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Chapter 11

Pyridoxine and Pyridoxal-Phosphate Dependent Epilepsies

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Abstract

Antiquitin (ATQ, *ALDH7A1*) and Pyridox(am)ine phosphate oxidase (*PNPO*) deficiency are two metabolic encephalopathies which respond to high dosages of pyridoxine and pyridoxal phosphate (PLP). Their common pathophysiological denominator is deficiency of PLP, the active form of all B6 vitamers.

In ATQ deficiency, the primary defect resides in lysine catabolism resulting in accumulation of α-aminoadipic semialdehyde (AASA)/pyrroline 6' carboxylate (P6C) and pipecolic acid. PLP is inactivated via a chemical binding with P6C. In PNPO deficiency, reduced synthesis of PLP from pyridoxine(phosphate) and pyridoxamine (phosphate) results in primary PLP deficiency.

Clinically both conditions result in neonatal/infantile epileptic encephalopathy with pharmaco-resistant seizures. AASA (and pipecolic acid) are reliable diagnostic markers for ATQ deficiency. In the untreated state, reduced PLP (measured in CSF) is characteristic but not specific for PNPO deficiency. Molecular analysis is required to establish the diagnosis. ATQ and PNPO deficiency are treated with up to 30mg/kg of pyridoxine and/or 30-60 mg/kg of PLP orally. Some patients with PNPO deficiency respond to pyridoxine. High pyridoxine and PLP dosages result in peripheral neuropathy and liver dysfunction. While patients with normal outcomes have been reported, a majority shows neurodevelopmental impairments. Lysine-restricted diet (supplemented with lysine-free amino-acid formula) aimed at reducing AASA/P6C accumulation is an additional treatment option for ATQ deficiency to optimize seizure control and developmental outcomes.

Introduction

The concept of vitamin B6 responsive seizures has been known for over six decades [1]. Prior to the elucidation of their molecular background, these conditions have been known as "pyridoxine dependent epilepsy (PDE)" and as "pyridoxal phosphate responsive epilepsy". In 2005 Mills et al., [2] identified Antiquitin (ATQ) deficiency as the gene defect underlying the majority of cases with PDE. Shortly thereafter the same group discovered pyridox(am)ine-phosphate oxidase (PNPO) deficiency as cause of pyridoxal phosphate responsive epilepsy [3]. ATQ and PNPO deficiency present with epileptic encephalopathy and have an overlapping response to treatment with pyridoxine and/or pyridoxal-phosphate.

More than 200 patients with PDE have been described in the literature and many more cases have been diagnosed in clinical practice. Depending on the degree of systematic ascertainment via a therapeutic trial with pyridoxine, reports of PDE frequency vary between 1:20.000 and 1: 600.000. [4-6]. ATQ deficiency likely accounts for the main proportion of PDE cases. PNPO deficiency might account for another proportion of patients originally diagnosed as PDE, as some respond to pyridoxine [7, 8].

Antiquitin (ATQ1, EC 1.2.1.3) is a member of the aldehyde dehydrogenase family. Its function resides in the pipecolic acid pathway of lysine catabolism. ATQ is encoded by *ALDH7A1*, located on chromosome 5q.31. The gene product is also known as α-aminoadipic semialdehyde dehydrogenase (AASADH). Throughout this chapter, the term Antiquitin (abbreviated as ATQ) will be used. Folinic acid responsive seizure disorder (FARS) is allelic to ATQ deficiency [9]. Pyridox(am)ine phosphate oxidase (PNPO, EC 1.4.3.5) is a flavin mononucleotide (FMN)-dependent oxidoreductase required for the rate-limiting synthesis of pyridoxal phosphate from pyridoxine and pyridoxamine and their phosphates. Its gene (*PNPO*) is located on chromosome 17q.21.32.

The common endpoint of ATQ and PNPO deficiency is intracellular deficiency of pyridoxal phosphate (PLP). While PNPO deficiency results in reduced synthesis of PLP, in ATQ deficiency PLP is normally synthesized but chemically inactivated by accumulating metabolites arising from defective lysine catabolism.

PLP acts as a co-factor for more than 140 enzymatic reactions, mostly transamination and decarboxylation of aminoacids [10]. PLP deficiency foremost affects the decarboxylation of mono-aminergic neurotransmitter precursors, mimicking the biochemical profile of aromatic amino-acid decarboxylase deficiency.

There are 3 other inborn errors affecting B6 vitamer metabolism and utilization: familial hypophosphatasia (tissue non-specific alkaline phosphatase [TNSALP] deficiency; familial hyperphosphatasia (phosphatidyl inositol glycan anchor biosynthesis type V [PIGV] deficiency; and pyrroline 5-carboxylate dehydrogenase [P5C] deficiency commonly known as hyperprolinemia type 2. Although in these conditions seizures can be part of the clinical picture, their major clinical manifestations include bone disease and symptoms of hypercalcemia (TNSALP deficiency), predominant intellectual developmental disability (P5C deficiency), and dysmorphic features (PIGV deficiency).

