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## Minireview

## Pyridoxine dependent epilepsy and antiquitin deficiency Clinical and molecular characteristics and recommendations for diagnosis, treatment and follow-up

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## ABSTRACT

Antiquitin (ATQ) deficiency is the main cause of pyridoxine dependent epilepsy characterized by early onset epileptic encephalopathy responsive to large dosages of pyridoxine. Despite seizure control most patients have intellectual disability. Folinic acid responsive seizures (FARS) are genetically identical to ATQ deficiency. ATQ functions as an aldehyde dehydrogenase (*ALDH7A1*) in the lysine degradation pathway. Its deficiency results in accumulation of  $\alpha$ -aminoadipic semialdehyde (AASA), piperidine-6-carboxylate (P6C) and pipercolic acid, which serve as diagnostic markers in urine, plasma, and CSF.

To interrupt seizures a dose of 100 mg of pyridoxine-HCl is given intravenously, or orally/enterally with 30 mg/kg/day. First administration may result in respiratory arrest in responders, and thus treatment should be performed with support of respiratory management.

To make sure that late and masked response is not missed, treatment with oral/enteral pyridoxine should be continued until ATQ deficiency is excluded by negative biochemical or genetic testing. Long-term treatment dosages vary between 15 and 30 mg/kg/day in infants or up to 200 mg/day in neonates, and 500 mg/day in adults. Oral or enteral pyridoxal phosphate (PLP), up to 30 mg/kg/day can be given alternatively. Prenatal treatment with maternal pyridoxine supplementation possibly improves outcome.

PDE is an organic aciduria caused by a deficiency in the catabolic breakdown of lysine. A lysine restricted diet might address the potential toxicity of accumulating  $\alpha$ AASA, P6C and pipercolic acid. A multicenter study on long term outcomes is needed to document potential benefits of this additional treatment.

The differential diagnosis of pyridoxine or PLP responsive seizure disorders includes PLP-responsive epileptic encephalopathy due to PNPO deficiency, neonatal/infantile hypophosphatasia (TNSALP deficiency), familial hyperphosphatasia (PIGV deficiency), as well as yet unidentified conditions and nutritional vitamin B<sub>6</sub> deficiency. Commencing treatment with PLP will not delay treatment in patients with pyridox(am)ine phosphate oxidase (PNPO) deficiency who are responsive to PLP only.

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## 1. Introduction

Pyridoxine dependent epilepsy (PDE) (MIM#266100) is an autosomal recessive epileptic encephalopathy characterized by a therapeutic response to pharmacological dosages of vitamin B<sub>6</sub> and resistance to conventional antiepileptic treatment. PDE was first described in 1954 in an infant with therapy-resistant seizures who showed prompt cessation of seizures after the administration of a multivitamin cocktail containing vitamin B<sub>6</sub> [1]. Since then, over 200 patients have been described in the literature. The underlying genetic defect remained unknown for a long time and diagnosis was limited to the demonstration of seizure remission and relapse after a controlled trial of pyridoxine administration and withdrawal [2]. Due to the lack of a biological diagnostic marker, diagnosis may have been missed in many cases. The variation in diagnostic hits is reflected in the considerable heterogeneity of published prevalence data, ranging from 1:20.000 in a German center with a pyridoxine trial routinely performed in all patients with epileptic encephalopathy, [3] to 1:400.000 in a survey focusing on diagnosed cases in Dutch neuropsychiatric clinics, [4] and 1:600.000 in the UK [5]. In a hospital based study 7.4% (6 out of 81) children with intractable seizures below 3 years of age, showed a clear response to pyridoxine [6].

Notably, despite the clear response of seizures to high dosages of vitamin B<sub>6</sub>, patients with PDE do not have biochemical evidence of vitamin B<sub>6</sub> deficiency [7,8]. For a long time deficiency of glutamic acid decarboxylase (GAD), catalyzing the conversion of glutamate to GABA and requiring vitamin B<sub>6</sub> (pyridoxal-phosphate) as cofactor, was considered the underlying cause of PDE [9]. However, conflicting results of glutamate and GABA studies in CSF [8,10,11] and negative linkage studies to the two GAD isoforms in the brain (Gad1 and Gad2) [12,13] made clear that GAD deficiency is not the primary cause of PDE.

Following the description of pipecolic acid as a first diagnostic marker of PDE [14] mutations in the gene for  $\alpha$ -aminoadipic-semialdehyde dehydrogenase and resultant enzyme deficiency were

identified as the major underlying genetic cause of PDE [15]. Since then this association has been confirmed in numerous cases ascertained clinically with PDE [16–27].

$\alpha$ -Aminoadipic-semialdehyde dehydrogenase (also known as ALDH7A1 or antiquitin, ATQ) is encoded by the *ALDH7A1* or *ATQ* gene, and its function lies in the catabolism of lysine. The direct link to amino acid metabolism provides new insights into the pathophysiology of PDE and clues for improved diagnostic and therapeutic options for this condition.

We reviewed the current state and new developments in diagnosis and treatment of PDE and ATQ deficiency. This article provides an overview of the current knowledge of clinical, biochemical, and molecular genetic characteristics of ATQ deficiency and summarizes recommendations for diagnosis and management.

## 2. Clinical presentation of antiquitin deficiency

The clinical phenotype of ATQ deficiency is that of pyridoxine dependent epilepsy, characterized by intractable seizures that are not controlled with conventional anticonvulsants but that respond clinically and electroencephalographically to pharmacologic doses of pyridoxine [28].

### 2.1. Classical presentation

Classically, PDE presents with neonatal or early infantile seizures or epileptic encephalopathy refractory to common anticonvulsants, but responsive to high dosages of pyridoxine. A single dosage of 50–100 mg administered intravenously often results in cessation of seizures within minutes. A transient coma concomitant with seizure cessation is characteristic for PDE, but does not always occur [29,30]. Coma has not only been reported after intravenous administration of pyridoxine, but in single cases also after oral administration [31]. Non-responders do not exhibit this feature. Repeat dosages may be needed in some patients to interrupt seizures while in others very small dosages may result in

complete response [29,31]. Seizures usually recur when pyridoxine is stopped either incidentally or for a diagnostic withdrawal. Time intervals between 1 and 51 days have been reported [22,32]. Although dramatic presentations consisting of prolonged seizures and recurrent episodes of status epilepticus are typical, recurrent self-limited events including partial seizures, generalized seizures, atonic and myoclonic seizures, and infantile spasms may occur.

Seizure types are variable even in the individual patient, ranging from myoclonic to clonic and bilateral tonic clonic seizures, and partial seizures, and a propensity to develop status epilepticus [33–35]. EEG patterns may vary from normal to high voltage delta activity, focal spike wave discharges, burst suppression patterns and rarely hypsarrhythmia [22,34,36,37]. In some patients movements suggestive of clinical seizures may not be accompanied by epileptic EEG changes [38]. This suggests that some of the movements may not be epileptic in origin or imply a very deep focus. Recent EEG studies by Naasan et al. [39] show that burst suppression pattern after cessation of seizures can persist for up to five days after initiation of pyridoxine. Neonates with PDE frequently have periods of encephalopathy with marked irritability, fluctuating tone, sleeplessness and emesis that precede the onset of clinical seizures. If hyperalertness is observed, misdiagnosis of drug withdrawal syndrome may occur [40]. A retrospective analysis of video EEG recordings from 140 neonates with seizures and 5 patients with PDE or PLP responsive epileptic encephalopathy (PNPO deficiency) showed that multifocal and generalized myoclonic jerks, abnormal eye movements, grimacing and irritability are characteristic features of PDE and PLP responsive epilepsy [41]. Intrauterine onset of seizures, [2] low Apgar scores and low cord blood gases at birth may result in a picture consistent with neonatal hypoxic ischemic encephalopathy [5,42]. In this instance, a preliminary diagnosis of hypoxic ischemic encephalopathy may prevent further diagnostic workup of neonatal seizures and responsiveness to pyridoxine may not be tested at all.

