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REVIEW

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Disorders affecting vitamin B_6 metabolism

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Abstract

Vitamin B_6 is present in our diet in many forms, however, only pyridoxal 5′-phosphate (PLP) can function as a cofactor for enzymes. The intestine absorbs nonphosphorylated B_6 vitamers, which are converted by specific enzymes to the active PLP form. The role of PLP is enabled by its reactive aldehyde group. Pathways reliant on PLP include amino acid and neurotransmitter metabolism, folate and 1-carbon metabolism, protein and polyamine synthesis, carbohydrate and lipid metabolism, mitochondrial function and erythropoiesis. Besides the role of PLP as a cofactor B_6 vitamers also play other cellular roles, for example, as antioxidants, modifying expression and action of steroid hormone receptors, affecting immune function, as chaperones and as an antagonist of Adenosine-5'-triphosphate (ATP) at P2 purinoceptors. Because of the vital role of PLP in neurotransmitter metabolism, particularly synthesis of the inhibitory transmitter γ-aminobutyric acid, it is not surprising that various inborn errors leading to PLP deficiency manifest as B_6-responsive epilepsy, usually of early onset. This includes pyridox(am)ine phosphate oxidase deficiency (a disorder affecting PLP synthesis and recycling), disorders affecting PLP import into the brain (hypophosphatasia and glycosylphosphatidylinositol anchor synthesis defects), a disorder of an intracellular PLP-binding protein (PLPBP, previously named PROSC) and disorders where metabolites accumulate that inactivate PLP, for example, ALDH7A1 deficiency and hyperprolinaemia type II. Patients with these disorders can show rapid control of seizures in response to either pyridoxine and/or PLP with a lifelong dependency on supraphysiological vitamin B_6 supply. The clinical and biochemical features of disorders leading to B_6-responsive seizures and the treatment of these disorders are described in this review.

KEYWORDS

ALDH7A1, epilepsy, PLPBP, PNPO, PROSC, pyridoxal phosphate, vitamin B6

1 | CHEMISTRY OF VITAMIN B_6 AND DIETARY SOURCES

Chemically, B_6 vitamers are derivatives of 2-methyl-3-hydroxy-5-hydroxymethyl-pyridine (Figure 1). The C4 substituent is a hydroxymethyl ($-CH_2OH$) group in pyridoxine, an aminomethyl ($-CH_2NH_2$) group in pyridoxamine, and an aldehyde group (-CHO) in pyridoxal. The 5′ alcohol group can be esterified to phosphate producing pyridoxine 5′-phosphate [PNP], pyridoxamine 5′-phosphate [PMP], and pyridoxal 5′-phosphate [PLP]) and in the case of pyridoxine, linked to glucose (pyridoxine β-glucoside, PNG). Sources of

Philippa B. Mills and Peter T. Clayton contributed equally to this study.

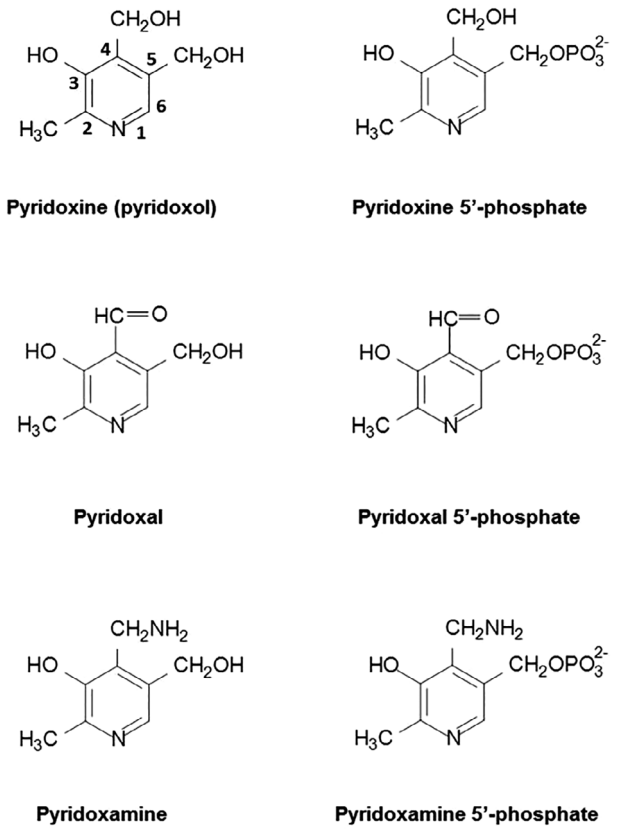

FIGURE 1 Chemical structure of the B_6 vitamers. The ring positions referred to in the text are as illustrated for pyridoxine

vitamin B_6 for man are the diet[1] and the gut microbiota. In fruit and vegetables, B_6 is present principally as pyridoxine and its phosphate and glucoside. In meat and fish, it is mainly present as PLP and PMP. Synthesis of B_6 by the gut flora can make a significant contribution to our B_6 intake[2] and may explain why dietary B_6 deficiency is rare. It can occur in the first year of life when the gut flora is not fully established; in the 1950s, infants fed a milk formula that had been overheated during production developed seizures due to B_6 deficiency.[3]

2 | REACTIVITY

PLP is the only B_6 vitamer that acts as a cofactor for enzymes, a role that is enabled by its reactive aldehyde group. It undergoes a condensation reaction with the amino group of amino acids to produce a Schiff base which forms the basis of most of the enzyme catalyzed reactions for which PLP is required.[4] However, because of the reactivity of its aldehyde group PLP can be involved in other, unwanted reactions with macromolecules within the cell (a form of aldehyde or carbonyl stress) analogous to the unwanted reactions of reactive oxygen species. Reactions of PLP with amino groups of proteins can alter their structure[5] and at concentrations of 10 to 100 μM inhibit enzyme activity[6] and aldehydes can damage DNA leading to mutagenesis.[7] PLP can also react with small molecules[8,9] including sulphydryl compounds such as cysteine resulting in the formation of thiazoliodines[10] and with sulfite producing a sulphonate. The aldehyde group of PLP can also react, via Knoevenagel condensation, with Δ^1-pyrroline 5-carboxylate and Δ^1-piperideine 6-carboxylate. This is the mechanism of PLP deficiency in ALDH7A1 (antiquitin) deficiency and hyperprolinaemia type II, respectively.[11,12] Hence, the cell maintains intracellular levels of free PLP at approximately 1 μM to avoid any unwanted reactions occuring.[13]

Consideration of the reactivity of the aldehyde group of PLP is important when treating patients with certain drugs. Drugs that can inactivate PLP include hydrazines (eg, isoniazid and hydralazine), sulphydryl compounds (eg, penicillamine), and substituted hydroxylamines (eg, cycloserine).

3 | REACTIONS CATALYZED BY PLP ENZYMES

The Enzyme Commission (http://www.chem.qmul.ac.uk/iubmb/enzyme/) lists more than 140 PLP-dependent activities, corresponding to ~4% of all classified activities[14]; about 70 of these occur in man. These reactions involve diverse pathways (Table S1) including amino acid and neurotransmitter metabolism, folate and 1-carbon metabolism, protein and polyamine synthesis, carbohydrate and lipid metabolism, mitochondrial function and erythropoiesis. The involvement of PLP in these pathways is often apparent in PLP deficiency states which can be associated with biochemical abnormalities, for example, raised plasma threonine and glycine or low cerebrospinal fluid γ-aminobutyric acid (CSF GABA) (Table 1) although these are not always/only transiently present in some patients.[15]

In humans, each holoenzyme contains PLP attached by a Schiff base link to the ε-amino group of a lysine residue at the active site.[4] The largest number of catalyzed reactions involve the transfer of PLP to produce a new Schiff base linkage with the amino group of an amino acid substrate. This increases the reactivity of the α, β, and γ carbons of the amino acid. Subsequent reactions at the α-carbon atom of the substrate include transamination, decarboxylation, racemisation, and elimination and replacement of an electrophilic group. Those occurring at the β- or γ-carbon atoms of amino acids include elimination or replacement.[4]

PLP is also instrumental in the reaction catalyzed by glycogen phosphorylase (EC 2.4.1.1). The aldehyde group of PLP forms a Schiff base linkage to lysine 680. However, unlike most PLP-dependent enzymes the reaction catalyzed

TABLE 1 Clinical and biochemical presentations caused by the aberrant function of PLP-dependent enzymes in a PLP-deficient state