This chapter focuses on ATQ and PNPO deficiency, in which epilepsy and encephalopathic presentations are the predominant clinical features. For a comprehensive overview of ATQ and PNPO deficiency see [7, 8, 11, 12]. For an overview of the pathways of Vitamin B6 metabolism and its disorders see [13].

ATQ Deficiency

Disease Characteristics

Clinical Presentation

ATQ deficiency typically presents with neonatal or early infantile seizures, which are refractory to pharmacologic anti-convulsive treatment. Seizures may occur intra-uterine with onset at the end of the last trimester. Seizures resolve after a single or repeat dosage of intravenous (50-100 mg) or enteral (20-30mg/kg) administration of pyridoxine. Transient coma requiring mechanical ventilation may occur after intravenous (and very rarely after oral) administration of pyridoxine.

The encephalopathy in neonates with ATQ deficiency may present as hyper-alertness, motor hyperactivity, insomnia, and feeding refusal, resembling withdrawal from intra-uterine substance exposure. Co-existing signs of birth asphyxia/perinatal stress are frequently encountered. Prolonged episodes of mixed multifocal myoclonic tonic symptoms, associated with grimacing and abnormal eye movements are typical [14] Neonatal lactic acidosis, hypoglycemia and electrolyte disturbances have been reported along with ATQ deficiency [12, 15].

Milder variants with self-limited seizures (generalized, partial, atonic, myoclonic, infantile spasms), initial response to common anticonvulsants and later childhood onset, up to 3 years of age, may account for up to 30% of cases.

Breakthrough seizures may occur during episodes of febrile illness/gastro-enetritis, which reduces bio-availability of pyridoxine.

Although there are no specific imaging findings in ATQ deficiency, mega cisterna magna, neuronal migration abnormalities and progressive hydrocephalus requiring shunting have been reported in several patients, and varying degrees of cerebral atrophy have been described in late-diagnosed patients [12, 16]. White matter abnormalities in neonates may resolve within the first year of life [16], while others are progressive over time [17, 18]. Intracerebral and retinal bleedings [19] as well as intrauterine sub-ependymal cysts [20] add to the myriad of changes. ATQ is expressed within glial cells in the brain, and its dysfunction in PDE is associated with neuronal migration abnormalities and other structural brain defects, which might explain the limited developmental outcomes [21].

Biochemical Markers

Elevated α amino adipic semialdehyde (AASA) is a characteristic marker for ATQ deficiency. AASA is in chemical equilibrium with piperidine-6-carboxylate (P6C). Both metabolites can be determined in urine, blood and CSF, serving as diagnostic markers. Physiologically, AASA levels decline during the first year of life from < 2mmol/mol creatinine between 0-0.5 years to < 0.5 mmol/mol creatinine after the age of 1 year in the urine [13]. Pathological values at any age are several-fold above the normal limits and levels remain elevated upon treatment.

Given the biochemical pathway (Figure 1), pipecolic acid is also elevated. Physiological levels are decreasing during the first year of life. Pathological values are only moderately elevated compared to normal values. Levels may come close to normal upon treatment [22, 23]. Single patients with normal pretreatment levels have been reported [24].