Neonatal lactic acidosis, hypoglycemia, profound electrolyte disturbances, hypothyroidism, and diabetes insipidus have been reported along with PDE [22,43]. As most of these disturbances have improved upon pyridoxine treatment, a direct relation to ATQ deficiency is plausible. A primary gluconeogenesis defect was initially suspected in one case with instantaneous correction of severe neonatal hypoglycemia and lactic acidosis after administration of intravenous glucose [43].

Cranial magnetic resonance imaging shows a spectrum of changes from normal to hypoplasia of the corpus callosum, megacisterna magna [44], enlarged ventricles and diffuse cerebral hemispheric gray and white matter atrophy [22,45]. Mesial temporal sclerosis [46] and cortical dysplasia [2] have been described in a few individual cases. White matter involvement includes pronounced supratentorial white matter changes in newborns with a tendency to resolve with treatment and periventricular dysmyelination is common in older children [47,48].

## 2.2. Atypical presentations

Atypical presentations may account for 1/3 of cases with PDE [49,50]. These include late onset of seizures at up to 3 years of age [51–53], autism, initial response to common anticonvulsants, especially phenobarbital [53] or response to extremely low doses of pyridoxine [1,31]. ATQ gene mutations have been confirmed in late onset cases with PDE [19] indicating that atypical presentations indeed are part of the phenotypic ATQ spectrum.

## 2.3. The outcome of treated patients

Outcome of treatment is variable. While individuals with normal intellectual function have also been reported, [21,25,42,50] most patients, despite seizure control have developmental delay and

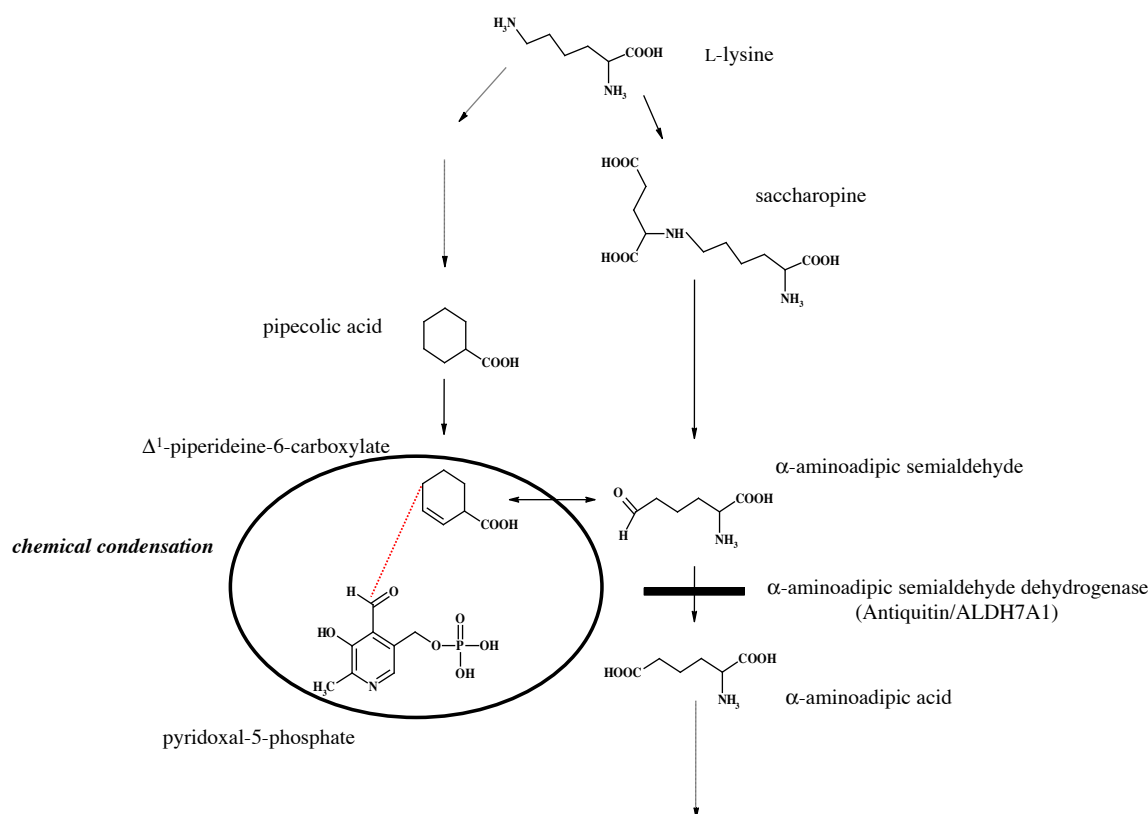
intellectual disability. It has been suggested that an earlier onset of clinical seizures has a worse prognosis for cognitive function, and the length of the delay in diagnosis and initiation of effective pyridoxine treatment correlates with increased handicaps [2,25,50]. Few formal psychometric assessments have been performed, but these limited studies indicate that verbal skills are more impaired than nonverbal skills [54,55]. In a cohort of 63 clinically ascertained North American cases surveyed by questionnaires, 47 provided data on mental outcome. In the absence of objective neuropsychological test results, 8 patients with PDE were termed normal, 16 had severe neurodevelopmental impairment and the remainder had some degree of impairment and required special education [50]. There is no clear correlation between outcome and time of diagnosis. Patients treated as early as in the neonatal period may have developmental delay and intellectual disability, whereas other patients with prolonged status epilepticus and later diagnosis may have a normal IQ [32]. Summarizing all these outcomes, Schärer et al. [21] distinguish three different phenotypes in pyridoxine treated patients: 1) complete seizure control and normal developmental outcome; 2) complete seizure control and developmental delay or intellectual disability; and 3) incomplete seizure control and developmental delay or intellectual disability.

## 3. Folinic acid responsive seizures (FARS) are genetically identical to ATQ deficiency

Recently FARS were shown to be genetically identical to ATQ deficiency [16]. FARS were first described in 1995, in patients with intractable seizures and encephalopathy who had two characteristic, but yet unidentified peaks (peak X) in the HPLC chromatogram for CSF monoamine neurotransmitter analysis. Patients showed an improvement of seizures upon administration of folinic acid (3–5 mg/kg/day), but the genetic basis of this condition remained elusive [56]. Two patients, whose CSF showed the marker of folinic acid-responsive seizures, responded clinically to pyridoxine. Both patients had increased levels of  $\alpha$ AASA and pipercolic acid in CSF, and known or presumably pathogenic mutations in the ATQ (*ALDH7A1*) gene, all being consistent with ATQ deficiency [16]. Retrospective analysis of the seven individuals described with this diagnostic entity [56–58] shows a clear response to pyridoxine in those tested. Outcome on folinic acid monotherapy was poor in two patients with early death, while the remaining five patients who were on pyridoxine comedication all survived with variable degrees of neurocognitive impairment. Interestingly one patient remained seizure free on pyridoxine monotherapy and had never received folinic acid [56]. Analysis of CSF samples from seven anonymous individuals, originally diagnosed with FARS, showed similar biochemical results and genetic testing confirmed ATQ deficiency [59]. Retrospectively peak X has been systematically observed in patients with PDE, indicating that this too can be a reliable biomarker (K Hyland, personal communication). The mechanism of folinic acid treatment in PDE is not understood to date, nor has the nature of peak X been elucidated. Experiments showed that peak X is neither  $\alpha$ AASA nor the P6C-PLP complexation product (Struys, Jakobs, Hyland unpublished).