Clinical or biochemical features identified in individuals with impaired vitamin B_6 metabolism	Implicated PLP-dependent enzymes	Mechanism
Seizures	Branched-chain amino acid aminotransferase (BCAT1 + 2; EC 2.6.1.42)	Major source of glutamate in the brain
	Glutamate decarboxylase (GAD1 + 2; EC 4.1.1.15) GABA-transaminase (GABA-T; EC 2.6.1.19)	Important for the regulation of neuronal GABA/glutamate interconversion and therefore neuronal excitability. Imbalances of these neurotransmitters are thought to explain the seizures caused by PLP deficiency. Low GABA has been found in *ALDH7A1* $^{-/-}$ zebrafish and in a mouse model of hypophosphatasia with epilepsy
Dystonic movements Low CSF HVA/5-HIAA; raised VLA/3-OMD	Aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.28)	Deranged regulation of serotonin and catecholamine neurotransmitters leads to dystonia and a characteristic CSF neurotransmitter profile seen in both primary AADC deficiency and that which is secondary to severe PLP deficiency
Neuronal migration defects	L-Serine racemase (SRR; EC 5.1.1.18)	Deficiency leads to an inability to form D-serine, implicated in neuronal migration and neurotransmission through NMDA receptors. Although rare, neuronal migration defects and dysplasia have been identified in individuals with perturbed vitamin B_6 metabolism
Anemia and lactic acidosis	Δ-Aminolevulinic acid synthase (ALAS1 + 2; EC 2.3.1.37) Cysteine desulfurase (NFS1; EC 2.8.1.7)	Disordered haem and Fe-S cluster synthesis due to severe PLP deficiency leads to hematological abnormalities and lactic acidosis
Hypoglycemia	Aspartate transaminase (AST or GOT; EC 2.6.1.1) Alanine transaminase (ALT or GPT1 + 2; EC 2.6.1.2) Serine/threonine deaminase (SDS; EC 4.3.1.17)	Important for the formation of pyruvate and therefore gluconeogenesis from amino acids. GOT important for the malate-aspartate shuttle
	Glycogen phosphorylase (PYG(M[uscle],L[iver],B[rain]); EC 2.4.1.1)	The rate-limiting step in glycogenolysis. Severe PLP deficiency leads to the inability to liberate sufficient glucose from stored glycogen through the action of hepatic phosphorylase
Disordered plasma and CSF serine, threonine and glycine	Serine hydroxymethyltransferase (SHMT1 + 2; EC 2.1.2.1) Glycine dehydrogenase (decarboxylating) (GLDC; EC 1.4.4.2; component of the glycine cleavage system) Serine/threonine deaminase (SDS; EC 4.3.1.17) Phosphoserine aminotransferase (PSAT1; EC 2.6.1.52) Glycine C-acetyltransferase (GCAT; EC 2.3.1.29)	These enzymes are important for the biosynthesis and catabolism of serine, threonine and glycine. Recently, an in vitro study of neuronal cells has reported reduced synthesis of serine and glycine cultured in B_6-deficient medium. The homeostasis of these amino acids is complex and tissue-specific. This manifests in severely PLP deficient humans as high concentrations of threonine, serine and glycine in plasma and CSF
Elevated urinary xanthurenic acid	Kynureninase (KYNU; EC 3.7.1.3) Kynurenine aminotransferase (KYAT1 & 2; EC 2.6.1.7)	Impaired tryptophan catabolism leads to elevated urinary xanthurenic acid excretion in cases of PLP deficiency

Abbreviations: 3-OMD, 3-methoxytyrosine, 3-O-methyldopa; 5-HIAA, 5-hydroxyindoleacetic acid; CSF, cerebrospinal fluid; GABA, γ-aminobutyric acid; HVA, homovallinic acid; NMDA, N-methyl-D-aspartate; PLP, pyridoxal 5′-phosphate; VLA, Vanillyl lactic acid.

does not involve formation of a new Schiff base, but instead involves transfer of a phosphate generating glucose-1-phosphate.[16]

4 | NONENZYMATIC FUNCTIONS OF PLP AND OTHER B_6 VITAMERS

Besides the role of PLP as a cofactor B_6 vitamers may also play other roles within the cell, for example, as antioxidants, quenching singlet oxygen at rates comparable to vitamins C and E.[17] PLP can also modify expression and action of steroid hormone receptors[18] and may have an effect on immune function.[19] Finally, of interest because of its antiepileptic activity,[20,21] PLP is an antagonist of ATP at P2 purinoceptor7 (P2X7).[22] It has been suggested that when neuroinflammation triggers cellular ATP release this can lead to epilepsy by activation of P2X7 receptors;[23] PLP has the potential to block this activation. This could explain the action of PLP on drug-resistant epilepsies besides those genetic disorders discussed in detail below.[24,25]

5 | CONVERSION OF B_6 VITAMERS TO THE ACTIVE COFACTOR

The intestine only absorbs nonphosphorylated B_6 vitamers (Figure 2). The phosphorylated forms of B_6 and the glucoside of pyridoxine are therefore first hydrolyzed by intestinal phosphatases and an intestinal glycosidase, respectively.[26,27] Once within cells of the intestine, pyridoxine and pyridoxamine can either be converted prior to transport to the liver[28] or once within the liver to pyridoxal phosphate. In the liver (or intestine) pyridoxamine, pyridoxine and pyridoxal, are first rephosphorylated by pyridoxal kinase (EC 2.7.1.35). The flavin mononucleotide (FMN)-dependent enzyme, pyridox(am)ine phosphate oxidase (PNPO; EC 1.4.3.5) converts the phosphates of pyridoxamine and pyridoxine to PLP. Although this occurs mainly in the liver, PNPO is expressed in all cell types. PLP is exported from the liver bound to the lysine 190 residue of albumin.[29]

When B_6 vitamer intake exceeds requirements, pyridoxal phosphate is dephosphorylated (mainly in the liver) and the pyridoxal is oxidized to pyridoxic acid prior to excretion in urine. Early reports suggested that in humans an aldehyde dehydrogenase (EC 1.2.1.4) or aldehyde oxidase (AOX; EC 1.2.3.1)[30] is responsible for this reaction. However, while in *Drosophila* pyridoxal has been shown to be a substrate of AOX1[31] it is not metabolized by mouse AOXs.[32]

For PLP to enter the brain, it must dissociate from albumin and be dephosphorylated to pyridoxal at the blood-brain barrier (BBB). Dephosphorylation is accomplished by tissue nonspecific alkaline phosphatase (E.C. 3.1.3.1), an ectoenzyme tethered to cell membranes at the BBB by a glycophosphatidylinositol (GPI) anchor. Pyridoxal crosses the BBB, probably by facilitated diffusion, and is "trapped" as PLP in the brain cells and the choroid plexus by the action of pyridoxal kinase.[33] The choroid plexus appears to be the only organ besides the liver which is able to readily release PLP which explains the relatively high percentage of PLP in CSF (25% of total B_6 in rabbit[33] and 38% in man[34]). Once within the CSF and the extracellular space of the brain phosphorylated B_6 vitamers must be dephosphorylated to enter the brain cells prior to being rephosphorylated by pyridoxal kinase. Similar mechanisms occur in other target cells. Within tissues such as the brain partial catalysis by PLP or catabolism of PLP enzymes can lead to production of

