: *ALPL* Hyperphosphatasia in GPI anchor disorders: *PIGO, PIGV,* PGAP2 and *PGAP3*	*MOCS1, MOCS2, MOCS3,* GPHN and SUOX	*PLPBP*
Mechanism of PLP deficiency	PLP inactivation (1)	PLP inactivation (1)	Reduced PLP formation (1)	Impaired PLP import into brain (2)	Inhibition of α-AASA dehydrogenase resulting in high AASA and PLP inactivation (1, 3, 4)	Impairs PLP intracellular homeostasis (2, 5)
Seizure onset:						
Neonatal	+++		+++	+++	+++	+++
Infantile	+	+ in 50% of patients (1, 2)	+	+++		+
Adulthood	+					
EEG pattern	Variable[+]	Non-specific	Burst suppression in 60% (1)	Burst suppression (1)	Non-specific	Mainly burst suppression (2, 6)
Developmental delay	+++	Variable (1)	++	+++	+++	Variable
ID	75% mild to moderate (1)	Variable	Variable	+++	+++	Variable
Others features	Movement disorder (2, 7)	Often responds to ASMs (1, 2)	Movement disorder (2, 7)	Hypophosphatasia *(ALPL):* Bone disease, enzyme replacement therapy available (1, 2). Hyperphosphatasia in GPI anchor disorders*:* HPMRS (8). In *PIGV* and *PIGO,* additional features of coarse facial and hypoplastic terminal phalanges (8).	Coarse facial features, post-natal microcephaly, brain MRI abnormalities (3)**	Movement disorder (6).
Response to vitamin B6	To pyridoxine (or PLP) (1).	To pyridoxine (or PLP) (1).	Mainly to PLP, in certain mutations to pyridoxine (1).	Partial response to pyridoxine (or PLP) in *ALPL* (1, 2). Partial response to pyridoxine in GPI anchor disorders (8).	Partial response to Pyridoxine (3, 4).	Partial response to pyridoxine and good response to PLP (5, 6), some patients need addition of folinic acid (6).
Biomarkers						
Urine	↑ α-AASA (1, 2)	↑ proline (1), Abnormal presence of P5C (1).	Inconstantly ↑ Vanillactate (1, 2).	↑ phosphoserine, ↑ phosphoethanolamine and inconsistent ↑ Vanillactate in hypophosphatasia (*ALPL) (2).*	↑ S-Sulfocyteine, ↑sulfite ↑ taurine,↑thiosulfate and ↑α-AASA (3) ↓ uric acid, ↑ xanthine and ↑ hypoxanthine in MoCD only (3, 4).	↑ Vanillactate (6, 9).
Plasma	↑ α-AASA, ↑P6C and ↑pipecolic acid (1).	↑ proline (1). Abnormal presence of P5C (1).	↑ PM and ↑PM/PA ratio (1).	↓ ALP, ↓ Ph,↑ Ca, ↑ PLP and ↑ PLP/Pyridoxal ratio in hypophosphatasia (*ALPL) (1, 2, 8).* ↑ ALP in GPI anchor disorders (8).	↑ S-Sulfocyteine, ↑ taurine and ↑ α-AASA (3, 4). -Low uric acid in MoCD only (3, 4).	↑ threonine, ↑ serine and ↑ glycine (2, 6, 9).
CSF	↑ α-AASA, ↓ PLP*, Inconsistent findings of NT changes (1).		↓ PLP* and Inconsistent findings of NT changes (1).		↑ α-AASA and ↓ PLP (4).	↑ glycine, ↑ L-DOPA, ↑3-methyltyrosine, ↑ threonine, ↓ HVA (6, 9).