## 4. Metabolic function of antiquitin

ATQ (*ALDH7A1*) takes part in lysine catabolism in the brain and in the liver (Fig. 1). In humans there are two biochemical pathways for lysine catabolism: the saccharopine pathway, which is dominant in the liver and many other tissues [60]; and the pipercolic acid pathway, which is dominant in brain (reviewed in [61]). There seems to be separation of both pathways in different cell compartments. While the saccharopine pathway is mitochondrial, the pipercolic acid pathway has been located mostly in the peroxisomal compartment [61]. Convergence of the two pathways occurs at the level of  $\alpha$ AASA formation, which occurs in the cytosol.  $\alpha$ AASA is in spontaneous



**Fig. 1.** Outline of the metabolism of L-lysine via the saccharopine and pipecolic acid pathways and biochemical pathophysiology of antiquitin (ATQ) deficiency: The two pathways converge where L-Δ<sup>1</sup>-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 (α-AASA-DH = ALDH7A1 = ATQ). P6C undergoes chemical condensation with pyridoxal phosphate (PLP) resulting in PLP deficiency. Pipecolic acid (PA) accumulates due to backpressure from the enzymatic block.

equilibrium with Δ<sup>1</sup>-piperideine-6-carboxylic acid (P6C) and is oxidized by ATQ to α-aminoadipic acid, which is eventually oxidized to produce acetyl CoA [62]. Developmentally, in fetal rat brain the pipecolic acid pathway is 2–3 folds higher compared to liver [63], whereas in the liver the saccharopine pathway is low prenatally but rises significantly after birth [63,64]. Lysine is regarded as a nitrogen donor allowing the formation of glutamate out of α-ketoglutarate in the central nervous system [65]. Pipecolic acid is formed in the pipecolic acid pathway and modulates the function of GABA, which is a major inhibitory neurotransmitter [66,67].

ATQ catalyzes the conversion of α-aminoadipic semialdehyde (αAASA) to α-aminoadipic acid (Fig. 1). The reaction requires NAD as cofactor. Like most aldehyde dehydrogenases, ATQ has relatively broad substrate specificity. In addition to its function in lysine catabolism, ATQ converts betaine aldehyde to betaine, an important cellular osmolyte and methyl donor (Brocker, 2010). It also protects from oxidative stress by metabolizing a number of highly reactive aldehydes derived from lipid peroxidation [68]. ATQ is also able to use other aldehyde substrates, such as acetaldehyde and benzaldehyde, but its affinity for these substrates is much lower than for αAASA indicating these are not preferred substrates [69]. Surprisingly, ATQ has recently been identified as a novel susceptibility gene for osteoporosis via genome-wide association studies [70]. The pathophysiological mechanism, the relation to known ATQ function, or the relation to PDE of this finding are unclear.

## 5. Molecular properties of antiquitin

ATQ is classified as a member of the aldehyde dehydrogenase family 7 (ALDH7) of which there are three subfamilies: ALDH7A for

humans and animals, ALDH7B for plants, and ALDH7C for *Drosophila* [71]. ATQ from seabream has been the prototype for most biochemical and structural work [69,72,73]. The name “Antiquitin” derives from the apparent ancient origin of the protein [74]. The human and rat cDNAs for ATQ were identified through their remarkable similarity to a plant turgor protein [74]. The amino acid sequence of human ATQ has 60% identity with plant ATQ and up to 98% identity with other mammalian ATQ. Plant ALDH7B1 protects against various forms of stress such as salinity, dehydration and osmotic stress. In humans the role of ATQ in lysine catabolism was recognized in 1990 [75], when the enzyme was referred to as L-α-aminoadipate-δ-semialdehyde oxidoreductase. Human ATQ is mitochondrial and cytosolic, a conclusion based on the presence of a potential mitochondrial targeting sequence and cleavage site, as well as localization studies [76,77]. There is also expression of ATQ in the nucleus, but nothing is known about its function at this location [68].

The human ATQ cDNA (NCBI #NM\_001182.3) has an open reading frame of 1620 bp divided among 18 exons. The human gene is located at chromosome 5q31 [12]. Three pseudogenes for ATQ are predicted on the basis of sequence homology [78], but they are unconfirmed. The human cDNA codes for a protein of 539 amino acids. The protein size is based on the NCBI predicted sequence (NP\_001173.2). There remains some uncertainty about the actual translational start site for the human ATQ as there are 3 in-frame potential translation initiation sites. Much of the early work with the recombinant enzyme used the most 3' site. However, analysis of the human sequence using MITOPROT (<http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html>) predicted a mitochondrial cleavage site 3 amino acids upstream from the most 3' site [79]. This would be consistent with the current reported mitochondrial location [77]. NCBI also predicts this site

based on sequence context. The crystal structure of the fish ATQ (PDB 2JG7) reveals an 8-chain asymmetric unit cell composed of two tetramers, each a dimer of dimers [73]. The ATQ tetramer is the functional unit. Each monomer (~58 kDa mass) has three domains: NAD<sup>+</sup> binding, catalysis, and oligomerization. Non-denaturing protein electrophoresis of the seabream ATQ demonstrated an ~250 kDa moiety consistent with the tetrameric structure [69]. The human ATQ structure available in the protein database (PDB 2J6L) is superimposable on that of the fish enzyme. The crystallographic coordinates of human ATQ structure (PDB code:2J6L) is obtained from the RCSB protein Data Bank (<http://www.rcsb.org>). Refinements of this structure are presented by Brocker et al. [68].

## 6. Mutational spectrum of antiquitin deficiency

To date more than 60 different mutations within the 18 exons of the ATQ gene have been published [15–27,80]. Of these, 50–60% are missense mutations, resulting in an altered amino acid in the protein sequence. Missense mutations cluster around exons 14, 15, and 16 [21]. The missense mutation p.Glu399Gln in exon 14 occurs in various populations and accounts for about 30% of published alleles [17,22]. The “silent mutation” p.Val250Val [18] leads to alternate splicing with some residual activity and is prevalent in Caucasian patients [22]. From a total of 36 kindred studied by Plecko et al. [17] and Bennett et al. [19], three cases had only one mutated allele despite elevated  $\alpha$ AASA and pipercolic acid respectively. Most likely this might be due to the limitations of the applied sequencing techniques which may not have picked up larger deletions or deep intronic mutations.

It should be noted that, to date, the mutations have been numbered according to a translational initiation at the most 3' site. If, indeed, as suggested above, translation is actually initiated at the second of the three potential sites, codon numbering may need to be revised by an additional 28 amino acid residues to conform with standardized nomenclature guidelines [81].

## 7. Pathophysiology

The pathophysiology of ATQ deficiency is determined by three different components. First, the accumulation of  $\alpha$ AASA and its heterocyclic form, L- $\Delta^1$ -piperideine-6-carboxylate (P6C) as the primary consequence ATQ deficiency; second, PLP deficiency as a consequence of  $\alpha$ AASA and P6C accumulation; third, the accumulation of pipercolic acid as a secondary consequence of ATQ deficiency (Fig. 1).

The accumulation of P6C leads to a spontaneous, type Knoevenagel, chemical reaction with PLP resulting in the formation of a P6C–PLP complex and thus to depletion of free PLP [15]. Low PLP concentrations have recently been reported by Footitt et al. [82] in patients with ATQ deficiency. Shin et al. [83] showed a significant reduction of PLP in CSF in patients with vitamin B6 related seizures while plasma PLP levels were not different from normal controls and from patients with non-vitamin B6 related seizures. Thus depletion of PLP seems to be mainly occurring in the brain, and CSF to plasma PLP ratio might be an adjuvant marker for diagnosis and treatment monitoring. Low levels of pyridoxal-phosphate were previously measured in the frontal and occipital cortex post mortem in one patient [46]. A comparable chemical inactivation of PLP occurs through accumulation of P5C in hyperprolinemia type 2 [84], and in isoniazid induced vitamin B<sub>6</sub> deficiency [85].