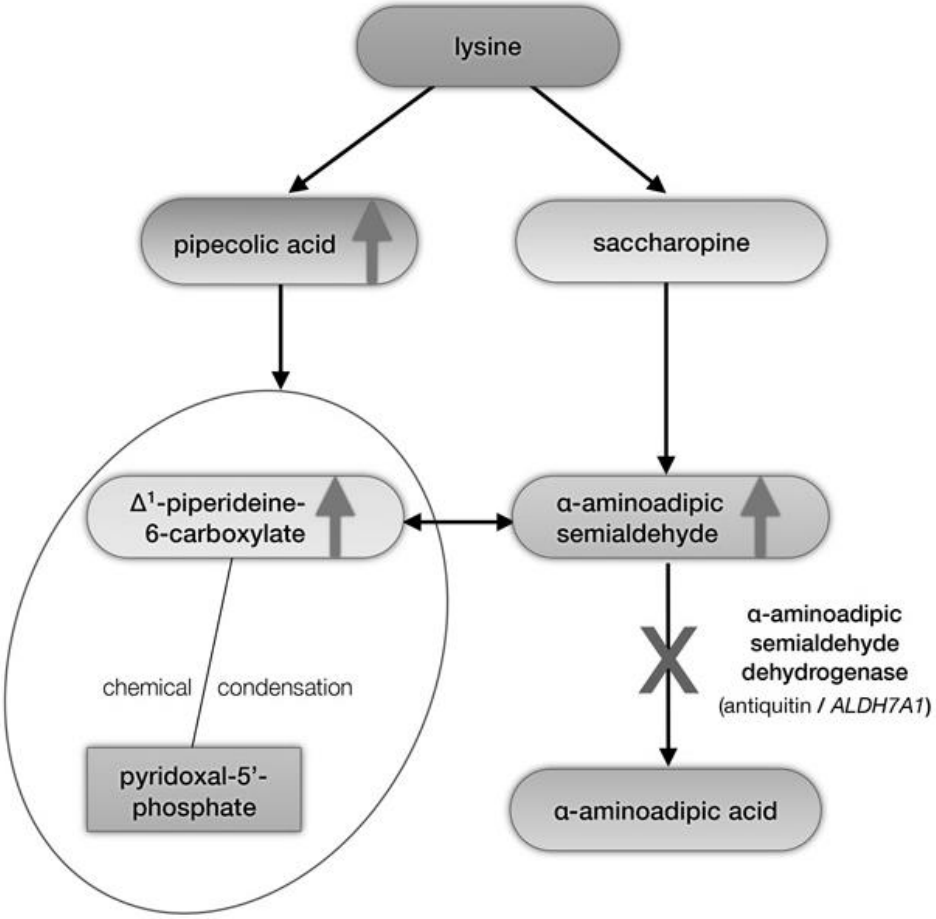

Figure 1. Schematic overview of the metabolism of L-lysine via the saccharopine and pipecolic acid pathways and biochemical pathophysiology of ATQ deficiency. The two pathways converge where L-Δ1-piperideine 6-carboxylate (P6C), produced via the pipecolic acid pathway and α-aminoadipic semialdehyde (α-AASA), produced via the saccharopine pathway, are in equilibrium. α-AASA is then converted to α-aminoadipic acid (α-AAA) by ATQ. In ATQ deficiency, P6C and α-AASA accumulate due to a block in α-aminoadipic semialdehyde dehydrogenase (Antiquitin, *ALDH7A1)*. P6C undergoes chemical condensation with pyridoxal phosphate (PLP) resulting in PLP deficiency. Pipecolic acid (PA) accumulates due to backpressure from the enzymatic bloc.

PLP levels are expected to be low as a result of chemical binding with P6C. Footitt et al., [25] measured plasma PLP levels in 2 pairs of siblings with ATQ deficiency on pyridoxine treatment. Interestingly they found low levels in one pair and high levels in the other. High PLP levels could be explained by an analytical 'artefact' caused by dissociation of the P6C-PLP complex through trichloracetic acid used for sample preparation.

Disease Mechanisms

ALDH7A1 encodes for α-aminoadipic-semialdehyde dehydrogenase (AASAD) (also known as ATQ), the function of which lies in the catabolism of lysine. AASAD catalyzes the

conversion of AASA and P6C into α-aminoadipic acid. In ATQ deficiency, accumulating P6C results in a spontaneous chemical reaction with pyridoxal phosphate (PLP) and its inactivation via formation of a P6C-PLP complex. The accumulation of AASA, a reactive semialdehyde, might undergo multiple chemical reactions within the cell and thus interact with various metabolic pathways. Finally the biochemical fate and potential toxicity of the PLP/P6C complex is unknown.

To date more than 80 mutations have been reported within the 18 exons of *ALDH7A1* on the HGMD website. Of these, 50-60% are missense mutations, resulting in an altered amino acid sequence. A high number of missense mutations cluster around exons 14, 15, and 16. The missense mutation, p.Glu399Gly in exon 14 occurs in various populations and accounts for about 30% of published alleles [12, 23, 26, 27].

Molecular modeling using human ATQ structure indicates that missense mutations can be divided into three categories: affecting NAD+ cofactor binding or catalysis, altering the substrate binding pocket, and potentially disrupting dimer or tetramer assembly. The mutations are expected to have different effects on enzyme activity, however no clear correlation has been established between the genotype, AASA/P6C levels and clinical severity [9, 26].

An example of the different outcomes is shown in three individuals homozygous for p.GluE427Gln. Two were in the neonatal onset group and one in the later-onset and clinically milder group [28].

PLP deficiency explains previous observations of increased levels of glutamate (neuroexcitatory) and decreased levels of GABA (neuroinhibitory) [29, 30] as well as hypoglycemia and lactic acidosis [12, 15] and numerous other metabolic abnormalities observed in patients with ATQ and PNPO deficiency [8, 12] such as hypoglycemias. Finally PLP deficiency has a negative impact on sphingosine-1-phosphatase lyase, which plays a role in the proliferation, differentiation, migration and apoptosis [31]; the expression of ATQ in glial cells may explain the cerebral dysgenesis found in patients with ATQ deficiency [21].