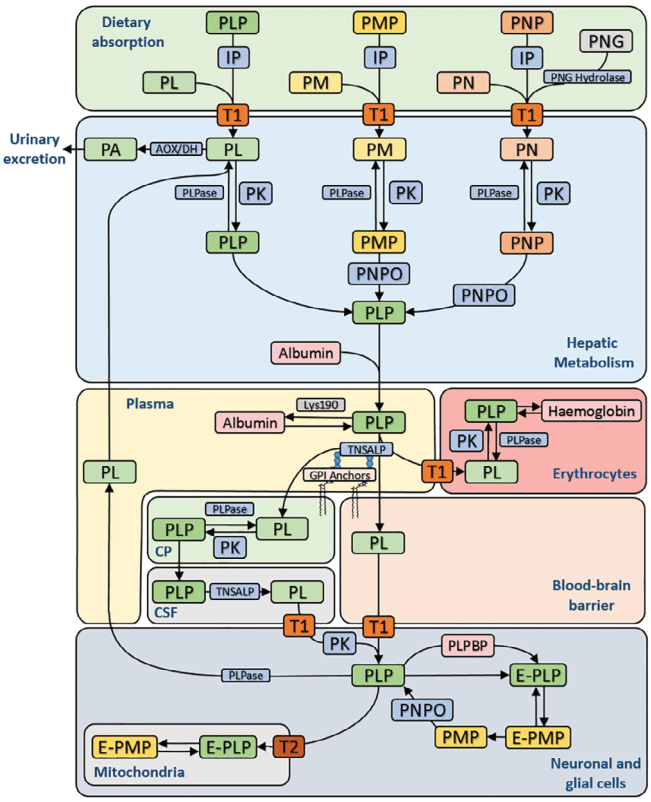

FIGURE 2 Enzymes and transporters involved in human PLP synthesis and homeostasis. AOX/DH, aldehyde oxidase (Mo cofactor)/β-NAD dehydrogenase; (identity unknown; T1), plasma membrane transporter; (postulated to be encoded by the *SLC25A39/40* genes; T2), mitochondrial membrane transporter; CP, choroid plexus; CSF, cerebrospinal fluid; IP, intestinal phosphatases; ; E-PLP, enzyme bound PLP; E-PMP, enzyme-bound PMP; GPI, glycosylphosphatidylinositol anchor; PA, 4-pyridoxic acid; PK, pyridoxal kinase; PL, pyridoxal; PLP, pyridoxal 5′-phosphate; PLPase, pyridoxal-phosphatase; PLPBP/PROSC, pyridoxal 5′-phosphate binding protein; PM, pyridoxamine; PMP, pyridoxamine 5′-phosphate; PN, pyridoxine; PNG, pyridoxine-5′-β-D-glucoside; PNP, pyridoxine 5′-phosphate; PNPO, pyridox(am)ine 5′-phosphate oxidase; TNSALP, tissue nonspecific alkaline phosphatase.

PMP which can be converted back to PLP by PNPO (salvage pathway).[13]

In man, the gene(s) and protein(s) responsible for uptake of B_6 vitamers have yet to be identified. In yeast, however, uptake of pyridoxine at the plasma membrane is mediated by Tpn1, a member of the purine-cytosine permease superfamily[35] and transport of PLP into the mitochondria is mediated by Mtm1p.[35]

6 | HOMEOSTASIS OF PLP CONCENTRATIONS IN CELLS AND BODY FLUIDS

Intracellular-free PLP concentrations are maintained at approximately 1 μM[13] to prevent inappropriate reactions ("aldehyde stress" or "carbonyl stress"). Several mechanisms are involved in maintaining low concentrations. Proteins that bind PLP and help maintain low-free PLP concentrations include glycogen phosphorylase in muscle,[36] hemoglobin in erythrocytes,[37] and albumin in plasma.[29] The enzymes that produce PLP (pyridoxal kinase and PNPO) are inhibited by PLP and in vitro studies suggest PLP is protected intracellularly by being transferred from these enzymes directly, to some PLP-dependent enzymes.[38–40] Free PLP can be degraded by phosphatases such as pyridoxal phosphatase (EC 3.1.3.74). Recently, it came to light that PROSC, an intracellular protein, renamed as PLP-binding protein (PLPBP), plays an important role in PLP homeostasis[41] and very recently it has been suggested that, in some species at least, free PLP levels might be affected by mutations in the NAD(P)HX epimerase enzyme[42] (EC 5.1.99.6).

7 | CAUSES AND CONSEQUENCES OF PLP DEFICIENCY

7.1 | Epilepsy

Because of the vital role of PLP in neurotransmitter metabolism, particularly synthesis of the inhibitory transmitter GABA, it is not surprising that inborn errors leading to PLP deficiency manifest as B_6-responsive epilepsy, usually of early onset. This includes PNPO deficiency (a disorder affecting PLP synthesis and recycling; MIM #610090), disorders affecting PLP import into the brain (alkaline phosphatase deficiency [hypophosphatasia; MIM #241500] and GPI anchor synthesis defects), PLPBP deficiency (MIM #617290), a disorder likely due to abnormal intracellular PLP transport and disorders in which metabolites accumulate that inactivate PLP. This last mechanism occurs in the commonest cause of pyridoxine-dependent epilepsy (PDE), that is, ALDH7A1 deficiency (also known as α-aminoadipic semialdehyde [AASA] dehydrogenase or antiquitin deficiency; MIM #266100) and in hyperprolinaemia type II (deficiency of ALDH4A1; MIM #239500). ALDH7A1 deficiency results in build-up of an intermediate with a nucleophilic carbon atom—Δ^1-piperideine 6-carboxylic acid (P6C) which reacts with PLP thereby inactivating it and resulting in PLP deficiency.[12] Accumulation of Δ^1-pyrroline 5-carboxylic acid has a similar effect in hyperprolinaemia type II.[11] The clinical and biochemical features of disorders leading to B_6-responsive seizures are summarized in Table 2 and some of these disorders are described in more detail below.

Acquired epilepsy can occur when PLP synthesis is impaired, for example, by pyridoxal kinase inhibitors such as 4-O-methyl pyridoxine in Ginkgo nuts[43] or aminophylline.[44] Drugs such as isoniazid that can bind to and inactivate PLP may also cause B_6-responsive seizures.[45]

The pathogenesis of seizures in a brain with low PLP levels is likely to be multifactorial. The changes in brain chemistry are easier to study in mouse models or cultured neuronal cells than in human subjects. In a mouse model of hypophosphatasia, low levels of the inhibitory neurotransmitter, GABA, were demonstrated in 1995.[46] However, seizures in patients with hypophosphatasia are refractory to pro-GABAergic drugs and other experiments on the hypophosphatasia mouse show downregulation of P2X7 receptors and reduction of seizures by blockade of P2X7 receptors, suggesting the anticonvulsive effects of vitamin B_6 may be due to its capacity to block P2X7R receptors.[47,48]

Recent metabolomic profiling of the brains of mice with hypophosphatasia showed a complex range of abnormalities including low levels of GABA and another important neurotransmitter, adenosine, as well as abnormalities of metabolites involved in myelin synthesis (N-acetylaspartate [NAA], N-acetylglutamate [NAG]), and in the methionine cycle and transsulfuration pathway (cystathionine and methionine).[49]

Studies on vitamin B_6-deprived Neuro-2a cells showed reduced synthesis of glycine, serine, and 5-methyl-tetrahydrofolate. The authors suggested that the low intracellular 5-methyl-THF concentrations observed in vitro may explain the favorable but so far unexplained response of some patients with ALDH7A1 deficiency epilepsy to folinic acid supplementation.[50]

There are some dangers in extrapolating from animal models and cultured neuronal cells to human subjects with inborn errors affecting vitamin B_6 metabolism. Some conclusions on the causes of epilepsy due to PLP deficiency can perhaps be drawn from human inborn errors affecting specific PLP-dependent enzymes[51](Table S1). Seizures are a major feature of disorders of the glycine-cleavage enzyme (nonketotic hyperglycinaemia), of phosphoserine aminotransferase deficiency and of mitochondrial glutamate oxalaoacetate amino transferase (GOT2) deficiency.[52,53] They

TABLE 2 Clinical and biochemical features of disorders leading to B_6-responsive seizures