Such chemically induced PLP deficiency has major consequences on a multitude of pyridoxine (PLP) dependent enzyme activities. The Enzyme Commission (EC; <http://www.chem.qmul.ac.uk/iubmb/enzyme/>) has catalogued more than 140 PLP-dependent activities, corresponding to ~4% of all classified activities [86]. Foremost, PLP acts as a cofactor in numerous enzyme reactions facilitating trans-

amination and decarboxylation of amino acids and neurotransmitter precursors. It is a cofactor for liver, muscle and brain glycogen phosphorylase isozymes, thus playing an essential role in the mobilization of carbohydrate reserves in a wide variety of tissues [87]. PLP further acts as a cofactor to serine palmitoyl transferase (SPT) which catalyzes the rate-limiting step in the de novo synthesis of sphingolipids [88]. Finally PLP is essential for sphingosine-1-phosphate (S1P) lyase activity, an enzyme involved in the degradation of S1P, a bioactive lipid molecule which regulates proliferation, differentiation, migration, and apoptosis [89]. Insufficiency of some of these reactions explains previous findings in PDE patients. For example, increased levels of glutamate [11] and decreased levels of GABA [10,46] seem to be secondary to deficient activity of PLP dependent glutamate decarboxylase. Although ATQ deficiency affects lysine catabolism, an accumulation of lysine has not been observed. An increased formation of the excitatory amino acid glutamate out of  $\alpha$ -ketoglutarate and lysine [65] might however contribute to the pathophysiology in the central nervous system. The subsequent imbalance between excitatory (glutamate) and inhibitory (GABA) neurotransmitters could in part account for the encephalopathy and seizure characteristics of PDE [8,9,11]. Elevated levels of threonine, glycine, taurine, histidine, and 3-methoxytyrosine in plasma and/or CSF [22] can also be attributed to secondary PLP deficiency. Increased plasma concentrations of alanine and glutamine suggest impaired ammonia detoxification and increased production of lactate [22]. Indeed severe neonatal lactic acidemia (blood lactate 11 mmol/L) and hypoglycemia (blood glucose 0.6 mmol/L) preceded intractable status epilepticus in a three day-old infant who finally was diagnosed with ATQ deficiency [43]. Notably similar changes have been found in patients with primary PLP responsive epileptic encephalopathy due to pyridoxamine-5'-phosphate oxidase (PNPO) deficiency [22]. Despite these apparent similarities, these changes are more prevalent and more marked in PNPO deficiency than in ATQ deficiency.

The accumulation of pipercolic acid deserves special attention. Pipercolic acid acts as a modulator of GABA with its accumulation potentially contributing to seizure pathophysiology [66,67]. A direct association of pyridoxine and pipercolic acid metabolism was ruled out in earlier studies in pyridoxine-deficient rats demonstrating normal pipercolic acid concentrations in brain tissue despite significantly reduced pyridoxal phosphate concentrations [90]. Currently, pipercolic acid accumulation is considered to be secondary to backpressure from the primary enzyme deficiency [15,17]. This would imply that pipercolic acid in ATQ deficiency is mainly produced in the brain, the site with highest pipercolic acid oxidase activity. This is supported by high CSF/plasma ratios of pipercolic acid in patients with ATQ deficiency [17], while the CSF/plasma ratio is low in peroxisomal disorders such as Zellweger syndrome. Recent research by Struys [91] however, indicates that pipercolic acid is also formed outside the brain in the absence of pipercolic acid oxidase. Formation of pipercolic acid was clearly detected in ATQ deficient fibroblasts, a tissue which naturally lacks pipercolic acid oxidase activity. The authors speculate that accumulating P6C can be converted into pipercolic acid via  $\Delta^1$ -pyrroline-5-carboxylate reductase, an enzyme, which primarily converts pyrroline-5-carboxylate, a metabolite occurring in proline metabolism. In the presence of high concentrations of P6C the affinity of the enzyme might be high enough to react with P6C as well and consequently convert it to pipercolic acid. The pathophysiologic impact to brain dysfunction of these small elevations of pipercolic acid has to be further elucidated before a clear pathophysiological significance can be attributed.

Finally, the accumulation of  $\alpha$ AASA per se may play a pathogenetic role. Due to its chemical nature as a reactive semialdehyde,  $\alpha$ AASA may undergo multiple chemical reactions within the cell and thus interact with various metabolic pathways. The fate of the P6C/PLP complex is unknown. It is not known to what extent this compound is eliminated from the cell or what are its further degradation

products. Thus potentially this complex could also be toxic and toxicity might paradoxically be enhanced by pyridoxine treatment. Finally, the impact of the enzyme deficiency on substrates other than AASA and their potential relation to pathophysiology have not been researched.

## 8. Diagnostic markers

Both  $\alpha$ AASA [15,17,22,92,93] and pipercolic acid [14,32,94] serve as diagnostic markers of ATQ deficiency.

### 8.1. Elevated $\alpha$ AASA

Elevated  $\alpha$ AASA is pathognomonic for ATQ deficiency. Levels are elevated in urine [92,93], plasma [94] and CSF [16]. Due to the unstable nature of  $\alpha$ AASA, it is in spontaneous equilibrium with P6C and exact quantification of  $\alpha$ AASA and P6C is not feasible. The original analytical procedure [15,22] was described in urine. After derivatization of the  $\alpha$ AASA molecule with fluorenylmethyloxycarbonyl chloride, the sample is directly analyzed by LC–MS/MS. Although this method is semiquantitative, it provides a sensitive and specific diagnostic tool [17,92,93]. Levels of urinary  $\alpha$ AASA in normal individuals decline during the first year of life: 0–0.5 years: <2 mmol/mol creatinine; 0.5–1 year:  $\alpha$ AASA <1 mmol/mol creatinine; >1 year  $\alpha$ AASA <0.5 mmol/mol creatinine. Pathological values for  $\alpha$ AASA are several folds above the upper limit of the appropriate reference range and seem to depend on the nature of the mutation, and the child's age, treatment with pyridoxine, and possibly on nutritional lysine intake. Since the description of the original tandem mass spectrometry based method, a gas-chromatographic mass spectrometric method as an alternative way to measure urinary  $\alpha$ AASA has been developed [95]. Recently, based on the originally described tandem mass spectrometry method [15], a method has been developed which allows simultaneous measurement of  $\alpha$ AASA, P6C and pipercolic acid in plasma [94]. In five previously confirmed patients, the concentrations of  $\alpha$ AASA and P6C were significantly elevated compared to those of controls (<0.2  $\mu$ mol/L, <1.7  $\mu$ mol/L respectively). In particular, plasma concentrations of P6C ranged from 3 to 28.4  $\mu$ mol/L. The stability study showed that  $\alpha$ AASA and P6C in blood samples were unstable at room temperature degrading within a few hours. A careful sample handling with immediate freezing is critical for reliable results.