Supplementary Table 1. α-AASA, Alpha aminoadipic semialdehyde. ALP, Alkaline phosphatase. ASM, Anti-seizure medications. Ca, Calcium. CSF, Cerebrospinal fluid. HPMRS, Hyperphosphatasia and Mental Retardation syndrome. HVA, Homovanillic acid. GPI, Glycophosphatidylinositol. ID, intellectual deficiency. MoCD, Molybdenum cofactor deficiency. NT, Neurotransmitters. PA, Pyridoxic acid. Ph, Phosphate. PDE, Pyridoxine dependent epilepsy. PLP, Pyridoxal 5'-phosphate. *PLPBP*, Pyridoxal phosphate-binding protein gene. PLPHP, Pyridoxal phosphate homeostasis protein. PM, Pyridoxamine. PNPO, Pyridoxamine 5'-phosphate oxidase. P6C, Piperideine 6-Carboxylate1. *Before treatment with Vitamin B61. **These include Cerebral atrophy, multicystic white matter lesions, symmetric involvement of globi pallidi, pontocerebellar hypoplasia with retrocerebellar cyst, Dandy-Walker malformation and dysgenesis of corpus callosum [17].$^{+}$ Variable, normal to bilateral high-voltage rhythmic delta waves, hypsarrhythmia or burst–suppression. (+++) Classic presentation. (++) Usually present. (+) Rarely.

Supplementary Table 2: Previously reported pathogenic *PLPBP* variants (NM_ 007198.3)

Report	Patients	Zygosity	Variant effect	Nucleotide variant	Amino acid change
This report	Subject 1	Compound heterozygous	Frameshift deletion Missense	c.370_373delGACA c.704 T>G	p.Asp124LysfsX2 p.Val235Gly
	Subject 2	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
	Subject 3	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
Darin *et al*. 2016	Subject 1	Homozygous	Nonsense	c.233C>G	p.Ser78X
	Subject 2	Homozygous	Nonsense	c.233C>G	p.Ser78X
	Subject 3	Homozygous	Nonsense	c.233C>G	p.Ser78X
	Subject 4	Homozygous	Nonsense	c.524T>C	p.Leu175Pro
	Subject 5	Compound heterozygous	Canonical splice site Canonical splice site	c.207+1G>A c.320-2A>G	- -
	Subject 6	Homozygous	Nonsense	c.211C>T	p.Gln71X
	Subject 7	Compound heterozygous	Missense Missense	c.260C>T c.722G>A	p.Pro87Leu p .Arg241Gln
Plecko *et al.* 2017	Subject 1	Compound heterozygous	Missense Missense	c.119C>T c. 722G>A	p.Pro40Leu p.Arg241Gln
	Subject 2	Compound heterozygous	Frameshift deletion Missense	c.249_252del c.614G>A	p.Ser84CysfsX21 p.Arg205Gln
	Subject 3	Homozygous	Missense	c.260C>T	p.Pro87Leu
	Subject 4	Homozygous	Missense	c.206A>G	p.Tyr69Cys
Kernohan *et al.* 2018	Subject 4	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
Shiraku *et al.* 2018	Subject 1	Compound heterozygous	Missense Missense	c.134T>A c.122G>A	p.Val45Asp p.Arg41Gln
	Subject 2	Homozygous	Missense	c.122G>A	p.Arg41Gln
	Subject 3	Homozygous	Missense	c.199G>A	p.Glu67Lys
	Subject 4	Homozygous	Missense	c.614G>A	p.Arg205Gln
Johnstone *et al*.2019	Subject 1	Homozygous	Missense	c.347C>T	p.Thr116Ile
	Subject 2	Homozygous	Missense	c.122G>A	p.Arg41Gln
	Subject 3	Homozygous	Missense	c. 199G>A	p.Glu67Lys
	Subject 4	Compound heterozygous	Canonical splice site Missense	c.320-2A>G c.671G>C	- p.Gly224Ala
	Subject 5	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
	Subject 6	Homozygous	Missense	c.347C>T	p.Thr116Ile
	Subject 7	Homozygous	Missense	c.280A>T	p.Ile94Phe
	Subject 8	Homozygous	Missense	c.122G>A	p.Arg41Gln
	Subject 9	Homozygous	Missense	c.122G>A	p.Arg41Gln
	Subject 10	Homozygous	Missense	c. 199G>A	p.Glu67Lys
	Subject 11	Homozygous	Missense	c. 199G>A	p.Glu67Lys
	Subject 12	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
Jensen *et al.* 2019	Subject 1	Homozygous	Canonical splice site	c.207+1G>A	-
	Subject 2	Homozygous	Missense	c.121C>T	p.Arg41Trp
Johansen *et al*. 2019	Subject 1	Homozygous	Missense	c.725T > C	p.Ile242Thr
Heath *et al.* 2020	Subject 1	Compound heterozygous	Canonical splice site Missense	c.207+1G>A c.722G>A	- p.Arg241Gln
Ahmed *et al.* 2020	Subject 1	Homozygous	Frameshift deletion	c.46_47insCA	p.Leu17Hisfs
Maitou Pal *et al.* 2021	Subject 1	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
	Subject 2	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
	Subject 3	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
	Subject 4	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2
	Subject 5	Homozygous	Frameshift deletion	c.370_373delGACA	p.Asp124LysfsX2