Inborn error	ALDH7A1 deficiency (pyridoxine-dependent epilepsy)	PNPO deficiency	PLPBP deficiency	Hyperprolinaemia type II	Hypo-phosphatasia	GPI anchor defects	Molybdenum cofactor deficiency
Genetic locus	*ALDH7A1*	*PNPO*	*PLPBP (PROSC)*	*ALDH4A1*	*ALPL*	*PIGO; PIGV* (+others)	*MOCS1; MOCS2; GPHN*
Neonatal/infantile B_6-responsive seizures	± Few patients with onset after the first year of life[8,9] and as late as adolescence[70]	± Few patients with onset after first year of life[90]	+	± (~50% of cases; seizure onset usually in infancy or childhood)[112]	± Only 7.5% of hypophosphatasia cases are infantile—not all of these present with seizures[113]	± Variable presentation according to specific GPI defect[114]	± Only isolated cases reported to respond to pyridoxine[66]
Prevalence of developmental delay	± 75% have developmental abnormalities[115]	± Can be developmentally normal—linked to early treatment (13/41 Guerin et al[116])	+ Broad spectrum[41]	± Asymptomatic individuals described[117] but in the absence of developmental delay behavioral problems/psychosis/anxiety are usually present[108]	± Not in milder cases[105]	+	+ Survival rare beyond early childhood[118]
Some cases with pyridoxine failure	− Ambiguous initial response is seen in about 15%	± ~60% of cases[116]	−	−	±[104]	± Proportion unknown[114]	± Only isolated cases reported to respond to pyridoxine[66]
Skeletal abnormalities	± Macrocephaly, facial dysmorphia in rare cases[119]	± Microcephaly in severe, untreated cases	± Microcephaly at birth 4/14; acquired microcephaly in an additional 4/14[41,109,110]	−	+ Similar to rickets; less pronounced in mild cases—some only dental[105]	+ Brachy-telephalangy	± Microcephaly common[118]
Abnormal MRI (including white matter changes)	± Including narrow corpus callosum (esp posterior), dilated ventricles, small cerebellum[8,9]	± May have changes (including white matter oedema) as a result of untreated NEE[88] normal in 37% (5/15)[116]	± Global underdevelopment (coarse gyri shallow sulci) + underdeveloped white matter[98]	−	−	± In one case of PIGO mutations with B_6-responsive seizures: hypomyelination; lesions in the bilateral basal ganglia and brainstem[106]	+ Delayed myelination, enlarged ventricles, cerebral atrophy in >40%; CC and cerebellar atrophy in >20%[118]
Parental history of infertility, miscarriage or prematurity	−	± 8/22 families[88]	−	−	−	−	−
Unique biochemical features (among B_6-responsive seizure disorders)	Raised plasma + urinary α-AASA[8,9] but normal urinary S-sulfocysteine	Raised plasma pyridoxamine/pyridoxic acid ratio[98]; low DBS PNPO enzyme activity (<40% below the normal range)[110]	N/A	Raised plasma proline and P5C; Raised urinary P5C and N-(Pyrrole-2-carboxyl) glycine[120,107]	Low plasma alkaline phosphatase; high plasma phosphate, urine phosphoserine and phosphoethanolamine	Raised plasma alkaline phosphatase	Raised plasma + urinary α-AASA AND raised urinary S-sulfocysteine/xanthine/taurine[65]

Abbreviations: +, usually present; ±, variably present; −, not present; α-AASA; α-aminoadipic semialdehyde; CC, corpus callosum; DBS, dried blood spot; GPI, gycolsylphosphatidylinositol; P5C, Δ^1-pyrroline-5-carboxylate; PLPBP, PLP-binding protein; PNPO, pyridoxamine phosphate oxidase; MRI, magnetic resonance imaging.

Presentation of B_6-responsive seizure disorders is often dependent upon prompt and appropriate treatment, for example, MRI abnormalities can be a consequence of NEE and are usually present only in patients with a more severe form of the disorder or those with delayed treatment.

are also common in adults with untreated cystathione β-synthase deficiency (homocystinuria). In aromatic amino acid decarboxylase deficiency, the clinical picture is dominated by hypotonia, dystonia, and oculogyric crises and it has been suggested that some of the paroxysmal involuntary movements observed in PNPO deficiency might also be due to impaired dopaminergic neurotransmission.[54]

7.2 | Peripheral neuropathy

In adults, PLP deficiency most commonly causes peripheral neuropathy. It can be seen in individuals treated with drugs that form a complex with PLP and inactivate it such as penicillamine, hydralazine, isoniazid, and cycloserine. We speculate that mechanisms protecting the brain from PLP deficiency are developed after infancy so that beyond this age the major effect is seen on peripheral nerves.

7.3 | Systemic manifestations

PNPO-, ALDH7A1-, and PLPBP-deficiency in their most severe forms (neonatal presentations) can have other expected consequences including anemia, lactic acidosis, and hypoglycemia. Isoniazid over-dosage causes lactic acidosis.

8 | DETECTING B_6-DEPENDENT EPILEPSY IN NEONATES

ALDH7A1 deficiency, PNPO deficiency, PLPBP deficiency, and hypophosphatasia can all present with antiepileptic drug resistant seizures (often myoclonic) in the first days of life. Although biomarkers and/or DNA analysis can provide fairly rapid diagnosis of these disorders, management of therapy-resistant seizures in the newborn is urgent and warrants an early standardized B_6 trial (Figure 3).[55] It is important to recognize that B_6-responsive patients may have shown poor adaptation (eg, low Apgar scores) and may have magnetic resonance imaging (MRI) scans suggestive of hypoxic ischemic encephalopathy which initially may deter clinicians from looking for inborn errors of metabolism. The B_6 trial must only be carried out in an intensive care setting with full resuscitation facilities as B_6-reponsive patients may show severe apnoea, hypotension, or coma. Apnoea can occur with i.v. and oral administration. Many patients show rapid and lasting control of seizures in response to either pyridoxine or PLP but in some neonates, the response may be ambiguous or delayed and so it is important to administer B_6 over three consecutive days and to continue with analysis of biomarkers/sequencing of genes. If seizures are controlled by pyridoxine or PLP, treatment should be continued; a withdrawal trial in the neonatal period or early infancy is not recommended.

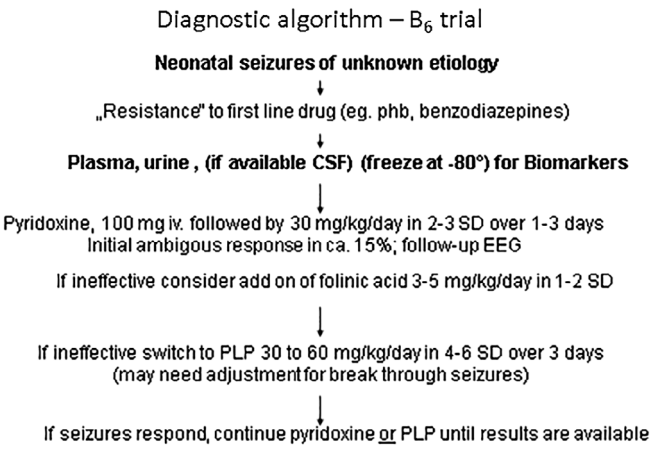

FIGURE 3 Algorithm for the detection and treatment of B_6-responsive epilepsies in the neonate. The duration of each step depends on the individual seizure frequency and seizure response. phb, phenobarbitone; SD, single doses

9 | B_6 VITAMER DOSE FOR THE TREATMENT OF PLP-DEFICIENCY AND VITAMIN B_6 TOXICITY

Pyridoxine hydrochloride is available as a licensed drug for i.v. as well as oral administration in most Western countries, while PLP, outside of Asia, is only available as a nutraceutical or chemical compound for oral administration. Vitamin B_6 trial doses are as recommended in Figure 3.

In the first description of PDE, Hunt et al[56] described complete control of seizures for 21 months with just 2 mg/day of pyridoxine. Over the ensuing years, the dose of pyridoxine used to treat PDE was gradually increased. One 10-year-old child with PDE, whose school performance was deteriorating showed a dramatic increase in intelligence quotient (IQ) when his pyridoxine dose was increased from a total of 50 to 150 mg/day. Subsequent testing of five other cases suggested some increase in IQ on increasing the dose from 5 mg/kg/d but no further increase was observed beyond 15 mg/kg/d.[57] These children were not characterized genetically and while it is likely that they had ALDH7A1 deficiency, the most prevalent form of PDE, we cannot be certain. Current guidelines suggest that total daily pyridoxine doses should, wherever possible not exceed 200 to 300 mg[55] and be divided into two single doses to avoid excess B_6 vitamer concentrations. Patients who experience break-through seizures during febrile infections while on pyridoxine, may receive a double daily dose for the first 3 days of future febrile illnesses.[55] For long-term treatment there is no evidence on the optimal pyridoxine dose to be used. In experimental animals, doses of pyridoxine >50 mg/kg/d cause ataxia, peripheral neuropathy and muscle weakness. Histological examination demonstrates widespread neuronal damage with loss of myelin and

degeneration of sensory fibers in peripheral nerves, the dorsal columns of the spinal cord and descending tract of the trigeminal nerve.[58,59] In human adults, peripheral neuropathy has rarely occurred at total doses <200 mg/d. For most cases where peripheral neuropathy has been documented the total dose was >1000 mg/d. In a neuronal cell line (SHSY5Y), pyridoxine has been shown to induce expression of apoptosis genes and cause dose-dependent cell death.[60] The mechanism may involve inhibition of PLP enzymes by pyridoxine.