### 8.2. L-Pipercolic acid (piperidine-2-carboxylic acid)

L-Pipercolic acid is another characteristic biomarker for ATQ deficiency [14,17,32,96,97] but elevated concentrations of this imino acid are also encountered in other inborn errors of metabolism, e.g. generalized peroxisomal dysfunction [98], hyperlysinemia, and defects of proline metabolism [99]. Furthermore pipercolic acid can be elevated due to liver disease or dysfunction, and in patients without any apparent cause, giving it a good sensitivity but low specificity. Pipercolic acid is measured quantitatively with GC–MS or tandem mass spectrometry based quantitative methods [99–101]. Reference values have been established for plasma: 2.5 + 1.25  $\mu$ mol/L; for urine: 0–6 mmol/mol creatinine; for CSF <12  $\mu$ mol/L [99]. Values are strongly age dependent [100]. Normal values have been established in CSF (mean = 0.041  $\mu$ mol/L, range 0.010–0.120  $\mu$ mol/L), in plasma of at term infants (age less than 1 week, mean = 5.73  $\mu$ mol/L, range 3.75–10.8  $\mu$ mol/L; age greater than 1 week, mean = 1.46  $\mu$ mol/L, range 0.70–2.46  $\mu$ mol/L), in urine of at term infants (age less than 6 month, mean = 32.5  $\mu$ mol/mol creatinine, range 9.81–84.5  $\mu$ mol/mol creatinine; age greater than 6 month, mean = 6.35  $\mu$ mol/mol creatinine, range 0.15–13.6  $\mu$ mol/mol creatinine) and in amniotic fluid (mean = 4.65  $\mu$ mol/L, range 2.24–8.40  $\mu$ mol/L). Plasma and CSF pipercolic acid are clearly elevated in patients with ATQ deficiency. Pipercolic

acid in plasma was 4.3- to 15.3 fold elevated compared to the upper normal range before pyridoxine and remained in the mildly elevated range while on pyridoxine in six unrelated patients with PDE finally confirmed as ATQ deficiency [32,17]. Pipercolic acid was even more markedly elevated in CSF. The extent of pipercolic acid elevation in CSF exceeded that of plasma by a factor of 2.2 to 4.8 [32]. Sadiilkova et al. [94] found pipercolic acid in plasma within normal range in one patient while on pyridoxine. This is in line with data from Plecko et al. [97] where single patients showed pipercolic acid levels within the upper range of normal while on treatment with pyridoxine.

Pretreatment plasma pipercolic acid levels decrease upon treatment with pyridoxine but usually remain moderately elevated [14,32]. Elevated urinary excretion of pipercolic acid has been found in four neonates with finally confirmed PDE and ATQ deficiency [14,15]. However, urinary pipercolic acid is not considered a reliable diagnostic marker as excretion may be unremarkable in older children and or normalize during treatment [14,97].

### 8.3. Peak X

Peak X originally described in folinic acid responsive seizures that is present on monoamine analysis using HPLC with electrochemical detection, is also present in all patients with PDE currently analyzed. A summary of diagnostic markers, methods and preferred body fluids is given in Table 1.

## 9. Screening and diagnosis

### 9.1. Patients at risk

The availability of diagnostic markers in urine and plasma makes low threshold screening possible. Typically, patients with unexplained early onset epilepsy poorly responsive to pharmacological treatment should be screened. As already recommended by Goutières and Aicardi [52], pyridoxine dependency should be considered as the cause of intractable seizures in the following situations:

- Seizures of unknown etiology in a previously normal infant without an abnormal gestational or perinatal history
- The occurrence of long-lasting focal or unilateral seizures
- Signs of encephalopathy (irritability, restlessness, crying, and vomiting) preceding the actual seizures
- A history of a severe epilepsy in a sibling, often leading to death during status epilepticus
- Parental consanguinity

In order not to miss milder and atypical presentations the following patients should also be considered for screening:

- Infants and children with seizures which are partially responsive to pharmacological anticonvulsive drugs (e.g. phenobarbital), in particular if associated with developmental delay and intellectual disability
- Neonates with hypoxic ischemic encephalopathy and difficult to control seizures.
- Patients with a history of transient or unclear response to pyridoxine
- Patients with a history of response to folinic acid and or with the characteristic unidentified peak X on CSF monoamine analysis
- Seizures in any child under the age of 1 year without an apparent malformative cause.

### 9.2. Biochemical tests

As a first step patients at risk should be tested for  $\alpha$ AASA (urine or plasma) and pipercolic acid (plasma). As both urinary and plasma  $\alpha$ AASA and plasma pipercolic acid are informative in both the untreated and treated states, initiation of therapy with pyridoxine

**Table 1**  
Laboratory findings in ATQ deficiency.

Finding		Comment
Diagnostic markers	AASA/P6C (U,P,CSF)	Due to spontaneous equilibrium between AASA and P6C quantitative values may not be accurate
Informative irrespective of treatment status	Pipecolic acid (P, CSF)	Plasma levels decrease but do not normalize upon pyridoxine treatment Age dependent normal values need to be taken into account in the interpretation of results Single patients beyond infancy may show plasma levels within the upper normal range while on treatment Can be elevated in peroxisomal disorders or liver disease
Diagnostic markers	Pipecolic acid (U)	Urine levels normalize upon treatment.
Not informative upon pyridoxine treatment		Age dependent normal values need to be taken into account in the interpretation of results
Disease-related markers	Peak X (peaks of unidentified compounds) (CSF)	Unidentified peak upon HPLC monoamine neurotransmitter analysis characteristic of ATQ deficiency
	PLP (CSF)	Low in untreated state. Secondary deficiency due to chemical reaction with P6C. PLP level alone does not allow distinction to PNPO deficiency.
Non-specific findings	Aminoacids (P,CSF)	Attributed to secondary PLP deficiency (see text). Can also be seen in PNPO deficiency.
Observed occasionally, reported in individual patients	Threonine, glycine, taurine, histidine, 3-methyltyrosine, alanine, glutamine	
	Lactic acidemia	Mimicking gluconeogenesis defects, may resolve upon IV glucose.
	Hypoglycemia	
	GABA, glutamate (CSF)	Low GABA and elevated glutamate most likely caused by impaired glutamate decarboxylase activity due to secondary PLP deficiency
	Electrolyte imbalance	Underlying mechanism unclear
	Diabetes insipidus	
	Hypothyroidism	

AASA/P6C = alpha amino adipic semialdehyde/L- $\Delta^1$ -piperidine 6-carboxylate; U = urine; P = plasma; CSF = cerebrospinal fluid; PLP = pyridoxal phosphate.

should not be delayed for diagnostic purposes, and diagnostic samples can be taken any time before and after treatment. Because  $\alpha$ AASA is unstable, samples should be frozen soon after collection. As only specificity, but not yet sensitivity has been established for both markers, ideally both biomarkers should be determined. This particularly applies to cases with atypical phenotypes. Determination of  $\alpha$ AASA is still limited to few laboratories worldwide. Thus in areas where access to this analysis is difficult, plasma pipecolic acid may serve as a first screening marker and  $\alpha$ AASA is determined in cases with negative or equivocal pipecolic acid results and persisting suspicion of ATQ deficiency. It should be taken into account that pipecolic acid levels decrease upon treatment with pyridoxine. Therefore, levels close to the upper normal range still should be considered potentially abnormal, and followed up with testing with a better specificity. If the pipecolic acid is highly elevated, very long chain fatty acid analysis should be considered to rule out peroxisomal disorders. Most patients receive pharmacologic anticonvulsants and pyridoxine at the same time. As in such situations where improvement or resolution of seizures cannot be clearly attributed to any specific intervention, pyridoxine should be continued until biomarker results are available.

### 9.3. Mutation analysis

Mutation analysis of the ATQ gene is recommended to confirm the diagnosis.

**Table 2**  
Diagnostic tests for ATQ deficiency.

Test	Material	Handling	Pitfalls/interpretation
AASA	U	Random urine sample Store at minus 20 degrees Celsius and ship frozen	Semiquantitative
AASA/P6C	P	Random plasma sample Store at minus 20 degrees Celsius and ship frozen	AASA and P6C are simultaneously quantified
Pipecolic acid	P	Random sample Store at minus 20 degrees C and ship frozen	Levels decrease upon pyridoxine treatment, but usually remain above normal values. Age dependent differences of normal values need to be considered in the interpretation of data Good sensitivity but low specificity
ATQ mutation analysis	Extracted DNA EDTA blood		Large deletions or duplications are not detected by standard sequencing methodology

AASA/P6C = alpha amino adipic semialdehyde/L- $\Delta^1$ -piperidine 6-carboxylate; U = urine; P = plasma; CSF = cerebrospinal fluid.