Management

Diagnosis

Patients with unexplained early onset epilepsy, which is poorly responsive to pharmacological treatment, should be assessed for ATQ deficiency. Diagnosis is based on determination of AASA/P6C in urine, plasma and/or CSF. Elevation persists upon treatment with pyridoxine. Thus treatment with pyridoxine should not be withheld until diagnostic samples are collected. Over time pipecolic acid has proven less reliable for diagnosis than AASA, given its limited sensitivity and specificity. Thus pipecolic acid is only a secondary marker, which should only be used in conjunction with AASA.

For diagnostic confirmation, *ALDH7A1* mutation analysis should be performed. If sequencing does not reveal point mutations, molecular testing for insertions/deletions should be performed.

PLP is low in CSF as a consequence of chemical binding with P6C, but low levels are found in various other conditions. Because of the capability of sulfite and sulfocysteine to

bind and inactivate PLP, low PLP levels are also found in molybdenum cofactor and sulfite oxidase deficiency [32].

Differential Diagnosis

AASA is not pathognomonic for ATQ deficiency. Both molybdenum cofactor deficiency and isolated sulfite oxidase deficiency should be considered as differential diagnosis of elevated AASA levels [33, 34], particularly in children with epileptic encephalopathy and atypical response to pyridoxine.

The latter two disorders can be diagnosed by measuring urinary levels of sulphite, sulphocysteine, and xanthine.

Pipecolic acid elevation is also encountered in other inborn errors of metabolism such as disorders of peroxisomal biogenesis, and hyperlysinemia. While pipecolic acid is more often severely elevated in peroxisomal disorders, the degree of elevation is moderate in ATQ deficiency.

First Line Treatment

At first presentation, in the acutely seizing neonate/infant, 100 mg of pyridoxine-HCl can be given intravenously to interrupt status epilepticus. Given the risk of apnea, adequate support for respiratory management is mandatory. EEG monitoring helps to determine the treatment effect. Oral/enteral administration of 30 mg/kg/day (during 3 days) of pyridoxine is an alternative strategy.

Most patients receive pharmacologic anticonvulsants and pyridoxine at the same time. As in such situations improvement or resolution of seizures cannot be clearly attributed to any specific intervention, pyridoxine should be continued until biomarker results are available.

Folinic acid (3-5 mg/kg/d) may be of additional benefit, particularly in case of incomplete response to pyridoxine [9], although the underlying mechanism remains unclear. High dose folinic acid may also exacerbate a seizure disorder, thus caution is required.

Standard Treatment

Standard treatment includes lifelong supplementation of pyridoxine [11]. Dosages between 15-30 mg/kg/day in infants or up to 200 mg/day in neonates and 500 mg/day in adults have proven effective and safe. Dosages down to 5 mg/kg/day have proven effective in single cases, thus assessment of the optimal therapeutic dosage may help to optimize treatment individually. Double dosages may be given during episodes of (febrile) illness to prevent breakthrough seizures. Long-term administration of higher doses may cause peripheral sensory and motor neuropathy and should be avoided. Adverse effects, including increased seizure activity have been reported in cases with high pyridoxine or PLP intake [35, 36].

In patients with incomplete seizure control, additional pharmacologic anticonvulsants may be required.

Supportive/Alternative Treatment

Although the mechanism underlying folinic acid responsiveness has not been elucidated, folinic acid (3-5 mg/kg/day in neonates; 10-30 mg/day in older patients) may have potential benefit in the presence of incomplete pyridoxine responsiveness or breakthrough seizures.

Pyridoxal phosphate (PLP) has no additional benefit in the treatment of ATQ deficiency, and is more expensive. As PLP has the potential to treat PNPO as well as ATQ deficiency, some centers advocate the use of PLP (30mg/kg/d divided into 3 dosages), as the first line B6 vitamer, while other centers advocate its consecutive use when pyridoxine, given over three consecutive days, has failed to control seizures [37].

Lysine Restricted Diet

While treatment with pyridoxine compensates chemical PLP inactivation, the accumulation of substrates from lysine degradation is not sufficiently reduced (Figure 1). These potentially neurotoxic compounds could be an explanation of the limited efficacy of pyridoxine, as 75-80% of patients suffer developmental delay or intellectual disability (IQ <70) despite excellent seizure control in the majority of patients [38].

In an observational study of 7 patients with ATQ deficiency, an up to 67% reduction in plasma pipecolic acid, 72 and 45 % in urinary and plasma AASA and 42% in plasma P6C levels was achieved upon a low protein (lysine) diet, supplemented with lysine free amino-acid protein equivalents [39].

The results from the study show that dietary lysine restriction (evidence level IV): 1) is tolerated without adverse events; 2) leads to significant decrease of potentially neurotoxic biomarkers in different body compartments; and 3) has potential benefit for seizure control and neurodevelopmental outcomes.

Recently, recommendations for implementation of this diet along with monitoring and follow-up have been developed [40], adapting treatment guidelines for glutaric aciduria type I [41], another inborn error of metabolism affecting lysine catabolism. Started as early in life as possible, daily lysine intake should be prescribed at an amount that maintains the plasma lysine level within the lower normal age-dependent reference range [42, 43] and should be supplemented by commercially available lysine-free amino acid formulas approved for use in conditions affecting lysine metabolism. In contrast to GA-I, tryptophan restriction is not indicated in the management of ATQ deficiency.