For the treatment of classical PNPO deficiency and PLPBP deficiency with insufficient stabilization on pyridoxine there is no pharmaceutical grade PLP available. Where PLP has been used for treatment in Europe and North America, it is a chemical compound or nutraceutical product where the PLP content may not be as stated.[61] PLP, when in solution, may be rapidly degraded by light and/or oxygen with the formation of dimers or pyridoxic acid 5′-phosphate and other products, respectively.[61] Hence, when a liquid formulation is required each PLP dose should be prepared from a tablet or capsule and given without delay. Usual doses range from 30 to 50 mg/kg/d divided into four to six single dosages. In order to avoid potential side effects the lowest effective dose should be used and no weight adjustment be performed in cases of seizure freedom. Some children taking high doses of PLP have developed persistently raised transaminases with progression to cirrhosis[62] and hepatocellular carcinoma (unpublished observation). The cause remains uncertain; possibilities included aldehyde stress from PLP and pyridoxal, or toxicity of PLP photodegradation products. In the case of molecularly proven diagnosis of any form of vitamin B_6-dependent epilepsy, parents and caregivers have to be informed that B_6 treatment is lifelong and that upon withdrawal, symptoms will re-occur; in some cases within a couple of hours, in others it may take several weeks.

10 | DEFICIENCY OF ALDH7A1

It was not until 2000 that findings of elevated pipecolic acid implied that PDE might be caused by a defect in the lysine catabolic pathway.[63] Subsequently, the defect in this pathway was identified as a deficiency of antiquitin (α-AASA dehydrogenase encoded by *ALDH7A1*), leading to the accumulation of α-AASA and its equilibrium partner, Δ1-piperideine-6-carboxylate (P6C)[12] (Figure 4) and was shown to be identical to folinic acid-responsive seizures.[64] P6C inactivates PLP as indicated above. An elevated urinary AASA/creatinine ratio is a more reliable biomarker for this disorder than pipecolic acid although it is also elevated in some patients with molybdenum cofactor (MIM #252150) and sulfite oxidase deficiency (MIM #272300).[65] Simultaneous determination of sulfocysteine is therefore recommended to avoid diagnostic pitfalls.[66] Recently 6-oxo-pipecolate has been reported as a novel biomarker for this disorder.[67] Electroencephalogram (EEG) recording during i.v. administration of pyridoxine is not a reliable way of identifying patients.[68]

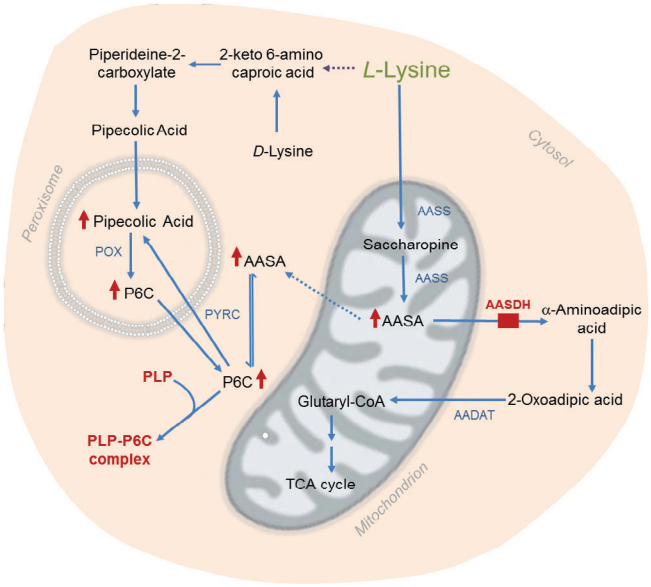

FIGURE 4 ALDH7A1 deficiency is a defect on the lysine catabolism pathway. Lysine catabolism occurs via two pathways; the saccharopine pathway (predominantly mitochondrial with two key steps within the cytosol) and the pipecolate pathway (predominantly cytosolic with a peroxisomal comparment).[121] Both pathways converge at 2-aminoadipic-6-semialdehyde (AASA) which is in a nonenzymatic equilibrium with 1-piperideine-6-carboxylate (P6C). The contribution of each pathway to lysine catabolism depends on the age of the individual and cell-type studied; studies in mice suggest that the saccharopine pathway is primarly reponsible for AASA/P6C prodcution.[80,81] POX, L-pipecolate oxidase; PYRC, pyrroline-5-carboxylate reductase (three homologous genes exist; *PYCR1* and *PYCR2* localize to mitochondria and possibly with the outer mitochondrial membrane and *PYCRL* (aka *PYCR3*) is cytosolic[122,123]); AASS, α-aminoadipate semialdehyde; AASDH, α-aminoadipate semialdehyde dehydrogenase; AADAT, α-aminoadipate aminotransferase. Patients with AASDH deficiency (depicted by the red block) due to mutations in *ALDH7A1* have raised levels of AASA and P6C. Some patients also have raised pipecolic acid. P6C complexes with PLP decreasing the bioavailable PLP. This manifests as a seizure disorder

The clinical spectrum of antiquitin deficiency extends from unspecific ventriculomegaly detected on foetal ultrasound, through abnormal foetal movements and a multisystem neonatal disorder, to onset of seizures and autistic features after the first year of life.[8,9,55] Clinical diagnosis can be challenging because: (a) there may be partial response to antiepileptic drugs (especially phenobarbitone); (b) in infants with multisystem pathology, response to pyridoxine may not be instant and obvious; and (c) structural brain abnormalities may coexist and be considered sufficient cause of epilepsy, whereas seizures are in fact a consequence of antiquitin

deficiency. An overview of the clinical features of patients with ALDH7A1 deficiency is as detailed in Figure 5 (adapted from Karnebeek et al.[69]). Phenotypes of 49 children with ALDH7A1 deficiency were summarized in a meta-analysis by van Karnebeek et al.[69] Since then the spectrum has been further extended, for example, ALDH7A1 deficiency presenting with adolescent onset epilepsy.[70]

An increased incidence of structural brain abnormalities has been reported in individuals with mutations in ALDH7A1.[71,72] Histologic analysis of PDE cortex in a deceased child revealed areas of abnormal radial neuronal organization consistent with type Ia focal cortical dysplasia. These malformations persist despite postnatal pyridoxine supplementation and probably contribute to neurodevelopmental