Supplementary References:

1. Plecko B, Steinfeld R. Disorders of Vitamin Metabolism. Swaiman's Pediatric Neurology: Elsevier (2017). p. 373-82.
2. Wilson MP, Plecko B, Mills PB, Clayton PTJJoimd. Disorders affecting vitamin B6 metabolism. J Inherit Metab Dis. (2019) 42:629-46. doi: 10.1002/jimd.12060.
3. Atwal PS, Scaglia FJMg, metabolism. Molybdenum cofactor deficiency. Mol Genet Metab. (2016) 117:1-4. doi: 10.1016/j.ymgme.2015.11.010.
4. Mills PB, Footitt EJ, Ceyhan S, Waters PJ, Jakobs C, Clayton PT, et al. Urinary AASA excretion is elevated in patients with molybdenum cofactor deficiency and isolated sulphite oxidase deficiency. J Inherit Metab Dis. (2012) 35:1031-6. doi: 10.1007/s10545-012-9466-1.
5. Darin N, Reid E, Prunetti L, Samuelsson L, Husain RA, Wilson M, et al. Mutations in PROSC disrupt cellular pyridoxal phosphate homeostasis and cause vitamin-B6-dependent epilepsy. Am J Hum Genet. (2016) 99:1325-37. doi: 10.1016/j.ajhg.2016.10.011.
6. Johnstone DL, Al-Shekaili HH, Tarailo-Graovac M, Wolf NI, Ivy AS, Demarest S, et al. PLPHP deficiency: clinical, genetic, biochemical, and mechanistic insights. Brain. (2019) 142:542-59. doi: 10.1093/brain/awy346.
7. Stockler S, Plecko B, Gospe Jr SM, Coulter-Mackie M, Connolly M, van Karnebeek C, et al. Pyridoxine dependent epilepsy and antiquitin deficiency: clinical and molecular characteristics and recommendations for diagnosis, treatment and follow-up. Mol Genet Metab. (2011) 04:48-60. doi: 10.1016/j.ymgme.2011.05.014.
8. Altassan R, Fox S, Poulin C, Buhas DJMg, reports m. Hyperphosphatasia with mental retardation syndrome, expanded phenotype of PIGL related disorders. Mol Genet Metab Rep. (2018) 15:46-9. doi: 10.1016/j.ymgmr.2018.01.007.
9. Wassenberg T, Molero-Luis M, Jeltsch K, Hoffmann GF, Assmann B, Blau N, et al. Consensus guideline for the diagnosis and treatment of aromatic l-amino acid decarboxylase (AADC) deficiency. Orphanet J Rare Dis. (2017) 12:1-21. doi: 10.1186/s13023-016-0522-z.