Prophylactic Pre-/Postnatal Treatment

Intra-uterine treatment of an affected fetus with supplemental pyridoxine (100mg/day) given to the mother during pregnancy has proven effective in preventing intrauterine and neonatal onset seizures in single cases [44, 45], but did not prevent poor cognitive outcome in two affected offspring in a family with a homozygous stop codon in exon 14 (p.Tyr380*).] In an unaffected child, intra-uterine and postnatal prophylactic treatment with pyridoxine was associated with a status epilepticus and encephalopathy, which reversed after treatment was discontinued at the time ATQ deficiency had been ruled out [46].

This observation indicates that in healthy newborns, high-dose treatment with pyridoxine may result in increased rather than decreased neuro-excitability. Thus postnatal prophylactic pyridoxine treatment of fetuses and neonates at risk for PDE should be limited to the shortest possible interval, via prenatal or immediate postnatal biochemical/genetic testing.

Outcome

On pyridoxine mono-therapy, despite resolution of seizures and initiation of treatment, 80% of affected individuals suffer developmental delay and various degrees of intellectual

disability [47]. The degree of intellectual disability can vary within the same family [48]. Pyridoxine-treated patients group into 3 types of outcomes [26]: 1) complete seizure control and normal developmental outcome (minority); 2) complete seizure control and developmental delay/intellectual disability; 3) incomplete seizure control and developmental delay/intellectual disability.

PNPO Deficiency

Disease Characteristics

Clinical Presentation

A comprehensive overview of the clinical, biochemical and genetic features is provided in two recent studies including 15 and 11 patients respectively [7, 8]. The clinical presentation of PNPO deficiency is indistinguishable from ATQ deficiency. Neonatal epileptic encephalopathy is frequently associated with prematurity and there seems to be a high rate of infertility and miscarriage in mothers of affected patients. Seizure onset is either in the neonatal period or within the first 6 months of life. Seizures may be tonic, clonic and myoclonic, focal or generalized, with burst suppression patterns and hypsarrhythmia in the EEG. Systemic comorbidities include anemia, coagulopathy, hypoglycemia and lactic acidosis giving rise to the suspicion of a primary mitochondrial disease. Based on clinical responsiveness there seem to be at least 3 groups of patients: patients who exclusively respond to PLP; patients who respond to pyridoxine and PLP; patients who respond to pyridoxine, but deteriorate upon PLP.

Biochemical Markers

Specific abnormalities are expected in B6 vitamer patterns in urine, plasma and CSF. Ormazabal et al., [49] identified clearly decreased PLP values in CSF of affected neonates. A distinct elevation of pyridoxamine, pyridoxamine-phosphate, pyridoxine and pyridoxine-phosphate was found in 2 patients with genetically proven PNPO deficiency on treatment with 30mg/kg/d PLP [25]. These changes are explained as a result of the enzyme defect resulting in accumulation of its B6 vitamer substrates and their precursors.

Because PLP is an essential cofactor of aromatic amino-acid decarboxylase (AADC), reduced synthesis in PNPO deficiency results in AADC-like neurotransmitter changes, including markers of low serotonin (5-hydroxyindolacetic acid, HIAA) and dopamine (homovanillic acid, HVA), and elevated 3-*O*-methyldopa (3OMD), vanillactic acid, and 5-hydroxytryptophan as sign of accumulating substrates [13].

Disease Mechanisms

PNPO catalyses the synthesis of PLP from pyridoxine and pyridoxamine/their phosphorylated forms (Figure 2). It requires flavin mononucleotide (riboflavin-5'-phosphate) as a tightly bound (prosthetic) cofactor. Because of the inability to produce sufficient amounts of pyridoxal out of pyridoxine and pyridoxamine, patients with PNPO deficiency have reduced capacity to generate PLP, which is the active form of vitamin B6. Currently less than 20 mainly missense mutations are known with various mechanisms of functional impairment

which potentially explain the various clinical response patters to pyridoxine and PLP. Mutations affecting the binding site of pyridoxine might explain why some patients respond to pyridoxine [8, 50].