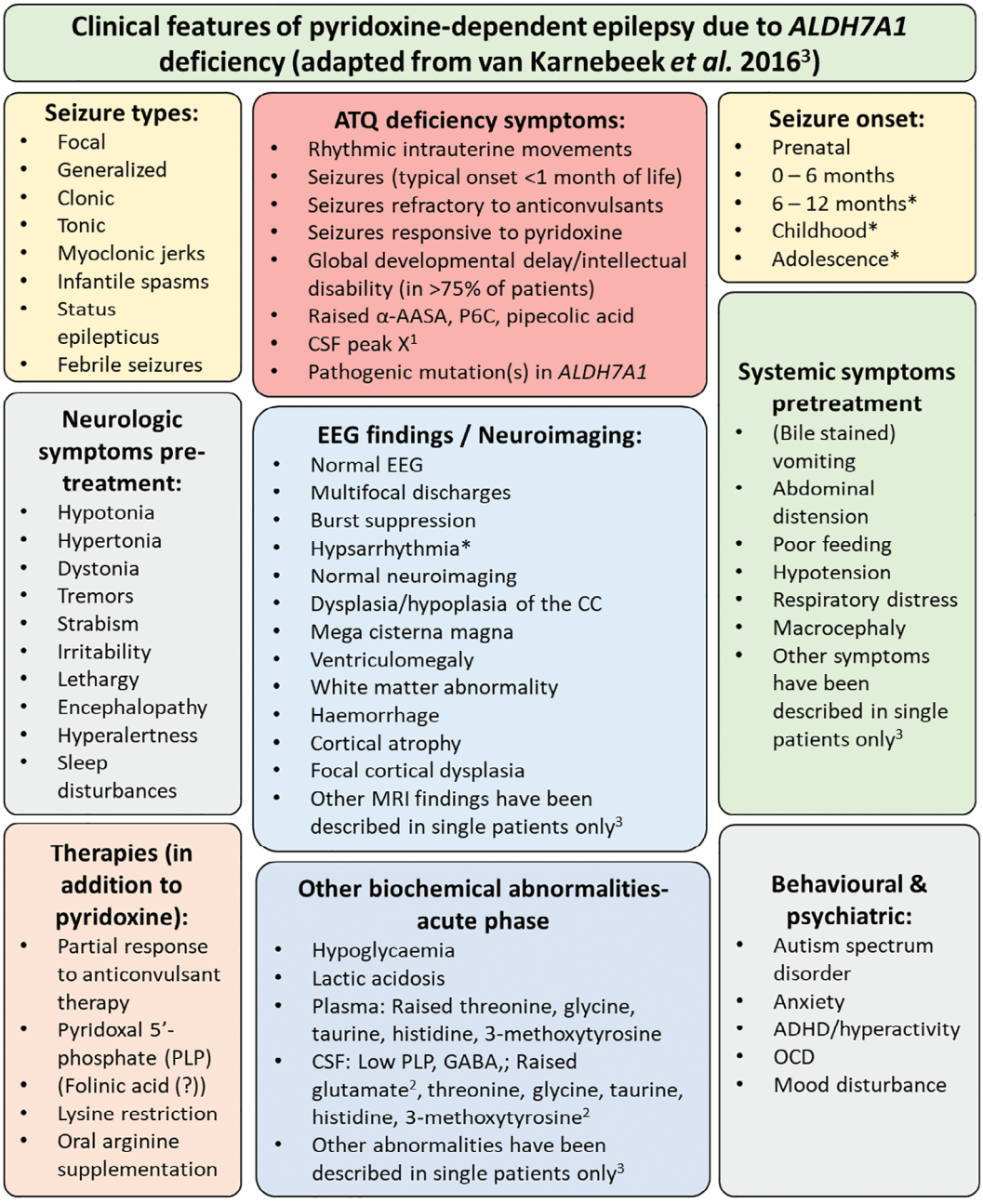

FIGURE 5 Overview of the symptoms reported in the literature for patients with ATQ deficiency. Red box: symptoms or biochemical features present in the majority of patients. All other boxes: symptoms present in the minority of patients. *Ultra-rare symptoms, reported in eight literature patients or fewer. [1]Unidentified peak in the HPLC chromatogram for CSF monoamine neurotransmitter analysis in ATQ deficiency patients. [2]Potentially normalize on pyridoxine therapy. [3]See van Karnebeek et al.[69] α-AASA, α-aminoadipic semialdehyde; ADHD, attention deficit hyperactivity disorder; ATQ, antiquitin; CC, corpus callosum; CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid; OCD, obsessive-compulsive disorder; P6C, L-Δ1-piperideine-6 carboxylate

impairments. Serine racemase, a PLP-dependent enzyme, is involved in neuronal migration.[73]

Coughlin et al[74] have recently published a comprehensive overview of 165 known *ALDH7A1* pathogenic variants which includes the genotypes of 185 individuals. It is estimated that the carrier frequency of *ALDH7A1* mutations is 1:127 and that the estimated incidence of ALDH7A1 deficiency is 1:64 352 live births.[74]

Seizures of patients with ALDH7A1 deficiency are well controlled on pyridoxine monotherapy in about 90% of cases, however, at least 75% of children have intellectual disability (ID) and developmental delay (DD). The degree to which they have ID/DD does not correlate with treatment delay, pretreatment symptomatic interval, or duration taken to achieve seizure control.[74] Two add-on treatment options have been trialed in relatively small numbers of patients. The first approach is the lysine-restricted diet to lower potentially toxic AASA concentrations. The second is the use of arginine which competes with lysine in the process of transport thereby reducing its intestinal absorption and transport into the brain.

van Karnebeek et al[75] reported the effect of a lysine restricted diet in seven patients with ALDH7A1 deficiency. Reduction in biomarker levels (measurement of the last value before and first value after initiation of dietary lysine restriction) ranged from 20% to 67% for plasma pipecolic acid, 13% to 72% for urinary AASA, 45% for plasma AASA, and 42% for plasma P6C. CSF data, available for one patient, showed decreases of 87% and 82% for pipecolic acid and AASA, respectively. Improvement in age-appropriate skills was observed in four of five patients showing prediet delays, and seizure control was maintained or improved in six of seven children. Mercimek-Mahmutoglu et al[76] reported add-on supplementation with L-arginine (400 mg/kg/day; 5 g three times a day) in a 12-year-old boy. CSF α-AASA was decreased by 57% and neuropsychological assessments revealed improvements in general abilities index from 108 to 116 and improvements in verbal and motor functioning after 12 months of therapy.

"Triple therapy" (pyridoxine and arginine supplementation, lysine restriction) has been reported for nine patients.[77–79] Treatment reduced levels of biomarkers such as plasma AASA, P6C, pipecolic acid, and threonine and improved development. Optimum results were reported for patients where treatment was started early, however, larger cohorts and long-term data with neurocognitive testing are needed to provide robust data. When a formula low in lysine and tryptophan (as manufactured for treatment of glutaric aciduria type I) is used there is a potential risk of low plasma tryptophan leading to central serotonin deficiency. Other therapeutic strategies currently being investigated, although still some way from clinical translation, include antisense therapy and substrate reduction therapy.[80,81]

11 | PYRIDOX(AM)INE PHOSPHATE OXIDASE DEFICIENCY

Kuo and Wang[82] described a neonate whose seizures were not controlled by pyridoxine but were well controlled by PLP. Clayton et al[83] described the same phenomenon in an infant whose pre-PLP biochemistry suggested deficient PLP-dependent enzyme activities: low CSF concentrations of HVA and 5HIAA with raised CSF 3-methoxytyrosine and urinary vanillactate (indicating aromatic amino acid decarboxylase deficiency) and raised plasma threonine and glycine indicating defects in the PLP-dependent pathways for catabolism of these amino acids. Other patients with similar findings were identified and were shown to have *PNPO* mutations leading to reduced enzyme activity.[84] Subsequently, PNPO-deficient patients were identified who did not have the "typical" biochemical changes.[15] Untreated patients who survived the neonatal period had anemia and failure to thrive.[85] Treatment of PNPO deficiency was found to be more difficult than that of ALDH7A1 deficiency in that PNPO-deficient patients may require a dose of PLP every 6 hours to avoid an epileptic prodrome or frank seizures. Reports have emphasized the possibility of normal developmental outcome with early treatment.[86,87] A summary of the clinical features of patients with PNPO deficiency are as detailed in Figure 6.

By 2014, it was clear that some patients with PNPO deficiency respond to treatment with pyridoxine and indeed PLP may aggravate seizures.[88,89] Mills et al[88] identified three groups of patients with PNPO mutations that had reduced enzyme activity: (a) those with neonatal onset seizures responding to PLP (n = 6); (b) one patient with infantile spasms (onset 5 months) responsive to PLP; and (c) patients with seizures starting under 3 months of age responding to pyridoxine (n = 8). Certain genotypes (R225H/C and D33V) appear more likely to result in seizures responsive to pyridoxine. Other mutations seem to be associated with infertility, miscarriage, and prematurity. To date 62 genetically confirmed PNPO-deficient patients have been reported with 27 different mutations in *PNPO*[90–95] (Table 3). R116Q, initially thought to be a polymorphism due to its prevalence in the general population and segregation in some instances with other known pathogenic PNPO homozygous variants, has now been confirmed to be pathogenic,[90,96] dramatically affecting erythrocyte PNPO activity. Five children have been reported that are homozygous for this variant and no other known pathogenic PNPO variants with seizure onset at 3 hours and 5 months[88] and 8 months, 24 months, and