Mutation analysis should be performed

- in cases with abnormal  $\alpha$ AASA or pipecolic acid screening results
- in case of unclear  $\alpha$ AASA or pipecolic acid screening results
- in patients with clear evidence of pyridoxine and/or folinic acid responsiveness, but normal biomarkers, or if biomarker screening is not possible.

Exons 4, 6, 9, 11 and 14 harbor 60% of reported mutations in Caucasian patients and are targets for an initial screen in Caucasian patients [22]. If sequencing does not reveal point mutations, molecular testing for deletions should be performed. If mutations of unknown significance are found, expression studies are the ultimate confirmatory tool. ATQ enzymology is possible in fibroblast homogenates, but currently this assay has not been established for diagnostic purpose (E.S. work in progress). An overview of biochemical and molecular genetic diagnostic tests is shown in Table 2.

### 9.4. Diagnostic value of response to intravenous administration of pyridoxine

Recently Bok et al. [102] have shown that in neonates with therapy-resistant seizures, intravenous pyridoxine can induce non-specific EEG responses that neither identify nor exclude PDE. Clinical and EEG responses were observed both in patients with confirmed PDE and without PDE. At the same time not all patients with PDE immediately showed the expected responses. The authors suggest

that neonates with therapy resistant seizures should receive oral pyridoxine until PDE is fully excluded by biochemical or mutation analysis.

### 9.5. Pyridoxine withdrawal

A diagnostic withdrawal of pyridoxine to demonstrate recurrence of seizures, that are again responsive to pyridoxine, had been recommended prior to the time when biomarkers were recognized. Now with the availability of biomarkers, this clinical diagnostic approach has become obsolete in most cases. In cases however where ATQ deficiency could not be confirmed on a biochemical and molecular level, a pyridoxine withdrawal might be needed to obtain directions for the workup of alternative differential diagnoses.

### 9.6. Prenatal diagnosis

Prenatal diagnosis is possible by mutation analysis in chorionic tissues and amniocytes.  $\alpha$ AASA was increased in amniotic fluid (collected at delivery) in one affected pregnancy, and findings were also confirmed by mutation analysis (B.P. and E.S. unpublished).

## 10. Treatment of ATQ deficiency

### 10.1. Pyridoxine

The standard treatment of ATQ deficiency includes lifelong supplementation of pyridoxine in pharmacological doses.

In the acutely seizing infant, an initial dosage of pyridoxine should be given without delay. For patients in the ICU setting pyridoxine can be administered intravenously, under EEG monitoring and with adequate support for respiratory management in case apnea occurs as an immediate treatment response. If EEG monitoring is not instantly available, the trial is done without EEG monitoring in order not to delay possible effective treatment. A dose of 100 mg of pyridoxine-HCl should be given intravenously. Additional doses may be administered over the course of 30 min while observing for both a clinical and possible electrographic response. As intravenous pyridoxine can cause apnea, IV administration should not be performed outside an ICU setting or without readiness to intubate. If IV administration of pyridoxine is not possible for a first trial, pyridoxine is given orally/enterally with 30 mg/kg/day. Staff has to be aware that apnea requiring intubation has even been observed in single patients after a first oral administration of pyridoxine. Treatment response might be delayed or masked with concomitant pharmacological treatments, and therefore a longer trial is needed. A delayed response upon continued oral pyridoxine administration is also possible [16,29]. Thus oral/enteral treatment with pyridoxine should be continued at least for several days, ideally until ATQ deficiency is excluded by negative biochemical or genetic testing.

For long term treatment there are no clear-cut dosing recommendations at present. The physiological recommended daily allowance (RDA) for pyridoxine, covered by regular diet, is 0.5 mg for healthy infants and 2.0 mg for healthy adults. In most patients with PDE, therapeutic pyridoxine dosages vary between 15 and 30 mg/kg/day in infants or up to 200 mg/day in neonates and 500 mg/day in adults. These dosages seem to be safe in long-term treatment. Higher pyridoxine doses may cause sensory and rarely also motor neuropathy, which may be reversible [23,103]. Some patients experience breakthrough seizures during febrile illness. In these cases, higher (e.g. double) dosages may be given during the first 3 days of febrile illnesses.

Nerve conduction studies are performed to exclude sensory neuropathy as an adverse effect of pyridoxine treatment. This test is performed upon clinical evidence of neuropathy, or as a regular monitoring (e.g. annually), in particular if high doses (>500 mg/day

or >30 mg/kg/day) are used. Brain MRI/MRS to monitor cerebral myelination and metabolism are performed as needed.

### 10.2. Pyridoxal phosphate

Some patients with medically intractable epilepsy and unresponsiveness to pyridoxine respond to the administration of pyridoxal phosphate (PLP), the active form of vitamin B<sub>6</sub>. In most of them PNPO deficiency has been identified as the underlying genetic condition, [104,105] (see “other vitamin B<sub>6</sub> responsive conditions”), but idiopathic PLP response occurs as well [106]. As PLP has the potential to treat PNPO as well as ATQ deficiency, some centers advocate the use of PLP (30 mg/kg/day divided into 3 dosages), as the first line vitamin B<sub>6</sub>, while other centers advocate the consecutive use when pyridoxine, given over three consecutive days, has failed to control seizures [104]. In any case treatment should be continued until results from appropriate metabolic investigations in urine, blood and CSF are available. Both vitamers (pyridoxine and PLP) are relatively inexpensive. Still PLP tablets are unlicensed outside Japan, cost six to ten times more than pyridoxine products, and are less readily available in North America. Therefore, from a practical standpoint, local availability of PLP may determine how this particular vitamer will be used and studied [107]. As shown in animal experiments, high PLP concentrations in the brain can lead to convulsions [108]. Although such effects have not been observed in humans, adverse effects of high pyridoxine or PLP dosages should be taken into consideration in particular if there is a deterioration of seizures upon supplementation.

### 10.3. Folinic acid

Some patients who were later proven to have ATQ deficiency, but had an unclear response to pyridoxine, have shown response to folinic acid. Although the mechanism underlying folinic acid responsiveness in ATQ deficiency has not been elucidated, folinic acid (3–5 mg/kg/day) may have potential benefit as an add-on treatment in neonates, especially in the presence of incomplete pyridoxine responsiveness or of breakthrough seizures. In older patients 10–30 mg/day should be tried [16]. It is unknown whether long-term folinic acid is of benefit once the seizures are stabilized. High dose folinic acid therapy can also exacerbate a seizure disorder, and the clinical benefit has to be closely monitored.

### 10.4. Prophylactic pre-and postnatal treatment

There is a 25% recurrence risk for PDE in subsequent pregnancies. Prenatal treatment of an at-risk fetus with supplemental pyridoxine given to the mother during pregnancy may prevent intrauterine seizures and improve neurodevelopmental outcome [49]. Prenatal treatment with high dose pyridoxine followed by postnatal treatment was effective to prevent seizures, but did not prevent poor cognitive outcome in two affected offspring in a family with a homozygous stop codon in exon 14 (Y380X) [23]. In contrast, a Dutch group reported good developmental outcome after prenatal treatment in three patients homozygous for the missense mutation (E399Q) [109]. In these pregnancies, pyridoxine was given to the pregnant women at a dosage of 100 mg/day from early pregnancy. The dosage of 100 mg pyridoxine/day seems to be safe, as it has been used for the treatment of emesis gravidarum without fetal side effects [5].