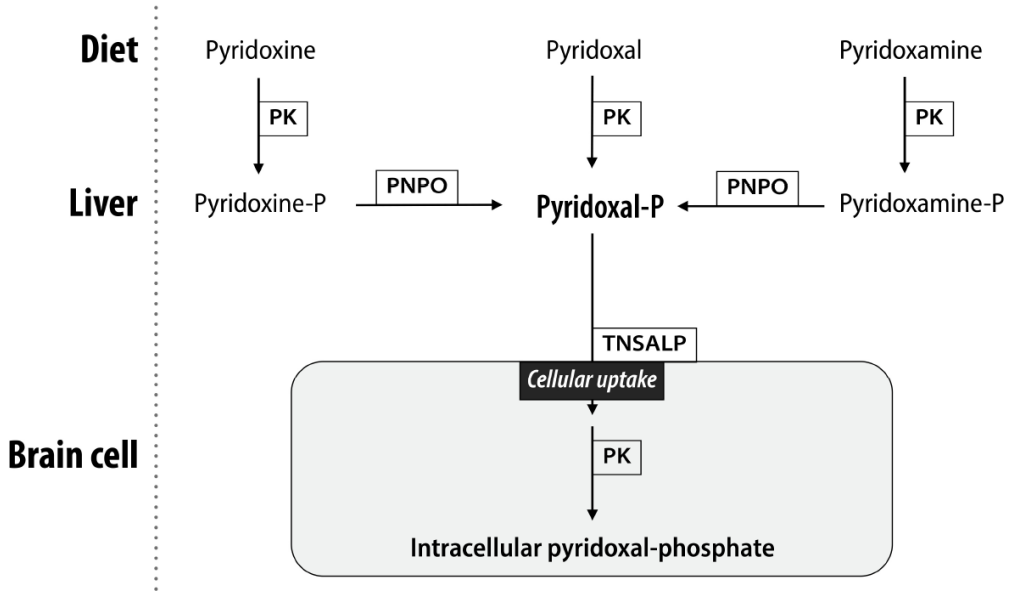

Figure 2. Metabolism of pyridoxine and its vitamers. Pyridoxal phosphate (PLP) is synthesized from dietary pyridoxine, pyridoxal, or pyridoxamine by pyridoxal kinase (PK) and pyridox(am)ine 5'phosphate oxidase (PNPO). Membrane-bound tissue non-specific alkaline phosphatase (TNSALP) is involved in cellular uptake. In PNPO deficiency, the inability to produce sufficient amounts of pyridoxal out of pyridoxine and pyridoxamine, leads to insufficient amounts of PLP, the active vitamer involved in numerous enzymatic reactions *(Source: adapted from [37]).*

Management

Diagnosis

There is no specific diagnostic marker for PNPO deficiency. Thus in every patient at risk, molecular analysis of the PNPO gene is required to establish the diagnosis. Clinical responsiveness to pyridoxine does not exclude PNPO deficiency. Thus patients with PDE negative for ATQ deficiency should be tested for PNPO deficiency. Because seizure exacerbation has been reported upon high PLP dosages, PNPO mutation analysis might be considered in this type of patients, too.

Measurement of B6 vitamers in plasma/CSF will play a future role in the diagnosis of PNPO deficiency [25]. More patient data are needed to describe the biochemical features of PNPO deficiency.

Table 1. Typical clinical characteristics of pyridoxine/pyridoxal-phosphate dependent epilepsies

System	Symptom	ATQ Def	PNPO Def
Seizures	Pharmaco-resistant seizures/status epilepticus	+	+
	Intrauterine/neonatal/early infantile onset	+	+
	Pyridoxine responsive	+	(+)
	PLP responsive	(+)	+
Encephalopathy	Restless/sleeplessness	+	(+)
	Vomiting	+	+
	Perinatal distress	+	+
Neurologic/Behavioural	Developmental delay/Intellectual disability	+	+
	Hypotonia/Spasticity	+	+
	Autism	+	+
Neuroimaging / Neuropathology	Hypo-/aplasia corpus callosum	+	(+)
	Cortical dysplasia	+	(+)
	White/grey matter atrophy	+	(+)
	White matter signal abnormalities	+	(+)
	Ventriculomegaly, Hydrocephalus	+	(+)
	Intracerebral hemorrhages	+	(+)
Routine Laboratory	Hypoglycemia	+	+
	Lactic acidemia	+	+
	Electrolyte disturbances	+	+
	Coagulopathy	+	+
	Anemia	+	+
Other	Prematurity	(+)	+
	Parental infertility	(-)	+

Antiquitin (ATQ) and Pyrox(am)ine 5'-phosphate oxidase (PNPO) deficiency (Def). ()= limited evidence.

Table 2. Biochemical markers of pyridoxine/pyridoxal-phosphate dependent epilepsies

Marker	Compound	Preferred Sample	ATQ Deficiency	PNPO Deficiency
Primary	AASA pretreatment AASA posttreatment	U,P,CSF /	↑ ↑	Normal Normal
	PLP pretreatment PLP posttreatment	P,CSF)	↓ (↓)	↓ Normal-↑
Secondary	PA pretreatment PA post treatment	P,CSF	↑ Normal/↑	Normal Normal
	3-OMT	CSF	?	↑
	Vanillactic acid	U	?	↑
	5-HIAA	CSF	?	↓
	HVA	CSF	?	↓
	Threonine	CSF	↑	↑

Antiquitin (ATQ) and Pyrox(am)ine 5'-phosphate oxidase (PNPO) deficiency. AASA= α–Aminoadipic semialdehyde; PA= Pipecolic acid; PLP= Pyridoxal phosphate; 3-OMT= 3-Orthomethyltyrosine/Methoxytyrosine; 5-HIAA= 5-Hydroxyindol acetic acid; HVA=Homovanillic acid; U= Urine; P= Plasma; CSF= Cerebrospinal fluid; ↑= elevated; ↓= reduced; (↑)= moderately elevated; (↓)= moderately reduced; ?= unknown.