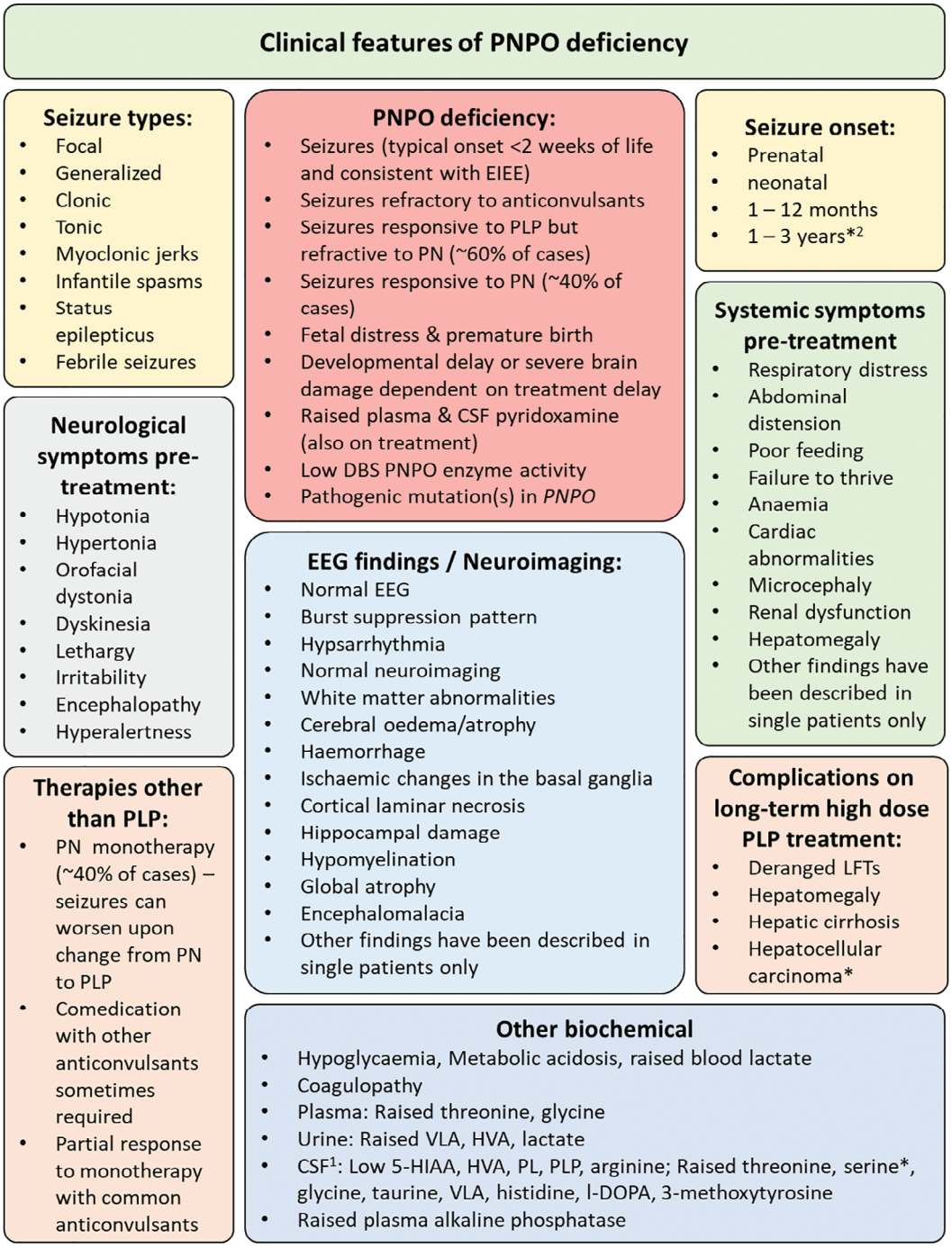

FIGURE 6 Overview of the symptoms reported in the literature for patients with PNPO deficiency. Red box: symptoms or biochemical features present in the majority of patients. All other boxes: symptoms present in the minority of patients. *Ultra-rare symptoms, reported in three literature patients or fewer. [1]Can normalize on effective treatment with PLP/PN. [2]Seizure onset after 1 year seen with patients homozygous for the p.R116Q variant in *PNPO*. α-AASA, α-aminoadipic semialdehyde; ADHD, attention deficit hyperactivity disorder; ATQ, antiquitin; CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid; OCD, obsessive-compulsive disorder; P6C, L-Δ1-piperideine-6 carboxylate; DBS, dried blood spot; VLA, vanillactic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; PL, pyridoxal; PLP, pyridoxal 5′-phosphate; L-DOPA, L-3,4-dihydroxyphenylalanine; EIEE, early infantile epileptic encephalopathy; LFT, liver function test; PN, pyridoxine

3 years 2 months.[90] R116Q appears to result in a milder phenotype with four of five of the children presenting later and three of five having a good developmental outcome. The story is likely to be complicated, however, as not all individuals homozygous for R116Q-PNPO present with seizures.[96]

While a low CSF PLP concentration is suggestive of a B_6-dependent epilepsy disorder it may also be found in

TABLE 3 Summary of PNPO and PLPBP pathogenic variants reported in the literature

Nucleotide	Position	Predicted effect	Number of patients		Number of families	
			Hz	Comp Het	Hz	Comp Het
PNPO (261 amino acids/7 exons) (62 patients/46 families)						
c.98A>T	Exon 1	p.(Asp33Val)	3	3	3	3
c.246delT	Exon 2	p.(Leu83TrpfsTer17)	—	1	—	1
c.263+2T>C[a]	Intron 2	Loss of exon 2[d]	1	—	1	—
c.264-21_264-1delinsC	Intron 2	Splice errors	—	1	—	1
c.279_290del	Exon 3	p.(Ser93Ser, Ala94_Leu97del)	—	1	—	1
c.283C>T	Exon 3	p.(Arg95Cys)	6	—	3	—
c.284G>A	Exon 3	p.(Arg95His)	1	1	1	1
c.347G>A	Exon 3	p.(Arg116Gln)[e]	12	2	9	2
c.352G>A	Exon 3	p.(Glu118Arg)	1	—	1	—
c.363+5G>A	Intron 3	Splice errors	2	—	1	—
c.364-1G>A[b]	Intron 3	Loss of exon 4[d]	2	—	1	—
c.413G>A	Exon 4	p.(Arg138His)	—	1	—	1
c.421C>T	Exon 5	p.(Arg141Cys)	—	1	—	1
c.445_448del	Exon 5	p.(Pro150ArgfsTer27)	—	1	—	1
c.448_451del	Exon 5	p.(Pro150ArgfsTer27)	—	2	—	1
c.481C>T	Exon 5	p.(Arg161Cys)	1	2	1	1
c.520C>T	Exon 5	p.(Gln174X)[f]	1	—	1	—
c.546+1G>A[c]	Intron 5	Splice errors	—	1	—	1
c.620delG	Exon 7	p.(G207VfsX215)	—	1	—	1
c.637C>T	Exon 7	p.(Pro213Ser)	2	—	1	—
c.641dupA	Exon 7	p.(Gln214fs)	—	1	—	1
c.673C>T	Exon 7	p.(Arg225Cys)	1	1	1	1
c.674G>A	Exon 7	p.(Arg225His)	13		9	
c.674G>T	Exon 7	p.(Arg225Leu)	4	—	3	—
c.685C>T	Exon 7	p.(Arg229Trp)	4	—	1	—
c.686G>A	Exon 7	p.(Arg229Gln)	2	—	2	—
c.784T>C	Exon 7	p.(Ter262GlnnextTer28)	2	—	1	—
PLPBP (275 amino acids/8 exons) (15 patients/13 families)						
c.119C>T	Exon 1	p.(Pro40Leu)	—	1	—	1
c.122G>A	Exon 2	p.(Arg41Gln)	1	1	1	1
c.134T>A	Exon 2	p.(Val45Asp)	—	1	—	1
c.199G>A	Exon 2	p.(Glu67Lys)	1	—	1	—
c.206A>G	Exon 2	p.(Tyr69Cys)	1	—	1	—
c.207+1G>A	Intron 2	Splice errors	—	1	—	1
c.211C>T	Exon 3	p.(Gln71Ter)	1	—	1	—
c.233C>G	Exon 3	p.(Ser78Ter)	3	—	1	—
c.249_252del	Exon 4	p.(Ser84Cysfs*21)	—	1	—	1
c.260C>T	Exon 4	p.(Pro87Leu)	1	1	1	1
c.320-2A>G	Intron 4	Splice errors	—	1	—	1
c.524T>C	Exon 6	p.(Leu175Pro)	1	—	1	—

(Continues)

TABLE 3 (Continued)

Nucleotide	Position	Predicted effect	Number of patients		Number of families	
			Hz	Comp Het	Hz	Comp Het
c.614G>A	Exon 8	p.(Arg205Gln)	1	1	1	1
c.722G>A	Exon 8	p.(Arg241Gln)	—	2	—	2

Abbreviations: Comp Het, compound heterozygous; Hz, homozygous; PLPBP, PLP-binding protein; PNPO, pyridox(am)ine phosphate oxidase.
Review was done using Pubmed.
[a]IVS2+2T>C.
[b]IVS3-1G>A; seen in conjunction with p.Glu50Lys however expression studies[84] showed that p.Glu50Lys does not affect activity.
[c]IVS5+1G>A.
[d]cDNA sequenced to confirm predicted effect on protein.
[e]Five individuals homozygous for only Arg116Gln (five families), one heterozygous for Arg116Gln and one known pathogenic heterozygous variant, one heterozygous for Arg116Gln and two known pathogenic heterozygous variants, seven homozygous for Arg116Gln and one known pathogenic homozygous variant (four families).
[f]In published paper,[85] the amino acid change for c.520C>T is reported as p.A174X.

epileptic encephalopathy with no specific diagnosis[97] and it should be noted that one PNPO-deficient child had PLP concentrations within the normal range.[91] A rapid LC-MS/MS-based dried blood spot assay, which measures PNPO enzyme activity, has been developed for diagnosis of PNPO-deficiency.[96] An elevated plasma pyridoxamine concentration can also be indicative of this disorder irrespective of vitamin B_6 treatment.[98–100]