If intrauterine molecular genetic testing is done, preferably in chorionic villi collected at gestational week 11 or 12, and ATQ mutations have been ruled out, antenatal treatment should be stopped. If intrauterine diagnosis is not attempted, continuation of antenatal treatment and, following delivery, postnatal prophylactic treatment may be considered. Adverse effects, including increased seizure activity [110] have been seen in cases with high pyridoxine or PLP intake [111]. A neonate with positive family history was on



prophylactic intrauterine treatment with pyridoxine. No prenatal diagnosis was performed and treatment continued postnatally until ATQ deficiency was ruled out. On day 15, still on treatment, the patient developed status epilepticus and encephalopathy. His condition only improved after pyridoxine treatment was discontinued at the time ATQ deficiency had been ruled out [112].

Thus biochemical (plasma pipercolic acid and urinary  $\alpha$ AASA) and genetic testing should be aimed for as soon and as quickly as possible in order to limit unnecessary high dose treatment with pyridoxine.

An overview of currently practiced treatments, based on supplementation of pharmacological dosages of vitamers, and for treatment monitoring is given in Tables 3 and 4.

### 10.5. Evidence and safety of high dose pyridoxine/PLP treatment

The accumulation of P6C and its condensation with PLP cause chemically induced pyridoxine depletion. Therefore, from a pathophysiological point of view, high pharmacological dosages of pyridoxine are needed to exceed ongoing condensation with P6C and increase the intracellular proportion of free PLP. This rationale does not explain why a few patients respond to very low dosages for pyridoxine. In the original PDE case [1] physiologic dosing of pyridoxine was therapeutic if used consistently. Controlled clinical studies to evaluate the optimal dose and shed light into adverse effects of high dose pyridoxine and PLP treatment have not yet been performed. In addition, there may be substantial inter-patient differences in the required effective dose related to the underlying ATQ mutation, or to other genetic causes, or to environmental influences such as the patient's lysine intake.

### 10.6. Lysine restricted diet

Pyridoxine supplementation aims at correction of PLP related pathophysiology. With the discovery of ATQ deficiency as the underlying cause, it has become clear that PDE is an organic aciduria by nature. Consequently, reduction of accumulating, potentially neurotoxic organic acids, such as  $\alpha$ AASA and related compounds, via a lysine restricted diet has become an option for additional treatment. To our knowledge less than 10 patients with ATQ deficiency so far have been treated with a lysine restricted diet. Intake of dietary

**Table 4**  
Treatment monitoring.

Supplementation of pyridoxine or PLP	Therapeutic effects	Seizures: Clinical and EEG Psychomotor/speech development: <4 years: Bayley Scale; >4 years: Kaumann ABC, Wechsler
	Side effects	Peripheral neuropathy: NCV Upon clinical evidence of neuropathy or as regular monitoring (e.g. annually) in particular if high doses (>500 mg/day or >30 mg/kg/day) are given

PLP = pyridoxal-phosphate; NCV = nerve conduction velocity.

lysine, and of a lysine free amino acid formula was calculated according to recommendations for treatment of glutaric aciduria type 1, another cerebral organic aciduria affecting the lysine pathway [113]. Catch-up of developmental delay and improvement of behavior, and a decrease in plasma pipercolic acid and in urinary  $\alpha$ AASA excretion have been observed upon treatment. Biochemical results have to be interpreted with caution because age dependent variations of both plasma pipercolic acid and urinary  $\alpha$ AASA and lack of exact quantification of  $\alpha$ AASA might be confounders. While the rationale for a lysine restricted diet is clear, the clinical effect on the outcome in ATQ deficiency still has to be documented before this can be universally recommended. Lysine restricted diets have the potential for side effects and risks, and impose a burden on the patient and family. This will have to be weighted to the evidence for the effect on ultimate outcome of seizure control and more importantly neurodevelopmental outcome. Given the wide spectrum of outcome in non-diet treated patients and the near impossibility of a placebo control in such dietary intervention, good stratification of patients and solid outcome measures both biochemically and cognitively will be required to document the effect of the dietary intervention.

## 11. Other vitamin B<sub>6</sub> responsive conditions

In addition to ATQ deficiency, three other autosomal recessive conditions with seizures responsive to pyridoxine or its vitamers are

**Table 3**  
Treatment of ATQ deficiency.

Medication	Route	Dosage	Indication	Monitoring	Side effects/precautions
Pyridoxine	IV	100 mg Single dosages	Interruption of initial status epilepticus, or of prolonged breakthrough seizures	EEG if available	May result in respiratory arrest. Administer upon availability of respiratory support
Pyridoxine	Oral/enteral	15–30 mg/kg/day Div in up to 3 single doses Up to 200 mg/day in neonates, and 500 mg/day 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/day	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. Consider IV pyridoxine in case of neonatal seizures.	Continue oral/enteral pyridoxine supplementation at 30 mg/kg/day immediately after birth and immediately initiate biochemical and molecular genetic investigations to prove or rule out ATQ deficiency.
Pyridoxal-phosphate	Oral/enteral	30 mg/kg/day divided in up to 3 single dosages	Interruption of initial status epilepticus: additional to IV pyridoxine in case pyridoxine initially failed to control seizures Long term treatment: Alternative to pyridoxine	Same as pyridoxine	Same as pyridoxine
Folinic acid	Oral/enteral	3–5 mg/kg/day divided in up to 3 SD	Additional therapy if pyridoxine or PLP failed to control seizures	No particular monitoring	None

IV = intravenous; SD = single dosage.

known [111,114]: Pyridoxal phosphate responsive epileptic encephalopathy (MIM#610090), caused by deficiency of Pyridoxamine 5'-phosphate oxidase deficiency (PNPO) (MIM603287), tissue non-specific alkaline phosphatase (TNSALP) deficiency (MIM#171760), and hyperprolinemia type II (MIM#239510). Mabry Syndrome (familial hyperphosphatasia with mental retardation, seizures and neurologic deficits) [115,116] is another condition with potentially pyridoxine responsive seizures [117]. A defect in PIGV (phosphatidylinositol glycan anchor biosynthesis class V) has been identified as the underlying gene defect [118]. An overview of clinical and biochemical features of pyridoxine-dependent and PLP-responsive seizures is shown in Table 5. By now ATQ deficiency seems to be the most frequent among these conditions, but exact incidence data are missing.

Pathophysiologically, PNPO and TNSALP deficiencies directly affect the conversion of B<sub>6</sub> vitamers to intracellular PLP. In ATQ deficiency and hyperprolinemia type II accumulating metabolites (P6C and P5C respectively) form a complex with intracellular PLP [15,84]. Similarly, chemical inactivation is the mechanism of action responsible for isoniazid induced pyridoxine deficiency, as this tuberculostatic drug combines with pyridoxine generating inactive isoniazid-pyridoxal hydrazones, thus depleting the supply of intracellular PLP [119,120].

Various cases with atypical PDE have not shown ATQ sequence alterations, elevated levels of biochemical markers, or linkage to the 5q31 locus [19,121,122]. One phenotype that can be distinguished, and is not related to ATQ deficiency, presents with infantile spasms. In four late onset cases including two siblings, and a clear response to pyridoxine, mutations were not detected in either allele, although in one case pipercolic acid was elevated (19; Gospe S, unpublished). Thus other gene defects causing PDE like presentations are still to be identified.

Apart from these clearly defined monogenic defects, pyridoxine may also have a non-specific therapeutic effect in patients with various types of cryptogenic and symptomatic epilepsies. In particular patients with infantile spasms or other catastrophic epileptic conditions may show a favorable response to treatment with pyridoxine in addition to conventional pharmacologic therapy [123–127].

Nutritional vitamin B<sub>6</sub> deficiency is rare, but can be seen in severely malnourished children or with severe underlying disease. Nutritional vitamin B<sub>6</sub> deficiency is systemic and besides epileptic seizures is associated with variety of systemic manifestations, foremost failure to thrive, anemia and eczema [128–130].