Table 3. Treatment of ATQ and PNPO deficiency (modified after *[11]*)

ATQ Deficiency				
Medication	Dosage	Indication	Monitoring	Pitfalls/Managment
Pyridoxine *IV*	100 mg Single dosages	Interruption of initial status epilepticus, or of prolonged break through seizures	EEG if available	May result in respiratory arrest. Administer only upon readiness of respiratory support
Pyridoxine *Oral/Enteral*	15-30 mg/ kg/d Divided in up to 3 single dosages Up to 200 mg/d in neonates, and 500 mg/d in adults	Long term treatment	Clinical and electrophysiological signs of neuropathy	Continue with dosages above the range only if high dosage has proven essential for effective seizure control
Pyridoxine *Prenatal Maternal*	100 mg/d	Prevention of intrauterine seizures and irreversible brain damage Start in early pregnancy, continue throughout pregnancy in case of positive prenatal diagnosis or if no prenatal diagnosis has been performed	Monitor for seizures and encephalopathy after delivery in NICU/SCN setting.	Continue oral/enteral pyridoxine supplementation at 30 mg/kg/d immediately after birth and immediately initiate biochemical and molecular genetic investigations to prove or rule out ATQ/PNPO deficiency.
Pyridoxal-phosphate *Oral/Enteral*	30 mg/kg/d Divided in up to 3 single dosages	Interruption of initial status epilepticus if pyridoxine has failed Long term treatment: Alternative to pyridoxine	Liver dysfunction No neuropathy reported upon PNP thus far	Continue treatment or switch to pyridoxine in confirmed ATQ deficiency.
Folinic acid *Oral/Enteral*	3-5 mg/kg/d Divided in up to 3 single dosages	Additional therapy if pyridoxine or PLP failed to control seizures	No particular monitoring	May cause/exacerbate seizures
Lysine restricted diet	Daily lysine intake at an amount that maintains the plasma lysine level within the lower normal age-dependent reference range, preferably in the lower quartile [40].	In ATQ deficient patients except those with normal development and seizure control. Goal: reduction of AASA/P6C accumulation; improvement of neurodevelopmental outcomes and seizure control.	AASA/P6C (U, CSF) Aminoacids (P) Pre-albumin, albumin, plasma amino acids; complete blood count; micronutrients Weight/Height	Lysine deficiency Tryptophane deficiency if lysine free aminoacid formulas for GAI are used (also low in tryptophane)
PNPO Deficiency				
Pyridoxal-phosphate *Oral/Enteral*	60 – 100 mg/kg/d Divided in up to 3 single dosages	Interruption of status epilepticus/epileptic encphalopathy	EEG if available	No reports about respiratory arrest known in PNPO deficiency
Pyridoxal-phosphate *Oral/Enteral*	30-60 mg/kg/d Divided in up to 3 single dosages	Long term treatment	Liver function test No neuropathy reported upon PNP thus far.	Abnormal liver function test and liver fibrosis/cirrhosis have been reported on high doses

IV= intravenous; SD=single dosage; PLP=pyridoxal phosphate.

Treatment

Supplemental pyridoxal is available as pyridoxal phosphate. The usual administered dosages of pyridoxal phosphate vary between 30 and 60 mg/kg/day. In a series of patients published by Mills et al. [8], dosages between 10 and 85 mg/kg/d were given at initiation of treatment, and 10-70 mg/kg/d as maintenance. Because PNPO is a riboflavin (flavin mononucleotide) dependent enzyme, patients might benefit from riboflavin supplementation, particularly in case of a mutation affecting the binding site to this co-factor. There are no reports about prenatal treatment of PNPO deficiency with PLP or pyridoxine.

Safety of PLP

High PLP dosages are toxic to the liver [51]. Abnormal liver function tests, liver fibrosis and portal hypertension have been observed in 3 patients upon PLP dosages up to 100 mg/kg [8, 34]. 3 patients in Mills' series [8] experienced deterioration of seizures once switched from pyridoxine to PLP.

Outcome

There is limited information about long-term outcomes of treated PNPO deficiency. Compared to ATQ deficiency, outcomes seem to be less favorable. Seizure control is variable. Most children show marked developmental delay/intellectual disability, but there are also patients with minor or absent cognitive impairments [7, 8, 37]. Of the 4 published adult patients [7, 8], one 26-year old has a normal IQ despite recurrent status epilepticus during childhood [7], while another 41-year old has dyslexia and high functioning autism [8].