12 | CONGENITAL HYPOPHOSPHATASIA DUE TO *ALPL* MUTATIONS

There is a very wide spectrum of disease caused by *ALPL* (*TNALP, TNAP, TNSALP*) mutations (hypophosphatasia) from skeletal hypomineralization and deformity detected in utero to presentation with premature loss of deciduous and/or permanent teeth. Only patients with the most severe neonatal form present with seizures in the neonatal period that can precede the detection of bone disease.[101]

Bone X-rays show features similar to rickets. Plasma alkaline phosphatase is below the normal range but it is important to compare to the correct age range. Urinary phosphoethanolamine is elevated. Plasma PLP and PLP/pyridoxal ratio are elevated (even prior to pyridoxine supplementation).[102] There may be biochemical evidence of intracellular PLP deficiency, for example, increased excretion of vanillactate.[101]

In some neonates with CHP, seizures are antiepileptic drug-resistant and respond dramatically to pyridoxine.[103] However, in other cases, there is only a modest transient response to pyridoxine and the encephalopathy is fatal.[104]

Most neonates and infants with severe CHP have severe restrictive thoracic deformity and most die of respiratory failure. Recently enzyme replacement therapy (ERT) that treats the bone disease has become available.[105] ERT reduces the plasma PLP concentration to a normal or mildly elevated level and the PLP-to-PL ratio to mildly reduced or low-normal levels.[102]

There are several reports of subacute brainstem degeneration in patients with CHP both on and off pyridoxine treatment. One hypothesis is that this is due to toxicity of very high extracellular PLP levels.[104] It is considered unlikely that ERT will be able to avoid this deleterious manifestation due to its inability to cross the BBB.

It should be noted that the TNSALP protein is anchored at the BBB to GPI anchors. Inborn errors of metabolism causing deficiency of the proteins responsible for GPI anchor processing (eg, PIGO [MIM 614730]; PIGE [MIM 610274]) can occasionally lead to brain PLP deficiency and B_6-responsive seizures.[106]

13 | HYPERPROLINAEMIA TYPE II

In hyperprolinaemia type II, mutations in *ALDH4A1* lead to a deficiency of pyrroline 5-carboxylate (P5C) dehydrogenase which catalyzes the second step in proline catabolism. P5C can react with PLP and inactivate it.[11] Plasma proline is markedly elevated (often >1000 μM) and P5C can be detected in the urine by its color reaction with ortho-aminobenzaldehyde. Urine organic acid analysis may show the presence of pyrrole-2-carboxyglycine.[107]

Only about 50% of individuals with HP2 develop seizures, onset of which is usually in infancy or childhood rather than in the first few days of life. The seizures can often be triggered by fever and controlled by antiepileptic drugs; B_6-dependency is not as evident as in deficiencies of ALDH7A1, PNPO, and PLPBP. In the case reported by Farrant et al,[11] urine obtained during the admission with prolonged seizures contained excess xanthurenic acid indicating PLP deficiency.

In the series reported by van de Ven et al,[108] all patients showed seizures and significant behavioral problems, including anxiety and hallucinations and all had low plasma PLP

levels. The clinical course was nonprogressive and independent of the B_6 concentration and B_6 therapy.

14 | PYRIDOXAL PHOSPHATE-BINDING PROTEIN (PROSC) DEFICIENCY

To date 15 patients with PLPBP-deficiency have been reported (Table 2). In 2016, Darin et al[41] reported seven children with pathogenic variants in proline synthetase co-transcribed homolog (bacterial), PROSC, which encoded a PLP-binding protein of hitherto unknown function. Pretreatment cerebrospinal fluid samples showed low PLP concentrations and evidence of reduced activity of PLP-dependent enzymes. However, cultured fibroblasts showed excessive PLP accumulation. The clinical picture was dominated by the seizure disorder (three of seven had intrauterine seizures, six of seven had seizures on day 1 of life, one of seven did not present until 1 month of age). However, systemic features (anemia, enterocolitis, electrolyte abnormalities) were sometimes present in the neonatal period. The birth head circumference was ≤ninth centile in four of seven. Brain MRI scans in early infancy showed global underdevelopment of the cortex (broad gyri and shallow sulci) and sometimes periventricular cysts, and later scans showed underdevelopment of white matter. Acquired microcephaly was present in six of seven and all had some degree of DD.

Plecko et al[109] confirmed that PROSC (PLPBP) deficiency was a cause of PDE but the four patients identified had a milder disease: no intrauterine seizures, neonatal onset of seizures on days 3 to 9, only one of four with burst suppression on EEG (compared to five of seven) no microcephaly, normal MRI scans and normal development in three of four. Recently, Shiraku et al[110] have also identified four patients with PLPBP deficiency while screening a cohort of 700 patients with childhood onset epileptic encephalopathies. Seizures responded to treatment with pyridoxine for three of four patients and with PLP for one of four. Two of these children presented with seizures after 1 month of age (3 months and 34 days). Birth head circumference was normal but acquired microcephaly was reported for two of four. Similar to the first cohort of PLPBP-deficient patients described, all of these patients had some degree of ID.

15 | B_6 TREATMENT OF PLP-ENZYMOPATHIES

Binding of PLP to apoenzymes is obviously essential for the catalytic function of the enzyme but it may also be important for correct folding of the protein and targeting it to the right organelle. In individuals with primary hyperoxaluria type I (alanine glyoxylate transaminase [AGT] deficiency; MIM #259900), binding of PLP can prevent the build-up of AGT monomers which inappropriately target to the mitochondria.[111] Thus, PLP can act as a chaperone as well as requiring a chaperone (PLPBP) itself. It is beyond the scope of this review to list all PLP-enzymopathies that might respond to pyridoxine supplementation, however, five important disorders in which it should always be tested are cystathionine β-synthase deficiency (classical homocystinuria; MIM #613381), δ-amino laevulinic acid synthase-2 deficiency (X-linked sideroblastic anemia; MIM #300751), primary hyperoxaluria type I (MIM #259900), hyperornithinaemia with gyrate atrophy of the choroid and retina (MIM #258870), and aromatic amino acid decarboxylase deficiency (MIM #608643).[51]

Recently, a patient with mutations in *GOT2* encoding the mitochondrial glutamate oxaloacetate transaminase (EC 2.6.1.1) has been described. The patient presented with serine- and vitamin-B_6-responsive epileptic encephalopathy, profound psychomotor delay and spastic paraparesis.[52,53]

16 | CONCLUSIONS AND FUTURE RESEARCH

- Most neonates with epileptic encephalopathy who respond dramatically to treatment with pyridoxine or pyridoxal phosphate can now be shown to have ALDH7A1 (antiquitin) deficiency, PNPO deficiency, or PLPBP deficiency.
- The challenge for the future in ALDH7A1-deficiency is to try and ensure that as few children as possible have learning difficulties. There may be a role for prenatal treatment to prevent brain structural malformations, consideration needs to be given to optimal pyridoxine dose and preliminary studies of dietary lysine restriction and arginine supplementation are encouraging.
- Some individuals can have a normal neurodevelopmental outcome, as is the case in 25% of patients with ALDH7A1 deficiency and in cases of PNPO deficiency which received prompt diagnosis and treatment with pyridoxine or pyridoxal phosphate.
- For subjects whose seizures cannot be controlled by pyridoxine and require pyridoxal phosphate, we need to understand why some develop abnormalities of liver function tests leading to cirrhosis and occasionally hepatocellular carcinoma and strive for improved methods of treatment.
- There are still big gaps in our understanding of PLP homeostasis: unidentified transporters, ill-defined regulation of expression of enzymes and transporters; the exact mechanism by which the cell gets PLP to holoenzymes while keeping free PLP levels low.

- There remain many subjects, either undiagnosed or with other genetic epilepsies, whose epilepsy is reduced in frequency and severity by vitamin B_6 treatment. We need to understand how this works. Do these subjects have more subtle defects in vitamin B_6 metabolism or is PLP simply blocking P2X7 receptors when epilepsy is caused by inflammation and ATP release?

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CONFLICTS OF INTEREST

M.P.W. declares that he has no conflicts of interest. B.P. has received an honorarium for a lecture on vitamin B_6-dependent epilepsy due to hypophosphatasia from alexion in 2017 and has received travel reimbursement from Nutricia in 2018. P.B.M. and P.T.C. have received consultancy fees, research funding, and travel reimbursement from Actelion (now owned by Johnson and Johnson) who market Miglustat for the treatment of Niemann-Pick C disease.

AUTHOR CONTRIBUTIONS

All authors contributed to drafting and reviewing the manuscript. This article does not contain any studies with human or animal subjects performed by any of the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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