## 12. Future perspectives

Due to limited awareness by clinicians of PDE and especially ATQ deficiency as a potentially treatable, albeit rare, cause of epilepsy, it may still be under-diagnosed. Given the efficacy of treatment with pyridoxine, one may even consider it a candidate condition for newborn screening once the analytical issues in identification have been resolved. Inherent to their rarity, patients are scattered throughout the world hampering systematic studies. Therefore, knowledge dissemination and international collaboration are required to obtain data in a time-efficient and reliable manner. There is also a need for a multicenter study to collect relevant information on biochemical and clinical outcomes of PDE patients. This will allow systematic evaluation of the pyridoxine dosages and of the effect of a lysine-restricted diet on seizure control, behavior and neurodevelopmental outcome, as well as possible side-effects, and may reveal relevant biomarkers for monitoring. Application of innovative methodologies applicable to small patient numbers will help to find evidence for the currently available and new treatments for ATQ deficiency. The creation and maintenance of a disease specific website may foster translation of obtained knowledge into useful diagnostic and therapeutic tools for physicians in the clinical setting as well as for patients and families.

## 13. Summary points

- Antiquitin deficiency is the main cause of pyridoxine dependent epilepsy. The prevalence of PDE is unknown with estimates varying from 1:20.000 infants with epileptic encephalopathy to 1:600.000 in patients in the UK.
- PDE caused by ATQ deficiency is characterized by intractable or difficult to treat seizures that are poorly controlled with pharmacologic anticonvulsants but that respond clinically and electroencephalographically to large dosages of pyridoxine. Seizures recur upon pyridoxine withdrawal.
- Typically, onset is in the neonatal period or shortly thereafter with irritability, status epilepticus sometimes mimicking hypoxic ischemic encephalopathy. Atypical presentations include cases with onset of seizures of up to 3 years, partial responsiveness to pharmacological anticonvulsants, and delayed response to pyridoxine treatment.
- Despite seizure control most patients have intellectual disability.

**Table 5**  
Monogenic defects causing vitamin B6 responsive/dependent epilepsies.

Gene defect	PDE (ATQ deficiency)	PLP responsive epileptic encephalopathy (PNPO deficiency)	Hypophosphatasia (TNSALP deficiency)	Familial Hyperphosphatasia (PIGV deficiency)	Hyperprolinemia type 2 (P5CD deficiency)
Gene function	Lysine degradation	Extracellular PLP formation	Bone mineralization, cellular pyridoxal uptake	Glycosyl phosphatidyl inositol anchor biosynthesis	Proline degradation
Clinical presentation	Neonatal/infantile epileptic encephalopathy	Neonatal epileptic encephalopathy	Osteomalacia, hypercalcemia, hypo phosphatemia, in severe forms also neonatal epileptic encephalopathy	DD/ID, seizures, dysmorphic facial feature, brachytelephalangy	Developmental delay, intellectual disability, seizures, mild ataxia
Diagnostic/biomarkers	U-AASA, P-Pip P-AASA, P-P6C	Urinevanillyl-lactate; CSF HVA, HIAA, Threonine, Glycine	P-ALP low, P-PLP high, U-Phosphatidyl-ethanolamine high	P-ALP high	P-Proline, U-P5C
Treatment	Pyridoxine; (low lysine intake?)	Pyridoxal-phosphate	Pyridoxine	Pyridoxine	Pyridoxine
Outcome on treatment	(Near) complete seizure control, DD/ID	Improvement of seizures, severe DD	Seizure control, (lethal) bone disease	Seizure control	Non-progressive DD/ID, occasional seizures

ALP = Alkaline phosphatase; ATQ = Antiquitin; CSF = Cerebrospinal fluid; DD/ID = Developmental delay/Intellectual disability; HIAA = Hydroxyindole acetic acid; HP1 = Hyperprolinemia type 1; HVA = Homovanillic acid; P-AASA = plasma  $\alpha$ -amino adipic semialdehyde; PIGV = Phosphatidylinositol glycan anchor biosynthesis type V; PLP = Pyridoxal-phosphate; PNPO = Pyridox(am)ine-phosphate oxidase; P-Pip = Plasma pipercolic acid; P5C = Pyrroline 5-carboxylate; P5CD =  $\Delta^1$ -pyrroline 5-carboxylate dehydrogenase; P-P6C = plasma  $\Delta^1$ -piperideine-6-carboxylate; TNSALP = Tissue non specific alkaline phosphatase; U-AASA = urinary  $\alpha$ -amino adipic semialdehyde.

- Folinic acid responsive seizures (FARS) are genetically identical to ATQ deficiency.
- ATQ functions as an aldehyde dehydrogenase (ALDH7A1) in the lysine degradation pathway and catalyzes the conversion of  $\alpha$ -amino adipic semialdehyde ( $\alpha$ AASA) to  $\alpha$ -amino adipic acid.  $\alpha$ AASA is in chemical equilibrium with P6C.
- Biochemically, ATQ deficiency is characterized by accumulation of  $\alpha$ AASA and P6C and by accumulation of pipercolic acid, which is formed proximal to the primary enzyme defect.
- Current known elements of the pathophysiology of ATQ deficiency are secondary PLP deficiency due to chemical inactivation of PLP via a condensation reaction with P6C, and possibly primary toxicity of pipercolic acid and of  $\alpha$ AASA and the P6C/PLP complex.
- Screening for ATQ deficiency is possible via determination of urinary or plasma  $\alpha$ AASA and P6C, and of plasma pipercolic acid. Diagnosis is confirmed by mutation analysis.
- Screening should be performed in neonates, infants and older children with unexplained intractable or poorly controlled seizures, particularly if in conjunction with encephalopathy, long lasting focal seizures and status epilepticus. With the availability of biomarkers in urine and blood, patients with later onset, and milder and atypical courses should be considered for screening in particular if parents are consanguineous and if there is a history of partial, transient or poorly documented pyridoxine responsiveness.
- To interrupt seizures pyridoxine-HCl is given intravenously with 100 mg, or orally/enterally with 30 mg/kg/day. First administration may result in respiratory arrest in responders, and thus treatment should be performed with support of respiratory management.
- In case of unclear response, and to make sure late and masked response is not missed, treatment with oral/enteral pyridoxine should be continued until ATQ deficiency is excluded by negative biochemical or genetic testing.
- Oral or enteral pyridoxal phosphate (PLP), up to 30 mg/kg/day can be given alternatively. This would make certain that treatment would not be delayed in patients with PNPO deficiency, who are responsive to PLP only.
- Long-term treatment dosages vary between 15 and 30 mg/kg/day in infants or up to 200 mg/day in neonates, and 500 mg/day in adults.
- Folinic acid may have an additional benefit as an add-on treatment.
- Prenatal treatment with maternal pyridoxine supplementation possibly improves outcome.
- PDE is an organic aciduria caused by a deficiency in the catabolic breakdown of lysine. Pyridoxine treatment does not address the potential toxicity of accumulating  $\alpha$ AASA, P6C and pipercolic acid. While the rationale for a lysine restricted diet is clear, the clinical effect on the outcome in PDE still has to be documented before this can be universally recommended. Lysine restricted diets have the potential for side effects and risks, and impose a burden on the patient and family.
- Multicenter studies comparing the outcomes of patients with standard treatment and lysine restricted diet are needed to determine the evidence of this additional treatment.
- Other pyridoxine or PLP responsive seizure disorders include PLP-responsive epileptic encephalopathy due to PNPO deficiency, neonatal/infantile hypophosphatasia (TNSALP deficiency), familial hyperphosphatasia (PIGV deficiency), as well as yet unidentified conditions and nutritional vitamin B6 deficiency.
- A technically more simple diagnostic method for the measurement of biomarkers with high sensitivity and specificity such as AASA or P6C would facilitate diagnosis and newborn screening for this treatable condition.

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