Step-by-Step Procedures and Pitfalls

In neonates and infants with epileptic encephalopathy/intractable seizures, the following step-by-step procedures are instrumental to provide adequate treatment to individuals affected by ATQ or PNPO deficiency:

1. Pyridoxine 15-30 mg/kg/d orally/enterally, divided into 3 single dosages (maximal 200 mg for neonates and infants) over several days, or in case of intractable status epilepticus: Pyridoxine hydrochloride 100 mg intravenously as single or repeated injection

   Pitfall 1: Risk of apnea particularly when pyridoxine is given intravenously.
   Pitfall 2: Peripheral neuropathy when given at high dosages over long time.
   Pitfall 3: Patients with molybdenum cofactor deficiency can have a similar clinical presentation and response to pyridoxine.

2. If no/insufficient response to pyridoxine: Pyridoxal phosphate 60 mg/kg/d orally/enterally, divided into 3 single dosages (dosages up to 100 mg/kg have been given) over several days.

   Pitfall 1: Adverse reaction to PLP with deterioration of seizures.

Pitfall 2: Liver dysfunction when given at high dosages over long time.

3. For diagnostic confirmation of ATQ deficiency, urinary AASA and/or plasma pipecolic acid is determined any time prior or during treatment with pyridoxine/PLP. Positive or ambiguous results are confirmed/followed-up by ATQ mutation analysis

   Pitfall 1: AASA is also elevated in molybdenum cofactor/sulfite oxidase deficiency
   Pitfall 2: Particularly upon ongoing treatment with pyridoxine, pipecolic acid is only moderately elevated and ATQ cases with normal levels have been reported.

4. PNPO mutation analysis is performed if ATQ deficiency has not been confirmed/if there is selective response to PLP.

5. Determine B6 Vitamers in plasma/CSF

   Pitfall 1: PLP is not informative once treatment has started
   Pitfall 2: Limited data are available in literature from patients with PNPO
   Pitfall 3: Numbers of labs offering B6 vitamers as diagnostic test are limited.

6. Consider institution of lysine restricted diet supplemented with lysine free aminoacid formula.

   Pitfall 1: Limited data are available to prove the clinical evidence of benefit. To facilitate evidence generation, treat/monitor your patient according to protocols previously suggested [40].
   Pitfall 2: Some commercially available lysine free aminoacid formulas are also free of tryptophan. Make sure you supplement tryptophan when using such formulas

7. Consider prenatal treatment in pregnancies at risk.

   Pitfall 1: High dosages of pyridoxine can result in seizures in unaffected neonates [46]. Aim at confirmation of diagnosis as soon as possible.

Conclusion

ATQ and PNPO deficiency are treatable causes of epilepsy and intellectual disability [52]. Untreated these conditions result in severe and irreversible developmental and neurologic deficits. Although rare, every child with unexplained epilepsy with onset in the neonatal period or early infancy should be tested for Vitamin B6 responsiveness. Treatment with pyridoxine/pyridoxal phosphate significantly improves their outcomes. Systematic testing of infants at risk will result in a better understanding of the frequency of these disorders.

Newborn screening has become technically feasible [53] for ATQ deficiency. Once implemented it will be instrumental to effectively determine the frequency of this treatable

condition. Collaborative observational studies are needed to better understand long-term outcomes and to create better evidence of the potentials of new treatment approaches, such as the lysine restricted diet for ATQ deficiency or prenatal treatment with pyridoxine/PLP.

Due to the complex metabolic pathway of Vitamin B6 and its importance in brain metabolism, it is likely that more genetic defects causing unexplained vitamin B6 responsive seizures are discovered in the future.

Disease Box

Disease name	Antiquitin deficiency/α-Aminoadipic-semialdehyde dehydrogenase deficiency
Abbreviation	AASADH
Alternative name	Pyridoxine-dependent Epilepsy
Affected protein	α-Aminoadipic-semialdehyde dehydrogenase (E.C. 1.2.1.3)
Gene	*ALDH7A1*
Chromosomal localization	5q31
Substrate(s)	α-Aminoadipic-semialdehyde Piperidine-6-Carboxylate
Product(s)	α-Aminoadipic acid
OMIM# protein	
OMIM# phenotype	266100
ICD+	

Disease name	Pyridox(am)ine-5'-phosphate oxidase deficiency
Abbreviation	PNPO
Alternative name	Pyridoxalphosphate responsive (dependent) epilepsy
Affected protein	
Gene	*PNPO*
Chromosomal localization	17q.21.32
Substrate(s)	Pyridoxine phosphate, Pyridoxamine Phosphate
Product(s)	Pyridoxal phosphate
OMIM# protein	
OMIM# phenotype	610090
ICD+	

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